



**Effects of livestock management and insecticide treatment
on the
transmission and control of human malaria**

by

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**In memory of
Clive Davies**

Declaration by Candidate

I, Ana Isabel de Oliveira Franco, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed:..... Date: 13/10/2019.....

Abstract

This thesis aims to clarify the different effects of livestock on human malaria in areas where the disease is transmitted by zoophilic mosquito vectors, to understand under which circumstances livestock-based interventions could contribute to malaria control. Namely, the impact of livestock abundance, availability and insecticide-treatment (ITL) were explored, by developing a comprehensive deterministic mathematical model and integrating it with data from Pakistan, where an ITL trial for malaria control has been performed, and from Ethiopia, where I conducted a field study to parameterize the model. The model allows explaining situations where livestock by itself can lead to an increase, decrease or no impact at all on malaria transmission, by combining the effects of livestock on decreasing the human blood index, while decreasing vector mortality and increasing vector density. The key explanatory factors are the: abundance and availability of livestock and human hosts, vector density in relation to the system's carrying capacity before livestock introduction, and time elapsed since livestock introduction. The overall findings indicate that ITL is likely to produce stronger decrease in malaria in settings with highly zoophilic vectors as in Asia, than in African settings with the more opportunistic vector *An. arabiensis*. Nevertheless, the results suggest that ITL is still likely to substantially decrease malaria incidence in the latter settings. The work highlights the importance of accounting for potential excito-repellency effects of the insecticide upon vectors, although only if excito-repellency is very strong would ITL become prejudicial. It is also important to understand the density-dependent regulation operating in the vector population, given its determinant effects upon the intervention outcome. It is hoped that this work may pave the way for the implementation of an ITL intervention trial in an African region with *An. arabiensis* where this strategy could contribute to the integrated control of malaria and livestock diseases.

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Glossary of Terms and Abbreviations

ACT	Artemisin Combination Therapy
CI	Confidence Interval
CQ	Chloroquine
DD	Density-Dependent
DDT	Dichloro-Diphenyl-Trichloroethane
DFE	Disease Free Equilibrium
EIR	Entomological Inoculation Rate (number of potentially infectious mosquito bites received by a human per unit time)
Eq.	Equation
Fora	Ethiopian Oromo pastoralist term for a cattle herd managed in satellite camps
GIS	Geographic Information System
GM	Geometric Mean
HBC	Human-Biting Catches
HBI	Human Blood Index (proportion of mosquito blood-meals on humans)
HBR	Human-Biting Rate (number of mosquito bites received by a human per night)
ITL	Insecticide-Treated Livestock (or Insecticide Treatment of Livestock)
ITN	Insecticide-Treated Net
KDA	Konso Development Association
kdr	Pyrethroid knockdown resistant gene
Kebele	Ethiopian administrative unit corresponding to a neighbourhood or group of villages
KHC	Karat Health Centre
LBI	Livestock Blood Index (proportion of mosquito blood-meals on livestock)
LLIN	Long-Lasting Insecticidal Net
LSHTM	London School of Hygiene and Tropical Medicine (London, UK)
MYHS	Mekane Yesus Health Station
OBET	Odour-Baited Entry Trap

Glossary of Terms and Abbreviations (cont.)

ODE	Ordinary Differential Equation
OR	Odds Ratio
PF	<i>Plasmodium falciparum</i>
PQ	Primaquine
PV	<i>Plasmodium vivax</i>
R₀	Basic reproduction number
RBM	Roll Back Malaria
s	Sporozoite prevalence: proportion of mosquitoes that are infectious, i.e. containing sporozoites in their salivary glands
SP	Sulfadoxine–Pyrimethamine
WHO	World Health Organization

State Variables used in the Malaria Model

Symbol	Definition
S_h	Number of susceptible humans
I_h	Number of infected humans
N_h	Total human population size
S_v	Number of susceptible vectors
L_v	Number of infected latent vectors
I_v	Number of infectious vectors
N_v	Total vector population size
U_l	Number of untreated livestock
T_l	Number of insecticide-treated livestock
N_l	Total livestock population size

Vector=adult female anopheline mosquitoes.

The subscripts h , v and l denote the human, vector and livestock populations, respectively.

Parameters used in the Malaria Model

1. Malaria transmission parameters

Symbol	Definition
D_{inf}	Average duration of infectiousness in humans
r	Human daily recovery rate from infectiousness ($=1/D_{inf}$)
T_{lat}	Duration of latent period in vectors (time from ingestion of gametocytes to presence of sporozoites in salivary glands)
ω	Daily rate at which latent mosquitoes become infectious ($=1/T_{lat}$)
b	Probability that humans become infected from the bite of an infectious vector
c	Probability that vectors become infected after biting on an infectious human
g	Average duration of vector gonotrophic cycle (i.e. average interval between vector blood-meals on any host)
a	Vector daily biting rate on any host ($=1/g$)
Pr	Proportion of parous vectors
p	Probability of daily vector survival ($=Pr^{1/g}$)
$surv$	Overall average vector life expectancy (days) ($=-1/\ln(p)$)
μ	Overall average vector daily mortality rate ($\mu = \mu_{min} + \mu_{search} + \mu_{txonly}$)
x	Proportion of the vector natural mortality that is unrelated with searching for a bloodmeal host
$surv_{max}$	Vector maximum average life expectancy when there are no hazards due to search for a bloodmeal host and no vector control intervention.
μ_{min}	Vector daily minimum mortality rate when there are no hazards due to search for a blood-meal host and no vector control intervention. ($=1/surv_{max}$)
μ_{search}	Vector daily mortality due to searching for a blood-meal host
μ_{txonly}	Vector daily mortality due to the lethal effect of insecticide applied on livestock
$surv_{notx}$	Vector natural average life expectancy; i.e. in absence of insecticide treatment of livestock
μ_{notx} or μ_0	Vector daily natural mortality rate; i.e. in absence of insecticide treatment of livestock ($= 1/ surv_{notx} = \mu_{min} + \mu_{search}$)
$surv_{nolive}$	Vector survival in absence of available livestock

1. Malaria transmission parameters (cont.)

Symbol	Definition
μ_{motive}	Vector daily natural mortality rate, in absence of available livestock
ρ	Vector daily average recruitment rate
ρ_0	Vector recruitment rate in the absence of density-dependence constraints
ρ_s	Strength of the density-dependence in recruitment ($= (\rho_0 - \mu) / K$)
<i>delay</i>	Time (days) from oviposition to the emergence of adult vectors (time-delay introduced to overcome circularity when calculating ρ_s)
$N_v(t)$	Initial number of vectors, prior to change in livestock abundance and/or availability
<i>n</i>	Initial relative density of vectors:humans ($= N_v / N_h$)
<i>K</i>	Carrying capacity of the vector population
rN_l	Relative density of livestock:humans ($= N_l / N_h$)
rA_l	Relative availability of livestock:humans ($= A_l / A_h$)
A_l	Proportional availability of livestock to vectors
A_h	Proportional availability of humans to vectors
<i>j</i>	Factor to scale the proportional availability (into absolute availability) values
<i>q</i>	Proportion of vector feeds on humans (Human Blood Index) The remaining, $1 - q$, are feeds on livestock
a_{iind}	Proportion of hosts of type <i>i</i> that is accessible to the vector indoors ¹
a_{iout}	Proportion of hosts of type <i>i</i> that is accessible to the vector outdoors ¹
v_h	Intrinsic preference of the vector for feeding on humans
v_l	Intrinsic preference of the vector for feeding on livestock
v_{in}	Intrinsic preference of the vector for feeding indoors
v_{out}	Intrinsic preference of the vector for feeding outdoors

Vector=adult female anopheline mosquitoes.

The subscripts h, v and l denote the human, vector and livestock populations, respectively.

¹ *i*= humans (h) or livestock (l) hosts.

2. Insecticide treatment of livestock parameters

Symbol	Definition
ϵ_0	<i>Application coverage</i> : Proportion of livestock population treated with insecticide, at each intervention round ($T_{i(t=0)}/N_i$)
ϵ	<i>Effective coverage</i> : proportion of the livestock population that has effective insecticide at a given point in time ($T_{i(t)}/N_i$)
<i>Frequency</i>	Number of treatment rounds
<i>Interval</i>	Interval (days) between rounds
k	Probability that vectors are killed due to exposure to insecticide-treated livestock on the day of treatment
LT	Lethal Time. LT_{50} , LT_{15} , LT_{10} , LT_5 , LT_1 = time (days) from insecticide application until there is only 50%, 15%, 10%, 5% and 1%, respectively, vector mortality due to exposure to insecticide-treated livestock (on bioassay)
$T_{i(LT_{50})}$	Number of animals that have active residual insecticide by day LT_{50}
t_{max}	Time (days) of the “maximum” estimated duration of the insecticide residual effect (on bioassay)
$perc_{max}$	Estimated percentage of vectors exposed to insecticide treated animals that are killed due to the insecticide at t_{max} (i.e. at t_{max} the $\mu_{i,only} = perc_{max}/day$, on bioassay)
d	Daily decay rate of insecticide residual activity (average duration of insecticide residual activity = $1/d$)
d_1	Decay rate of the insecticide residual activity acting until LT_{50}
d_2	Decay rate of the insecticide residual activity acting after LT_{50}
α	Repellence probability: probability that, when attempting to bite an insecticide-treated animal, a mosquito will be diverted to search another animal or human host
ρ_l	Livestock daily recruitment rate
μ_l	Livestock daily removal rate

Chapter 1

General Introduction and Literature Review

Summary

Background: Animals play an important role in the epidemiology of many human diseases, where they can act as a reservoir source for infectious pathogens, and/or a source of blood-meal to disease vectors. The recognition of this relationship has led to the implementation of human disease control strategies targeted at animal populations. These control opportunities have been investigated both empirically and theoretically. Yet, our knowledge on what determines the public health benefits of many of these veterinary interventions remains limited. This PhD thesis addresses this question, focusing on human malaria, where animals can act as an important source of blood-meal to the anopheline mosquito vectors. This insight has led people to propose control interventions based around the use of animals to divert vector biting from humans ('zooprophylaxis') as well as baits to attract vectors to insecticide (insecticide-treated livestock). This thesis aims to clarify the different effects that livestock can have on human malaria in areas where the disease is transmitted by zoonophilic mosquito vectors, in order to understand under which circumstances livestock-based interventions could play a role in malaria control programmes. In particular, this thesis assesses the impact of livestock presence, abundance, and management practices – focusing on insecticide treatment of livestock (ITL) –, through the development of a theoretical framework and its integration with empirical data, from a field study I conducted in Ethiopia and from elsewhere.

Methods: This Chapter sets the basis for the work that was developed through the thesis by reviewing: (1) the use of veterinary interventions to control disease transmission to humans, (2) the use of mathematical models to describe disease transmission dynamics and to investigate the relative effectiveness of different disease control interventions; (3) the global malaria burden and epidemiology, vector behaviour, and prevention and control strategies; (4) empirical and theoretical studies of the impact of untreated and insecticide-treated livestock on malaria; and (5) vector ecology determinants related with the density-dependent regulation of vector populations. An introduction is then

given to the African scenario of the Konso District in Southwest Ethiopia where the field study was conducted. Finally, a summary is given of specific gaps identified in the current understanding of the effects of livestock on malaria, together with how these questions will be addressed in this thesis.

Findings: Despite the large number of empirical studies that have examined the role of untreated livestock on malaria transmission, apparently contradictory results have been obtained, both between and within settings, and no consensus exists about their zooprophylactic effect. The insecticide treatment of livestock appears to be a promising strategy, although much of its potential remains to be uncovered. Mathematical modelling has also emerged as a way of informing field trials and interventions. The Konso District of Ethiopia is an area of typical malaria transmission in Africa, where people's subsistence depends largely on livestock which have occasionally been treated with insecticide to control tsetse flies and ticks. Recent studies in the area have shown that the main malaria vector is *An. arabiensis*, and that it feeds considerably on cattle. Findings from nearby experimental behavioural studies suggested that, under some circumstances, ITL might be effective against *An. arabiensis* and therefore reduce malaria transmission.

Interpretation: A theoretical framework is needed to foster the understanding of the role of both untreated and insecticide-treated livestock on malaria transmission. Namely, a framework is required that allows the translation of findings from settings where livestock-based interventions have been conducted into settings where those interventions have not been tested. The development of such framework is the focus of this thesis. A mathematical model will be developed to allow exploring in detail the potential effects of untreated and insecticide-treated livestock on malaria transmission in various scenarios. The theoretical platform will be applied to hypothetical ecological settings (Chapter 4 and 5) and also to two specific settings (Chapters 5 and 6): in Asia, where a trial of ITL has successfully reduced malaria cases, and in Africa, where malaria burden is the greatest but the impact of ITL on this disease at the community level has not been formally tested yet. The African scenario is the Konso District in Southwest Ethiopia, which was briefly introduced in this Chapter, and where a field study was conducted to parameterize the mathematical model, as described in the next Chapter.

1.1. The One Medicine: Human & Animal Health

We live in a time when the border between the determinants of human and animal health is increasingly fading (Zinsstag and Weiss, 2001). This reality has given birth to “the one medicine” concept, coined two decades ago by the US epidemiologist Calvin Schwabe (1984), to stress the need of an integrated public health and veterinary medical approach to face the present disease threats. Broadly speaking, animals may play either one or all of the following roles in the epidemiology of several of the most important diseases of man. Animals may act as reservoirs and amplifiers of human disease pathogens, may be a source of blood-meals, or may create breeding sites for arthropod vectors of human disease, thereby amplifying the disease vectors.

The human pathogens that are capable of “natural transmission between vertebrate animals and humans” are classified as zoonotic (W.H.O., 1959). It has recently been acknowledged that 61% of all human pathogens are zoonotic (Cleaveland et al., 2001; Taylor et al., 2001). Moreover, zoonotic pathogens are twice as likely as non-zoonotic pathogens to be associated with emerging diseases (Cleaveland et al., 2001; Taylor et al., 2001; Jones et al., 2008). This has been recently illustrated by the emergence of several arthropod-borne diseases, such as: West Nile fever Virus (WNV) in the USA (Asnis et al., 2001), Rift Valley fever virus in east Africa (CDC, 1998) and in the Arabian Peninsula (CDC, 2000a, b), Hendra virus in Australia (Barclay and Paton, 2000), Nipah virus in Southeast Asia (Chua et al., 2000; Enserink, 2004), Japanese encephalitis in Southeast Asia and Australia (Chevalier et al., 2004), and Crimean-Congo hemorrhagic fever in several regions of the world including Africa, southern and eastern Europe, the Middle East, and western Asia (Chevalier et al., 2004). Amongst the non-arthropod-borne diseases, three examples are the recent outbreaks of swine flu, highly pathogenic avian influenza, and severe acute respiratory syndrome (SARS).

Despite the potential problematic role of animals as determinants of disease transmission to humans, animals may also provide a solution, as they can be a target for disease control interventions, and for integrated medical and veterinary surveillance systems. Such effective integration has been exemplified in the UK response to the bovine spongiform encephalopathy (BSE) epidemic in cattle, where stringent measures were taken to decrease the transmission of infection to humans several years before the detection of the first cases of a new variant of Creutzfeldt-Jakob disease (vCJD) (Wilesmith, 1994; Anderson et al., 1996). Another example has been the use of animals

as sentinels (i.e. predictors) of human disease. For instance, sentinel birds have been involved in the surveillance systems for WNV in the US (Eidson et al., 2001; Langevin et al., 2001; Mostashari et al., 2003) and for highly pathogenic avian influenza worldwide (OIE, 2007).

The extent to which veterinary interventions can be used to control the rate of disease transmission to human populations depends partly on the degree to which the animal hosts act as maintenance reservoirs. If animals are the main reservoir of a zoonotic disease, then there are important opportunities for identifying interventions that, through controlling the infection in the animal population, will potentially decrease the disease in humans (Coleman, 2002). For example in the case of rabies, effective control strategies targeted at the animal reservoirs across the developed world has almost eliminated the human cases (W.H.O., 1999). In most of Europe and North America rabies has been virtually eliminated in domestic dogs through widespread dog vaccination and control laws (movement restriction and removal of unvaccinated dogs). The number of human cases has radically decreased accordingly. In these areas the wildlife reservoirs have taken on increased importance. Large-scale oral vaccination programmes in Western European countries have been successful at eliminating the disease in red foxes, as well as in Canada and Texas (USA), targeting coyotes and foxes respectively. In some developing countries (China, Thailand, Sri Lanka and Latin America), the recent implementation of policies for dog vaccination allied to improved post-exposure treatment of humans has also significantly dropped the number of human rabies cases (King, 1998; W.H.O., 2001). Many other examples could be given where veterinary interventions have been used to prevent and/or control zoonotic public health diseases, such as brucellosis, leishmaniasis, African trypanosomiasis (*Trypanosoma brucei rhodesiense*), echinococcosis-hydatidosis, BSE/vCJD, Nipah virus, and avian influenza (Table 1.1), not to mention the broad spectrum of food safety issues, such as leptospirosis, toxoplasmosis, salmonellosis, listeriosis, campylobacteriosis, cryptosporidiosis, trichinellosis, and verotoxin-producing *Escherichia coli* (Collins and Wall, 2004; Schlundt et al., 2004).

Table 1.1. Examples of veterinary interventions used against zoonotic diseases.

Disease	Intervention	(Reference)
Rabies	- Vaccinate dogs and control laws - Vaccinate wildlife (foxes, racoons, coyotes)	(Cleaveland et al., 2006) (Cross et al., 2007)
Brucellosis	- Vaccinate cattle, sheep, goats	(Minas, 2006; Seleem et al., 2009)
Leishmaniasis	- Cull rodents - Cull dogs - Insecticide treatment of dogs	(Faulde et al., 2009) (Xu, 1989; Dietze et al., 1997; Ashford et al., 1998) (Xiong et al., 1994; Maroli et al., 2001; Gavvani et al., 2002; Reithinger et al., 2004; Ferroglio et al., 2008)
Echinococcosis-hydatidosis	- Worm dogs	(Moro and Schantz, 2006; Craig et al., 2007)
BSE/vCJD	- Cull cattle	(Donnelly et al., 1997; Smith and Bradley, 2003)
Nipah virus	- Cull pigs	(Enserink, 1999; Ahmad, 2000)
Avian influenza	- Cull poultry - Vaccinate poultry	(Snacken et al., 1999; Alexander and Brown, 2009) (Capua et al., 2009; Domenech et al., 2009)
African trypanosomiasis	- Chemotherapy and chemoprophylaxis of cattle - Insecticide treatment of cattle	(McDermott and Coleman, 2001)

In addition to the zoonotic scenario, veterinary interventions have also been useful where animals are not a reservoir of the infectious pathogen, but provide a blood-meal source to the human disease vector. Notably, applying insecticide to the surface of livestock has been shown to significantly reduce the incidence of *Plasmodium falciparum* and *P. vivax* malaria (by 56% and 31%, respectively) transmitted by anopheline mosquitoes to humans in Pakistan (Rowland et al., 2001).

The process of deciding which amongst the alternative disease control interventions should be sanctioned is not straightforward. One key component of this process is the evaluation of the relative effectiveness of the available control strategies. Ideally, the assessment should be made on empirical basis, by testing each strategy in a given setting, under a range of intervention regimes. However, in reality such endeavour is often not realistic, due to logistic, time and economic constraints. A complementary tool to evaluate these control opportunities relies on the use of mathematical models describing the disease transmission dynamics within and between human and animal populations.

This thesis will be exploring the different effects that livestock can have in the transmission of human malaria, initially looking at the effects of livestock by itself, and then looking at the potential benefits of the insecticide-treatment of livestock as a tool for malaria control. This will be addressed through the development of a comprehensive mathematical model of malaria transmission and its integration with empirical data from a field study I conducted in Ethiopia, and from elsewhere.

The remaining of this introduction chapter sets the basis for the work that was developed through the thesis, by reviewing: firstly, the use of mathematical models to describe disease transmission dynamics and to investigate the relative effectiveness of disease control interventions; secondly, an overview of the global malaria burden and epidemiology, vector behaviour, and prevention and control strategies; thirdly, empirical and theoretical studies of the impact of untreated and insecticide-treated livestock on malaria, with a brief mention also to other diseases; this is followed by a review of vector ecology determinants related with the density-dependent regulation of vector populations; and finally, an introduction to the African scenario of the Konso District in Southwest Ethiopia, where a field study was conducted to parameterize the mathematical model developed in this thesis.

1.2. Mathematical Models

The first mathematical model of epidemiological processes was developed by Sir Ronald Ross about 100 years ago to explain the transmission of malaria (Ross, 1911). Subsequently, the first model of Ross was followed by several other analytical models on malaria (e.g. Macdonald, 1952; Macdonald, 1957; Molineaux and Gramiccia, 1980; Aron and May, 1982; Bailey, 1982; Gupta et al., 1994; Smith et al., 2006; Smith et al., 2008) and on a diversity of other vector and non-vector borne diseases (see, e.g. Anderson and May, 1991; Gomes et al., 2004; Michael et al., 2007). For instance, mathematical models describing the disease transmission dynamics within and between human and animal populations have been used to clarify the role of animal hosts in the persistence of disease in humans.

Such theoretical frameworks have also been applied to investigate the relative effectiveness of alternative veterinary and/or medical interventions for a range of zoonotic infections. Amongst these, a few models have assessed control strategies for non-vector borne diseases, such as: rabies (Anderson et al., 1981; Dye, 1996; Kitale et al., 2002), BSE/vCJD (Anderson et al., 1996) and tapeworms (Roberts, 1994); while more models have contemplated interventions for vector-borne diseases: West Nile Virus (Thomas and Urena, 2001; Wonham et al., 2004; Bowman et al., 2005; Lewis et al., 2006); Japanese Encephalitis virus (Saul, 2003); Bubonic plague (Keeling and Gilligan, 2000b), Leishmaniasis (Dye, 1996; Burattini et al., 1998; Courtenay et al., 2002; Reithinger et al., 2004); and African Trypanosomiasis (Welburn et al., 2001).

One of the strengths in modelling is that it enables the evaluation of a control strategy under a different set of conditions and scenarios, requiring much less time, logistic and financial effort than experimental or field trials. Moreover, it can provide valuable insights towards the identification of critical parameters for intervention success, thereby informing data collection in empirical trials. On the other hand, by their nature, epidemiological models do not consider all the biological complexities, providing an approximate picture of reality. Apparently, Picasso once said "*Art is a lie that helps us to discover the truth*" (Segel, 1984). Similarly, notwithstanding the simplifications, models can provide important insights into disease transmission dynamics and the relative impact of alternative control interventions (Anderson and May, 1991, pp. 6-8).

Given the limited resources for most disease control programmes, the process of “policy making” typically depends not only on the relative effectiveness of alternative control strategies, but also on the costs and benefits associated with each intervention strategy. Consequently, the need for integrating mathematical models of disease-transmission dynamics with economic models has been increasingly recognized. Such an integrated approach has been illustrated in a recent study to evaluate a brucellosis control program through mass vaccination of livestock, in Mongolia (Roth et al., 2003). A conceptual framework was developed to estimate the campaign cost-effectiveness and economic benefit to human health and the agricultural sector. It was concluded that if the costs of vaccinating livestock against brucellosis were allocated to all sectors in proportion to the benefits, the intervention might be profitable and cost-effective for both the agricultural and public health sectors.

Amongst the wide range of epidemiological models developed to date, only a minority has been dedicated to vector-borne transmission of diseases involving more than one host species. To the best of my knowledge, all of these multi-host models have been developed within the two decades years, and are listed in Table 1.2. Such a framework was first devised for African trypanosomiasis and for Malaria. Other diseases followed, namely: Leishmaniasis, Chagas disease, Bubonic plague, West Nile Virus, Japanese encephalitis, several tick-borne viral and parasitic infections, and African Horse sickness. Most multi-host models are deterministic, except for Bubonic plague where both deterministic and stochastic models have been developed (Keeling and Gilligan, 2000a, b). Nearly all multi-host models considered only two vertebrate hosts (Human and/or Animals), except for one model of Malaria/Japanese encephalitis virus (Saul, 2003) and another of Chagas disease (Cohen and Gurtler, 2001) where three types of blood source hosts were explicitly included.

Table 1.2. Multi-host models of vector-borne diseases transmission.

Disease	Vector	Hosts	(Reference)
African trypanosomiases	Tsetse flies	Humans/Wildlife & Cattle → Wildlife & Cattle → Humans & Cattle →	(Rogers, 1988) (Milligan and Baker, 1988) [§] (Welburn et al., 2001) [§]
Leishmaniasis	Sandflies	Humans & Dogs	(Burattini et al., 1998) [§] ; (Dye, 1996) [§]
African horse sickness	<i>Culicoides</i> midges	Horses & Donkeys/Zebras	(Lord et al., 1996)
Bubonic plague	Fleas	Humans & Rats	(Keeling and Gilligan, 2000a); (Keeling and Gilligan, 2000b) [§]
West Nile Virus	Culicine mosquitoes	Humans & Birds	(Thomas and Urena, 2001); (Bowman et al., 2005) [§]
Japanese encephalitis virus	<i>Culex</i> mosquitoes	Humans [†] & Animal reservoir	(Ghosh and Tapaswi, 1999); (Saul, 2003) [§]
Malaria	Anopheline mosquitoes	Humans & Livestock [†]	(Sota and Mogi, 1989; Killeen et al., 2001); (Saul, 2003; Kawaguchi et al., 2004; Killeen and Smith, 2007) [§]
General models of tick-borne infections	Ticks	Two animal species: A reservoir host and a dead-end host [†]	(Norman et al., 1999; Rosà et al., 2003; Rosà and Pugliese, 2007)
Looping ill virus	Ticks	Red grouse & Hares [†]	(Norman et al., 2004)
Lyme disease	Ticks	Roe deer & Rodents [†]	(Ghosh and Pugliese, 2004)
Chagas disease	Triatomine bugs	Humans & Dogs & Chicken [†]	(Cohen and Gurtler, 2001)

The table lists the vector and hosts populations that were explicitly considered in each model.

[§] Models that incorporate disease control strategies, targeted at the vector and/or host population(s).

[†] Blood-meal host for the vector but that is not infectious (i.e. dead-end host for the disease pathogen).

[‡] The model by Saul 2003 assumes that humans can also be infected by the Japanese encephalitis virus but are not infectious to vectors.

These diseases represent epidemiological scenarios where domestic animals can play different roles. While in malaria the role of livestock is restricted to provision of blood-meals for the disease vectors (host for the vector), in the other multi-host vector-borne diseases modelled, animals act also as a reservoir for disease pathogens (host for the vector and for the pathogen). A particularity occurs with the epidemiology of Chagas disease, where humans and dogs are both a host for the vector and for the disease pathogen, while chickens cannot sustain infection being only a host for the vector (Cohen and Gurtler, 2001). Similarly, the models for tick-borne infections also include two types of animal hosts – a reservoir and another that cannot sustain infection - and, in addition, two of the models also account for the possibility of non-viraemic transmission between ticks while co-feeding on the same host (Rosà et al., 2003; Norman et al., 2004).

This thesis focuses on malaria which, despite continued research efforts, remains amongst the most important vector-borne diseases, due to its global distribution, the high number of people affected, and the high number of deaths.

1.3. Malaria overview

This section provides an overview of: 1) the general malaria burden, causative agent and life cycle; 2) the diversity of the vector behaviour and its implications on the disease transmission dynamics and on design of vector control programmes; and 3) the strategies used for malaria prevention and control.

1.3.1. Global disease burden and epidemiology

Malaria is a major constraint for the health and socioeconomic development of a large percentage of the world population. Almost half of the world's population are at risk of malaria (Hay et al., 2004), mainly those living in the poorest countries, where people can least afford to pay for prevention and treatment of disease. About 300-500 million cases of clinical malaria are reported every year, causing more than 1 million deaths, with 90% of malaria deaths occurring in sub-Saharan Africa, where malaria is the main cause of death for children under five years of age (Breman et al., 2004; W.H.O., 2007).

Human malaria is usually caused by infection with one or more of four species of the *Plasmodium* parasite: *P. falciparum* (tropics), *P. vivax* (tropical and temperate zones), *P. ovale*, *P. malariae*. The first two species are the main causes of disease, with most deaths being due to infection with *P. falciparum*. Recent evidence has shown that a fifth species, *P. knowlesi* which naturally infects macaques in Southeast Asia, can also routinely infect humans living near the monkeys, being potentially life threatening (Singh et al., 2004; Cox-Singh et al., 2008; Cox-Singh and Singh, 2008).

Although some animal species (including non-human primates, birds, reptiles, rodents and bats) can also contract malaria, generally animal malaria cannot spread to humans, nor can human malaria spread to animals (Warrel and Gilles, 2002)¹.

The life cycle of the malaria parasite involves both a vertebrate host and an insect vector. The parasite is transmitted to humans by the bite of a blood feeding female mosquito of the genus *Anopheles* (Diptera: Culicidae), infected with sporozoites of *Plasmodium spp.* Only female anopheline mosquitoes are involved in transmission, as the males do not feed on blood. Following infection of the human host, the parasite undergoes two multiplication phases. After multiplying in the liver, *Plasmodium spp.* invades the red blood cells, where it develops into gametocytes (gametogony) which are the infective form for the mosquito. Within the mosquito, the parasite must also go through a developmental phase (sporogony, also called extrinsic incubation period), during which sporozoites are formed and the mosquito becomes infectious to other human (Warrel and Gilles, 2002).

1.3.2. Vector behaviour

There are about 70 species of anopheline mosquitoes involved in malaria transmission, amongst which 40 are thought to be of major importance (Warrel and Gilles, 2002).

¹ The main exception is *P. knowlesi* which was previously considered a disease of macaques occurring only sporadically in humans and has recently been recognized as a zoonosis that is widely spread in south-east Asia, where it is the main cause of human malaria, and that has been often misdiagnosed as *P. malariae* (Cox-Singh and Singh, 2008). The rare exceptions involve *P. malariae* which can also infect chimpanzees and some *Plasmodium* species of nonhuman primates that have been transmitted naturally or experimentally to humans (*P. simium* from monkeys in Brazil; *P. brasilianum* from monkeys in Brazil, Peru, Colombia, Venezuela and Panama; *P. cynomolgi*, *P. inui*, *P. eylesi* from primates in several Asian countries; and *P. schwetzi* from chimpanzees in Africa) (Acha and Szyfres, 1987; Carter and Mendis, 2002).

More importantly, distinct behaviours can be exhibited between and even within anopheline species. For instance, the main vector of malaria in sub-Saharan Africa, *Anopheles gambiae* Giles *sensu strictu* (*s.s.*), is generally highly anthropophilic, preferring to feed on humans than on animals, and endophilic, resting predominantly indoors after feeding. In contrast, another important vector in drier savannah regions of Africa, *An. arabiensis* Patton, is considered to be more zoophilic and opportunistic, feeding more frequently on non-human hosts such as livestock. *An. arabiensis* also tends to be more exophilic, resting predominantly outdoors. The type of host and/or resting site selected by these and other vector species can, however, vary greatly, under the influence of factors such as local variation in host abundance and accessibility and genetic features of the local vector (Coluzzi et al., 1979; Hewitt et al., 1994; Bøgh et al., 2001). Noteworthy, regarding intra-specific variation of vector behaviour, both *An. gambiae s.s.* and *An. arabiensis* were shown to display intraspecific heterogeneities in the selection of resting sites (namely, indoors versus outdoors), which were linked to chromosomal polymorphisms (Coluzzi et al., 1979; Hewitt et al., 1994; Bøgh et al., 2001).

Such diversity of vector behaviours has major implications on malaria transmission dynamics as well as on the design of vector control programs. On one hand, it contributes to the high complexity of the disease transmission dynamics. On the other hand, it opens up the possibility of applying diverse control strategies; namely, it enables the implementation of alternative strategies targeted at non-human hosts of the mosquito.

1.3.3. Prevention and Control

The current primary measures for malaria prevention are long-lasting insecticidal nets (LLINs), spraying the indoor walls of homes with residual insecticides, and intermittent preventive treatment for pregnant women to prevent malaria transmission in areas with high transmission (RBM, 2009).

Other vector control strategies such as larviciding (Walker and Lynch, 2007) and environmental management are applied when suitable, based on scientific evidence (RBM, 2009). Environmental management encompasses: physically reducing mosquito

breeding sites (W.H.O., 1982; Van der Hoek et al., 2004), and modifying peoples houses. Examples of modifications of people's houses include: improving house construction (Lindsay et al., 2002; Lindsay et al., 2003), locating houses away from mosquitoes breeding grounds and/or raised above ground level (Lindsay et al., 2002; Habtewold, 2004; Tirados et al., 2006), use of house smoke (reviewed in Biran et al., 2007), and zooprophylaxis (see section 1.4).

Research is undergoing on the development of genetically modified anophelines (Alphey et al., 2002) and a vaccine (e.g. Richie and Saul, 2002; Sharma and Pathak, 2008; Targett and Greenwood, 2008). Yet, in the meantime, efforts should concentrate on the sustainable implementation of the tools presently available and on integrated vector management, as these are amongst the priorities for health research recently established by the World Health Organization (WHO) (Remme et al., 2002). The strategy of integrated vector management encourages: selection of methods based on knowledge of local vector biology, disease transmission and morbidity; a multidisease control approach and integration with other disease control methods; use of a range of interventions, often in combination and synergistically; and collaboration within the health sector and with other public and private sectors that impact on vector breeding, as well as community participation (W.H.O., 2004a).

Case management relies on an early diagnosis and treatment with medicines. Malaria can be routinely confirmed by parasitological diagnosis with microscopy or, preferably, by a rapid diagnostic test (RBM, 2009). The recommended treatment against *P. falciparum* (PF) malaria is being shifted to artemisin-based combination therapies (ACTs), which is the only effective treatment in regions where PF is resistant to other drugs, namely, where PF is resistant to sulfadoxine-pyrimethamine (SP) and to chloroquine (CQ). The choice treatment against chloroquine-sensitive *P. vivax* malaria are CQ and primaquine (PQ) (RBM, 2009).

Following an abandoned first global campaign to eradicate malaria in the 1950s-1970s, malaria became low-profile on the international health agenda until recently. In 1998 a new global framework to implement coordinated efforts against malaria was born: the Roll Back Malaria (RBM) Partnership. Its overall strategy aims at reducing malaria morbidity and mortality by reaching universal coverage for all populations at risk with locally appropriate interventions for prevention and case management, and strengthening health systems (RBM, 2009). The RBM initiative has promoted

increasing awareness about malaria and stimulated the development of large funding bodies to fight the disease. The Global Fund to Fight AIDS, Tuberculosis and Malaria¹, is the largest international financier for malaria control programs. Other important funding resources include: The U.S. Government President's Malaria Initiative (PMI)²; the World Bank Booster Program for Malaria Control in Africa³; and the UNITAID⁴.

The concentrated efforts over the last decade are bearing fruits. According with the latest World Malaria Report (W.H.O., 2008), following expanded coverage with LLINs and ACTs, malaria cases and deaths in at least 7 of 45 African countries/areas with relatively small populations and good surveillance dropped by 50% or more between 2000 and 2006 or 2007. In 22 more countries in other parts of the world, malaria cases declined by 50% or more from 2000-2006. Yet, many countries are still far from the desired coverage of effective tools for malaria prevention and case management, and sustained efforts are required to reach that goal.

1.4. Zooprophylaxis

Zooprophylaxis was defined by the WHO as “the use of wild or domestic animals, which are not the reservoir hosts of a given disease, to divert the blood-seeking mosquito vectors from the human hosts of that disease” (W.H.O., 1982). It can be active or passive. Active zooprophylaxis consists of strategically placing animals between mosquito breeding sites and people's houses, while passive zooprophylaxis is the protective effect of the normal presence of animals within a community.

Since in the early 1900s zooprophylaxis has been recognized as an important tool to decrease malaria transmission to people in certain places of the world, and the approach has also been evaluated against other vector-borne diseases (Service, 1991). Cattle have been considered the most appropriate hosts for this strategy, because they are a blood-meal source for various important vectors and are frequently “dead-end” hosts; i.e. several vector-borne pathogens that infect humans are not sustained in cattle, and therefore cattle are not sources of those pathogens for vectors. There are, however,

¹ <http://www.theglobalfund.org/en/malaria/>

² <http://www.fightingmalaria.gov>

³ <http://siteresources.worldbank.org/EXTAFRBOOPRO/Resources/MALARIAREPORTfinalLOWRES.pdf>

⁴ <http://www.unitaid.eu/en/Malaria.html>

exceptions, for example some arboviruses such as Rift Valley Fever (Service, 1991) and Crimean-Congo haemorrhagic fever (Ergonul, 2006), and some parasites such as *Schistosoma japonicum* (Pesigan et al., 1958) and *Trypanosoma brucei rhodesiense* (Maudlin, 2006), can infect both humans and cattle.

1.4.1. Zooprohylaxis and malaria

The potential of zooprohylaxis for malaria control was originally envisaged in 1903 in Italy by Bonservizi (Escalar, 1933). Since then, several studies have been performed worldwide to try to assess the value of this strategy in the fight against malaria, and a few reviews have been done of the subject (Hackett, 1937; Brumpt, 1944; Service, 1991; W.H.O., 1991; Bettini and Romi, 1998). Despite the large number of studies that have been performed for over a century, the available evidence is still contradictory and no consensus exists on the prophylactic effect of animals. Indeed, although in several situations the presence of livestock has been referred to as a protective factor for malaria, the opposite has been reported in various other studies, where livestock were shown to be a risk factor or even to have no impact at all on malaria transmission.

A brief mention to historical anecdotic reports about the prophylactic effect of animals for malaria is given in the next section, followed by a review of more rigorous studies that have been performed to further examine this matter.

1.4.1.1. Historical Reports

There are a number of historical reports where the *decrease in the number of animals* seemed to be associated with an *increase in malaria cases*. For example, in British Guyana, subsequently to eradication of *An. darlingi* and malaria in the Demerara river estuary, there was an increase in the human population while agricultural mechanization resulted in livestock depletion from the area (cattle, horses, donkeys, and mules). Such change led *An. aquasalis*, that was originally zoophagic and not a malaria vector, to start feeding on humans. This, together with the reintroduction of malaria parasites by infected workers who returned from neighbouring areas, was suggested as the most likely cause of a malaria outbreak that occurred fifteen years after it had been eradicated (Giglioli, 1963). Similar switching in vector feeding from animals to humans have also

presumptively been associated with reduction in livestock density in Indonesia (Muir, 1981) and Vietnam (Warrel and Gilles, 2002), and with depletion of wildlife in Malaysia (Loong et al., 1990) and Brazil (PAHO, 1988 in Service 1991). Likewise, there have been studies reporting situations where *increase in the ratio of livestock:human density* was likely to have *decreased malaria transmission*. For instance, it has been proposed that the increase in the ratio of livestock:human density and the consequent deviation of the local *Anopheles* vectors from humans to animals were partially responsible for the reduction in malaria cases from Northern Europe and much of North America, in the early 20th century (e.g. Warrel and Gilles, 2002; Kuhn et al., 2003).

On the other hand, the opposite has also been reported. For instance, in Italy, Fermi (1928) reported that communities with a *higher ratio of livestock:human density* were found to have *higher malaria prevalence*, and vice-versa. He presumptively attributed this to the fact that livestock contributed to increase anopheline density, and that, if livestock sheds were poorly built and located near the human dwellings, the anophelines would not remain in the cold and ventilated animal sheds, but instead would easily escape and enter the nearby houses to bite humans, especially when temperatures were lower and it had rain for long. Accordingly, Fermi (1928) stated that for zoonophylaxis to be effective against malaria, livestock sheds should be placed at a distance of at least 100 meters from human dwellings, ideally, between the anopheline breeding sites and the edge of the village, and should be built properly, with little openings.

These reports, although mostly speculative, have laid the foundations for further investigations of the impact of animals on malaria transmission to people, through more rigorous entomological and/or parasitological studies, as reviewed below.

1.4.1.2. Empirical evidence

I identified and reviewed 45 empirical studies that have examined the role of livestock on malaria transmission, amongst which the most relevant are described here. Most studies are observational (69%, n=31/45) and only a few (31%, n=14/45) are experimental. Among the observational studies, the great majority have been cross-sectional, mostly purely entomological, examining anophelines collected in different biotopes. Most of these studies

were based on blood-meal analysis to identify the host source, and a few works additionally or alternatively looked at vector density and/or sporozoite prevalence. Within the longitudinal studies, a few repeated cross-sectional parasitological surveys and matched case-controls studies have been conducted. These have been designed to assess the effect of livestock related factors on the risk of clinical malaria, among several other potential risk factors. Only one cohort study has been performed, and this was designed specifically to investigate the effects of passive zooprophylaxis on both entomological and parasitological outcomes. The findings of the key studies are summarized below.

1.4.1.2.1. Vector biting on people

Several *cross-sectional studies* have found a *reduction in the proportion of human blood-fed malaria vectors* (assessed by the human-blood index, HBI) with *increase in cattle:human density*. This has been observed at the biotope level, i.e. comparing human dwellings vs. mixed dwellings with humans and cattle vs. cattle only dwellings (Garrett-Jones, 1964; Boreham, 1975; White et al., 1980; Adugna and Petros, 1996; Hadis et al., 1997; Habtewold, 1999; Mahande et al., 2007a), and also at the village level, i.e. comparing villages with different cattle:human density (White, 1971; White and Rosen, 1973; Highton et al., 1979; Garrett-Jones et al., 1980; Charlwood et al., 1985; Joshi et al., 1988). Although the authors of these studies tend to argue that these findings could be an indication of the zooprophylactic effect of livestock, i.e. diverting mosquitoes feeding from humans to cattle, the evidence from blood-meal analysis of resting mosquitoes is limited and inconclusive. Namely, in the first set of studies that compared different biotopes, due to possible sampling bias it is not known whether the mosquitoes collected resting in a given dwelling had actually fed in that dwelling or elsewhere. Additionally, in the second set of studies that compared different villages, the reported differences in HBI between villages might have resulted from other factors at the village level that were not accounted for, such as the proximity to breeding sites, type of house construction, and usage of protective measures against mosquitoes. Such biases have been greatly overcome by experimental host preference studies that controlled for equal accessibility of hosts to mosquitoes.

In a number of these *experimental studies*, *malaria vectors* were shown to *prefer biting on animals than on people*, leading the authors to suggest the potential of zooprophylaxis for malaria control. Such studies have been conducted by a variety of methods, such as:

- by comparing the proportion of human vs. animal fed mosquitoes collected inside a bednet under which a human and animal host were sleeping (e.g. *An. farauti* in Papua New Guinea (Charlwood et al., 1985));
- by comparing mosquito collections on human and animal baits outdoors, either in open air (e.g. *An. aconitus* in Thailand (Harrison, 1980; Junkum et al., 2007)), or under bed net traps (*An. arabiensis* and *An. gambiae s.s.* in Senegal (Diatta et al., 1998)); and, more recently,
- by using odour-baited entry traps (OBETs) containing human or animal hosts (e.g. *An. arabiensis* and *An. gambiae s.s.* in Madagascar (Duchemin et al., 2001); and *An. arabiensis* in Tanzania (Mahande et al., 2007a)).

On the other hand, the opposite has been observed in OBET experiments in Burkina Faso (Costantini et al., 1998), Zimbabwe (Costantini et al., 2005) and in Ethiopia (Habtewold, 2004; Tirados et al., 2006), where *An. arabiensis* was much more attracted to human than to cattle odour. Both Ethiopian studies used further sampling methods, which revealed mixed results about the effect of cattle upon malaria risk to people. For instance, in the study by Tirados et al. (2006), which was performed in the Konso District, although the OBET experiments showed that *An. arabiensis* was highly anthropophilic (the human-baited traps collected ~6 times more mosquitoes than the cattle-baited traps), a high proportion of cattle-fed mosquitoes was found in cross-sectional resting catches indoors (59% - including mixed blood meals from cattle and humans; 45% - including only single-host meals from cattle), and outdoors (71% to 91% - including mixed meals; 63% to 85% - only single-host meals). As proposed by the authors, this was likely to have been due to the combination of the high tendency of the vector to feed and rest outdoors (exophagy and exophily, respectively), and the greater abundance and accessibility of cattle than humans outdoors at night. The same study found no relation between the proportion of cattle-fed mosquitoes and the ratio of cattle: human density in the compounds where mosquitoes were collected. The combination of findings from this work illustrates the importance of combining different sampling methods for better understanding the impact that livestock may have on malaria risk. Namely, it is not enough to determine the vector's intrinsic preference for feeding upon livestock vs. humans, but it is also important to account for the place where the vector prefers to feed (exophagy vs. endophagy) and for the relative density and accessibility of livestock:humans in different places.

Likewise, in the study by Habtewold (2004), near Sille town, despite the high anthropophily of *An. arabiensis* observed in OBETs, the presence of an ox next to a person indoors, reduced by 38% the human-biting catch (HBC) of *An. arabiensis* on that person and

decreased by 40% its HBI, while it had no significant effect on the HBC of the highly zoophilic *An. pharoensis*. The reverse was obtained outdoors, where the presence of an ox next to a person, did not change the HBC of *An. arabiensis*, while it halved the catches of *An. pharoensis*. The same study also assessed the effect of the presence of ox-odour only near a collector. The indoor catches of *An. arabiensis* and *An. pharoensis* were virtually unaffected by the presence of ox-odour, suggesting that sharing a hut with cattle did not increase the number of mosquitoes attracted into the house. Conversely, the outdoors catches of *An. arabiensis* were increased in the presence of ox-odour, suggesting that being close to an ox outdoors attracts more *An. arabiensis*, although these did not land on the person unless ox-odour only was present. From the combination of experiments Habtewold (2004) concluded that close proximity to cattle, indoors or outdoors, did not seem to increase the biting rate on people, nor was likely to increase malaria risk to people.

Mark-release-recapture experiments have also been conducted to investigate zoophylaxis. Using this approach, Charlwood et al (1985) showed that introducing one buffalo in Maraga village (that previously had no buffaloes) in Papua New Guinea, diverted *An. farauti* mosquitoes feeding on humans and/or other animals (pigs, dogs, cats). Around 40-45% buffalo-fed mosquitoes were found up to 10 m away from the buffalo, decreasing up to 60 m where there was no diversion caused by the buffalo, indicating that the vectors tended to fly less than 50 m from the site of their bloodmeal. The HBI varied considerably between villages, with lower HBI in the villages with more animals available as alternative hosts. In Maraga, with a high number of animals, mostly pigs, which slept under the villagers' houses at night, the HBI was 9.2 times lower than in a nearby village with very few animals. Also, in a simple host choice experiment with a person and a dog sleeping under the same bednet, in another nearby village, 2.3 times more *An. farauti* mosquitoes fed on the dog than on the man (Charlwood et al., 1985). The overall findings of this study suggest that keeping domestic animals near human dwellings could reduce human-mosquito contact.

1.4.1.2.2. *Vector density*

Cross-sectional mosquito catches have also been applied to look at the effect of host availability upon the *density of larvae and adult vectors*. For instance, the density of *An. crucians* and *An. quadrimaculatus* adults in USA Louisiana rice lands were shown to be positively correlated with cattle density ($r^2=68$ and 74% , respectively), and similar correlation was found for other pest mosquito species (McLaughlin and Focks, 1990). Similarly, in Guinea Bissau, the presence of pigs in a house was associated with increased mosquito density (mostly *An. gambiae s.s.*) collected resting in the bedrooms of the same house ($\chi^2=17.63$, $p=0.0001$) (Pålsson et al., 2004).

Conversely, a study in Kenya (Minakawa et al., 2002) found that the density of *An. gambiae s.s.* larvae was lower when a breeding site was farther away from a house but closer to a cowshed ($r_1=-0.69$, $p<0.01$; $r_2=-0.75$, $p<0.01$)¹, and when the nearby housing compounds had lower ratio of human:cow density ($r_1=0.40$, $p=0.02$). The density of anopheline adults in a house was negatively associated with the distance from the house to its nearby larval habitats ($r_1=-0.50$, $p<0.05$; $r_2=-0.51$, $p<0.01$), but it was independent on host availability. These findings suggest that the relative abundance of *An. gambiae* larvae in the area depended on livestock and human host availability, while the density of anopheline adults in houses was determined by the distance from houses to larval habitats.

1.4.1.2.3. *Vector biting on people and/or disease*

Repeated cross-sectional parasitological surveys in India, where *An. fluviatilis* and *An. culicifacies* are the main malaria vectors, found a *negative association* between *cattle:human density* and the risk of *malaria infection* in the household ($\chi^2=15.32$, $p=0.018$), and no association between proximity of cattle sheds to human dwellings (Subramanian et al., 1991).

In contrast, there are studies where keeping *animals nearby* (or far from) *human dwellings* was associated with *increased* (or decreased) *vector biting on people and/or disease*. For instance, in a cross-sectional study in Pakistan, higher prevalence of malaria was observed

¹ r_1 = correlation coefficient in the beginning (r1) or end (r2) of the rainy period.

in villages where a higher proportion of households owned cattle than in villages with fewer cattle-owners ($r=0.79$, $p=0.036$) (Bouma and Rowland, 1995). This “village effect” was thought to be due to higher vector densities resulting from the readily accessible blood-meals from cattle. The same study also found that, within a village, malaria prevalence was 1.6 times higher in children from families which kept cattle within their housing compounds than in those without cattle ($\chi^2=9.6$, $p<0.005$), which the authors designated as the “compound effect”. Similarly, two separate repeated cross-sectional studies in Ethiopia found that keeping animals in the house at night (mixed dwellings) resulted in about 1.8 times higher prevalence (Seyoum et al., 2002) and 1.9 times higher incidence (95%CI 1.29-2.85) (Ghebreyesus et al., 2000) in children than when animals were kept in a separate animal shelter. The latter study also assessed the effect of the number of animals owned but this was not associated with malaria risk (Ghebreyesus et al., 2000). The explanation proposed by the authors of these studies for the “compound effect” observed in Pakistan and the similar effects observed in Ethiopia was that cattle were attracting mosquitoes but, when in close proximity to humans, a proportion of these mosquitoes might be deviated from their route towards cattle and bite on humans instead of cattle.

This hypothesis was supported by experimental work where the presence of cattle or goats in close proximity to humans increased the indoor HBR by about 1.4 times for *An. stephensi* in Pakistan (the HBR of other highly zoophilic anophelines also increased, Hewitt et al. 1994), and by 2.4 times for *An. arabiensis* in Ethiopia (Seyoum et al. 2002). Similar findings had been observed in two previous studies in the Philippines (Russel, 1934; Schultz, 1989). Namely, as early as 1934, Russel reported that the presence of buffaloes outdoors next to an experimental hut increased by 16.6 times the total number of anophelines and by 3 times the number of unbloodfed female anophelines collected from inside the hut (including the main local vector *An. flavirostris*). Resembling findings were reported in an analogous later study by Schultz (1989) (increased HBR by 1.9 times), although when he repeated the experiment outdoors the reverse, i.e. a protective effect, was obtained: the presence of buffaloes near man (vs. no animals nearby) decreased the HBR by 1.6 times when both man and animals were outdoors.

Taken together, these findings indicate that, in these areas, keeping animals near a house potentially increased malaria risk, while deploying animals around a village, ideally between vector breeding sites and human houses, could have a protective effect against malaria. Such a protective effect did indeed seem to occur in some experimental

entomological and parasitological works that looked at the *type and location of animal shelters*. For example, in an early study in Italy in mid 1932 (Escalar, 1933), following the change in the location of pig shelters from the centre to the periphery of Ardea village (i.e. between human dwellings and vector breeding sites), the proportion of the total anophelines catches (mainly *An. maculipennis s.l.*) that invaded humans dwellings decreased by 7.2 times, and the annual incidence of malaria cases and the spleen rate reduced by 3.6 and 2.1 times, respectively. The decrease in malaria was not due to a spontaneous attenuation of transmission nor due to more intense chemoprophylaxis or treatment, as during the study period malaria levels in the areas surrounding the intervention village were higher than in the previous year, while in Ardea the opposite was observed despite a lower consumption of the anti-malarial quinine. All this indicates that the reduction in malaria was due to the active zooprophyllaxis with the pigs.

1.4.1.2.4. Null or ambiguous association

Furthermore, a few studies have revealed a *null or ambiguous association between livestock and malaria*, namely: two cross-sectional (Joshi et al., 1975; Mbogo et al., 1993) and two case-control (Snow et al., 1998; Mbogo et al., 1999) studies in Kenya, a case-control study in Peru (Guthmann et al., 2001), and a case-control (Adiamah et al., 1993) and a paired-cohort study (Bøgh et al., 2001; Bøgh et al., 2002) in The Gambia.

The paired-cohort study in The Gambia was the first entomological and parasitological study designed to specifically investigate zooprophyllaxis in Africa. No significant differences were found between the total number, sporozoite prevalence or entomological inoculation rates (EIR) of *An. gambiae s.l.* (*An. arabiensis s.s.*, *An. melas* and *An. arabiensis*) collected resting in the bedrooms of children where cattle were either present or absent from the immediate the vicinity (<20m or >50m, respectively). Additionally, the presence of cattle did not significantly change the proportional abundance of any of the sibling species, or the HBI of *An. gambiae s.s.* or *An. melas*, but it did significantly reduce the HBI of *An. arabiensis* by 37%. However, since *An. arabiensis* was the least abundant (accounting for only 10%) of the three vector species, no significant difference was observed on the overall HBI of *An. gambiae s.l.* (Bøgh et al., 2001). Similarly, no significant difference was found in the prevalence of *P. falciparum* parasitaemia and anaemia between the two groups. Despite the presence of cattle initially appeared to be a protective factor against high parasitaemia, its statistical significance vanished after adjusting the

data for differences in wealth. However, given that wealth was estimated with a financial index calculated from the total values of cattle and other animals present, which were the farmer's main valuable assets, it could not be ruled out that the loss of statistical significance might have been due to inclusion of cattle ownership in the generation of the wealth rank (Bøgh et al., 2002). This study highlights the importance of taking into account socioeconomic variables when investigating the prophylactic effect of domestic animals, and the need to use a method for wealth ranking that does not include the ownership of the same animals.

Theoretical evidence also suggests that increases in livestock density/accessibility might increase malaria transmission. Mathematical models have been developed which predict that by providing readily accessible blood-meals, the presence of livestock might increase mosquito density (Sota and Mogi, 1989) or increase mosquito survival (Saul, 2003), and consequently enhance the risk of transmission (see detailed review in Section 1.6 below).

In summary, the apparently contradictory outcomes of the numerous studies conducted result from the combination of several possible effects of livestock presence. Firstly, livestock may divert the blood-seeking mosquito vectors from humans, thereby decreasing the biting on people (e.g. Charlwood et al., 1985; Burkot et al., 1989; Habtewold, 2004), and as a result i) decreasing the transmission of the malaria parasite (Subramanian et al., 1991), and ii) preventing its amplification in people (i.e. the basis for the zooprophylaxis concept). Secondly, livestock can provide additional blood-sources and/or larval breeding sites (Gillies and De Meillon, 1968; White et al., 1972; Service, 1993; Charlwood and Edoh, 1996; Minakawa et al., 1999), which can increase vector survival and density (McLaughlin and Focks, 1990), consequently increasing the probability of the vector surviving the parasite extrinsic incubation period and becoming infectious, as well as increasing biting on people (Escalar, 1933; Service, 1991; Saul, 2003). And finally, livestock may attract more mosquitoes which, once in the vicinity of the human dwellings, may end up biting humans rather than animals (Russel, 1934; Schultz, 1989; Hewitt et al., 1994; Seyoum et al., 2002).

The disparity in reports highlights the complexity and the significant gaps in our knowledge of zooprophylaxis in malaria. The need to further investigate this conundrum is clear and has been increasingly recognized (see, e.g. Sota and Mogi, 1989; Hewitt et al., 1994; Mutero et al., 1999; W.H.O., 2001; Seyoum et al., 2002). Research efforts are needed to clarify the impact of livestock on the human biting rate of local anopheline vectors and on the risk of malaria transmission and infection according to local conditions.

1.4.2. Zooprophyllaxis and other vector-borne diseases

Besides malaria, there are several human diseases transmitted by arthropod vectors that can feed considerably on animal hosts which cannot sustain infection, and therefore act as dead-end hosts of pathogen transmission.

Additional examples where cattle can potentially have a zooprophyllactic effect by decreasing vectors feeding on humans and pathogen amplification are listed in Table 1.3 and include: (1) several arboviroses transmitted by *Culex* mosquitoes, such as Japanese Encephalitis (Carey et al., 1968 in Service 1991; Reuben et al., 1992), and West Nile Virus (Reuben et al., 1992); (2) Human Onchocerciasis transmitted by *Simulium* flies (Seidenfaden et al., 2001); and (3) Visceral Leishmaniasis transmitted by zoophilic sandflies, *Phlebotomus argentipes* in Asia (Mukhopadhyay and Chakravarty, 1987; Bern et al., 2000; Bern et al., 2005), and *Phlebotomus martini* in Africa (Kolaczinski et al., 2008).

Table 1.3. Examples of vector-borne diseases of humans where cattle are dead-end hosts.

Vector	Disease	References
<i>Culex</i> mosquitoes	Western Equine Encephalitis	(Hess and Hayes, 1970; W.H.O., 1982)
	St. Louis Encephalitis	(Hess and Hayes, 1970; Reisen et al., 1990)
	Japanese Encephalitis	(Carey et al., 1968 in Service 1991; Reuben et al., 1992)
	West Nile Virus	(Reuben et al., 1992)
<i>Simulium</i> flies	Human Onchocerciasis	(Seidenfaden et al., 2001)
Sandflies	Visceral Leishmaniasis	
In Asia: <i>Phlebotomus argentipes</i>		Bangladesh (Bern et al., 2005), India (Mukhopadhyay and Chakravarty, 1987), Nepal (Bern et al., 2000)
In Africa: <i>Phlebotomus martini</i>		Kenya and Uganda (Kolaczinski et al., 2008)

Other domestic animals with a potential zoonophylactic effect are poultry. For instance, in Latin America, chicken are the favourite peri-domestic blood-meal host for the sandflies vector of Visceral Leishmaniasis (Alexander et al., 2002) and for the triatomine bugs that transmit Chagas disease (Gurtler et al., 1998), while being dead-end host for both disease pathogens. Also, in Nepal a cross-sectional study has found that people who owned small animals, mainly fowl, seemed to have decreased risk of Visceral Leishmaniasis (Odds Ratio: 0.4, 95% CI: 0.2-1.1) (Schenkel et al., 2006).

As with malaria, also with these diseases there is the possibility that, by providing additional blood sources and/or larval breeding sites, the presence of animals could increase vector densities and consequently increase disease transmission. For example, the abundance of cattle has been shown to be positively associated with the density of adult Japanese Encephalitis vectors *Culex spp.* in Vietnamese rice-cultivating villages (Hasegawa et al., 2008), as well as with the density of *Psorophora columbiae* adults (Focks et al., 1988; McLaughlin and Focks, 1990), larvae (Chambers et al., 1981; McLaughlin and Vidrine, 1987) and eggs (Meek and Olson, 1977; Chambers et al., 1981) in USA ricelands. Transforming ricelands into pasture lands after harvesting the crops produces many hoofprints in the moist soil which have been shown to be important sources of oviposition sites for *Ps. columbiae* in Texas (Meek and Olson, 1977; Welch et al., 1986). These detrimental effects can, however, be potentially counteracted by the application of insecticide on animals and/or on the animals' shed.

Zoonophylaxis has also been applied in veterinary medicine. For instance, cattle have been used as a zoobarrier to protect sheep from *Culicoides immicola* that transmits bluetongue virus (Nevill, 1978), since it has been shown that the vector preferentially feeds on cattle rather than on sheep, when the former are abundant (Walker and Boreham, 1976).

1.5. Insecticide treatment of livestock (ITL)

In areas where the presence of livestock near people increases malaria transmission, an apparently simple solution could be to change livestock management in order to deploy the animals away from people's houses, between village and vector breeding site (W.H.O., 1991). However, in Pakistan as well as in some Ethiopian regions, for instance, this is not likely to be a feasible strategy, given that livestock are such an important source of household income that people prefer to keep the animals near their houses (Bouma and

Rowland, 1995; Habtewold et al., 2001). An alternative solution has therefore been proposed: target the non-human host of the zoophilic mosquito, by treating livestock with insecticides (Bouma and Rowland, 1995).

This strategy has since long (Freeborn and Regan, 1932; Moerman, 1998) been widely and effectively used to control several ectoparasites and the diseases and/or nuisance they cause to animals (and also often to humans), and to reduce the associated economic losses, as reviewed by USDA (1976) and Strong&Wall (1990). See Table 1.4 for a list of examples where the treatment of animals with insecticides/acaricides (hereafter referred globally as ‘insecticides’ for simplicity) has been applied to control ectoparasites of veterinary importance, many of which with zoonotic potential. Namely, this strategy has been used against tsetse flies transmitted animal trypanosomiasis in Africa (e.g. Whiteside, 1949; Thomson, 1987) and tick-borne diseases worldwide (e.g. Barnett, 1961), as well as against a variety of other biting and/or nuisance flies (e.g. Foil and Hogsette, 1994), mosquitoes (e.g. Nasci et al., 1990; Focks et al., 1991; Schmidtman et al., 2001), biting midges (Standfast et al., 1984), mites, lice, and fleas. Among the zoonotic diseases, for example applying insecticide on dogs is an efficient measure for Leishmaniasis control (Xiong et al., 1994; Maroli et al., 2001; Gavgani et al., 2002; Reithinger et al., 2004; Ferroglia et al., 2008) and has recently started being tested for Chagas disease control (Gentile et al., 2004; Reithinger et al., 2005, 2006).

For human malaria, Table 1.5 lists the bioassays that tested the effect of insecticide-treated cattle upon anopheline malaria vectors. As early as in the 1950s, a study in Tajikistan demonstrated that treating livestock with Dichloro-Diphenyl Trichloroethane (DDT) could decrease the density and survival of the vector *An. superpictus* (Lysenko et al., 1957 in Hewitt and Rowland 1999). However, the formulations then available required DDT treatments to be repeated every 10 days, making the cost of the strategy prohibitive in comparison with indoor spraying. Better prospects emerged with the development of new insecticide formulations and of synthetic pyrethroids with high insecticidal action and low mammalian toxicity (Elliot et al., 1978).

In Pakistan, following the studies that identified that keeping livestock in residence compounds was associated with increased malaria transmission (Hewitt et al., 1994; Bouma and Rowland, 1995), a bioassay was conducted to test the effect that applying pyrethroid insecticides (deltamethrin, permethrin or lambda-cyhalothrin) on cattle had upon the local

malaria vectors (Hewitt and Rowland, 1999). Deltamethrin had the strongest and longest-lasting effect and was selected to be used in a community-randomised trial that tested the effectiveness of ITL on malaria transmission in refugee settlements in Pakistan (Rowland et al., 2001). In the intervention villages virtually all domestic animals (cattle, sheep and goats) were treated with deltamethrin solution applied by a sponging method (Figure 1.1). Three rounds of sponging were done with 6 week interval between rounds. The trial took place over a three year period, with villages being alternately allocated as an intervention or control in different years. Encouraging results were obtained. Namely, the incidence of malaria caused by *P. falciparum* decreased by 56% (95% CI 14%-78% p=0.02), and *P. vivax* by 31% (5-50% p=0.03) over a 4 month period after the second round of livestock treatment. Malaria prevalence was also reduced, as well as the density and life expectancy of the main vectors populations, *An. stephensi* and *An. culicifacies*, which are strongly zoophilic and endophilic (Reisen and Milby, 1986). Efficacy was comparable to that of traditional indoor insecticide spraying but with 80% less costs. Moreover, significant improvements were obtained in productivity (weight and milk yield) of livestock previously infested with ectoparasites, enhancing community uptake of the programme (Rowland et al., 2001).



Figure 1.1. Sponging cattle with insecticide in a Pakistan community trial.

It is important to point out a remarkable particularity about the impact of ITL on malaria control. As opposed to the majority of situations where insecticide treatment of animals has been used (for example, the insecticide treatment of dogs to control Zoonotic Visceral Leishmaniasis in dogs and in humans), in the case of malaria the intervention is targeted at a host which is not a reservoir of infection, and yet, decreases in disease incidence in the human host have been achieved.

Additional studies have been conducted to explore the potential use of ITL as a tool for malaria control in areas of sub-Saharan Africa where the zoophilic vector *An. arabiensis* is an important vector. Namely, bioassays have been performed to test the effects of deltamethrin-treated cattle upon malaria vectors in Sille Valley in Ethiopia (Habtewold et al., 2004) and in Lower Moshi in Tanzania (Mahande et al., 2007b), where *An. arabiensis* is the main vector. Despite the encouraging results from these bioassays, no study has yet been conducted to assess the impact of ITL on malaria transmission at the community level in Africa.

Table 1.4. Veterinary ectoparasites and diseases that are controlled by treating the animal host(s) with insecticides/acaricides.

Treated Animal Host	Ectoparasite	Disease
Cattle	<ul style="list-style-type: none"> • Biting flies: - Tsetse flies (<i>Glossina spp.</i>) - Horn flies (<i>Haematobia irritans</i>) • Mosquitoes: - <i>Culex</i>, <i>Mansonia</i>, <i>Anopheles</i>, <i>Aedes</i>. - <i>Psorophora</i>, <i>Aedes</i>. 	<ul style="list-style-type: none"> → African trypanosomiasis² → Intermediate host for <i>Stephanofilaria stilesi</i> (filarial dermatitis) → Human Lymphatic Filariasis → Anaplasmosis in cattle
Cattle & <u>Horses</u>	<ul style="list-style-type: none"> • Biting flies: - Stable flies (<i>Stomoxys calcitrans</i>) - Horse flies (<i>Tabanus spp.</i>) and Deer Flies (<i>Chrysops spp.</i>) 	<ul style="list-style-type: none"> → Mechanical vectors of anthrax², surra, equine infectious anaemia virus; <i>Trypanosoma evansi</i>. Intermediate host for the nematode <i>Habronema muscae</i> (horses). → Mechanical vectors of anthrax², anaplasmosis, tularaemia², equine infectious anaemia virus, <i>Trypanosoma spp.</i> etc.
Livestock (all classes, including poultry)	<ul style="list-style-type: none"> • Biting flies: Black flies¹ (<i>Simuliidae</i>) 	<ul style="list-style-type: none"> → Leucocytozoonosis (poultry) → Onchocerciasis²¹ (cattle)
Cattle, Sheep, Horses	<ul style="list-style-type: none"> • Biting midges (mostly <i>Culicoides spp.</i>) 	<ul style="list-style-type: none"> → Bluetongue (cattle and sheep) → Onchocerciasis (horses)
<u>Sheep</u> (& Goats)	<ul style="list-style-type: none"> • Biting wingless dipterans: Sheep keds (<i>Melophagus ovinus</i>) 	
Livestock (Cattle, Sheep, Goats, Horses, Swine)	<ul style="list-style-type: none"> • Non-biting flies: - face fly (<i>Musca autumnalis</i>) 	<ul style="list-style-type: none"> → Transmit <i>Thelazia spp.</i> (eye worm) to cattle and sheep/goats; <i>Parafilaria bovicola</i> (subcutaneous lesions) and Infectious bovine keratoconjunctivitis to cattle, Hog cholera to swine, and cause irritation in horses.
Livestock	<ul style="list-style-type: none"> • Non-biting flies: - <i>Musca sorbens</i> 	<ul style="list-style-type: none"> → Transmits eye and diarrhoeal diseases to livestock and humans. E.g. infectious bovine keratoconjunctivitis in cattle; trachoma in humans.
Livestock (Cattle, Sheep, Goats, Horses, Swine, Poultry)	<ul style="list-style-type: none"> • Non-biting flies: - house fly (<i>Musca domestica</i>) 	<ul style="list-style-type: none"> → Transmit several diseases affecting animals and humans. → Transmit <i>Thelazia spp.</i> (eye worm) to cattle and sheep/goats; Hog cholera to swine, cholera, tapeworm and Newcastle disease to poultry, and cause irritation in horses.
Livestock (Cattle, Sheep, Goats, Horses, Pigs, Buffalo), Dogs & Cats, Rabbits	<ul style="list-style-type: none"> • Non-biting flies: - larvae of several species: Screw-worm flies², Human bot fly², <i>Hypoderma spp.</i>², Sheep nose bot fly², blow flies, Equine bot fly. 	<ul style="list-style-type: none"> → Myiasis²

Table 1.4. (Continued)

Treated Animal Host	Ectoparasite	Disease
Livestock (Cattle, Sheep, Goats, Horses, Pigs, Poultry), Dogs&Cats	• Lice (several species)	
Livestock (Cattle, Sheep, Goats, Horses, Pigs, Rabbits, Poultry), Dogs&Cats	• Mites (several species)	→ Mange (in most animals except poultry) ²
	• Ticks (<i>Argasidae</i> and <i>Ixodidae</i>)	→ Several diseases affecting animals and humans, e.g:
Dogs		Tick bite fever ²
Dogs, Cattle, Sheep, Goats, Horses		Erllichiosis ² , Anaplasmosis ² , Babesiosis ²
Cattle, Sheep, Goats		Crimean-Congo hemorrhagic fever ² , Theileriosis
Sheep, Goats		Tick-borne encephalitis ²
Horses	• Mosquitoes: - <i>Culex spp.</i> (mostly) - <i>Culex tarsalis</i> (mostly) - <i>Aedes spp.</i> and others - <i>Culex spp.</i> , <i>Aedes spp.</i> , <i>Culiseta spp.</i> - <i>Aedes spp.</i> , <i>Psorophora spp.</i>	→ Viral equine encephalitis (EE): - West Nile Virus ² - Western EE ² - Eastern EE ² - Highlands J EE - Venezuelan EE
Cattle, <u>Sheep</u> , Goats	• Mosquitoes (<i>Aedes spp.</i> and others)	→ Rift Valley Fever ²
Dogs&Cats	• Mosquitoes (several species e.g. from genera: <i>Aedes</i> , <i>Anopheles</i> , <i>Culex</i> , <i>Psorophora</i>) • Fleas (mostly: <i>Ctenocephalides felis</i>)	→ Dirofilariosis ² (canine heartworm) → Dipylidiasis ²
Dogs	• Phlebotomine sandflies (<i>Lutzomyia</i> or <i>Phlebotomus spp.</i>) • Triatomine bugs (<i>Triatoma spp.</i>)	→ Leishmaniasis ² → Chagas disease ^{2,3}

With the exception of the non-biting flies, all the other arthropods listed are blood-sucking.

² Zoonotic potential.

¹ *Simulium spp.* black flies are co-vectors for *Onchocerca volvulus* which causes human onchocerciasis, and for the *Onchocerca* species which cause disease in cattle. However, Onchocerciasis is not a “true zoonosis”, because humans are immune to the *Onchocerca* species that cause disease in livestock and vice-versa.

² Mites: Among the several types of mange in domestic animals, the only ones with zoonotic potential are the Sarcoptic mange from cattle and dogs (high risk) and the Demodexic mange from dogs (low risk).

³ Trials have recently started to test the effect of applying pyrethroid-impregnated collars in dogs upon Triatomine bugs.

References: The Merk Veterinary Manual (Merk, 2008) and USDA (1976), complemented with additional information from several published studies (some referred in the main text).

Table 1.5. Bioassays assessing the effects of insecticides applied on cattle upon malaria vectors.

Insecticide (application method)	Anopheles species (country)	(Reference)
DDT: Dichloro-Diphenyl Trichloroethane (dipping)	<i>An. superpictus</i> (Tajikistan)	(Lysenko et al., 1957)
Permethrin (spray and pour-on)	<i>An. quadrimaculatus</i> (USA)	(McLaughlin et al., 1989)
Permethrin (spray)	<i>An. quadrimaculatus</i> (USA)	(Nasci et al., 1990)
Cyhalothrin	<i>An. maculatus</i> Theobald, <i>An. dirus</i> Peyton (Malasya)	(Vythilingam et al., 1995)
Deltamethrin, permethrin, lambdacyhalothrin (sponging)	<i>An. stephensi</i> , <i>An. culicifacies</i> (Pakistan)	(Hewitt and Rowland, 1999)
Deltamethrin (spot-on)	<i>An. arabiensis</i> , <i>An. pharoensis</i> , <i>An. tenebrosus</i> (Ethiopia)	(Habtewold, 2004; Habtewold et al., 2004)
Deltamethrin (spray)	<i>An. arabiensis</i> (Tanzania)	(Mahande et al., 2007b)

1.5.1. Possible pitfalls from using ITL for malaria control

Notwithstanding the observed beneficial effects of ITL on both humans and livestock, the possibility of vector selection for (1) *insecticide resistance* and for (2) *anthropophily* cannot be ruled out. Caution should also be taken to minimize additional possible side-effects of ITL upon (3) the *cattle's immunity to tick-borne diseases*, (4) the *soil flora*, and (5) the *people* who will consume products from the treated animals.

(1) The mechanisms and implications of *insecticide resistance* in human disease vectors have recently been reviewed by Hemingway (2000) and Nauen (2007); and by Takken (2002) for ITNs and malaria. With regards to malaria vectors, it has been argued that the treatment of livestock with pyrethroids is not likely to induce stronger selection pressure for resistance than insecticide-treated nets or indoor residual spraying of houses and cattle sheds (Hewitt et al., 1994; Hewitt and Rowland, 1999; Rowland et al., 2001). However, since the insecticide dosage applied in livestock is lower than in the other methods (Hodjati and Curtis, 1997; Curtis et al., 1998; Rowland et al., 2001; Habtewold, 2004), that may make resistance more likely to develop with ITL. Given the development of pyrethroid resistance in anophelines from Africa, Asia and South America (Curtis et al., 1998), as well as in other arthropods that feed on livestock, such as ticks (Beugnet and Chardonnet, 1995; Rodriguez-Vivas et al., 2006) and horn flies (Byford et al., 1999), the importance of monitoring resistance as part of a large scale field trial of ITL has been acknowledged (Hewitt and Rowland, 1999; Rowland et al., 2001; Habtewold, 2004). Moreover, it has been recommended that research efforts should target the identification of an alternative non-pyrethroid insecticide for livestock treatment (Hewitt and Rowland, 1999).

A possible non-pyrethroid candidate could be ivermectin which is used in veterinary and human medicine against several helminths and arthropod pests (Wilson, 1993), and its use in animals (Pampiglione et al., 1985; Iakubovich et al., 1989; Jones et al., 1992; Fritz et al., 2009) and humans (Bockarie et al., 1999; Foley et al., 2000) has been shown to be toxic to anopheline mosquitoes. Ivermectin could overcome the potential problems of pyrethroid resistance as well as excito-repellence upon malaria vectors, and could be administered as part of mass livestock vaccination campaigns. In a recent study (Fritz et al., 2009), the survivorship and fecundity of *An. gambiae s.l.* were reduced in the laboratory (*An. gambiae s.s.* and *An. arabiensis*) after feeding on bovine blood treated

with ivermectin, and also in the field (*An. gambiae s.s.*) after feeding on cattle that had been injected with ivermectin although at a dose three times higher than the recommended. Additional laboratory studies showed that feeding on ivermectin-treated dogs (Jones et al., 1992) decreased the survivorship of *An. quadrimaculatus*, and feeding on ivermectin-treated mice (Pampiglione et al., 1985) and rabbits (Iakubovich et al., 1989) had similar impact on *An. stephensi*. A downside of ivermectin applied to cattle is that, because of its persistence, it should not be administered in lactating animals for a certain period before calving. An alternative could be the more recent eprinomectin which has similar antihelminthic and ectoparasitocidal action as ivermectin in cattle, but has much less mammary excretion, and therefore does not require a milk withholding period in dairy cows (Bishop, 2004). Further studies are needed to assess the insecticidal effect upon malaria vectors from treating livestock with the recommended dose of ivermectin or eprinomectin.

(2) Selection for *anthropophily* could occur assuming that host preference is determined by genetic polymorphisms (Coluzzi et al., 1979; Donnelly and Townson, 2000; Petrarca et al., 2000). The shift for anthropophily could be exacerbated by excito-repellence effects of the insecticide used, particularly pyrethroids. However, bioassays in Pakistan (Hewitt and Rowland, 1999) and in Ethiopia (Habtewold, 2004; Habtewold et al., 2004), have found no diversion of host-seeking mosquitoes from pyrethroid-treated cattle to nearby humans. Nevertheless, shifting host-preferences may be only observable in the long term, and therefore changes in the HBI (as a proxy for host preference) should be monitored in regions where repeated campaigns are undertaken (Hewitt et al., 1994; Rowland et al., 2001). Additionally, as suggested by Habtewold (2004), selection for anthropophily could be prevented by combining ITL with other strategies to control anthropophilic and endophilic mosquitoes, like insecticide-treated nets and indoor spraying with residual insecticides.

(3) A common practice in the veterinary management of *tick-borne diseases* (e.g. babesioses, anaplasmoses, theilerioses) is to allow young cattle to be exposed to the bites of infected ticks, since young cattle suffer only mild and transitory disease and develop long-lasting *immunity*, which prevents them from more serious illness if infected later when adults. Such immunity can build up an epidemiological state of the cattle population, named *enzootic stability*, in which clinical disease is rare in spite of high levels of infection (Coleman et al., 2001; Peter et al., 2005). When treating

livestock with insecticides that impact not only on mosquitoes and/or tsetse flies but also on ticks, it is therefore important to prevent disruption of enzootic stability of tick-borne diseases in cattle (Van den Bossche and Mudenge, 1999; Peter et al., 2005). Preventive measures that have been suggested for that purpose include: a) restricting insecticide application to adult cattle and to selective areas of the animal's body, in accordance with the preferred feeding sites of the target vectors' species, and b) timing interventions to specific periods of the year, in accordance with the dynamics of the vector species involved (Habtewold, 2004; Bourn et al., 2005; Peter et al., 2005; Torr et al., 2007).

(4) The excretion of insecticide residues in the dung of treated animals can also affect the *soil flora* responsible for decomposing the dung, and consequently impair soil fertilization in mixed crop-livestock systems (Wardhaugh et al., 1998; Vale et al., 1999; Bourn et al., 2005). Such collateral effects could be minimized by the restricted application of currently available insecticides, as mentioned above, and by the development of highly degradable insecticides with selective toxicity (Bourn et al., 2005; Peter et al., 2005).

(5) Finally, ITL is recognized as a safe practice for both animals and people, as long as one respects the *safety restrictions* that may be prescribed to ensure that unacceptable residues are not present in the meat and/or milk used for human consumption. Such restrictions depend on the chemical and formulation of the insecticide and on the treated animal species. Most insecticides require that animals are not slaughtered for a certain period after treatment, and some insecticides required also that milk is not used for human consumption during a certain period. There are, however, certain compounds which do not require a milk withholding period, such as some topical formulations of pyrethroids and the above-mentioned eprinomectin (Bishop, 2004).

1.6. Mathematical models of zooprophyllaxis and ITL for malaria

The use of mathematical models integrating mosquito population dynamics with disease transmission dynamics has been suggested as a useful tool for exploring the links between livestock and the web of malaria epidemiology under different scenarios (Sota and Mogi, 1989). However, despite the extensive list of malaria epidemiological models published in the last 100 years, only a minority have considered systems with multiple blood-meal hosts and only five models have addressed the question of zooprophyllaxis (Sota and Mogi, 1989; Killeen et al., 2001; Saul, 2003; Kawaguchi et al., 2004; Killeen and Smith, 2007). Here, a review is presented of those five models and of the frameworks they used to understand the effects of untreated and insecticide-treated livestock on malaria transmission.

All the existing malaria models of zooprophyllaxis are deterministic and consider the proportion of vector blood-meals taken upon humans (HBI) to be a function of the human and animal hosts abundance and availability to the vector (the term “availability” is named differently depending on the authors, although all share a similar underlying concept, as detailed next). All the models explore the effects that untreated livestock can have on human malaria. Additionally, some models look at the impact of treating cattle (Saul, 2003), cattle-sheds (Kawaguchi et al., 2004) or bednets (Killeen and Smith, 2007) with insecticide. The latter two account not only for the insecticidal effects of the intervention, but also for the potential of insecticide resistance (Kawaguchi et al., 2004) or excito-repellency (Killeen and Smith) effects upon the malaria vectors. The key features of each model are summarized in Table 1.6 and further detailed in the text below.

Table 1.6. Key features of the existing mathematical models of zoophylaxis and ITL for malaria.

Author (Year)	Model focus	Time unit	Vector population					
			Number of blood-meal hosts	Extrinsic incubation period	Adult survival	Blood-feeding success	Density-dependent regulation	Pop. density
Sota & Mogi (1989)	- Untreated livestock	- Vector: Discrete (time between ovipositions) - Malaria transmission: Continuous	2	No	Constant	Yes (in adult stage)	Yes (in larval stages)	Variable
Killeen et al. (2001)	- Untreated livestock	NA	2 or more	No	Constant	No	No	Constant
Saul (2003)	- Untreated livestock - Insecticide-treated livestock	Discrete (time between blood feeds)	3	Yes	Variable	No	No	Constant
Kawaguchi et al. (2004)	- Untreated livestock - Insecticide-treated livestock sheds (resistance)	Continuous	2	No	Constant*	No	Yes (in larval stages)	Variable
Killeen & Smith (2007)	- Untreated livestock - Insecticide-treated bednets (excito-repellency)	Discrete (time between blood feeds)	2	Yes	Variable	No	No	Constant

NA: Non-applicable. * Under no insecticide spraying.

1.6.1. Sota and Mogi

The first of these attempts was undertaken by Sota and Mogi (1989). The authors devised a model for vector population dynamics with two blood-meal hosts (human and a domestic animal) to investigate the potential impact of domestic animals on the vector population, the human biting rate, and the endemicity of malaria. The *Anopheles* population is described with a discrete time model, where the time unit is the duration of one gonotrophic cycle, i.e. time between ovipositions (as opposed to the continuous time Ross-Macdonald malaria model), assuming density-dependent regulation in the larval stage. Most importantly, feeding success in the adult stage is included as a limiting factor for the increase of mosquito population, a feature that had been ignored in most previous malaria models. Namely, the model incorporates a satiation effect for mosquito blood-feeding as a function of host density. It assumes that each female mosquito satiates with one single bite per blood-meal and per gonotrophic cycle, after which they lay a constant number of eggs. Therefore, in situations where there is a sufficiently high number of animals for the vector to feed upon, the rate of successful blood-feeding reaches a maximum, so called “satiation” level. Above this level, increasing the number of livestock would simply attract mosquitoes to dead-end hosts, with a negligible effect on the rate of successful blood feeding, while it would continue to decrease the HBI, with consequent reduction in malaria prevalence. A key parameter in the model behaviour is the *biting efficiency* on each host type. This is defined as the instantaneous rate at which a single female vector finds and then successfully bites on each host type. The biting efficiency parameter may encompass the following factors: (1) intrinsic probability of choosing to bite a host type, (2) accessibility of the host to the blood-seeking mosquitoes, and (3) behavioural interaction between mosquitoes and hosts. Accordingly, the concept of *effective number* of a host type is introduced as the product of the host abundance and the biting efficiency upon that host.

The endemicity of malaria infection was analysed after combining the vector model with an adaptation of the Ross-Macdonald model (Ross, 1911; Macdonald, 1952), consisting of a Susceptible-Infectious-Susceptible (SIS) model for humans, and a Susceptible-Infectious (SI) model for mosquitoes, thereby ignoring the extrinsic incubation period of the parasite in humans and vectors. According to the Sota-Mogi model, the introduction of domestic animals can lead to increasing vector densities, due to enhanced blood-feeding success upon the easily accessible animal blood source,

although decreasing the proportion of blood-meals taken on humans. The model predicts that this could either increase or decrease the vector biting rate on humans and the malaria transmission, depending on the effective number of animals introduced as well as on conditions prior to the introduction of animals, namely the mosquito population size and prevalence of malaria infection. Human biting rate could increase in situations where the ratio of humans to animal is high (i.e. low effective number of animals) and growth in the mosquito population is unconstrained. If this is the case and if the prevalence of human malaria is low, then endemicity is predicted to increase. Conversely, the introduction of domestic animals could decrease human biting rate and lower malaria endemicity (as proposed by the zoophylaxis concept), but only if very large numbers of animals are introduced for which the vector has high biting efficiency (i.e. high effective number of animals) or if the vector population had achieved its maximum level before the introduction. Yet, the authors point out that “even if the average number of bites on man is reduced owing to the introduction of domestic animals, mosquito density will be kept at high levels so that there will still be a risk of intensive mosquito bites for some part of the human population”. A decrease in endemicity was also predicted in scenarios where the prevalence of infection prior to the introduction is very high.

One of the limitations of the Sota and Mogi (1989) model is that it assumes at most a single bite per mosquito blood-meal and per gonotrophic cycle, while this will not apply in scenarios with considerable rates of multiple/mixed blood-feeding (e.g. Macdonald, 1952; Boreham, 1975; Briegel and Horler, 1993; Koella et al., 1998; Tirados et al., 2006). Additionally, the model assumes that the adult vector survival per gonotrophic cycle is constant, and independent of the effective number of animal or human hosts, and ignores the extrinsic incubation period of the malaria parasite in the vector. Moreover, despite performing a sensitivity analysis to estimate the impact of different parameter values, the model was not validated against empirical data.

1.6.2. Killeen et al.

More recently, Killeen et al. (2001) presented a simple model to relate vector feeding behaviour to the availability of potential blood-meal hosts, and moved a step further applying the theory to empirical data. Host *availability* is modelled based on the *biting*

efficiency described by Sota and Mogi (1989), except that the parameter is explicitly deconstructed to allow the analysis of two or more species of blood-meal hosts besides humans (e.g. humans, cattle and other alternative hosts). The availability of a given host to the vector population is defined as the “rate at which a typical individual host-seeking vector encounters and feeds upon that host in a single feeding cycle”.

The authors estimate the relative availabilities of humans, cattle and other potential blood-meal hosts to African malaria vectors in Segera, Tanzania (*An. funestus*, *An. gambiae s.s.* and *An. arabiensis*), and in The Gambia (*An. gambiae s.l.*), using human blood indices and ratios of cattle:human population density from published work in those countries (White et al., 1972; and Lindsay et al., 1993, respectively). The fitted model was tested by regression analysis of the predicted human blood indices and cattle blood indices against those obtained in the field. Based on the fitted estimates for relative host availabilities, they further explored the potential effect of increasing the relative cattle:human density on the EIR as a proxy to malaria transmission intensity. As in two previous studies (Koella, 1991; Killeen et al., 2000), the EIR was derived by a method alternative to the classical one, and was considered to be proportional to the square of the HBI.

The impact of zooprophylaxis on the EIR was modelled assuming that increased cattle density and/or availability simply reduce the proportion of vector blood-meals on humans (HBI), due to diversion of host-seeking mosquitoes to feeding on cattle. However, as recognised by the authors and elsewhere, the density and/or availability of hosts can also influence other key parameters of the EIR, namely, vector emergence rate (Focks et al., 1988) (as accounted for in the Sota and Mogi model), feeding cycle length (Charlwood et al., 1986; Charlwood and Graves, 1987), survival per feeding cycle (Charlwood, 1986; Graves et al., 1990) and dispersal (Gillies, 1961; Burkot et al., 1989; Service, 1991; Trape et al., 1992; Manga et al., 1993; Thompson et al., 1997). The assumption that all these parameters are constant is the main shortcoming of the Killen *et al.* (2001) model, for it fails to predict situations where increased livestock density and/or availability may enhance malaria transmission. The influence of host density, and particularly availability, on these other key determinants of EIR, and on its distribution, has been acknowledged as an issue that requires further investigation (Service, 1991; Mutero et al., 1999; Killeen et al., 2001).

1.6.3. Saul

The third published modelling approach to zoonophylaxis was undertaken by Saul (2003). He builds on previous models described above (Sota and Mogi, 1989; Killeen et al., 2001) and elsewhere (Saul et al., 1990), allowing for 3 types of blood source: the host of disease (e.g. humans, in malaria), a refractory or strongly immune host (e.g. livestock for human malaria); and a “bait” (e.g. livestock treated with insecticide or humans under bednet). The expanded structure of the model enables one to simulate not only malaria but also several types of vector borne diseases involving multiple host species for the pathogen, such as Japanese encephalitis virus.

Saul’s work is a ‘cyclic feeding model’, which assumes that after mosquitoes feed they will not feed again for a period, while the Ross-Macdonald model assumes a constant feeding rate. The term *attraction rate constant* of vectors is used instead of *availability* and *biting efficiency* from the previous two models (Sota and Mogi, 1989; Killeen et al., 2001), although the underlying concepts are the same. The main differences from the previous models rely on assuming a constant vector population size, and most importantly, a variable vector survival per feeding cycle, dependent on relative host abundance and accessibility. The model was applied to analyse the relationships between attraction rate of vectors to humans / animals and disease transmission potential under the scenarios of stable endemicity and epidemic outbreaks.

Despite the useful insights provided by Saul’s model, a drawback of his approach, recognised by the author himself, is that model validation is difficult due to the lack of available data to estimate the model’s most critical parameter, which was the mosquito mortality while searching for a blood-meal host, not least because of the problems associated with collecting such data.

1.6.4. Kawaguchi et al.

An additional model was developed by Kawaguchi et al. (2004), to explore the effects of combining zoonophylaxis with insecticide spraying of the human and/or cattle sites, and the potential of these strategies to limit the development of insecticide resistance in the vector. Different scenarios are investigated, regarding a) the vector relative

preference for feeding upon humans vs. cattle – anthropophilic or zoophilic; and b) the relative location of the human and cattle populations – mixed or separated from each other by a distance greater than the mosquito's daily blood-searching range.

Contrarily to other models that detailed the cyclic blood-feeding behaviour of the malaria vector (Graves et al., 1990; Saul et al., 1990; Killeen et al., 2000; Killeen et al., 2001; Saul, 2003; Killeen et al., 2004; Killeen and Smith, 2007), the work by Kawaguchi et al. (2004) uses a classical epidemiological approach to model the blood feeding process. The demographic dynamics of the mosquito population is explicitly modelled, assuming that the recruitment rate of emerging adult vectors is i) proportional to the daily biting rate and to the fecundity of each mosquito, and ii) is limited by density-dependent competition between larvae. The daily biting rate is assumed to be proportional to the host density, and the survival of the adult vectors is set to be constant under no insecticide spraying. These two assumptions regarding the vector biting rate and survival are similar to the ones by Sota and Mogi (1989), except that in the Kawaguchi *et al.* (2004) model the number of blood-meals per gonotrophic cycle is not limited to be one at the most – i.e. there is no satiation effect incorporated in the blood feeding.

The dynamics of the malaria transmission on the human and mosquito populations are modelled based on the classical Ross malaria model (Ross, 1911), similarly to the approach used by Sota and Mogi (1989). The effect of insecticide spraying is to increase the mosquito natural mortality by a factor proportional to the insecticide induced mortality and the vector visiting rate to the sprayed site(s). The model assumes that mosquitoes with insecticide resistance incur no additional mortality due to insecticide spraying but suffer a decrease in their fecundity compared to the wild-type mosquitoes, due to the resistance cost. The authors derive the conditions to achieve malaria control, with and without the development of insecticide resistance in the vector.

A limitation of the model is that, similarly to the Sota and Mogi (1989) model, it assumes a constant adult vector survival per gonotrophic cycle and ignores the extrinsic incubation period of the parasite in the vector. Although the Kawaguchi model does not account for a blood-host satiation effect, as Sota and Mogi (1989), the authors recognise that that effect is a factor which can determine the protective effect of zoophylaxis in a mixed habitat. It is suggested that in scenarios where the blood feeding rate is below

the satiation level, malaria could be controlled by zooprophylaxis only if livestock were placed at a distance from the human dwelling that was greater than the vectors daily blood feeding range.

1.6.5. Killeen and Smith

Finally, Killeen and Smith (2007) extended on the previous models of the malaria vector cycle feeding behaviour (Graves et al., 1990; Killeen et al., 2000; Saul, 2003; Killeen et al., 2004), to investigate the insecticidal and excito-repellent effects of personal protection methods, namely, pyrethroid treated bednets, on malaria transmission in four scenarios: either by *An. gambiae s.s.* or *An. arabiensis*, in the presence or absence of cattle as alternative host for the vectors. As the previous models, this also accounts for the vector feeding preferences and relative availability of the human and cattle hosts to the vector population. An improvement to Killeen's previous work (Killeen et al., 2000) was to allow for the availability of hosts to impact on the vector feeding cycle length and survival per feeding cycle, similarly to Saul's model. For that, the processes of host- seeking, encounter and attack to feed, as well as the associated vector mortality, are explicitly modelled.

Transmission intensity was expressed as the EIR, similarly to Killeen et al. (2000). Baseline mosquito behaviour, host availability and survival parameters were based on published field studies in three different areas of Tanzania (Segera, Namawala and Muheza), while the insecticidal and repellence parameters were based on trials conducted not only in Tanzania but also in other areas of East and West Africa.

The key assumption is that by repelling a mosquito away from a person who is protected under a bednet, the foraging period will be extended until the mosquito finds another host to feed, increasing the associated mosquito mortality. Insecticide-treated bednets therefore decrease transmission intensity by i) reducing survival per feeding cycle, for either mosquito species in the presence or absence of cattle, and ii) increasing the feeding cycle length for either mosquito species, except for the zoophilic *An. arabiensis* when cattle are present and mosquitoes can therefore be diverted to cattle. In the latter circumstance, although the feeding cycle length remains unchanged, the HBI is reduced, producing an even stronger decrease in transmission intensity.

A limitation of this model, as of previous models by the same first author (Killeen et al., 2000; Killeen et al., 2001; Killeen et al., 2004), is not accounting for the possibility that presence of cattle may increase vector density, which can counteract the decrease in the HBI and therefore result in either no net effect (if there is perfect compensation) or an increase (if there is overcompensation) on malaria transmission. Additionally, although this work has investigated both the insecticidal and excito-repellent effects of ITNs on malaria transmission, with the findings suggesting that excito-repellency would be beneficial, it did not explore the possible existence of excito-repellency thresholds above which the intervention might become deleterious by increasing malaria risk.

A limitation common to the five zoophylaxis models reviewed is the assumption of genetic homogeneity for host preference, despite genetic heterogeneity having been documented in feeding patterns within vector species (Gillies, 1964; Coluzzi et al., 1979; Hii, 1985). This factor should be taken into account in long term predictions of the effects of the introduction of livestock on malaria transmission to humans (Sota and Mogi, 1989). Additionally, all models assume that female mosquitoes are randomly dispersed in the local searching for hosts, although other studies have pointed out the possibility of non-random biting (Dye and Hasibeder, 1986; Hasibeder and Dye, 1988; Kelly and Thompson, 2000). Also, none of the presented works accounts for the effects of the spatial arrangement of livestock (e.g. uniform vs. clumped), and the distance to the human dwellings, on the level of attraction of mosquitoes to livestock, or on the effect of insecticide (Kawaguchi et al., 2004). Finally, the only two models that investigated the impact of applying insecticide on animals (Saul, 2003) or on animal sheds (Kawaguchi et al., 2004) have not accounted for possible excito-repellency effects of the insecticide upon malaria vectors, neither for the decay of the insecticide residual activity, nor for a fluctuation in vector population density following exposure to insecticide.

1.7. Density-dependent regulation of vector population

An important aspect when modelling the effects of untreated and/or insecticide-treated livestock on malaria transmission is to fully capture the ecological determinants of the mosquito population dynamics. A key ecological driver is the density-dependent regulation of the vector population, due to competition between the larval stages within breeding sites, and/or between the adult stages for blood-feeding success.

The way how this has been integrated into models varies in degrees. Among the five zooprophylaxis malaria models reviewed, only two incorporate density-dependence effects upon the larval (Sota and Mogi, 1989; Kawaguchi et al., 2004) and/or the adult stages (Sota and Mogi, 1989) of the vector population, allowing for vector density to fluctuate, while the remainder models do not, assuming constant vector population density.

1.7.1. Density-dependent feeding success of adult blood-sucking arthropods

Several laboratory and field studies have shown that **increased density of blood-sucking arthropods can result in two forms of density-dependent competition**, mediated either by the blood-meal host or by the arthropods. **Host mediated competition** is due to increased defensive and/or avoidance behaviour of the hosts being attacked by the arthropods, such as: (1) using a fly swat (active defence) or bed-nets (avoidance) by people (Charlwood et al., 1995, for anopheline mosquitoes), and (2) grooming by non-human vertebrates. The latter has been observed for various arthropods in different non-human vertebrates, such as: *Culex* mosquitoes in Ciconiiform birds (Edman et al., 1972; Webber and Edman, 1972); *Aedes* mosquitoes in several small mammal species (Klowden and Lea, 1978; Walker and Edman, 1986); horse-flies in horses (Waage and Davies, 1986); tsetse flies (Torr and Mangwi, 2000) and ticks (Norval et al., 1988) in cattle; and triatoma bugs (Schofield, 1985). **Arthropod mediated competition** is due to increased physical interference between the individual arthropods that are trying to feed on the hosts (e.g. Waage and Nondo 1982 for *Aedes aegypti* and rabbits). **These two forms of competition** lead to a decrease in the residence time of the arthropods on the host, which can **compromise** their:

- (1) **blood-feeding success** – for example, as shown for *Culex* mosquitoes in Ciconiiform birds (Edman and Kale, 1971; Edman et al., 1972; Webber and Edman, 1972), for sandflies (Coleman and Edman, 1987; Kelly et al., 1996) and triatoma bugs (Schofield, 1982) in laboratory mice and chicken, for horse-flies in horses (Waage and Davies, 1986), and for ticks in cattle (Norval et al., 1988);
- (2) **survival** - as seen for mosquitoes (Edman and Kale, 1971), and for triatoma bugs (Schofield, 1985);
- (3) and **host choice** - as shown for *Culex* mosquitoes feeding on different species of birds (Edman and Kale, 1971), and on birds vs. mammals (Edman et al., 1974; Nelson et al., 1976).
- (4) As a consequence, these effects can impact **disease transmission** – as explored theoretically by Kelly & Thomson (2000) for insect vectors in general, and by Basanez et al. (2007) for black flies and Onchocerciasis.

Most of the evidence for density-dependent blood-feeding success of arthropods comes from studies performed on animals – as reviewed above -, while only a few studies have concerned interactions with human hosts. The latter have focused on the vectors of malaria (Burkot et al., 1989; Lindsay et al., 1992; Charlwood et al., 1995) and Chagas disease (Schofield et al., 1986; Gurtler et al., 1997).

For the malaria mosquito vectors, it has been argued that, in the absence of bednets, density-dependent feeding success is likely to be negligible, since the vectors tend to blood feed at night, when the hosts are likely to be asleep, and therefore less responsive against bites (Charlwood et al., 1995). Such absence of density-dependent feeding success has been shown in a field study in Tanzania (Charlwood et al., 1995), although at least two other studies, one in The Gambia (Lindsay et al., 1992), and the other in Latin America (Davies C., unpublished observations), seemed to suggest the contrary.

With regards to bednets, if these are available, people tend to use them more often when mosquito densities are higher, as shown in different parts of The Gambia (Aikins et al., 1993; Thomson et al., 1994; Clarke, 2001), in Pakistan (Rowland et al., 2002), and Ethiopia (Franco A.I.O., unpublished observations). This could decrease feeding success and eventually increase vector mortality, namely if bednets are treated with insecticide. Indeed, in a study in Papua New Guinea where a very high percentage (67-98%) of villagers were sleeping under bednets, the proportion of the local malaria vector, *An. punctulatus*, that blood-fed on humans (HBI) decreased as the biting density

of the local malaria vector (*An. punctulatus*) increased (Burkot et al., 1989). In some circumstances, bednet usage may also lead to a change in vector behaviour to start biting earlier in the evening. Such change in biting pattern has been observed in Papua New Guinea (Charlwood and Graves, 1987), Tanzania (Magesa et al., 1991) and Kenya (Mbogo et al., 1996), where it was attributed to widespread and long term usage of insecticide-treated nets. However, early night biting pattern has also been observed in the Konso District of Ethiopia in an area where, despite bednet usage was scarce, a considerable proportion of bites on humans was shown to occur in the early hours of the evening, when humans were outdoors unprotected (Tirados et al., 2006).

1.7.2. Density-dependent competition between larvae stages

Larval crowding may result in intra-specific and/or inter-specific competition between larvae within mosquito breeding sites, due to food shortage, physical interference between individual larvae, and chemical interference due to production of growth retardant factor by larvae (Reisen, 1975; Reisen and Emory, 1977a, b; Schneider et al., 2000; Koenraadt and Takken, 2003) These mechanisms can limit the breeding site's carrying capacity to an extent that it may not be enough to sustain the additional larvae. Such density-dependent processes have been frequently observed in laboratory and have also been reported to occur in nature, for several species of culicines and also anophelines. The majority of studies of density-dependent competition between mosquito larvae have been in single-species populations, while fewer studies have been dedicated to competition between different species.

In single-species population of mosquitoes, high larvae density has been shown to result in:

- (1) increased **larval mortality**;
- (2) retarded **larval development**; and/or
- (3) decreased **size of pupae and emerging adults** (Table 1.7).

Adult mosquito body size, typically determined by wing-length, has been shown to be positively correlated with several factors in mosquito populations, namely: (a) **adult survivorship**¹; (b) **blood-feeding success**; (c) likelihood of being **inseminated**; and (d) **fecundity** (Table 1.8). The impact of fecundity can be threefold: firstly, smaller mosquitoes may have an increased need of additional blood-meals for the development of the first batch of eggs (e.g. Lyimo and Takken, 1993); secondly, they may lay fewer eggs per oviposition cycle, (Reisen, 1975; Briegel, 1990; Lyimo and Takken, 1993; Renshaw et al., 1994; Takken et al., 1998), and/or smaller eggs (Reisen, 1975); and thirdly, if their life expectancy is reduced, they will lay fewer egg batches throughout their life than larger mosquitoes (Amenshewa and Service, 1996).

Although the general trend is as mentioned above, exceptions have also been observed. For instance, a study in field populations of mosquitoes in Louisiana (Nasci, 1987) found that while for *An. crucians* and *Ae. taeniorhynchus* larger size females had lower survival probability, the opposite trend was observed for *Ae. sollicitans*. Also, laboratory studies (Lyimo et al., 1992) have shown that the adult body size of *An. gambiae* was affected by the interaction between density and temperatures: at certain temperatures, larval crowding could result in higher larval survival and larger adults.

4) In addition to influencing the mosquito population dynamics, these density-dependent effects can also impact the **vector competence and disease transmission dynamics**. For instance, if the survival of adult mosquitoes is compromised, then they are less likely to survive the extrinsic incubation period of *Plasmodium spp.* and become infectious to humans; i.e. the expectant infective life of vectors will also be shortened. Body size can also influence the likelihood of a mosquito acquiring infection, as shown by Lyimo & Koella (1992) who found that, among catches of wild *An. gambiae s.l.*, females of intermediate body size had a higher probability of being infected by *P. falciparum* in nature than larger or smaller ones. Additional studies have looked at relationships between body size and vector competence in other anophelines, in nature (e.g. *An. maculatus*: Kittayapong et al., 1992), and in laboratory (e.g. *An. quadrimaculatus*: Wing et al., 1985; *An. dirus*: Kitthawee et al. 1990). Similar studies have also been conducted for other vectors: e.g. *Ae. triseriatus* and LaCross encephalitis virus (Grimstad and Haramis, 1984; Paulson and Hawley, 1991), and *Ae. aegypti* and Ross River virus (Nasci and Mitchell, 1994).

¹ Usually measured by the proportion parous, i.e. proportion of females that have laid at least one batch of eggs (Service, 1993).

Table 1.7. Effects of increased larval density in single species populations of mosquitoes.

	Species	(Reference)
↑ Larval mortality	<i>An. gambiae</i> s.s. §	(Schneider et al., 2000)
	<i>An. arabiensis</i> §	(Schneider et al., 2000)
	<i>An. stephensi</i> §	(Reisen, 1975)
	<i>Ae. triseriatus</i> §	(Mahmood et al., 1997)
↓ Larval development	<i>An. gambiae</i> s.s. †	(Gimnig et al., 2002)
	<i>An. stephensi</i> §	(Reisen, 1975)
	<i>Ae. triseriatus</i> §	(Mahmood et al., 1997)
	<i>Ae. albopictus</i> ‡	(Lord, 1998)
	<i>C. pipiens</i> §	(Roberts, 1998)
↓ Size of pupae and emerging adults	<i>An. gambiae</i> s.s. †	(Gimnig et al., 2002)
	<i>An. stephensi</i> §	(Reisen, 1975)
	<i>Ae. triseriatus</i> §	(Mahmood et al., 1997)
	<i>Ae. albopictus</i> ‡	(Lord, 1998)
	<i>Ae. sierrensis</i> #	(Hawley, 1985)
	<i>Ae. cantans</i> #	(Renshaw et al., 1994)

Studies conducted in: § laboratory; † artificial habitats; ‡ semi-natural habitats; # nature.

Table 1.8. Factors positively correlated with adult mosquito body size in single species populations.

	Species	(Reference)
Adult survivorship	<i>An. arabiensis</i> [#] (Ethiopia)	(Ameneshewa and Service, 1996)
	<i>An. gambiae s.s.</i> [§]	(Takken et al., 1998)
	<i>An. stephensi</i> [§]	(Reisen, 1975)
	<i>An. dirus</i> [§]	(Kitthawee et al., 1990)
	<i>Ae. sierrensis</i> [#]	(Hawley, 1985)
	<i>Ae. cantans</i> [#] (England)	(Renshaw et al., 1994)
	<i>Ae. triseriatus</i> [#] (Indiana)	(Haramis, 1983)
Blood feeding success	<i>An. stephensi</i> [§]	(Reisen, 1975; Briegel, 1990)
	<i>An. albimanus</i> [§]	(Briegel, 1990)
	<i>An. quadrimaculatus</i> [§]	(Briegel, 1990)
	<i>An. dirus</i> [§]	(Kitthawee et al., 1990)
Likelihood of being inseminated	<i>An. arabiensis</i> [#] (Ethiopia)	(Ameneshewa and Service, 1996)
Fecundity	<i>An. gambiae s.l.</i> [#] (Tanzania)	(Lyimo and Takken, 1993)
	<i>An. stephensi</i> [§]	(Reisen, 1975)
	<i>Ae. cantans</i> [#] (England)	(Ameneshewa and Service, 1996)
	<i>An. crucians</i> [#] (Louisiana)	(Nasci, 1987)
	<i>Ae. taeniorhynchus</i> [#] (Louisiana)	(Nasci, 1987)
	<i>Culex tarsalis</i> [#]	(Bock and Milby, 1981, cited in Ameneshewa and Service 1996)

Studies conducted in: [§] laboratory; [#] nature.

Among the studies on **inter-specific competition**, many have considered culicines, particularly container-breeding species (*Aedes albopictus*, *Ae. aegypti* and *Ae. triseriatus* (e.g. Black et al., 1989; Livdahl and Willey, 1991; O'Meara et al., 1995; Lounibos et al., 2002; Alto et al., 2008), while only a couple of studies have investigated interspecific interactions between anopheline mosquitoes (Schneider et al., 2000; Koenraadt and Takken, 2003). The laboratory study by Schneider et al. (2000) revealed inter-specific larval competition between *An. gambiae s.s.* and *An. arabiensis*, with a detrimental effect only on *An. arabiensis*, which larvae had higher **mortality** rate in the mixed-species populations than when only this species was reared. Additional laboratory investigations of *An. gambiae s.s.*, *An. arabiensis* and *An. quadriannulatus* by Koenraadt and Takken (2003) have shown that older larvae of the *An. gambiae* complex can consume younger larvae of the same species (**cannibalism**) and also larvae of other sibling species (**predation**). Moreover, for *An. arabiensis*, even when larvae were not consumed, the presence of older larvae retarded the **development time** of younger larvae. Although predation was induced by food deprivation, cannibalism and retarded development times were not food dependent.

1.8. Introduction to the African Scenario: Konso District in Ethiopia

This section provides a general introduction to the African scenario of the Konso District in Southwest Ethiopia, where the field study described in Chapter 2 was conducted to parameterize the mathematical model developed in the subsequent chapters of this thesis.

1.8.1. Ethiopia

Ethiopia is one of the poorest countries of the world. According with the United Nation's Human Development Report, in 2004 more than three quarters (77.8%) of the population was living under 2\$ a day, and about a quarter (23.0%) under 1\$ a day¹. The total population was then 75.6 millions², most of which (84.3%) lived in rural areas.

The life expectancy at birth was 47.8 years, with an under-five years old mortality rate of 166 per 1,000 live births (UNDP, 2006; pp. 286, 294, 300).

Malaria is a major cause of morbidity in the country. During 2004, a total of 11,499,244 clinical malaria cases were reported, which accounted for 93.8% of all the morbidity causes. There were 4,662 reported deaths due to malaria, corresponding to 32.3% of all the mortality causes. About 30% of malaria deaths were in children under five years old (AFRO, 2006).

The main malaria parasites are *Plasmodium falciparum* and *P. vivax* and the predominant vector is *An. arabiensis* (e.g. Krafsur, 1977; Krafsur and Armstrong, 1978; White et al., 1980; Mekuria et al., 1982; Lulu et al., 1991; Abose et al., 1998). Additionally, *An. funestus* is the second most common malaria vector (e.g. Rishikesh, 1966; Gillies and De Meillon, 1968; Krafsur and Armstrong, 1978), and *An. pharoensis* Theobald is also considered a secondary vector (e.g. Rishikesh, 1966; Gillies and De Meillon, 1968; Nigatu et al., 1992; Abose et al., 1998). *An. nili* has also been implicated in malaria transmission (Krafsur, 1970, 1971; Krafsur and Armstrong, 1978). No *An. gambiae s.s.* has been found in the country (White, 1974).

¹ Data refer to the most recent year available during 1990 to 2004.

² The annual population growth rate, based on medium-variant projections for 2004-2015 is 2.3%.

Despite its poverty, Ethiopia is the African country with highest density of cattle per sqkm of land area (35.5 heads of cattle/Km²; 17.8 Livestock Units¹ of cattle/Km²), and with the second highest number of Livestock Units (LU) when considering all livestock (ca. 20.3 million LU, following Sudan which is in the top of the rank). In 2004 the livestock population included 35.5 million cattle, 11.4 million sheep and 9.6 million goats.² One of the main diseases affecting cattle is trypanosomiasis. Control strategies for its tsetse vector include the use of insecticide impregnated artificial targets and insecticide treatment of livestock as baits.

Administratively, Ethiopia is divided into nine ethnically-based states, and two self-governing administrative councils, one of which is the capital city (Addis Ababa). Each state is further sub-divided into administrative units which include, in descending order: *zones*, districts (*woredas*), neighbourhoods (*kebeles*), villages, and sub-villages (*kantas*). Figure 1.2 shows maps with the administrative regions of the country as well as the temperature, rainfall, elevation, and duration of the malaria transmission season.

1.8.2. Konso District

The Konso District (*Konso Special Woreda*) where the study described in Chapter 2 was conducted is located in the Southern Nations, Nationalities and Peoples State of Ethiopia (SNNP), ~600Km Southwest of Addis Ababa. The Konso land coverage is about 2,276.25 Km², characterized by hilly mountains intersected by gullies and valleys, most of which have been cultivated for hundreds of years. It is designated a *Special Woreda* due to the ethnic homogeneity and cultural identity of the local people (Torr et al., 2001), namely due to the particularity that all its inhabitants belong to the same tribe, the Konso tribe. Its Capital is the most centrally located kebele, called “Karat town” and it hosts the main administrative, educational and health care facilities in the District.

¹ One Livestock Unit = 250Kg.

² Source: Global Livestock Production and Health Data – GLiPHA
<http://www.fao.org/AG/AGAINFO/resources/en/maps.html> (accessed 31st March 2008).

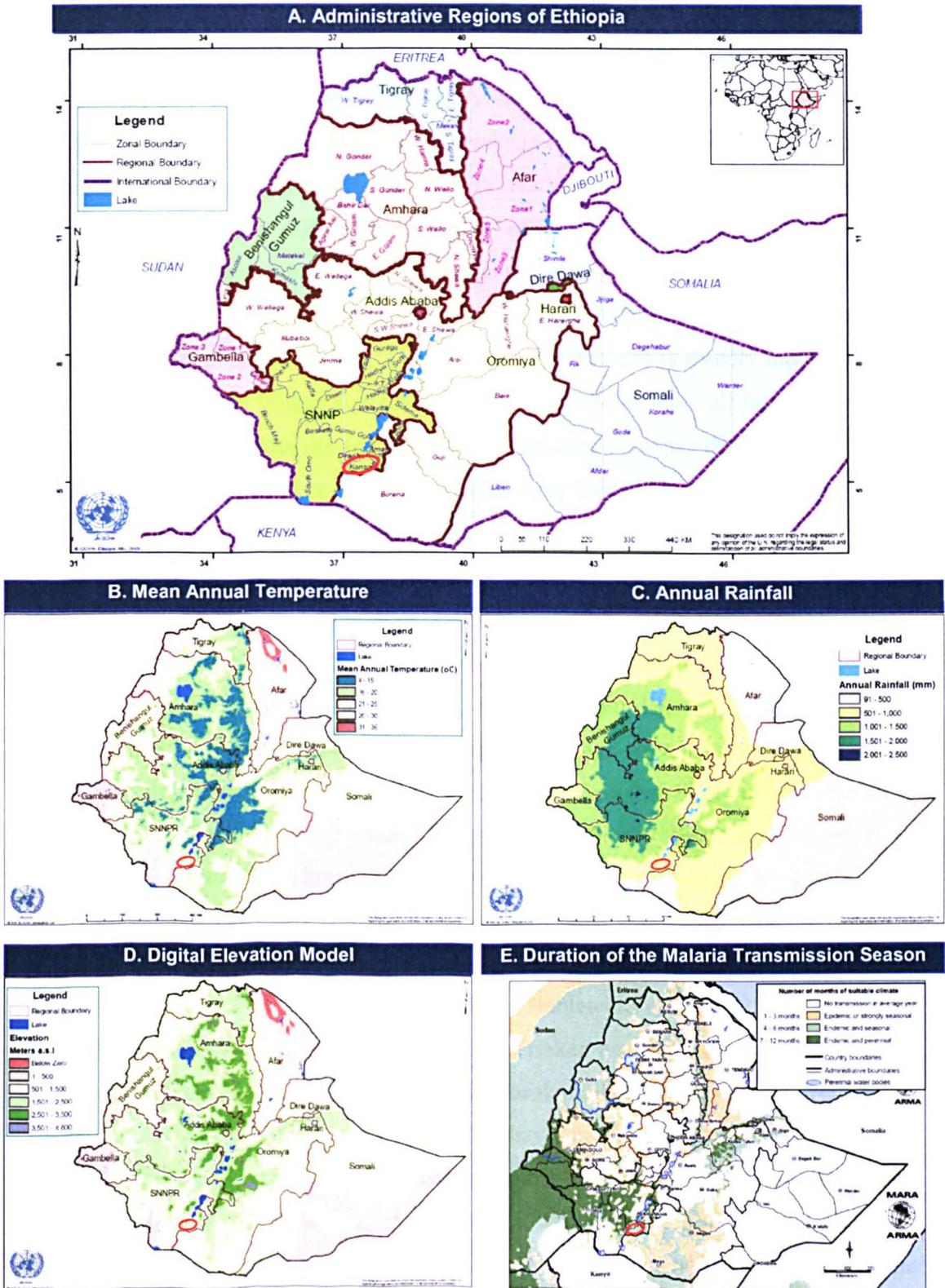


Figure 1.2. Maps of Ethiopia showing administrative regions (A), temperature (B), rainfall (C), elevation (D), and duration of the malaria transmission season (E).

The red ellipse highlights the Konso District, where the field study described in Chapter 2 was conducted, ~600 Km southwest from the capital Addis Ababa (12h drive in jeep). Mosquitoes are usually absent from areas at altitudes > ~2,500m. The lower lying area of eastern Ethiopia, bordering Somalia, is also too arid (<500 mm mean annual rainfall) to maintain mosquito populations. (Sources: A-D: United Nations Office for the Coordination of Humanitarian Affairs (UNOCHA)

<http://ochaonline.un.org/ethiopia/MapCentre/ReferenceMaps/tabid/2953/Default.aspx>;

In Konso, people live in two distinct types of settlements: (1) established villages, in mid-high altitude sites, and (2) temporary settlements in lowland sites, called ‘fora’¹, usually near a river, >5 km from the home villages. In the established villages at night, most people sleep inside a hut, while livestock stay outside but nearby, within the residence compound. Conversely, in the ‘fora’ cattle are brought to graze for months at a time, being tethered at night within a “fence” of thorn bush, while people tend to sleep outdoors near the cattle: during the dry season people sleep under small open huts, with thatched roof and no walls, while in the rainy season they sleep in platforms built on trees, a few metres above ground. The ratio of cattle/humans is usually much higher in the ‘fora’ than in the village settlements. Some households have all their cattle in the village where the animals are fed under a zero-grazing system; other households have all their cattle free-grazing in a ‘fora’; and some keep part of their animals in the village and the remainder in the ‘fora’.

The Konso communities keep high densities of livestock which, since 1994, have been sporadically treated with pyrethroid insecticides to control animal trypanosomiasis transmitted by tsetse flies (*Glossina spp.*) and tick-borne diseases (Torr et al., 2001). Following a trial of tsetse control using deltamethrin pour-on on livestock, local farmers apparently noticed a decrease in the incidence of malaria and in the mosquitoes biting rate (personal observation by local communities to Tibebe Habtewold) (Torr et al., 2001). This anecdotic evidence led to studies in the area to explore the question into more detail. Namely, two entomological studies have been conducted in Konso to determine the malaria vector feeding behaviour on humans and livestock, in relation with different livestock management practices (Habtewold et al., 2001; Tirados et al., 2006). Additionally, an experimental study undertaken near Konso has investigated the effect of insecticide-treated cattle upon the local malaria vectors (Habtewold et al., 2004). Evidence from these experimental behavioural studies suggested that, under some circumstances, ITL might be effective against *An. arabiensis* and therefore reduce malaria transmission. However, these studies have looked only at the effects of livestock on the vector component of malaria transmission, while the effects on the

In Figure 1.2, maps B&C were produced by the UNOCHA using data layers from WorldClim (<http://www.worldclim.org>). The data layers are on a 1Km² resolution and were generated through interpolation of average monthly climate data collected by weather stations from ~1950 to 2000. For a complete description, see: Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones and A. Jarvis, 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965-1978. Map D was produced by the UNOCHA using NASA - SRTM 90 meter Raster.

¹ ‘fora’ is an Oromo pastoralist term for a cattle herd managed in satellite camps (Habtewold, 2004).

actual disease in humans remained to be explored, making Konso remarkably appropriate for such a study.

1.8.2.1. Malaria vector species and feeding behaviour

As throughout Ethiopia, the predominant vector species in Konso District is *An. arabiensis* (Habtewold, 1999; Habtewold et al., 2001; Tirados et al., 2006), followed by *An. funestus* and *An. pharoensis* (Tirados et al., 2006).

In 2003 Tirados et al. (2006) undertook a comparative study in two settings that represent the two main types of human settlements in Konso district: an established village (Fuchucha) and a temporary settlement, i.e. a 'fora' (Jarso) (as briefly mentioned in section 1.4.1.2.1). The study found that in the established village of Fuchucha, where the ratio of cattle to humans was 0.6:1, ~66% of mosquitoes resting indoors (in huts) and ~51% of those outdoors (pit shelters) had fed on humans. The feeding ratio¹ on humans vs. cattle was approximately the same as expected when accounting for the relative proportion of human to cattle hosts present. Almost 2.5 times more biting mosquitoes and 5 times more resting mosquitoes were collected outside a hut than indoors, suggesting a strong exophagic and exophilic behaviour. In the other setting, the lowland temporary cattle camp site of Jarso, where the ratio of cattle to humans was 17:1, ~46% of the blood-meals had human origin (only mosquitoes resting outdoors, in pit shelters, could be sampled). The feeding ratio on humans was 8.3 times higher than expected in an area where cattle were 17 times more frequent than humans, suggesting a strong inherent preference of the *An. arabiensis* population for feeding on humans in this area.

This strong anthropophily was confirmed experimentally in Jarso using odour-baited entry traps (OBETs) baited with the odour of either one human or one ox. The human-baited traps collected ~6 times more mosquitoes than the cattle-baited one. Despite the strong anthropophily of *An. arabiensis*, a high number of blood-meals from cattle was observed, which ranged from 59% (indoors) to 71% (outdoors) in Fuchucha, and was as high as 91% (outdoors) in Jarso. According to the authors (Tirados et al., 2006), these

¹ Feeding ratio is the proportion of blood-meals from host A in relation to the proportion of host A in the host population. (Hess et al., 1968)

apparent contradictory results might be explained by the vector's high exophagy and exophily. A relatively high proportion of mixed human+cattle blood-meals was observed in all the scenarios, varying from 22% - 25% in Fuchucha (indoors - outdoors) to 37% in Jarso's cattle camps (outdoors).

In Fuchucha, there were no significant differences in the proportion of mosquitoes containing cattle blood-meals and the abundance of cattle and humans within the compounds. For example, in compounds containing only humans, 56% of the mosquitoes sampled had bovine blood compared with 54% of those sampled from compounds that had more cattle than humans.

Also, the sporozoite infection prevalence in mosquitoes fed on humans did not differ significantly from that in mosquitoes fed on cattle. This was concordant with findings from a previous study in Konso (Habtewold et al., 2001), and also from a later study in Sille, near Konso (Taye et al., 2006). As both Tirados et al. (2006) and Habtewold et al. (2001) mention, this suggests that it is not likely that there are behavioural subpopulations of *An. arabiensis* with different host preferences.

The nightly pattern of mosquito bites on humans was also studied in Fuchucha and Jarso, revealing that humans are exposed to a considerable proportion of bites in the early evening (19:00-22.:00) when they are outdoors and unprotected (Tirados et al., 2006).

1.8.2.2. ITL and control of the malaria vector

In 2001 experimental studies were conducted in the Sille Valley, near Konso, to determine the effect that treating cattle with the pyrethroid formulation used to control ticks and tsetse (deltamethrin 1%, spot-on) could have on the mortality and feeding behaviour of the main malaria vector, *An. arabiensis* (Habtewold, 2004; Habtewold et al., 2004).

Contact bioassays demonstrated that deltamethrin remained effective against *An. arabiensis* for ~ 4 weeks (>50% killed), when mosquitoes were exposed for 3 minutes to the flanks of treated cattle (cup bioassay). However, the duration of the effect was reduced to ~1 week under field conditions, where mosquitoes land on the animals for less than 1 minute to feed (whole animal bioassay). The explanation proposed by the authors is, not only the shorter

exposure time of mosquitoes to treated cattle, but also the fact that >90% of the mosquitoes tended to feed on the legs of cattle where the insecticide dose is lower. The active compound is less retained in the legs because these are further away from the application point, and also the insecticide may be more easily washed off from the legs when the cattle walks through wet areas.

Treatment of cattle with deltamethrin did not seem to have a short- or long- range effect on the attraction of *An. arabiensis* to their hosts; in particular, the vector did not appear to be diverted from the treated cattle to a nearby human host. Namely, there was no significant difference between the numbers of *An. arabiensis* biting on humans near a treated and an untreated ox. However, there was a decrease in the mosquitoes' landing times and feeding success on treated animals, which the author (Habtewold, 2004; Habtewold et al., 2004) suggests might have been due to contact with the sub-lethal dose of insecticide on the legs.

1.8. Gaps in current knowledge

As can be concluded from the literature review, several factors underlying the effects of untreated and insecticide-treated livestock on malaria remained poorly understood. For example:

1. How does host abundance and availability influence the feeding behaviour, survival and density of the malaria vector, and the overall disease transmission dynamics?
2. How do vector feeding behaviour, survival and density impact on the potential success of an ITL intervention?
3. How does density dependent regulation of adult and/or larval mosquito vector population influence the effects of untreated and insecticide-treated livestock on malaria transmission?
4. How relevant are coverage treatment levels? Namely, what proportion of the livestock population and which animal species should be preferentially treated, accounting for the potential differential abundance, accessibility and irritability of animals species vs. humans to the malaria vector, and the intrinsic host preference of vectors?
5. How does livestock demography influence the intervention outcome?
6. How frequently should animals be treated?
7. What is the best timing for the intervention in relation with the seasonality of malaria transmission?
8. What is the impact of using an insecticide with longer lasting residual activity?
9. If the insecticide used has possible excito-repellency effects upon the malaria vector, how may that impact malaria transmission?
10. How should one maximize the ITL cost-effectiveness while minimizing the opportunities for development of vector resistance to insecticide and shifting host preferences for humans?
11. Given that ITL was successful at reducing malaria cases in a Pakistan community trial, could the intervention also reduce the risk of malaria in humans at the community level in Africa, where the disease burden is the greatest, but the dynamics and determinants of infection differ from Asia and the intervention is yet to be formally tested? And in which areas of Africa could ITL potentially cause larger reductions on malaria transmission?

This thesis attempts to answer most of these questions, with the exception of questions 5, 7 and 10, which will be addressed in the discussion sections.

1.9. Thesis aims and structure

This thesis aims to clarify the different effects that livestock can have on human malaria in areas where the disease is transmitted by zoonophilic vectors, in order to understand under which circumstances livestock-based interventions could play a role in malaria control programmes. In particular, it explores the impact of livestock presence, abundance, and management practices – focusing on insecticide treatment of livestock. This was achieved through the development of a comprehensive mathematical model of malaria transmission and its integration with empirical data from the field study I conducted in the Konso District of Ethiopia and from elsewhere.

Chapter 2 describes the socioeconomic and environmental factors that influence malaria transmission in the Konso District of Southwest Ethiopia, an area of typical malaria transmission in Africa, with a particular focus on livestock ownership and management practices. Data were collected through systematic search of the literature and through structured interviews conducted during my field study in Konso. The information collected will allow parameterization of the mathematical model developed in the next Chapters to this African scenario.

Chapter 3 sets the bases for the theoretical framework, and outlines the general structure and analysis of the deterministic mathematical model for the transmission dynamics of malaria that was built expanding on the Ross-Macdonald model. The expanded model discriminates the feeding behaviour of the mosquito vector on its alternative hosts: livestock and human populations, and incorporates the treatment of livestock with insecticide as a potential *novel* method to control human malaria. The threshold dynamics of the system is investigated, and the basic reproduction number is analytically derived, as well as its sensitivity to parameter values.

In Chapter 4, the basic model is extended to explore under which circumstances can untreated livestock potentially increase, decrease or have no impact at all on malaria transmission to humans. This is done by explicitly modelling the possible effects that varying the abundance and/or availability of livestock could have on the malaria vector feeding behaviour, mortality and density and on the overall disease transmission dynamics. Several hypothetical ecological settings are considered. The framework also incorporates the density-dependent regulation of the adult vector population due to

larval competition within breeding sites, enabling to simulate scenarios where vector density can fluctuate.

Chapter 5 moves to investigate the impact of treating livestock with an insecticide that has a lethal and possible excito-repellent effect upon malaria vectors. Analyses are also performed of the threshold coverage of treatment with a non-repellent insecticide required for interruption of transmission, and how that depends on the vector blood feeding behaviour and density. Simulations are initially done in three hypothetical ecological settings, where livestock are much more, as much, or much less available to vectors than humans. The model is then applied to explore how an ITL intervention that successfully reduced malaria transmission in a Pakistan community trial could be best translated into the Ethiopian setting described in Chapter 2. Accordingly, the model is fitted to these specific Asian and African settings in Chapters 5 and 6. Simulations are done under different scenarios of density-dependent regulation of the adult vector population, where vector density is allowed to fluctuate following exposure to ITL.

Finally, Chapter 7 highlights the key findings of the thesis and discusses their implications, pointing out additional challenges and future directions.

Chapter 2

The African scenario:

Malaria epidemiology, livestock ownership and insecticide treatment in Konso, Ethiopia

Summary

Background: Despite ITL having reduced malaria cases in a Pakistan trial, the effectiveness of this intervention is yet to be formally tested in Africa, where malaria burden is the greatest, but where the dynamics and determinants of infection differ from Asia. Mathematical modelling is a powerful tool that can be used to explore and compare the potential impact on malaria of different livestock-based intervention scenarios within and between ecological settings. However, rigorous application of mathematical modelling for this purpose depends on a detailed understanding of the epidemiology of malaria, livestock ownership and insecticide treatment. This Chapter characterizes these aspects in the Konso District of Southwest Ethiopia, an area of typical malaria transmission in Africa, where people's subsistence depends largely on livestock which are highly abundant and have occasionally been treated with pyrethroid insecticides to control animal trypanosomiasis transmitted by tsetse flies and tick-borne diseases. Additionally, entomological studies have recently been conducted in the area and nearby, that investigated the effects of untreated and insecticide-treated livestock on the feeding behaviour and mortality of the main local malaria vector, *An. arabiensis*, although the effects on the actual disease in human remained to be assessed.

Methods: Three data collection methods were employed: (1) systematic search of the literature; (2) interviews to several Ethiopian national and local agencies; and (3) structured interviews to 214 households in 8 kebeles, and to the chairman of each of those kebeles, during a field trip to Konso in 2004. Data were gathered on: human and livestock demography; malaria control activities; seasonality, gender, age and geographical distribution of malaria cases; livestock management and insecticide treatment practices; and on additional factors that may affect the disease epidemiology.

Findings: Malaria is the most frequent human health problem in the Konso District, occurring throughout all year. During 2004, most cases were concentrated from May to December, peaking in July and August (~2 months after the rainfall peaks). Most cases were

due to *P. falciparum* and only a minority due to *P. vivax*. The incidence of clinical malaria was higher in males and in the 'over 15 years' age-group, suggesting that the adult population in Konso is not-immune to infection. Most malaria cases in the District were diagnosed only on clinical symptoms due to insufficient laboratory diagnosis facilities. The reported treatment failure of uncomplicated *P. falciparum* malaria to CQ and SP ranged from <25% to >70%, indicating high resistance of this parasite to the antimalarials being used. Spraying of houses with residual insecticide was limited to a small minority of villages in the District, of which only one of the study kebeles, Fuchucha, was included. The insecticide mostly used was DDT, despite *An. arabiensis* having shown considerably resistant to this insecticide throughout Ethiopia. The distribution of bednets had just recently started in the study area, and only a minority of the study population were using insecticide-treated nets. Most households interviewed (88% -189/214) had at least one type of domestic animal present in their village compound, where sheep/goat were the most frequent animal (n=167), followed by zebu cattle (199), and chicken (72). There was an estimated mean of 1.13 (95%CI=0.61-1.64) animals/person, with sheep/goats being usually more than three times the number of cattle, except in Fuchucha where cattle were the most frequent species. ITL with Deltamethrin 1% pour-on was provided free of charge by the government from February to July 2004, in 21 out of the 46 kebeles in the District. The free treatment was then interrupted although some persons still continued treating their livestock. ITL was more commonly reported for cattle, while only a minority of the people with sheep/goats had them treated. Amongst the households interviewed with cattle and/or sheep/goats in the village, 34% (68/128) reported that at least one animal type had been treated with insecticide. Amongst the households with treated animals 93% reported that only less than half of the number of each of the animal types present had been treated. The most reported frequency of treatment was once a month (61%), as recommended by the governmental programme, although it ranged from three times a month, to once a year.

Interpretation: The information collected will allow parameterization of a comprehensive mathematical model that will be developed in the next Chapters to explore the potential effects of untreated and insecticide-treated livestock on malaria transmission. The model will be applied, initially, to several hypothetical scenarios (Chapters 4 and 5), and, subsequently, to two specific ecological settings: the area of the Ethiopian study described in this Chapter and the area of the Pakistan ITL trial (Chapters 5 and 6).

2.1. Introduction

Malaria transmission is influenced by different levels of heterogeneity, at the village, family or individual level. A complex network of interactions between the human host, parasite, mosquito vector and environmental factors plays a key role in such heterogeneity (Greenwood, 1989). However, the relative contribution of each of these elements is still unclear.

As reviewed (section 1.4.1), in areas where malaria vectors feed both on livestock and humans, the presence of livestock close to the household may affect the probability of vector-human contact, and consequently the risk of malaria transmission to humans. The nature and extent of this impact are likely to be vector and site specific. Nevertheless, despite the several studies that have been conducted, its determinants remain uncertain. It has recently been suggested that in those areas, the insecticide treatment of livestock (ITL) could be a complementary tool for controlling malaria vectors, and hence reducing the incidence of human malaria. This strategy was tested in a community trial in Pakistan where encouraging results were obtained, with significant reductions in the number of malaria cases, as well as on vector survival and density (Rowland et al., 2001). Additional studies followed to explore whether the strategy could also be applied in sub-Saharan Africa, for integrated control of malaria and animal trypanosomiasis and tick-borne diseases. Namely, bioassays have been conducted in Ethiopia (Habtewold et al., 2004) and in Tanzania (Mahande et al., 2007b) to assess the effects of ITL on the mortality and behaviour of malaria vectors. Among the studies that investigated the effect of livestock management practices on malaria transmission in Africa and other continents, most have been restricted to the effects that presence of domestic animals near human dwellings can have on the malaria vector-human contact, and only a minority of studies has examined the impact on the risk of clinical disease. Moreover, no study has yet investigated the impact of ITL on the risk of clinical malaria at the community level in Africa, where malaria burden is the greatest, but the dynamics and determinants of infection differ from Asia. In the meanwhile, a useful alternative approach to a large scale community trial in Africa is to explore the potential effects of untreated and insecticide-treated livestock on malaria transmission using a theoretical framework, namely mathematical modelling, which will be the focus of the next Chapters.

Rigorous application of the mathematical modelling depends on a detailed understanding of the epidemiology of malaria, cattle ownership and insecticide treatment. Accordingly, this Chapter characterizes these aspects in the Konso District of Southwest Ethiopia, an area of typical malaria transmission in Africa, where people's subsistence depends largely on livestock which are highly abundant and have occasionally been treated with pyrethroid insecticides to control animal trypanosomiasis transmitted by tsetse flies and tick-borne diseases. Additionally, entomological studies have recently been conducted in the area and nearby, by researchers from the Natural Resources Institute (NRI, University of Greenwich, UK) and FARM-Africa (Food & Agricultural Research Management – UK based Charity working in Ethiopia), that investigated the effects of untreated and insecticide-treated livestock on the feeding behaviour and mortality of the main local malaria vector, *An. arabiensis* (Habtewold et al., 2001; Tirados et al., 2006). Evidence from these studies suggested that, under some circumstances, ITL might be effective against *An. arabiensis* and therefore reduce malaria transmission. However, these studies have looked only at the effects of livestock on the vector component of malaria transmission, while the effects on the actual disease in humans remained to be explored, making Konso remarkably appropriate for this study.

During 2004 I conducted a field trip to the Konso District of Ethiopia with the aims of (1) parameterize the mathematical model of malaria presented in the next Chapters and (2) improving existing knowledge about the effects of livestock on malaria risk. The ethical approval I was given by the Ethiopian authorities allowed only a non-invasive study where no biological samples could be collected. Obtaining approval for an invasive study would have required a longer waiting period, resulting in missing that year's malaria transmission season, and therefore a non-invasive study was designed. Accordingly, a retrospective population-based case-control study was performed to investigate the association between the likelihood of residents in Konso villages of Ethiopia being diagnosed with malaria and the livestock management practices in their residence compound. Namely, the study aimed to assess the effects on malaria risk of livestock: (1) presence, (2) abundance, (3) treatment with insecticide; and (4) shelter type and location. Additionally, other potential risk factors for malaria were also accounted for. To assist the design of the study and to improve the existing knowledge of malaria and livestock in Konso, additional data were collected on several socioeconomic and environmental aspects that may influence the disease transmission.

Since the study had to be non-invasive, cases for the case-control study were selected amongst the patients who: (1) had clinical symptoms of malaria confirmed by a positive blood slide for *Plasmodium spp.* - as read on microscope at the only two health facilities in the Konso District that had laboratory facilities: Karat Health Centre (KHC) and Mekane Yesus Health Station (MYHS) -, and (2) were resident in one of the 8 kebeles chosen for the study. The study included a total of 107 cases diagnosed during the main malaria transmission season (1st June to 3rd December 2004) and 107 matched controls. The reliability of the blood slide readings at the KHC was assessed from a second reading of a sub-sample of slides at the LSHTM Malaria Reference Laboratory. Unfortunately, this quality control showed that 33% (95% CI: 24-42%) of the slides diagnosed as positive for *Plasmodium spp.* in the KHC were diagnosed as negative in the LSHTM Malaria Reference Laboratory¹ (Appendix A5), and therefore it was likely that 33% of the 107 case-control pairs selected for the study consisted not of one malaria case and one control but of two controls, which invalidated the case-control study findings. Nevertheless, some of the findings from the interviews conducted could still be used to parameterize the model, and are therefore presented in this Chapter, together with socio-economic and environmental data that were compiled to build up a detailed characterization of the area.

2.2. Materials and Methods

2.2.1. Selection of the study area

A subset of eight kebeles amongst the 46 kebeles of the Konso District was selected for the study based on meeting the following criteria²: i) highest number of parasitological confirmed malaria cases from 1st June to 31st August 2004; ii) most people and livestock were staying in the village overnight; iii) travelling distance to the laboratory diagnosis health facilities was less than 30 Km; and iv) insecticide treatment of livestock was

¹ n=105 slides; Sensitivity: 70% (16/23; 95% CI= 61-79%); Specificity: 90% (74/82; CI= 84-96%); Positive predictive value: (67% - 74/81; CI=58-76%); Negative predictive value: (91% - 16/24; CI=86-96%); Overall accuracy: 86% - 90/105.

² Information sources for the selection criteria i) laboratory records from the only two health facilities that had a laboratory in the District: Karat Health Centre and Mekane Yesus Health Station; ii) and iii): interview to the Konso Agriculture Office; iv) interview to the Konso Government Veterinary Clinic.

taking place in some of the kebeles. A map of the Konso District highlighting the eight kebeles selected for the study is presented in Figure 2.1.

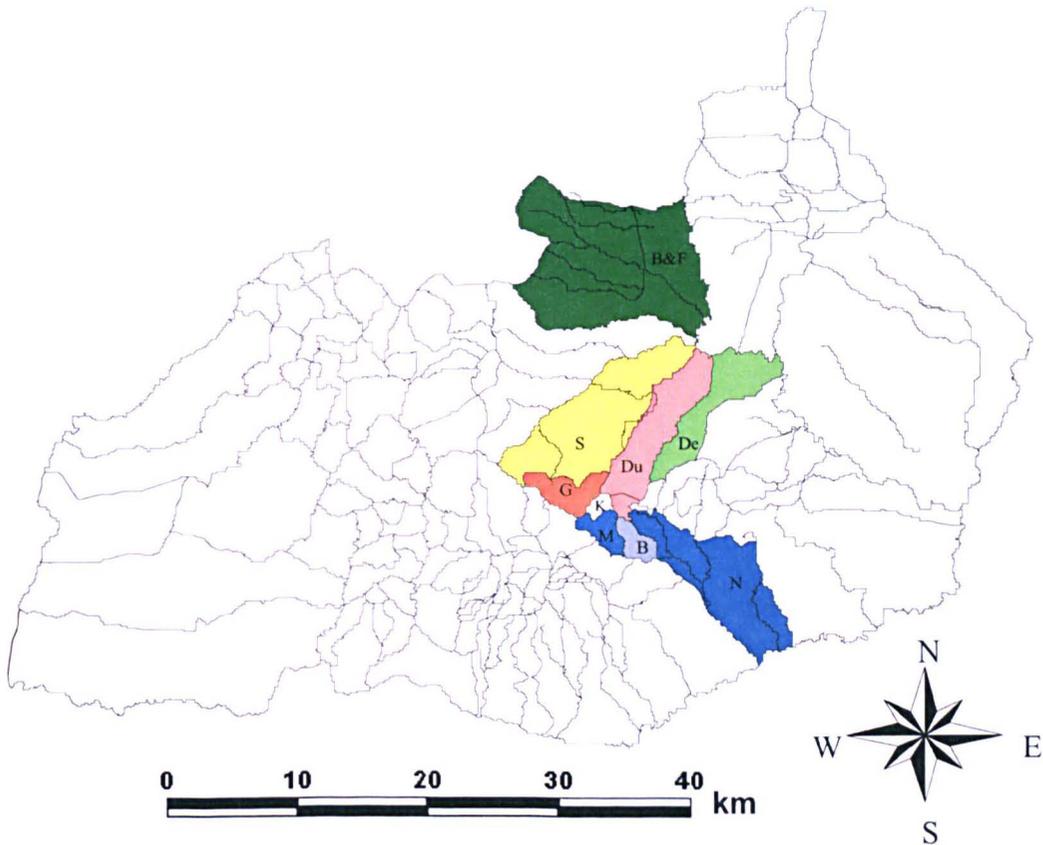


Figure 2.1. Map of the Konso District.

The eight kebeles selected for the study are highlighted with colours: Du=Dokatu, De=Duraite, N=Nalaya Segen, S=Sorobo, G=Gamole, B=Buso, M=Mechelo, B&F=Baide and Fuchucha. The central white area (K) is Karat, the capital town of the District, where I was based. (Source: Adapted from a map provided by the Ethiopian Government to Steve Torr / NRI).

2.2.2. Data and sources for the characterization of the study area

Data were collected for the years previous to the study and also for the whole year of the study. *Secondary data* was gathered from previous studies conducted in the area by others and also from me contacting/interviewing Ethiopian national and local agencies, namely: United Nations Office for the Coordination of Humanitarian Affairs in Ethiopia, Ethiopian National Meteorological Services Agency, FARM-Africa, Konso Development Association (KDA), Konso Health Office, Konso Agriculture Office, and the Konso Government Veterinary Clinic. Examples of secondary data obtained include: maps, temperature and rainfall, census data on humans and livestock, available

health services for humans and livestock, human diseases, records of malaria patients diagnosed in the two main health facilities, malaria control activities, and ITL practices. *Primary data* were collected through structured interviews to the chairman of each of the eight study kebeles and to the 214 participants of the case-control study (107 cases and 107 neighbourhood and age¹-matched controls), using specifically designed questionnaires. Examples of primary data gathered include: number of livestock kept in the study villages, details about ITL practices, household demography, characteristics of the sleeping room, and knowledge and usage of protection methods against mosquito bites.

2.2.2.1. Number of malaria cases

Data on the incidence of all malaria cases diagnosed during 2003 in the whole District were collected from Summary Statistics at the Konso Health Office. Data for the whole 2004 were collected from the main health facility in the District, Karat Health Centre² (KHC). For the incidence of all suspected malaria cases there diagnosed, data were detailed by gender, age-group, type of malaria (*P. falciparum*, *P. vivax*, or unspecified) and month; and for the number of laboratory confirmed cases³, data were detailed by gender and month.

Additionally, for the duration of the study period (1st June to 3rd December) and for the study kebeles only, data were collected on the incidence of all suspected malaria cases diagnosed at the KHC⁴, and the number of laboratory confirmed cases at the KHC or at the Mekane Yesus Health Station (MYHS), detailed by gender, age-group, type of malaria, month and kebele⁵.

¹ Age groups: ≤ 5, 6-9, 10-14, 15-44, 45-64, ≥65 years old.

² Outpatient and Inpatient Summary Statistics Monthly Reports.

³ Outpatient and Inpatient Laboratory Statistics Monthly Reports

⁴ Outpatient Records Book for ≥5 years old, and the Records Book for <5years old.

⁵ Laboratory log book.

2.2.2.2. Livestock data

Livestock data were collected separately for each animal type from: the Konso Agriculture Office, the Konso Government Veterinary Clinic, the chairman of each of the eight study kebeles, and the participants of the case-control study. The data collected from the interviews to the participants of the case-control study consisted of: number of animals present at night in the village compound where the study subject was sleeping; type of roof and wall of the animals' shed; distance from animals to subject sleeping room; and treatment of animals with insecticide. If animals had been treated with insecticide, the proportion treated was recorded as well as the interval between treatments. Information was also gathered regarding the number of animals kept outside the village compound (in the 'fora'), and the treatment of these animals with insecticide.

2.2.2.3. Conducting the interviews

The interviews were conducted under my supervision and in two rounds: from 9th September to 8th October 2004, and from 29th November to 11th December 2004. In Konso District many people do not speak or read the official National language, Amharic, and only speak the local dialect, Konsigna, which is not a written dialect. Therefore, the questionnaire was written in English and the interviews were conducted in the local dialect. Six local persons with knowledge of English were selected and trained by me to conduct the interviews. Several preliminary versions of the questionnaires were tested during the training period of the interviewers, before a final version was defined. Potential differences between interviewers in the method of selecting the case-control study participants and of collecting the information were minimized by the use of written guidelines for the interview procedure and standardized questionnaires. The field assistants were given information about malaria epidemiology and prevention, so that after concluding an interview they could advise the study participants on prevention methods¹.

¹ For example, when the interviewee replied not knowing how to be protected from mosquito bites, in the end of the interview, he would be advised to: close any openings in the house (e.g. close holes in the walls with mud; seal windows with journal/paper), and that people should cover them selves at night, and sleep under a bednet.

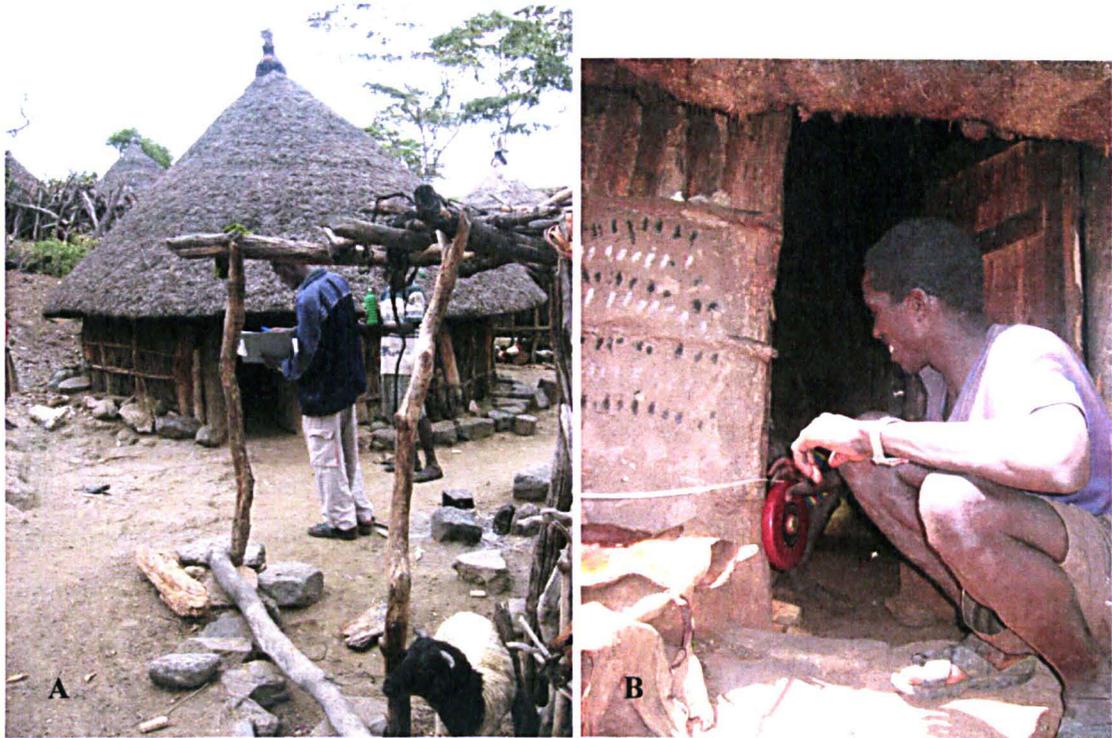


Figure 2.2. Conducting an interview in a Konso village in Ethiopia.

A) One of the six local persons that I selected and trained to assist me in the study is concentrated filling the study questionnaire. B) Another helper measuring the distance between the sleeping room of a study participant and the livestock shed.

2.2.3. Ethical Considerations

The study was authorized by the Ethiopian Southern Region Health Office in Awassa, and the Ethical Committee of the LSHTM. Additionally, consent was requested from the chairman of each of the eight study kebeles and from each study participant. The study subjects (malaria cases/controls, or their parents/guardians if the subject was < 15 years), had the purpose of the study explained to them in the local *Konsigna* language and were asked if they were willing to participate. An informed consent was obtained for each participant, confirmed by signature or thumb print for illiterate subjects.

After the interview, a soap bar was offered as acknowledgment for the time and help provided. Previous studies in the area have used as acknowledgment method to offer the interviewees a round of the local beer. Instead, offering a soap bar was chosen since this was a public health study, and there is strong evidence showing that a high percentage of infectious diseases can be prevented by the simple act of washing hands with soap (Curtis and Cairncross, 2003).

2.3. Results

2.3.1. General characteristics of the study population

2.3.1.1. Demography of the human and livestock populations

In 2004 the Konso District had an estimated population of 206,607 inhabitants, of which 49.1% were males, and 54% of people were below 18 years old¹. The total livestock population in 2003/04 was 338,221. The most abundant species were sheep/goats (50.46% - 44.50% goats and 5.95% sheep), followed by Zebu cattle (42.18% - 38.63% mature and 3.56% calves). There were also some chickens (6.64%) and only a small number of mules/donkeys/horses (0.73%)². Animal health was provided by a veterinary clinic, based in Karat town, and by community animal health workers, promoted by FARM-Africa.

The distribution of the human and livestock population in the study kebeles is in Tables 2.1 and 2.2.³ The total human population in the 9 study kebeles was 28,710 persons, with a mean of 3,190 persons/kebele (range: 5,459 in Dokatu to 1,065 in Fuchucha) (Table 2.1). The overall livestock population was 97,011 animals, with a mean of 10,779 animals/kebele (range: 19,676 in Duraite to 1,695 in Gamole). In most kebeles, the more frequent livestock species were cattle, followed by sheep/goats. In Gamole sheep/goats were the most frequent species, and in Dokatu and Duraite there was about the same number of cattle as of sheep/goats (Table 2.1).

To estimate the relative abundance of livestock to humans in the study area, an initial attempt was made using census data of the human and livestock populations in each of the study kebeles. However, these data do not inform about how many animals or persons stayed overnight in the village versus outside in the 'fora' cattle camps. Since some of the animals were kept in the 'fora', the true number of livestock in the village

¹ Source: Projections from previous Census done in 1994, provided by the Konso Rural Development Office; the data for each kebele is in Appendix A1.

² Source: Census by the Konso Rural Development Office; the data for each kebele is in Appendix A2.

³ Although Baide and Fuchucha are two villages that together constitute one single Kebele, hereafter in this Chapter they were described separately as if they were two distinct kebeles, for which the study area will be described as consisting of 9 kebeles (instead of 8). This is because i) the two villages are located considerably apart and ii) since a number of previous studies have been conducted in Fuchucha, describing this village separately from Baide facilitates relating the results from previous studies with this study.

would be smaller than the numbers from the census. On the other hand, some people might also have been spending the night in the 'fora', and therefore the true number of persons in the village would also be smaller than the figures in the census projections. Given that the animals staying in the 'fora' usually outnumber the persons staying there, it was considered necessary to adjust the number of animals in the villages. This was done using information from the interviews to the 214 eligible participants in the attempted case-control study.

Overall for the 9 study kebeles, the mean livestock:human ratio was 3.6:1 (range: 0.76 - 7.04) calculated from unadjusted census data (Table 2.1), which decreased to 1.13:1 (95% CI 0.61- 1.64) when considering only the estimated proportion of livestock kept in the village at night. The latter figure is a sampling weighted mean calculated using data from interviews to the participants in the attempted case-control study (summarized in Table 2.2).

Table 2.1. Total number of persons and livestock in each of the study kebeles.

Kebele	Households	Persons	All Livestock		Cattle		Sheep/Goat		Mule/Donkey (& 1Horse)	
			n	(%)	n	(%)	n	(%)		
Dokatu	1136	5459	14154	7204	(50.9)	6932	(49.0)	18	(0.1)	
Duraite	854	3939	19676	9587	(48.7)	9992	(50.8)	97	(0.5)	
Nalaya Segen	713	3305	8447	5831	(69.0)	2612	(30.9)	4	(0.0)	
Sorobo	771	4056	9420	5798	(61.5)	3577	(38.0)	45	(0.5)	
Gamole	447	2221	1695	594	(35.0)	1087	(64.1)	14	(0.8)	
Buso	528	2821	10435	5778	(55.4)	4620	(44.3)	37	(0.4)	
Mechelo	447	2412	9549	6527	(68.4)	2993	(31.3)	29	(0.3)	
Fuchucha	375	1065	7502	6651	(88.7)	828	(11.0)	23	(0.3)	
Baide	495	3432	16133	12200	(75.6)	3846	(23.8)	87	(0.5)	
TOTAL	5766	28710	97011	60170	(62.0)	36487	(37.6)	354	(0.4)	

Livestock=Cattle+Sheep/Goat+Mule/Donkey/Horse; Cattle=Cow+Ox/Bull+Calf. In brackets is the percentage out of the total number of livestock in each kebele. Source: Census data.

Table 2.2. Number of persons and livestock in the village compound of all the households interviewed in each kebele.

Kebele	N. interviewed Households	Total N. in all households interviewed					Mean N. Animals/Person in all households interviewed			
		Persons	Livestock	Cattle	Sheep/goat	Donkey	Livestock	Cattle	Sheep/goat	Donkey
Dokatu	62	386	256	63	193	0	0.65	0.15	0.50	0.00
Duraite	45	274	188	43	145	0	0.64	0.15	0.49	0.00
Nalaya	26	168	174	23	151	0	0.98	0.15	0.83	0.00
Sorobo	20	155	106	40	66	0	0.73	0.30	0.42	0.00
Gamole	12	60	54	7	47	0	0.97	0.14	0.83	0.00
Busso	18	119	96	25	71	0	0.84	0.20	0.64	0.00
Mechelo	8	49	56	14	42	0	1.14	0.30	0.84	0.00
Fuchucha	18	97	106	63	40	3	0.91	0.53	0.36	0.02
Bayde	4	25	75	14	59	2	4.50	0.69	3.74	0.07
TOTAL	213	1333	1111	292	814	5	1.13^w (0.61-1.64)	0.26^w (0.20-0.32)	0.86^w (0.40 - 1.33)	0.01^w (0.00 - 0.02)

Livestock = cattle + sheep/goat + mule/donkey; Cattle=cow+ox+bull+calf.

^w Sampling weighted mean (95% CI). The weight of each kebele was calculated dividing the total n° of households in a kebele by the n° of households interviewed in that kebeles (i.e. the weight corresponds to the number of houses in the whole kebele that are represented by each interviewed house). Source: data from interviews to the participants in the attempted case-control study. The total number of interviewed households equals the total number of participants in the case-control study minus one¹.

¹ One household in Duraite was interviewed twice; because both mother and son were cases of malaria included in the case-control study (both had laboratory confirmed malaria in different dates).

2.3.1.2. Characteristics of the sleeping rooms in the village compounds

Construction materials of the sleeping room

The sleeping room of most of the case-control study participants (75% - 160/213) had a thatched roof made with logs of wood and grass. The walls of the sleeping room were almost always (99% - 210/213) made of wood or branches, either completely or partially covered with mud and/or dung. One subject had walls made of local bricks of straw mixed with mud/dung. The most common type of floor was simply soil and/or stones covered with manure. Only 4 out of the 214 subjects interviewed had the floor covered with cement. The sleeping room of the majority of subjects (15% - 32/211) had no ceiling. The ceiling was usually made of wood logs and/or branches, and only in 7 subjects it was made of plastic or a nylon bag.

Openings in the sleeping room

The entrance of the room was usually closed with a door (86% - 179/209), which frequently had open spaces (77%). Only 23% (48/212) of the subjects had window opening(s) in the wall. Overall, the majority of subjects (92% - 197/213) had some type of opening(s) somewhere in the room.

2.3.2. Climate

The temperature and rainfall data recorded by the Meteorological Station in Karat town (altitude 1640m, longitude 37°.3, latitude 5°.15'), in the centre of the Konso District, for the periods of 2003-2004, and 1988-2004, respectively, were obtained from the Ethiopian National Meteorological Services Agency.

During the year of this study (2004) the mean day temperatures (average of mean daily maximum and mean daily minimum temperatures shown in Figure 2.3) in Konso were highest in March (25.3°C) and lowest in June (21.9°C). A similar pattern was observed in the previous year.

In Southern Ethiopia, the wet and dry seasons are usually considered to be the periods of April-October and November-March, respectively (Habtewold, 2004). There is a main rainy season usually from the end of March to May and a secondary one from

September to early November. There is, however, considerable interannual variation in the pattern and also in the magnitude of rainfall, as seen in Figure 2.4 (and detailed in Appendix A4). In the period from 1988 to 2004, the total annual rainfall was on average 762mm (SD=154mm), ranging from 962mm in 1997 to less than half (451mm) in 1999. During the year before this study (2003), the total annual rainfall was 763mm, peaking from mid April to mid May, and there was no formal secondary rain season, except for a peak in August. During the year of this study (2004), the total rainfall was considerably less, 563mm, although distributed around two peaks: a main peak from April to early May, followed by a dry period until the end of August, and a secondary peak from September to November (Fig. 2.3 and additional daily rainfall data not presented here).

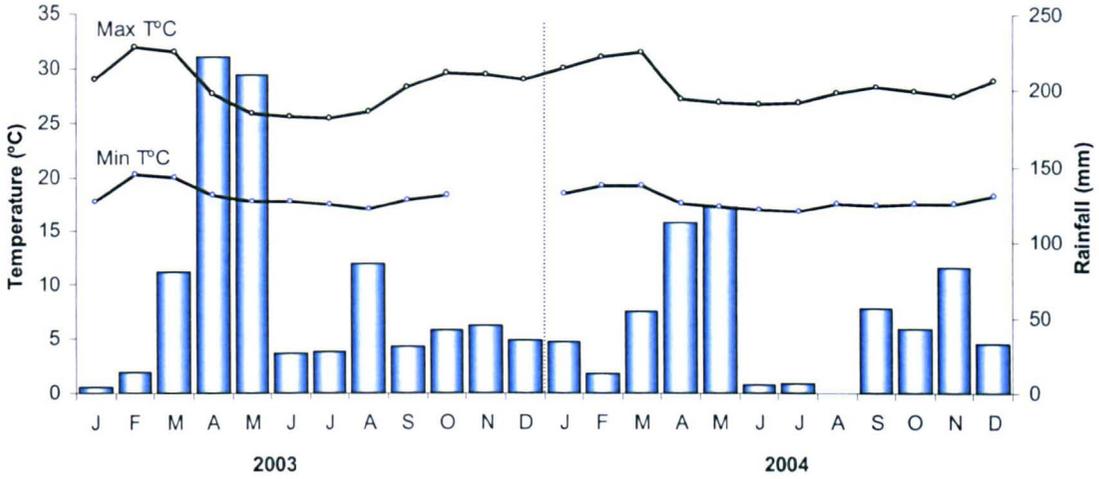


Figure 2.3. Changes in the monthly average minimum and maximum temperatures (lines) and in the total precipitation (bars) through time during 2003 and 2004, as recorded by the Meteorological Station in Karat, Konso District.

The minimum temperature was not recorded from the 1st November 2003 to the 26th January 2004. Source: Ethiopian National Meteorological Services Agency.

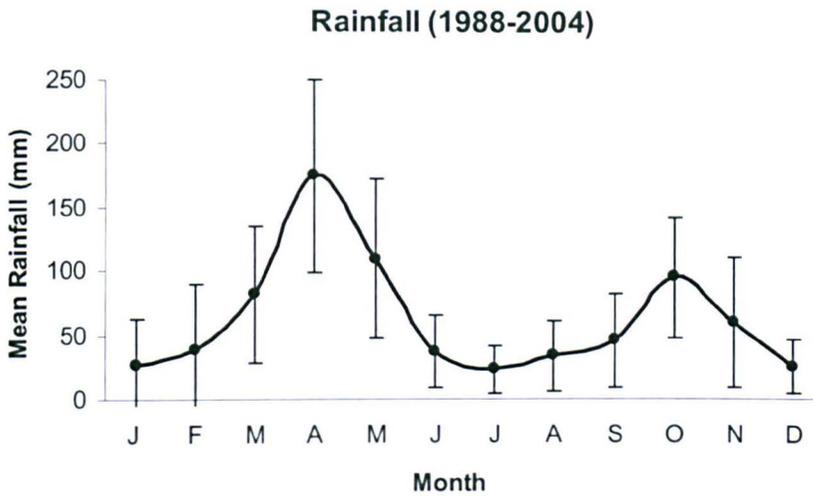


Figure 2.4. Changes in the total monthly precipitation through time from 1988 to 2004, as recorded by the Meteorological Station in Karat, Konso District.

The bars show the standard deviation of the mean. Source: Ethiopian National Meteorological Services Agency. The data from 1988 to 2001 were kindly provided by Iñaki Tirados and Steve Torr, who had previously obtained it. No data were available for 2002.

2.3.3. Epidemiology of malaria in Konso District

2.3.3.1. Malaria control

The malaria control programme in Konso District relies on the passive detection and treatment of all the symptomatic cases (including the ones with negative laboratory diagnosis) and on intermittent vector control activities that include: spraying houses with residual insecticide, distributing bednets and environmental management.

2.3.3.1.1. *Diagnosis and Treatment of malaria cases*

Diagnosis

The standard malaria case definition used in Ethiopia is presented in Table 2.3.

Table 2.3. Standard Malaria case definition from the Ministry of Health of the Federal Democratic Republic of Ethiopia.

Uncomplicated malaria	Confirmed uncomplicated malaria	Malaria with severe anaemia	Severe Malaria
fever or fever with: headache, back pain, chills, sweats myalgia, nausea and vomiting	Fever or fever with : headache, back pain, chills, sweats myalgia, nausea and vomiting + laboratory confirmation of diagnosis by malaria blood film or other diagnostic test for malaria parasites	A child 2 months up to 5 years with malaria and, if an outpatient, with severe palmar pallor, or if an inpatient, with a lab test confirming severe anaemia	A person hospitalised with a primary diagnosis of malaria and confirmed by a positive blood smear or other diagnostic test for malaria.

Most malaria cases in the Konso District were diagnosed based simply on clinical symptoms, since in the whole District there were only two health facilities where laboratory diagnosis of malaria (blood film) was done: Karat Health Centre (KHC, run by the government) and Mekane Yesus Health Station (MYHS, privately run by a Protestant

Church), both located in the capital, Karat town. The malaria cases for the attempted case-control study were selected from both these facilities. The KHC is a reference centre for the whole Konso District, and its catchment area (25,224 people) is about double that of the MYHS (12,945 people)¹. These two facilities perform ~40% of the total malaria cases diagnosed in the District, with 30% of cases diagnosed in the KHC, and 10% in the MYHS². Halfway through the present study (from 24th August 2004 onwards) the MYHS laboratory stopped working because its only laboratory technician was transferred to the KHC, and therefore, most malaria cases in the study were diagnosed at the KHC.

The criteria to send a patient with clinical symptoms of malaria for laboratory confirmation varied depending on the clinicians. One of the clinicians interviewed at the KHC reported that, as a general guideline, patients whose condition did not seem too serious would receive immediate treatment without laboratory diagnosis. Conversely, patients in a more serious condition and/or patients who were referred from other health facilities due to treatment failure would be sent for laboratory diagnosis. However, another KHC clinician reported different criteria for sending patients to laboratory diagnosis: coming from an endemic area, having fever $\geq 38^{\circ}\text{C}$ and chills, and availability of sufficient manpower and reagents in the laboratory. Additionally, sometimes the patient's history was also considered, namely whether previous to symptoms onset he/she had been in a lowland area where malaria transmission is higher.

Slides preparation and reading:

At the KHC all the laboratory diagnoses were made by two junior laboratory technicians (both had been working as lab technicians for ~2 years and 8 months, as of 4th August 2004). About 20-40 patients/day were received to be screened for malaria parasites. Additionally, other tests were also made in the laboratory.

By routine, thick blood films for malaria were done from a finger prick. Thin blood films were done only when it was difficult to identify the parasite species. Although the laboratory technicians should ideally spend ~5 minutes reading each slide, the high work load often imposed restricting the time per slide to 2-3 minutes. When a malaria patient had severe clinical condition both technicians reported spending more time per slide, to better identify the parasite species and quantify the parasitaemia load, so that a more accurate diagnosis and treatment could be made.

¹ Source: Konso Health Office; data for mid Sept 2002 to mid Sept 2003.

² Source: Summary statistics from the KHC; data for mid Sept 2002 to mid Sept 2003.

Treatment Policy

The choice of treatment depended on the patient's condition. Generally, when laboratory diagnosis was not available, or the laboratory result was negative, or when the parasite was *P. falciparum*, the first line drug for uncomplicated malaria was a combination of Chloroquine (CQ) and Sulfadoxine–Pyrimethamine (SP), while the second line drug for treatment failure and also for severe malaria was Quinine. *P. vivax* cases were treated with CQ.

The first line choice was a combined therapy of CQ and SP as an attempt to prevent drug resistance. Yet, there was high resistance of *P. falciparum* to these drugs. The perceptions about the level of drug resistance varied, between and within health facilities. At the KHC, one clinician reported that with those combined antimalarials <50% of the patients returned without improvement, due to either vomiting and/or drug resistance, while another clinician reported that only slightly <25% of the patients did not respond to treatment. Conversely, at the MYHS, a much higher failure rate of >70% was reported. Despite the high resistance to the first line drugs, Quinine was reserved only for the most severe cases because it was not always available.

2.3.3.1.2. Vector control

Insecticide spraying of houses

Since 1996, there has been a programme for spraying houses with residual insecticide in Konso District. From 1997 until 2002 only DDT had been used. In 2003 both DDT 75% and malathion 50% were used. However, in the year of the present study only DDT 75% was applied. Houses were sprayed every 6 months: the first round of spraying was from December 2003 to January 2004, and the second round was from June to July 2004. Only 6 villages in the whole District were sprayed, which were selected for having high vector densities in the rainy and dry seasons: Fuchucha (included in the study area), Waito, Segen town, Gete, Gerche, and Abaya. In each of these villages, more than 90% of the houses were sprayed.¹

¹ Source: Konso Malaria Office, in the Konso Health Office.

Insecticide-treated bednets

The distribution of bednets had only recently started in the District, and only a small coverage had been obtained at the time of this study. From September 2003 to June 2004 (1996 EC) 2,800 bednets were distributed among 4 kebeles/villages (1,200 in Karat town, 400 in Fuchucha, 600 in Waito and 600 in Segen town). Of these, 43% of bednets had been sold, at 18 Birr (~1.25£) each. In Karat town, at the Mekane Yesus Health Station bednets were given to people free of charge (within a project by the non-governmental organisation *Save the Children US*), while at the Karat Health Centre the bednets were sold. In both places, the nets were distributed untreated; insecticide was however provided and people were explained how to impregnate the net at home. At the Fuchucha Health Post bednets were also sold, but they were treated with insecticide at the time of purchase.

Environmental management

Environmental management activities were done to decrease larval breeding sites. For instance, awareness was raised among the Konso population to drain wet areas, and also artificial ponds were constructed using rocks and cement, creating steep slopes and shores that prevent the development of *An. arabiensis* larvae (Torr et al., 2001).

Knowledge and usage of protection methods against mosquito bites

Amongst the persons interviewed in the case-control study, 56% (120/213) were aware of at least one potential breeding site for mosquitoes. Additionally, 43% (91/212) of the interviewees revealed knowing one or more methods for protection against mosquito bites. Of these, the most frequently known method was covering with blanket or clothing very well at night (60%), followed by sleeping under bednet (40%). Other methods included drying wet areas or preventing swamps (5%), closing windows and other openings in the house (5%), and using insecticide (3%). Of the persons who used any of the known methods, most (68%) reported usage only in the rainy season, while only a quarter used the method(s) in the rainy and dry seasons.

Considerations on bednet usage

The distribution of bednets had only recently been introduced in the study area, and was limited to some of the study kebeles. Of the interviewees who knew a protection method, only about a third (36% - 33/91) mentioned bednet, and of these, only half (52% - 17/33) were using the net.

Of the 16 persons who new about bednets but were not using them, the most frequent reason for not having one was not being able to afford it (n=8) or “My husband doesn’t buy it to me” (n=1). Two interviewees did not know where the bednet was sold, and an interviewee in the household of another case claimed that bednets were not popular. Furthermore, there were also situations where people had a bednet but were not using it (!) due to the net being too big to fit in their small sleeping space (n=2), or due to not knowing how a net should be set up (!) (n=1).

2.3.3.2. Number of malaria cases***2.3.3.2.1. Malaria cases in the Konso District during the year previous to the study***

The results of household surveys previously conducted in Konso have shown that malaria was perceived to be the most important human disease (Torr et al., 2001), which is confirmed by clinical records from the local health facilities. In the year before this study (mid Sept. 2002 to mid Sept. 2003; i.e. 1995 EC), malaria was the major cause of morbidity in the Konso overall population, accounting for 37.8% (11,083 / 29,280) and 35.04% (9,147 / 26,107) of all diagnosed causes of morbidity in males and females, respectively. In children under five years old, malaria was the third major cause of morbidity, accounting for 21.5% (3,546 / 16,500) of the morbidity diagnosis. On the top of the list were acute respiratory infections, followed by diarrhoea. The annual incidence of reported malaria cases was 97.91 cases/1000 persons. In the KHC, out of the 3675 suspected malaria cases sent for laboratory diagnosis during 2002/03 (1995 EC), 27.40% were confirmed as PF and only 2.12% as PV¹.

¹ Source: Summary statistics from the KHC.

2.3.3.2.2. Malaria cases in the whole catchment area of the Karat Health Centre, during the year of the study

During the year 2004 there were 9054 malaria cases diagnosed in outpatients and inpatients at the KHC. Among these, about half (47.93%) were diagnosed simply by clinical symptoms, the remaining (52.07%) were referred for laboratory diagnosis, and only few (13.93%) were parasitologically confirmed (source: KHC summary forms). Overall, only 26.8% of the suspected malaria cases sent for laboratory diagnosis were confirmed.

Malaria cases were diagnosed throughout all months of the year, with most cases concentrated from May to December, peaking in July and August. The diagnosis of malaria comprised 35.2% of all the morbidity causes diagnosed at the Karat Health Centre during 2004, ranging from 46.3% in July to 25.3% in December.

There were more malaria cases in males than in females, in the overall year (56% of all the suspected malaria cases were males), as well as in each individual month of the year. The difference in the number of cases diagnosed between genders was larger from May to October and also in December. The age distribution between genders was similar, as well as the distribution of the type of malaria diagnosed. Most cases were in adults ≥ 15 years old (58%), notably in the age group of 15-44 years (49%).

The majority of malaria cases (92%) were unspecified; only a minority was specified as caused by *P. falciparum* (6%) or *P. vivax* (2%)¹. Most of the unspecified cases were diagnosed from May to December, while PF cases were more frequently diagnosed from June to October, and PV peaked in July.

2.3.3.2.3. Malaria cases in the study area, during the study period

During the study period (1st June to 3rd December 2004) there were 620 suspected malaria cases diagnosed at the KHC from the 9 kebeles of the study area². Out of the

¹ According with the KHC outpatient and inpatient morbidity statistics monthly report.

² For the malaria cases sent for laboratory diagnosis, detailed data per kebele, month, age and sex were extracted from the lab log book only for the positive and not for the negative cases. Therefore, it is not known

total number of suspected malaria cases in the study area, 41.1% (255/620) were laboratory confirmed. The proportion of suspected malaria cases in the study area that were laboratory confirmed as PF, PV and mixed PF&PV was 35.8%, 4.5% and 0.8%, respectively. Mix infections were diagnosed only in three kebeles (Dokatu: 1 case, Duraite; 2 cases and Gamole: 2 cases).

Overall, 58% of the laboratory confirmed malaria cases at the KHC were males. In the study kebeles there were more laboratory confirmed cases in adults (55%), notably in 15-44 years old (46%), followed by the age groups of 10-14 (15%), 1-4 years (14%), 45-64 years (9%) and 5-9 yrs (8%), and <1 (7%). There were no laboratory confirmed cases in >65yrs old. When looking at the age distribution by kebeles, in each of the study kebeles the age group with the highest proportion of laboratory confirmed malaria cases was in adults (≥ 15 yrs old), except in Fuchucha, where there were proportionally more confirmed cases in 1-4 years old. The distribution among other age groups was variable between kebeles.

There were only 37 malaria cases laboratory confirmed at the MYHS, of which only 16 were from the study kebeles. All the cases confirmed at the MYHS were PF, except one PV from a patient outside the study kebeles.

For males and females overall, the number of suspected malaria cases was highest in June–July, decreased in August, and then increased from September to November. In males, the peak months were June and July, while in females it was June and October. The total number of laboratory confirmed malaria cases throughout the study period varied in a similar trend as the total number of suspected malaria cases.

The proportion of the suspected malaria cases in males that was sent for laboratory confirmation was highest in June (56.1%) and in October (57.6%) and lowest in November (28.6%). For females, the distribution throughout the year was more homogenous. In both genders, the number of PF confirmed cases varied in the same pattern as the total number of laboratory confirmed malaria cases. The number of PV confirmations was highest from June to August, peaking in July for males and in June for females.

the proportion of the total suspected cases in the study area that were sent for laboratory diagnosis, and all its known is the proportion of the total suspected that were laboratory confirmed.

2.3.4. Livestock management and ITL practices in the households interviewed in Konso

2.3.4.1. Livestock presence

Most households interviewed (88% -189/214) had at least one type of domestic animal present in their village compound. The animal types most frequently present within the household compounds were sheep/goat (n=167), followed by cattle (199) – cow (79), ox/bull (69), calf (51) –, and chicken (72). Only a minority of persons kept donkey (3), cat (18) and/or dog (10).

Only 22% (48/214) of households interviewed kept animals in their ‘fora’ compound. Contrarily to the village scenario, in the ‘fora’ the presence of cattle (n=41) was more common than the presence of sheep/goats (n=31). Only 2 persons kept cat and/or dog, and none kept chicken or donkeys.

2.3.4.2. Number of livestock present

Among the households who had cattle in the village compound, most kept only about one cow, and/or ox/bull, and/or calf. Amongst the individuals who had more than one head of a given animal type, the maximum number of animals present per compound differed between animal type and was: 13 for cows, 9 for oxen/bulls, and 4 for calves. Among the households who kept sheep/goats in the village compound, most kept 3 to 4 sheep/goats; with a maximum number of 35 sheep/goats.

The geometric mean (GM) of the numbers of each type of animals present in the ‘fora’ compound was slightly higher than the GM in the village compound, although the maximum number of animals in the ‘fora’ was lower than in the village.

2.3.4.3. Place where livestock were tethered at night (in the village compound)¹

Amongst the subjects who had animals in their village compound, most places where cows (49% - 39/79) were tethered at night had some sort of roof and wall (“total shed”). On the contrary, most places where the other animal types were tethered (ox/bull: 54% -

¹ Percentages in the text were calculated with the denominator being the number of subjects interviewed who had animals of the given type present in their village residence compound.

37/69; calves: 57% - 29/51; sheep/goat: 62% -103/167) only had either roof or wall ("partial shed").

Roof: The majority of subjects had their animals tethered under a roof of some sort: 80% for cows (63/79) and bulls (55/69), 86% for calves (44/51), and 95% for sheep/goats (159/167). Overall, the most common type of roof was made of logs of wood as part of a store for cereals. Other types of roof include: a less solid structure also made of logs of wood but not as part of a store; logs of wood with soil/manure/grass; thatched roof of wood and grass; and sheets of iron/zinc.

Wall: Most subjects had their cows (67% - 53/79) and ox/bull (55% - 38/69) tethered within a wall of some sort, while calves (55% - 28/51) and sheep/goats (58% - 96/165) were most frequently kept without wall. Overall, the most common type of wall for all the animal types was a fence made of logs/wood. Other types of wall include: wood/branches; fence made with bush (thorns); and sheets of iron/zinc.

2.3.4.4. Distance between livestock shed and subject sleeping room (in the village compound)

The median distance between the place where animals were tethered at night and the study subject (case/control) sleeping room, was less than 5 meters for all animal types, ranging from 3.8m (ox/bulls) to 4.9m (cows). Only 11 of the interviewed compounds had animals tethered inside or under the subject room. Four subjects reported having kept animals inside their room: goats + chicken (n=1), only goats (n=1); only chicken (n=1); and cat (n=1). Seven subjects reporting having kept animals under their "room" (these subjects were sleeping inside a store for cereals, under which animals were kept): sheep/goats (n=6); and cows (n=1).

2.3.4.5. Insecticide treatment of livestock

Several intermittent programmes of ITL for tsetse flies control have taken place in Konso since 1994 (Torr et al., 2001; complemented with interviews to the KDA and the Konso Veterinary officers conducted during this study). Before the present study, ITL initiatives were promoted by FARM-Africa and implemented locally by its subsidiary KDA, under a cost-recovery approach. Only cattle were treated, costing 9 Birrs and 75

cents (~£0.70). At the kebele level, the KDA organized committees which would register the number of cattle to be treated, collect money from farmers, and train individuals who would apply the insecticide on animals. Although the benefits of ITL were highly appreciated by the farmers, many could not afford the insecticide, which compromised the programme's sustainability.

During the year of my field study, insecticide was provided free of charge from February to July 2004, as part of an Ethiopian Government programme for control of tsetse flies and animal trypanosomiasis (Southern Regions Rift Valley Tsetse Eradication Project Sterile Insect Technique Project¹). The insecticide was Deltamethrin oil base 1% pour-on, applied along the spine of the animals, using the kebeles' committees that had been organized by KDA. All cattle regardless of age were treated; additionally, sheep and goats were also treated but mostly after 3 years of age. Horses, mules and donkeys were not included, since priority was given to the other animal species. Animals were treated in 21 out of the 46 kebeles in the District. The kebeles were selected for the programme based on: having more animal trypanosomiasis and/or tick-borne diseases, being located in the lowlands, and having more cattle. According with the information reported by the Konso veterinary officers, in the selected kebeles, all the above mentioned animals in the villages and in the 'foras' were treated, generally once a month. However, smaller treatment coverage and frequency were reported in the interviews to the participants of the attempted case-control-study (as detailed below). The Government Project phased out in July 2004, which stopped the free distribution of the Deltamethrin spot-on. Notwithstanding, some persons still continued treating their animals.

Amongst the households interviewed, insecticide treatment was reported for cattle (cow, ox/bull, calf) and for sheep/goats kept in the village compound as well as for animals kept in the 'fora'. The proportion of households with animals who reported having them treated, varied depending on the animal type and, for a given animal type, it varied depending on whether the animals were kept in the village or in the fora.

The percentage of households interviewed with cattle and/or sheep/goats which reported that at least one animal type had been treated with insecticide in the villages was 34%

¹ The Project covered the regions south of the Rift Valley, from Shashamene town to the Kenyan border. It aimed at reducing the population density of tsetse flies by 95%, using ITL and insecticide treated baits, to enable the subsequent release of sterile male tsetse flies.

(68/128) and in the 'foras' was 54% (13/24). Insecticide treatment in the village was most frequent for cows (54% - 43/79), followed by ox/bull (49% - 34/69) and calves (31% - 16/51). Only a few (8% - 13/167) of the persons who kept sheep/goat in the village had them treated.

Most (93%) of the households interviewed with treated animals in the village reported that only less than half of the total number of each animal type present had been treated. Only a minority of households reported that a higher proportion (half or more) of their animals had been treated. In the 'fora', all the households with treated animals reported that only less than half of the total number of each animal type present had been treated. Overall, amongst the households with treated animals in the village, the most reported frequency of treatment was once a month (62% - 57/92 for cattle and 54% - 7/13 for sheep/goat). Nevertheless, the reported frequencies of treatment ranged considerably, from three times a month (treatment of cow and calf reported by 1 household), to once a year (treatment of ox/bull reported by another household). A similar pattern was observed in the 'fora'.

2.3.5. Summary characterization of the study kebeles

A summary for some of the above mentioned and additional characteristics of the study kebeles is presented in Table 2.4. The kebeles were located at an altitude ranging between 1400 and 1650 meters above sea level. For 4 of the kebeles the closest health facilities were in Karat town (KHC or MYHS), for 3 kebeles there was a health post located in an intermediate kebele (i.e. in a kebele nearer than Karat town), and only 1 of the study kebeles (Fuchucha) had its own health post.

In all the study kebeles the chairman reported that mosquitoes were considered a problem, mostly during/after the rainy season. Additionally, in Nalaya Segen mosquitoes were considered a problem also in the dry season. When asked about what methods people used for protection from mosquitoes, in most kebeles the chairman answered that no method was used, except in Fuchucha and in Mechelo. In Fuchucha, when people lived near ponds they tended to cover them with soil, as recommended by the Karat Health Centre; additionally, some people also burned fires inside their house. In Mechelo the methods mentioned were going home before getting dark, and covering with a blanket when sleeping outside.

Most kebeles had either none or very few compounds with bednets, except in Fuchucha where at the date of interview to the chairman (8 August 2004), about 200 households had insecticide-treated nets. They had started being sold in the Fuchucha Health Post about 3 month previously to that date. Before being sold the nets were treated with insecticide at the Health Post. Spraying of compounds with residual insecticide had also only taken place in Fuchucha (~ 95% of its compounds had been sprayed).

The subsistence of most people was based on farming and livestock herding in the lowlands 'fora'. In Dokatu, Duraite, Gamole and Buso, some livestock were kept in the villages in a zero grazing system (people bring the crops to feed the animals at home). Also, in Dokatu and Gamole there were some merchants, and in Duraite there were a few people in salaried jobs. The main crops were sorghum and maize.

Table 2.4. Selected characteristics of the 9 Konso kebeles chosen for the study.

Kebele	N. villages ^a	Altitude ^b (m)	Distance to Karat (Km)	Location of Nearest Health Facility			N. compounds with Bednets			Insecticide spraying of compounds	Insecticide treatment of Livestock ^f	Nearest standing water place ^g				Job ^h		Crops ⁱ
				Karat (KHC or MYHS) ^c	Intermediate kebele ^d	In situ	None	Very few	Some			Type	Distance (mints walking)	Rainy	Dry	Main	2ary	
Dokatu	1	1530	3								2 rivers 1 pond	60 60	• •		F	F&ZG, M	S, Ma, B	
Duraite	3	1500	1.5 to 5	•							Drill water pump	10 to 60	• •		F&H, F&ZG	S	S, Ma, B	
Nalaya-Segen	1	1438	4	•							River Well/Spring	30 90	• •		F&H		S, Ma	
Sorobo	2	1518	10		•						River Pond	5 5	• •		F&H		S, Ma, Mi, B, F	
Gamole	1	1603	5		•						River Pond	30 10	• •		F&H	F&ZG, M	S, Ma, Mi	
Buso	1	1553	5				•				River Pond	10 to 15 25	• •		F&ZG	F	S, Ma	
Mechelo	1	1646	7					•			River Pond Drill water pump	20 30 25	• • •		F&H		S, Ma	
Fuchucha	1	1499	22			•					River	5	•		F&H		S, Ma	
Baide	1	1521	28					•			River	35	•		F&H		Ma	

Source: Interviews to the chairman of each kebele. All the information refers to the year 2004.

^a Number of villages in each kebele.

^b Altitude was measured in the Kebele Chairman's Office, except in Fuchucha (measured at its Health Post), and in Sorobo (measured at its Primary School).

The distances from each kebele to Karat town are only approximations. See Figure 2.2 for a map with the location of the study kebeles and Karat.

^c KHC=Karat Health Centre; MYHS=Mekane Yesus Health Station.

^d There was a Health Facility somewhere between the indicated study kebele and Karat town.

^e Fuchucha Health Post.

^f Animals treated: In all the kebeles where ITL was conducted the chairman reported that only adult cattle were treated. Additionally, in Sorobo it was added that with the government programme sometimes also sheep/goats were treated. In Fuchucha it was further mentioned that lactating cows and calves were not treated to avoid residues in the milk. Frequency: In Fuchucha, Baide, Sorobo and Nalaya Segen the government treatment started March 2004, animals were treated twice a month; in Dokatu the Government treatment had started in August 2004; and in Duraite it was reported that only twice a year animals were treated.

^g Standing water place: Rainy=water is present in the rainy season; Dry=water is present also in the dry season.

^h Main and secondary jobs: F=farming only ; F&H=farming and livestock herding; F&ZG=farming and zero grazing (livestock are kept at home and people bring the crops to feed the animals at home); M=merchant; S=salaried.

ⁱ Crops: S=sorghum; Ma=maize; Mi=millet; B=hard coat beans; F=fruits. Crops irrigation: Most kebeles reported that only rain water was used; only Fuchucha and Baide added that also river water was used.

2.3. Discussion

Summary statistics of the causes of visits to health facilities in the Konso District of Ethiopia have shown that malaria is the most frequent human health problem in the area, and occurs throughout all year. During the study year (2004), most cases were concentrated from May to December, peaking in July and August.

In Konso, there are usually two rain seasons throughout the year, with peak rainfall in April and October. There is however, considerable inter-annual variation. In the study year there was a slight shift with peak rainfall occurring in May and November, i.e. preceding the peak of malaria cases by about two months.

Most of the malaria cases were diagnosed only on clinical symptoms, since in the whole District there were only two health facilities where laboratory diagnosis of malaria (blood film) was done: KHC and MYHS, and even in these facilities only a minority of the suspected malaria cases were sent for laboratory diagnosis. Most cases were due to *P. falciparum* and only a minority due to *P. vivax*.

The records of the main health facility in the District (KHC) show that during the study year the incidence of clinical malaria in the whole of its catchment area was higher in males (56%), and in the 'over 15 years' age-group (58%), followed by the 'under 5 years old' (29%). The observed age distribution suggests that the adult population in Konso is not-immune to infection. When considering only the nine study kebeles, a similar gender distribution was observed amongst the laboratory confirmed malaria cases: 58% were males, and most were in the 'over 15 years' age-group (55%), followed by the '5-14 years old' (23%). A careful interpretation of these figures is however needed because, since the denominator is unknown (i.e. the number of persons in each age-group that attended the health facility) and since most of the suspected cases were not parasitological confirmed, a parasitological prevalence survey across age groups would be required for a more accurate age-distribution of malaria.

Malaria control in Konso relies on the passive detection and treatment of all the symptomatic cases and on intermittent vector control activities that include environmental management, indoor spraying with residual insecticides, and more recently, insecticide-treated bednets.

At the only two health facilities with laboratory diagnosis, the criteria to send a patient with clinical symptoms of malaria for laboratory confirmation varied depending on the clinicians. Since most diagnoses in the District were based on clinical symptoms only, this may have led to under or over reporting of malaria cases. Of all the malaria cases diagnosed at the main health facility, KHC, during the whole of 2004, only about half (52%) were sent for laboratory confirmation, and of these only less than a third (27%) were confirmed, suggesting that a large proportion of the cases presumptively diagnosed as malaria based on clinical symptoms only did not have malaria but instead other febrile illness. Additionally, the findings from the quality control performed in this study indicate that about a third of the malaria cases laboratory confirmed at the KHC were likely to have been false positives.

The first line treatment for uncomplicated malaria in Ethiopia from 1998 to 2004 was a combination of CQ and SP if no microscopy was available, otherwise CQ for *P. vivax* and SP for *P. falciparum*. The second line drug was quinine. The local practice in the study health facilities was however slightly different, as CQ and SP were used also when the parasite was identified as *P. falciparum* or when the lab result was negative.

There were high levels of resistance of *P. falciparum* to the antimalarials being used. The clinicians of the studied health facilities reported treatment failure of uncomplicated *P. falciparum* malaria to CQ and SP ranging from <25% to >70%, which is consistent with findings from drug efficacy studies conducted in the region and also elsewhere in Ethiopia. In the SNNPR, treatment failure of uncomplicated malaria has been found to be 76% for CQ (study in 2000, n=101; W.H.O., 2004b), and 14.3% after 14-days follow up for SP (study in 2002, n=70; Abebe, 2006), with higher failure rates found also in other areas of the country. A nationwide study done in 2003 in 11 sentinel sites found a mean treatment failure of SP of 36% on 14 days follow-up increasing to 72% on the 28 days follow-up, (Jima et al., 2005). This study led to a change in the national antimalarial drug policy by mid 2004 replacing the first-line treatment of unconfirmed and confirmed *P. falciparum* from SP to artemether-lumefantrine (AL, Coartem™) (Federal Ministry of Health, Ethiopia 2004; cited in: Balasegaram, Dejene et al.2006). However, one year after the release of the new policy, it was yet to be implemented in Konso, as AL was expensive and not readily available (Tirados, 2005).

Spraying of houses with residual insecticide was limited to a small minority of villages in the District, of which only one of the study villages (Fuchucha) was included. DDT was the insecticide generally used, although there is evidence that *An. arabiensis* is considerably resistant to this insecticide (5%-76% DDT resistance was found in various areas of Ethiopia during 1986 -95 (Abose et al., 1998); a more recent study has found 30%-42.5% in two localities in Eastern Ethiopia (Balkew et al., 2003). The distribution of bednets had only recently started in the study area, and only a minority of the study population were using insecticide-treated nets.

Livestock are extremely abundant in the District, being a major source of subsistence for many of the Konso people. In the village compounds of the kebeles chosen for this study it was estimated that there was a mean of 1.13 (95%CI=0.61-1.64) animals/person, with sheep/goats being usually more than three times the number of cattle, except in Fuchucha where cattle were the most frequent species. Mature cattle (cows/oxen) were more abundant than younger cattle (calves).

The treatment of livestock with insecticide had been occurring intermittently in Konso, with the aim of controlling tsetse transmitted animal trypanosomiasis and tick-borne diseases, which are one of the major constraints affecting livestock production and consequently people's livelihoods in Konso. During the year of this field study, from February to July 2004, insecticide treatment of livestock was provided free of charge, as part of an Ethiopian Government programme to control tsetse flies and animal trypanosomiasis. The free treatment was interrupted in July 2004 due to lack of funding, although some persons still continued treating their livestock. The insecticide used was Deltamethrin oil base 1% pour-on, applied along the spine of the animals. Livestock were treated in 21 out of the 46 kebeles in the District. According with the information reported by the Konso veterinary officers, the kebeles were selected for the programme based on: having more animal trypanosomiasis and/or tick-borne diseases, being located in the lowlands, and having more cattle. According with the same source, in the selected kebeles all the above mentioned animals in the villages and in the 'foras' were treated, generally once a month. However, smaller treatment coverage and frequency were reported in the interviews to the villagers conducted in this study. From the data collected from the interviews to the study population it is evident that, in the villages where treatment took place, not all the compounds had their livestock treated, and not all the livestock in a given compound had been treated. Insecticide treatment was more commonly reported

for cattle (cows, ox/bulls, calves), while only a minority of the people with sheep/goats had them treated. Most (93%) of the persons with treated animals reported that only less than half of the number of each of the animal types present had been treated. Moreover, although the most reported (61%) frequency of treatment was once a month, as recommended by the governmental programme, it ranged from three times a month, to once a year.

Despite studies have been conducted in Konso and in areas nearby that looked at the interaction between livestock and the vector component of malaria transmission, the effect that treating livestock with insecticide has upon malaria in humans has never been assessed, in this area of Ethiopia, nor elsewhere in Africa. These questions will be addressed in the next four Chapters by developing a comprehensive mathematic model of malaria transmission and integrating it with empirical data from the African scenario of Konso and from elsewhere. The model will be applied to explore the potential effects of untreated and insecticide-treated livestock on malaria, initially in several hypothetical settings, and then in the specific settings of this Ethiopian scenario and of the Pakistan ITL trial.

Chapter 3

Basic framework to model the effects of livestock on malaria transmission to humans

Summary

Background: Mathematical models can be a useful tool to assist on public health recommendations, particularly when empirical evidence is limited. This Chapter sets the basis of a theoretical framework that was developed to examine the possible roles of livestock on human malaria.

Methods: A deterministic mathematical model for malaria transmission dynamics was built expanding on the classical work of Ross and Macdonald which modelled malaria transmission between humans and mosquitoes. The new model builds upon their work by discriminating the feeding behaviour of the mosquito vector on its alternative hosts: livestock and human populations, and incorporating the treatment of livestock with insecticide as a potential *novel* method to control human malaria. After outlining the basic structure of the model, the threshold dynamics of the system was investigated: the basic reproduction number (R_0) was analytically derived with the next generation operator approach (using Jacobian matrices methodology), and an analytical sensitivity analysis was performed.

Findings: The parameters that have a greater impact on R_0 are the mosquito biting rate on humans (proportion of vector bites on humans \times vector biting rate on any host) and the adult mosquito mortality rate, which is in accordance with the insights provided by the Ross-Macdonald model. The treatment of livestock with insecticide further increases the sensitivity of R_0 to the proportion of vector bites on humans, although it decreases the sensitivity of R_0 to the vector biting rate on any host. The analysis also indicates that by increasing the insecticidal activity a smaller proportion of livestock would need to be treated to achieve the same potential level of malaria control.

Interpretation: The refined model provides a flexible framework to evaluate the impact of ITL in varying transmission settings. In the next Chapters the model will be extended and applied to explore in more detail the possible effects of untreated livestock (Chapter 4), and of ITL (Chapter 5 and 6), on the malaria vector feeding behaviour, mortality and density, and on the overall disease transmission dynamics, in several hypothetical ecological settings and in two specific settings in Asia and in Sub-Saharan Africa.

3.1. Introduction

In this Chapter, a mathematical model for the transmission dynamics of human malaria is developed based on the seminal theoretical works of Ross and Macdonald (Ross, 1911; Macdonald, 1952) which have remained a cornerstone for many existing epidemiological studies (see, e.g., Molineaux and Gramiccia, 1980; Aron and May, 1982; Bailey, 1982; Anderson and May, 1991; Gupta et al., 1994). The Ross-Macdonald framework is a deterministic model with human hosts and mosquito vectors divided into epidemiological compartments according to their disease status. The first malaria model developed by Ross (1911) did not include latent periods, while Macdonald (1952) added the latent period for the malaria parasite in mosquitoes. Therefore, in the Macdonald model humans are compartmentalized into either susceptible (uninfected and not immune), or infected/infectious (SIS model: Susceptible–Infected–Susceptible), and mosquito vectors are divided into susceptible (uninfected and not immune), exposed/latent (have been infected but are not yet infectious) or infectious (SEI model: Susceptible–Exposed–Infectious).

Here, the Ross-Macdonald model is extended by discriminating the feeding behaviour of the vector on its alternative hosts: livestock and human populations, and by incorporating the treatment of livestock with insecticide as a potential *novel* method to control human malaria. After outlining the basic structure of the new model, the threshold dynamics of the system is investigated, and the basic reproduction number (R_0) is analytically derived, as well as its sensitivity to parameter values.

The theoretical framework here presented will form the basis for the subsequent Chapters, where the model will be extended and applied to explore in more detail the possible effects of untreated livestock (Chapter 4), and of insecticide-treated livestock (Chapters 5 and 6), on the malaria vector feeding behaviour, mortality and density, and on the overall disease transmission dynamics, in several hypothetical ecological settings and in two specific settings in Asia and in Sub-Saharan Africa.

3.2. Methods and Results

3.2.1. Malaria model basic structure

By their nature, epidemiological models do not consider all the biological complexities, but they can still provide important insights into the transmission dynamics and control of diseases (Anderson and May, 1991, pp. 6-8). Accordingly, a number of assumptions are made, to simplify the structure of the model and the subsequent analysis (a diagrammatic flow chart of the model is presented in Figure 3.1, and the parameters are specified in Table 3.1).

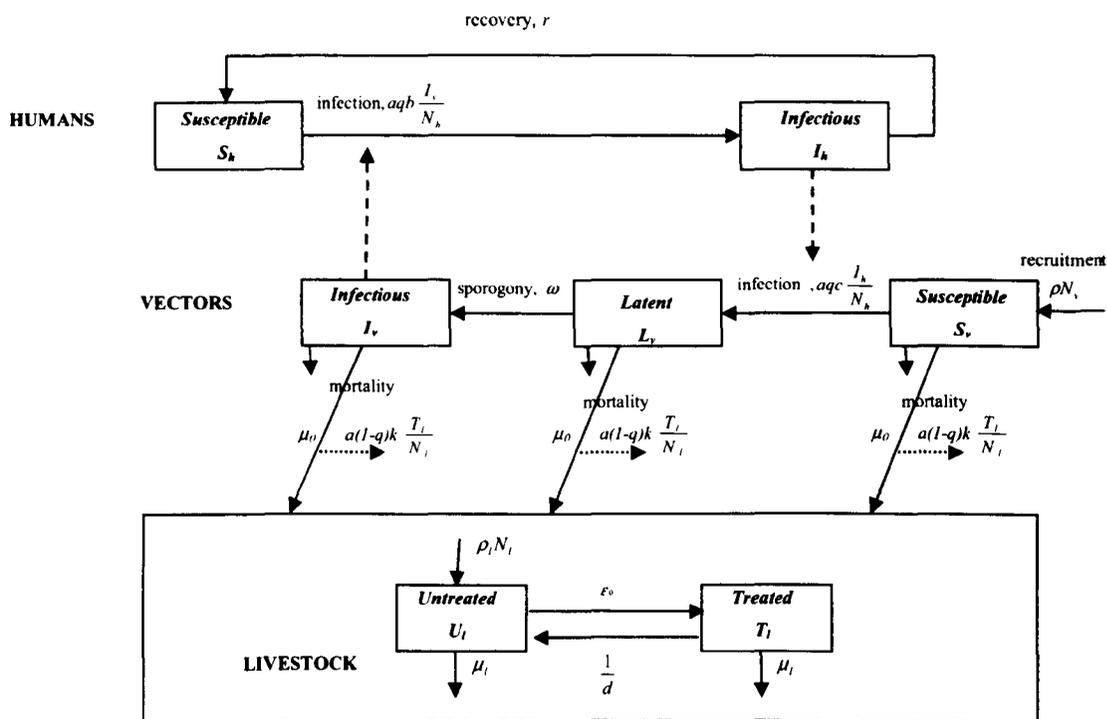


Figure 3.1. Schematic representation of the malaria model.

Horizontal solid lines denote transitions between epidemiological states, and dashed lines represent transmission of infection between human hosts and mosquito vectors. Diagonal solid lines denote vectors feeding on livestock, and dotted lines represent the effect of insecticide-treated livestock on vectors mortality.

Table 3.1. Parameters used in the malaria model.

Symbol	Definition
A_l	Proportional availability of livestock to vectors
A_h	Proportional availability of humans to vectors
N_v / N_h	Number of vectors per human host
a	Vector daily biting rate on any host (average interval between blood-meals = duration of gonotrophic cycle = $1/a$)
q	Proportion of vector feeds on humans (Human Blood Index) The remaining, $1-q$, are feeds on livestock
b	Probability that humans become infected from the bite of an infectious vector
c	Probability that vectors become infected after biting on an infectious human
ω	Daily rate at which latent mosquitoes become infectious (duration of latent period in surviving vectors = $1/\omega$)
r	Human daily recovery rate from infection [or infectiousness] (duration of infection [or infectious period] = $1/r$)
ρ	Vector daily recruitment rate
μ_0	Vector daily natural mortality rate, in absence of insecticide treatment of livestock (life expectancy = $1/\mu_0$)
k	Probability that vectors are killed due to exposure to insecticide-treated livestock on the day of treatment
ϵ_0	Proportion of livestock population treated with insecticide, in each intervention round (application coverage)
d	Decay rate of insecticide residual activity (daily) (average duration of insecticide residual activity = $1/d$)
ρ_l	Livestock recruitment rate (daily)
μ_l	Livestock removal rate (daily)

Vector=adult female anopheline mosquitoes.

Contrary to most other malaria models that used a fix parameter (e.g. m) to refer to the number of vectors per human hosts, we refer explicitly to N_v/N_h , to allow for situations where the vector population size will change after the insecticide intervention, and therefore, the number of vectors per human hosts will not remain constant but will instead be a function of time ($N_{v(t)}/N_h$).

Throughout the thesis, the human, vector and livestock populations will be referred to with the subscripts h , v and l , respectively.

First, let us consider the dynamics of infection in the human population, which is modelled by the following set of ordinary differential equations (ODE):

$$\begin{aligned}\frac{dS_h}{dt} &= -\left(aqb\frac{I_v}{N_h}\right)S_h + rI_h, \\ \frac{dI_h}{dt} &= \left(aqb\frac{I_v}{N_h}\right)S_h - rI_h,\end{aligned}\quad (1)$$

where $N_h = S_h + I_h$ (total human population size).

Transmission of infection from vectors to humans depends on the number of infected vectors per human, I_v/N_h , the vector blood feeding rate on any host, a , the proportion q of feeds taken on humans; the probability b that a human will become infected following the bite of an infectious vector; and the number of susceptible hosts (S_h). Once susceptible humans are infected the parasite undergoes a latent period required for development of the infective gametocytes. Nevertheless, since the latent period is short compared to the duration of infection, the latent period can be ignored, and we therefore assume that all the infected humans will become infectious, similarly to other malaria models (e.g. Näsell, 1985). Infected individuals (I_h) recover from infection at a rate r , becoming fully susceptible to re-infection (the average duration of infectiousness is $1/r$). It is therefore assumed that there is no acquired boosting immunity due to repeated infections, as done for simplification in the previous malaria zoonophylaxis models (reviewed in Section 1.6). The natural human demography (mortality and reproduction) is omitted from the model because humans have a long life expectancy relative to all other time periods in the model (such as the latent period, infectious period and vector life span). Moreover, we assume no disease-induced death and therefore, the human population size remains constant.

The disease dynamics in the vector population may be similarly described by the following system of ODEs:

$$\begin{aligned}\frac{dS_v}{dt} &= \rho N_v - \left(aqc\frac{I_h}{N_h} + \mu_0 + a(1-q)k\frac{T_l}{N_l}\right)S_v, \\ \frac{dL_v}{dt} &= \left(aqc\frac{I_h}{N_h}\right)S_v - \left(\omega + \mu_0 + a(1-q)k\frac{T_l}{N_l}\right)L_v, \\ \frac{dI_v}{dt} &= \omega L_v - \left(\mu_0 + a(1-q)k\frac{T_l}{N_l}\right)I_v,\end{aligned}\quad (2)$$

where $N_v = S_v + L_v + I_v$ (total vector population size).

The vector population comprises only adult female anopheline mosquitoes, since males do not feed on blood. Transovarial transmission does not occur in the malaria vector, thus all the emergent adult female mosquitoes are considered susceptible to infection. Transmission of infection from humans to vectors depends on the proportion of infectious humans, I_h/N_h , the vector feeding rate on humans, aq , and the probability c that a vector will become infected after feeding upon an infectious human. Infected latent mosquitoes (L_v) become infectious following a period for sporozoites maturation (latent period = $1/\omega$). The duration of the latent period can be modelled using a constant rate (Lord et al., 1996; Ngwa and Shu, 2000; Chitnis et al., 2006; Wyse et al., 2007; Chitnis et al., 2008; Mtisi et al., 2009), or a fixed time delay (as originally used by Macdonald (1952)). Here, a constant rate is used for mathematical simplicity in later expansions. Anophelines usually remain infectious throughout their life, not recovering from infection. Infection is assumed to have no impact on vector feeding behaviour, reproduction, nor mortality, despite recent evidence suggesting that this may not be always the case (Hurd, 2003).

The vector life expectancy is short relatively to other time periods in the model, and is often about the same order of magnitude as the latent period in the vector. Consequently, vector demography and the class of latent vectors must be incorporated. In the absence of any vector control intervention, the abundance and age structure of the vector population is limited only by the recruitment rate, ρ , of newly emerged female adults entering the susceptible class, the average natural mortality rate of an adult vector, μ_0 (assumed to be age independent, such that average vector life-span in absence of vector control interventions = $1/\mu_0$), and by the carrying capacity, K .

As done for simplification in previous malaria models, we assume that vectors take one blood-meal per gonotrophic cycle, and therefore, the interval between blood-meals corresponds to the length of the gonotrophic cycle. Likewise, for simplification female mosquitoes are assumed to have a homogenous feeding behaviour, and feed with a fix preference on humans and/or animals. The proportion of vector feeds on humans, q , therefore corresponds to the probability of feeding on humans, for any particular feed of each vector. Data on the proportion of vector blood-meals on humans, the so-called human blood index (HBI, which corresponds to the model parameter q), is easier to obtain than the proportion of blood-meals on a given animal species. Therefore, we

assume that the proportion of vector feeds on livestock can be approximated by the value $(1-q)$ although, in reality, this figure corresponds to the proportion of vector feeds on non-human hosts (livestock and other animals). This means that in a scenario where the HBI is 0.10 (e.g. in regions of Southeast Asia), at any given point in time 90% of the mosquitoes blood-meals will be taken on livestock and 10% will be on humans. Additionally, we assume that vectors have no preference for a particular livestock species (e.g. cows *versus* goats).

When mosquitoes feed or try to feed on insecticide-treated livestock, their mortality rate will be increased by the factor

$$\mu_{txonly} = a(1-q)k \frac{T_l}{N_l},$$

which is a function of the vector biting rate on livestock, $a(1-q)$, the proportion of the livestock population with active insecticide at a given point in time, T_l/N_l , also referred hereafter as effective coverage, and the insecticidal probability, k . The average mortality rate of the vector population is therefore $\mu = \mu_0 + \mu_{txonly}$, such that average vector lifespan = $1/\mu$.

The final section of the model is the livestock population, modelled by U_l and T_l , the number of untreated and treated livestock, respectively. The equations are given by:

$$\frac{dU_l}{dt} = \rho_l N_l - \varepsilon_0 U_l + dT_l - \mu_l U_l, \quad (3)$$

$$\frac{dT_l}{dt} = \varepsilon_0 U_l - dT_l - \mu_l T_l,$$

where $N_l = U_l + T_l$ (total livestock population size).

Notice that since the malaria parasite is not infective to livestock, this system is linear, as opposed to the human and vector systems. We consider the case where the livestock recruitment rate (ρ_l) equals the rate at which animals are removed from the livestock population (μ_l), giving a constant population size. In the absence of insecticide treatment of livestock, all recruited animals remain in the untreated class (U_l), until their removal by death or sold. At each pulse intervention, a proportion ε_0 of the livestock population is treated with insecticide, thus moving into the treated class (T_l). The insecticide activity is assumed to be maximal on the day of the intervention and is subject to exponential decay, d , with average duration $1/d$. The value of d will depend

mainly on the type of insecticide chemical, formulation (e.g. 'spot-on' vs. sponging or spraying), concentration, dosage, and area of the animal covered by the insecticide, as all these factors affect the insecticide's pharmacokinetics (absorption, distribution, metabolism and excretion) within the animal. Additionally, in the case of formulations where insecticide washes off, the insecticide effect will last longer on sheltered than on grazing animals (Hewitt and Rowland, 1999), particularly during the raining periods. Performing simulations with various d values may provide insights into the election of the most cost-effective insecticide.

3.2.2. Threshold dynamics

The average number of secondary cases generated by a single infectious individual introduced in a population of fully susceptible individuals, is known as the basic reproduction number, denoted by R_0 (Macdonald, 1952; Anderson and May, 1991). This threshold quantity expresses the maximum transmission potential of an infectious disease and must exceed unity for the infection to be maintained in the population. Here, we determine the threshold conditions required for persistence of malaria, by analyzing the equilibriums of the model represented by Systems (1) - (3).

The R_0 was derived by linearization around the disease-free equilibrium (DFE), using the next-generation operator approach (Diekmann et al., 1990; van den Driessche and Watmough, 2002), which is a mathematical rigorous framework for the derivation of R_0 in the context of infectious diseases models (Dietz, 1993).

The DFE for the malaria model with insecticide-treated livestock is

$$\text{DFE} = (S_h^*, I_h^*, S_v^*, L_v^*, I_v^*) = (N_h, 0, N_v, 0, 0),$$

where the entire population consists of susceptible humans and vectors, and

$$R_0 = \frac{N_v (aq)^2 bc}{N_h r} \frac{\omega}{\left(\mu_0 + a(1-q)k \frac{T_l}{N_l} \right) \left((\omega + \mu_0) + a(1-q)k \frac{T_l}{N_l} \right)}.$$

The mathematical details for deriving R_0 are in Appendix B1.

To allow comparison with the majority of previous malaria models, the expression of R_0 without the square root will be used hereafter:

$$R_0 = \frac{N_v}{N_h} \frac{(aq)^2 bc}{r} \frac{\omega}{\left(\mu_0 + a(1-q)k \frac{T_l}{N_l} \right) \left((\omega + \mu_0) + a(1-q)k \frac{T_l}{N_l} \right)}.$$

Note how this expression for R_0 explicitly incorporates the different terms that compose the additional vector mortality due to exposure to ITL: $a(1-q)kT_l/N_l$. Having this expression for R_0 is important as it will allow deriving thresholds for disease control. For example, it will enable deriving the critical proportion of the livestock population that needs to be treated with insecticide to decrease R_0 below unity, and therefore interrupt malaria transmission, as will be done later in Chapter 5 (see Section 5.2.4). Additionally, it will also enable exploring how the critical proportion of ITL varies with the vector feeding behaviour and with vector density (Sections 5.2.4.3.2 and 5.2.4.3.3).

It is also possible to derive the R_0 for malaria heuristically. To illustrate this simpler derivation, consider one infectious human coming into a population where everyone is susceptible (e.g. an individual with malaria infection, immigrating to an isolated area in the UK). The human host will remain infectious for the period $1/r$, during which time he will suffer an average of $(N_v/N_h)aq$ bites by susceptible mosquitoes. Of these bites, a fraction c will lead to infection in the vector, producing a total of $(N_v/N_h)aqc/r$ infected mosquitoes. A proportion $\omega/(\omega + \mu_0 + a(1-q)kT_l/N_l)$ of these will survive the latent period and become infectious. These mosquitoes will survive, on average, $1/(\mu_0 + a(1-q)kT_l/N_l)$ days and bite humans at a rate aq during this period. A fraction b of these bites will lead to infection of susceptible humans.

When no animal is treated with insecticide ($T_l/N_l=0$) or when the vector feeds exclusively on humans, ($q=1$, either due to strict vector anthropophily or due to the absence of accessible animals), the insecticide treatment of livestock has no impact on the disease transmission dynamics. This is easily seen from R_0 , since the terms relating to livestock treatment (T_l/N_l and k) are cancelled, and the expression reduces to:

$$R_0 = \frac{N_v}{N_h} \frac{(aq)^2 bc}{r\mu_0} \left(\frac{\omega}{\omega + \mu_0} \right).$$

This expression is similar to the classical Ross-Macdonald R_0 for a model with no control intervention

$$R_0 = \frac{ma^2bc}{r\mu} \exp^{-\mu\tau} ,$$

(where $m=N_v/N_h$, $a=aq$ and $\mu=\mu_0$), with the exception that the term $\omega/(\omega+\mu_0)$, where latency is modelled with a constant rate ω , replaces the Ross-Macdonald's term $\exp^{-\mu\tau}$, where latency is modelled with a fixed time delay τ . The assumption that the rate at which latent vectors become infected is constant would, in a stochastic formulation of the model, imply that the time spent in the latent state has an exponential distribution, while modelling latency with a fixed time delay assumes that the time spent in the latency state is constant (Nåsell, 1985). In both approaches, latency decreases the endemic equilibrium number of infectious vectors by a factor $\omega/(\omega+\mu_0) \approx \exp^{-\mu\tau}$, which equals the proportion of infected vectors that survive the latent period. The magnitude of the decrease is slightly different, but the qualitative effect is the same (Nåsell, 1985). For instance, modelling latency with a constant rate produces only slightly smaller estimates of R_0 than with the Ross-Macdonald's fixed time delay.

If R_0 is greater than 1, the DFE is unstable and we are in the presence of an endemic equilibrium, where the disease can invade and persist (Figure 3.2A). However, if R_0 is smaller than 1, then the DFE is stable, and the disease dies out (Figure 3.2B). Small changes in vector life expectancy, $1/\mu_0$, and interval between blood-meals, $1/a$, may originate a drastic shift in disease dynamics. For example, decreasing $1/\mu_0$ by one day, while increasing $1/a$ by the same amount, can produce a reduction in R_0 to <1 , thereby shifting the disease dynamics from persistence to extinction (Figure 3.2).

The model equations were solved by numerical integration using the Berkeley Madonna™ package (Macey and Oster, 2006), with the built-in method *fourth order Runge-Kutta*. For illustration, the initial conditions in the number of individuals in each class were set to simulate the situation where one infected human is introduced into a fully susceptible population of humans and vectors ($S_h=99$, $I_h=1$, $S_v=1000$, $L_v=I_v=0$). The model was run for different sets of initial data and the final results were qualitatively the same.

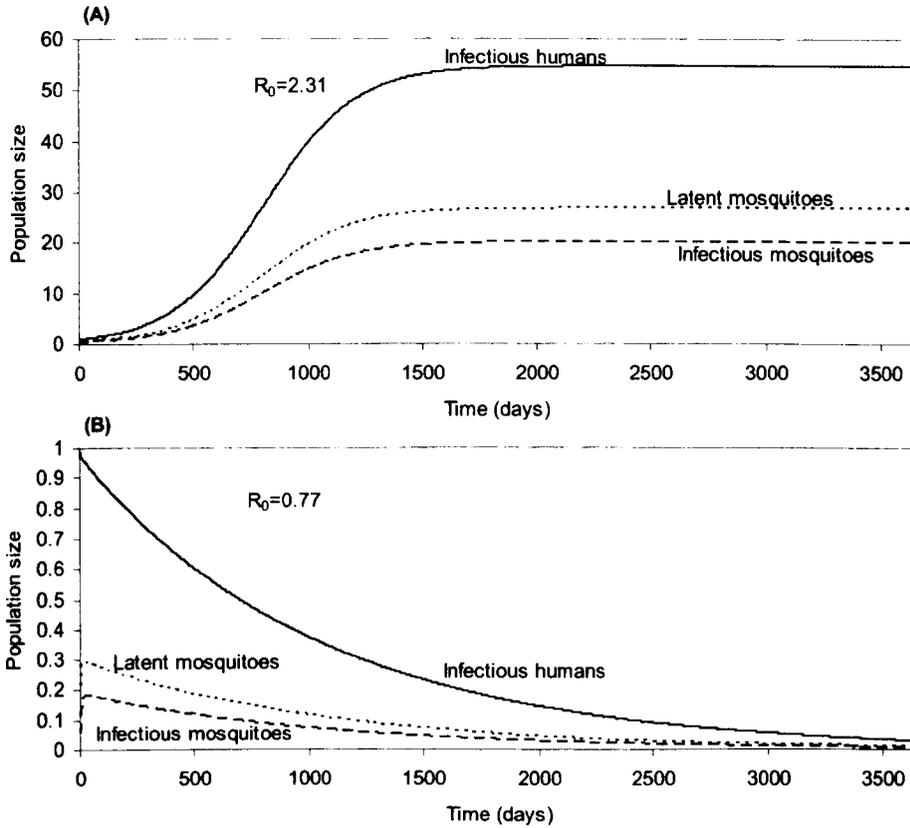


Figure 3.2. General behaviour of the malaria model prior to a control intervention. (A) Scenario where $R_0 > 1$ ($R_0 = 2.31$), and therefore the disease persists ($N_v/N_h = 10$, $a = 1/2$, $q = 0.05$, $b = 1$, $c = 0.6$, $r = 1/240$, $\mu = \mu_{\sigma} = \rho = 1/6$, $\omega = 1/8$). (B) Scenario where $R_0 < 1$ ($R_0 = 0.77$), and thus the disease dies out (all parameters are kept fixed as in (A) except for $a = 1/3$ and $\mu = \mu_{\sigma} = \rho = 1/5$).

3.2.3. Endemic equilibrium expressions

The endemic equilibrium that exists prior to a control intervention is given by

$$(S_h^*, I_h^*, S_v^*, L_v^*, I_v^*)$$

where

$$S_h^* = N_h - I_h^*$$

$$I_h^* = \frac{N_h(-N_h\omega r\mu^2 - N_h\mu^3 r + a^2 q^2 b\omega\rho N_v c)}{(N_h\omega r\mu + N_h\mu^2 r + aqb\omega\rho N_v)aqc}$$

$$S_v^* = \frac{N_h\omega r\mu + N_h\mu^2 r + aqb\omega\rho N_v}{aqb\omega(aqc + \mu)}$$

$$L_v^* = \frac{-N_h\omega r\mu^2 - N_h\mu^3 r + a^2 q^2 b\omega\rho N_v c}{aqb(aqc + \mu)(\omega + \mu)\omega}$$

$$I_v^* = \frac{-N_h\omega r\mu^2 - N_h\mu^3 r + a^2 q^2 b\omega\rho N_v c}{aqb(aqc + \mu)(\omega + \mu)\mu}$$

and $\mu = \mu_0$.

These expressions were derived after simplifying the human system by assuming that $S_h = N_h - I_h$, which enabled to omit the differential equation for S_h and solve the remaining model equations (ODEs 1 and 2 in Section 3.2.1) in Maple 7 (© Waterloo Maple Inc. 2001). Having the expressions for endemic equilibrium is a powerful tool, as it greatly facilitates exploring how the predicted number of humans and vectors in each epidemiological class in the equilibrium will be affected by changes in any of their parameter values (see for e.g. Chapter 4, Section 4.2.3.1).

3.2.4. Sensitivity of malaria R_0 to parameter values

Sensitivity analyses allow estimating the parameter(s) that most impact the outcome(s) under study, with the aims of: (1) informing control strategies to target those parameters, and/or (2) informing the design of empirical studies to collect data that can allow more accurate estimation of such parameters.

The R_0 for malaria is determined by several parameters, and here, we investigate the sensitivity of R_0 to each of its parameter. The sensitivity index S of R_0 to a parameter P is defined conventionally as:

$$S_{(P)} = \frac{P}{R_0} \frac{\partial R_0}{\partial P}$$

The definition shows that the sensitivity measures the proportional change in R_0 for a small proportional change in the parameter P . When R_0 changes linearly as the parameter alters, the sensitivity index S is equal to (+ or -) unity (Keeling and Gilligan, 2000a; Arriola and Hyman, 2007).

Table 3.2. Sensitivity analysis of R_0 in the malaria model without and with insecticide treatment of livestock.

Parameter	Without Treatment		With Treatment	
	$S = \frac{P}{R_0} \frac{\partial R_0}{\partial P}$	$ S $	$S = \frac{P}{R_0} \frac{\partial R_0}{\partial P}$	$ S $
a	2	= 2	$0 < \frac{2\mu_0(\omega + \mu_0) + ak \frac{T_l}{N_l} (\omega + 2\mu_0)(1-q)}{\left((\omega + \mu_0) + ak \frac{T_l}{N_l} (1-q) \right) \left(\mu_0 + ak \frac{T_l}{N_l} (1-q) \right)} \leq 2$	≤ 2 If $T_l/N_l=0, k=0,$ or $q=1$, then =2
q	2	=2	$2 \leq \frac{2\mu_0(\omega + \mu_0) + ak \frac{T_l}{N_l} (\omega + 2\mu_0)(2-q) + 2(ak \frac{T_l}{N_l})^2 (1-q)}{\left((\omega + \mu_0) + ak \frac{T_l}{N_l} (1-q) \right) \left(\mu_0 + ak \frac{T_l}{N_l} (1-q) \right)} < 17$	≥ 2 If $T_l/N_l=0, k=0,$ then =2
μ_0	$-2 < -\frac{\omega + 2\mu_0}{\omega + \mu_0} < -1$	< 2	$-6 < -\frac{\mu_0 \left(\omega + 2\mu_0 + 2ak \frac{T_l}{N_l} (1-q) \right)}{\left((\omega + \mu_0) + ak \frac{T_l}{N_l} (1-q) \right) \left(\mu_0 + ak \frac{T_l}{N_l} (1-q) \right)} < -1$	> 1 If $T_l/N_l=0, k=0,$ or $q=1$, then <2
$\frac{N_v}{N_h} = b=c$	1	= 1	1	= 1
r	-1	= 1	-1	= 1
ω	$0 < \frac{\mu_0}{\omega + \mu_0} < 1$	< 1	$0 < \frac{\mu_0 + ak \frac{T_l}{N_l} (1-q)}{\omega + \mu_0 + ak \frac{T_l}{N_l} (1-q)} < 1$	< 1
$\frac{T_l}{N_l} = k$	---	---	$-2 < -\frac{ak \frac{T_l}{N_l} (1-q) \left(\omega + 2\mu_0 + 2ak \frac{T_l}{N_l} (1-q) \right)}{\left((\omega + \mu_0) + ak \frac{T_l}{N_l} (1-q) \right) \left(\mu_0 + ak \frac{T_l}{N_l} (1-q) \right)} \leq 0$	< 2

The values of the sensitivity of R_0 to each of its parameter were obtained by algebraic manipulation of the expressions for the sensitivity index S of R_0 and by varying each of the parameter values in the expression for S within a conservative range: $a=1/2$ to $1/3$; $q=0$ to 1 ; $\mu_0=1/2$ to $1/30$; $\omega=11/(T-16)$, where $T=18^\circ\text{C}$ to 39°C ; $T_l/N_l=0$ to 1 ; $k=0$ to $1-\mu_0$.

The sensitivity analysis (Table 3.2) shows that the parameters that have a greater impact on R_0 are the proportion of vector feeds on humans, q , followed by the vector biting rate on any host, a , (which multiplied together correspond to the mosquito biting rate on humans, aq), and the adult mosquito natural mortality rate, μ_0 , as opposed to other parameters such as the size of the mosquito population (N_v/N_h). These findings are in accordance with the insights provided by the Ross-Macdonald model, which were the rationale behind the shift in malaria control strategies from traditionally concentrating on reducing the size of the mosquito population (by preventing mosquito breeding), into promoting strategies that increase the mortality rate of adult vectors (e.g. residual insecticides), or decrease the human biting rate (e.g. use of bednets, screens or repellents) (Macdonald, 1956). Interestingly, although in the absence of ITL R_0 is equally sensitive to changes in q as in a , in the presence of ITL R_0 becomes considerably more sensitive to changes in q than in a .

Note that in the model without ITL the sensitivity of R_0 to μ_0 ($|S| < 2$) is slightly smaller than the sensitivity of R_0 to a or to q ($|S| = 2$). This may seem contrary to the insights from the classical Ross-Macdonald model where R_0 was more sensitive to changes in μ than to changes in aq . However, this slight difference results simply from the fact that in the present work latency is modelled with a constant rate ω , while Ross-Macdonald modelled latency with a fixed time delay τ (as mentioned in section 3.2.2). Accordingly, the proportion of mosquitoes that survives the latent period and becomes infectious is given by the term $\omega/(\omega + \mu_0)$ in the present model, in contrast with the term $\exp^{-\mu\tau}$ in the Ross-Macdonald model. Thereby, in the Ross-Macdonald model, R_0 is more sensitive to changes in μ , since this parameter is exponentiated, than to changes in aq , since these parameters are squared. Note, however that, independently of this, the present model still reflects the main insights of the Ross-Macdonald model, namely that: (1) halving the mosquito biting rate on humans reduces R_0 by a factor of four while halving the mosquito population reduces R_0 by a factor of two; and that (2) decreasing the adult mosquito mortality rate also causes a stronger reduction in R_0 than decreasing the mosquito population size.

The analysis also indicates that, under the model assumptions, changes in the proportion of livestock population with active insecticide, T_1/N_1 have the same impact in R_0 as changes in the vector mortality induced by the insecticide, k . In practical terms, this

means that by increasing the insecticidal activity, a smaller proportion of livestock would need to be treated to achieve the same potential level of disease control. For example, the impact of having 40% of the livestock population treated ($T_l/N_l=0.4$) in a theoretical situation where there is no decay on insecticide residual activity, i.e. an average constant treatment coverage, is therefore predicted to be the same as when 80% of the livestock population has been treated ($\epsilon_0=0.8$) but the insecticide has lost 50% of its original activity, k . Note also that, when the proportion of livestock population with active insecticide is allowed to decay, and/or when temporal variation is introduced into the mosquito density, R_0 remains formally unchanged, except that it becomes a function of time, as ' T_l ' becomes ' $T_{l(t)}$ ' and/or ' N_v ' becomes ' $N_{v(t)}$ '.

3.3. Discussion

This Chapter has set the basis of a theoretical framework that was developed to examine the possible roles of livestock on malaria transmission dynamics. A deterministic mathematical model for malaria transmission dynamics was built expanding on the basic model of Ross and Macdonald for malaria transmission between humans and mosquitoes. The new model has built upon their work by discriminating the feeding behaviour of the mosquito vector on its alternative hosts: livestock and human populations, and incorporating the treatment of livestock with insecticide as a potential *novel* method to control human malaria. After outlining the basic structure of the model, the threshold dynamics of the system was investigated: the basic reproduction number (R_0) was derived with the next generation operator approach, and an analytical sensitivity analysis was performed.

The parameters that have a greater impact on R_0 are the mosquito biting rate on humans (proportion of vector bites on humans \times vector biting rate on any host) and the adult mosquito mortality rate, which is in accordance with the insights provided by the classical Ross-Macdonald model (Macdonald, 1956). The treatment of livestock with insecticide further increases the sensitivity of R_0 to the proportion of vector bites on humans, although it decreases the sensitivity of R_0 to the vector biting rate on any host. The analysis also indicates that by increasing the insecticidal activity a smaller proportion of livestock would need to be treated to achieve the same potential level of malaria control.

The model here presented is a robust and flexible framework than can serve as a platform for exploring into more detail several particularities of the effects of untreated and insecticide-treated livestock on malaria transmission dynamics in various ecological settings. For instance, the proportion of vector feeds on humans, q , and the vector mortality rate, μ , can be expanded to be a function of the density and availability of alternative hosts to the vector population. Also, the assumption of constant vector population density can be modified to reflect the more realistic scenarios of variable vector population density, by introducing explicit density dependence compensation of the vector population. In addition to allowing exploring the lethal effect of ITL upon malaria vectors, the model can also be expanded to account for possible excito-repellent effects of the insecticide applied on livestock, and investigate its impact on the vectors and on the overall disease dynamics. Moreover, the model can be applied to scenarios where ITL trials have been conducted, namely Pakistan, to explore the impact of alternative intervention regimens. And most importantly, the model can be applied to assess the potential impact of ITL in settings where the intervention has not been formally tested yet, such as in Africa, where malaria burden is the greatest and many communities live in close proximity with large numbers of livestock, but where the determinants of infections differ from Asia.

In the next Chapters the basic theoretical framework here presented will be expanded to address all the above mentioned particularities regarding the role of livestock on malaria transmission dynamics. The model will be applied to describe firstly, the possible effects of untreated livestock (Chapter 4), and secondly, the effects of ITL (Chapters 5), on the malaria vector feeding behaviour, mortality and density and on the overall disease transmission dynamics in several hypothetical ecological settings. Finally, the impact of ITL interventions will be further investigated in two specific ecological settings: in Pakistan, where a community-based trial of ITL has been performed (Rowland et al., 2001), and in Ethiopia, where I conducted a field study (described in Chapter 2) to parameterize the model (Chapters 5 and 6).

Chapter 4

Modelling the effects of untreated livestock on malaria transmission dynamics – Exploring different ecological settings –

Summary

Background: Field studies show that the presence of livestock near human dwellings is associated with varying effects on malaria transmission, both negative and positive. Livestock may confer protection by diverting mosquito bites from humans, although they may also increase malaria risk by sustaining mosquito populations. Here, both these features will be incorporated in the basic theoretical framework presented in the previous chapter, with the aim of capturing and explaining the inherent paradox of livestock in different ecological settings from a theoretical point of view.

Methods: A theoretical framework was built that allows the potential effects of untreated livestock on malaria transmission to be explored, for different scenarios of livestock and human hosts' abundance and availability to the mosquito vectors, and different assumptions about the vector population carrying capacity. The model incorporated the effects of livestock on (1) decreasing the human blood index, while (2) decreasing vector mortality, and (3) increasing vector population density. A range of simulations were performed, focusing mainly on scenarios of endemic malaria, both at equilibrium and also the dynamic effects following a perturbation.

Findings: It is shown that the impact of livestock on malaria transmission depends not only on the relative abundance and availability of alternative hosts, but also on whether vector population density was at its maximum level previous to livestock introduction, and on the time elapsed since livestock introduction. In situations where introduction of livestock occurred when the vector population was at its carrying capacity level (or where sufficient time has past after introduction of livestock for a new endemic equilibrium to have been established such that the vector population density will then have reached its carrying capacity), the introduction of livestock will cause no observable change in vector density and is expected to have always a zooprophylactic effect, i.e. decrease malaria transmission, as long as the relative availability of livestock

to the host seeking vector is greater than zero. However, before the vector population has reached its carrying capacity, the introduction of livestock can lead to increases in vector density and malaria transmission; furthermore, increasing livestock numbers could actually cause apparently different impacts on malaria transmission depending on the outcome that is being measured and on how long following introduction of livestock the impact is measured. Also, for a given carrying capacity of the vector population, at a level equal or higher to the initial vector population density, the higher the density and/or availability of livestock introduced, the quicker will the new endemic equilibrium be established, and the stronger will be the zooprophylactic effect of livestock on malaria transmission in the new endemic equilibrium. Conversely, for a given density and availability of livestock, the higher the carrying capacity is in relation to the initial vector population density, the longer it will take for the new endemic equilibrium to be established, and the higher will be malaria transmission levels in the new endemic equilibrium.

Interpretation: By combining the effects of livestock on decreasing the human blood index, while decreasing vector mortality and increasing vector population density, the model allows us to explain situations where the presence of livestock by itself can lead to an increase, decrease, or no impact at all on malaria transmission. The theoretical framework here described will form the basis for the next two Chapters, where the mathematical model will be expanded to examine the potential effects on malaria transmission of treating livestock with an insecticide that has lethal and possible excito-repellency effects on the disease vectors. After initial explorations in hypothetical ecological settings (Chapter 5), the model will be applied to specific settings (Chapters 5 and 6) to assess how the ITL intervention that successfully reduced malaria transmission in the Asian scenario of Pakistan, during a community-based trial, could be best translated into an African scenario, exemplified by the Ethiopian setting described in Chapter 2.

4.1. Introduction

The presence of livestock near human dwellings has been associated with an increased risk of malaria vector-human contact and/or disease in countries such as Ethiopia (Ghebreyesus et al., 2000; Seyoum et al., 2002), Pakistan (Hewitt et al., 1994; Bouma and Rowland, 1995) and Philippines (Russel, 1934; Schultz, 1989). Conversely, other studies have reported a protective zooprophylactic effect, such as in Papua New Guinea (Charlwood et al., 1985; Burkot et al., 1989) and in Sri Lanka (van der Hoek et al., 1998), or even no overall effect on malaria risk, as in The Gambia (Bøgh et al., 2001; Bøgh et al., 2002), Kenya (Snow et al., 1998), and Peru (Guthmann et al., 2001) (see comprehensive review in Section 1.4).

The protection conferred by the presence of livestock has been explained by the diversion of mosquito bites from humans to animals, with consequent decrease in the proportion of vector blood-meals on humans (human blood index, HBI). On the other hand, the increased risk could be due to an increase in the survival and/or overall density of malaria vectors. Here, both these features will be incorporated in the basic theoretical framework presented in Chapter 3, with the aim of capturing and explaining the apparently contradictory outcomes that have been associated with the presence of livestock in different ecological settings. The extended model will be applied to describe the possible effects of untreated livestock on the malaria vector:

- (1) feeding behaviour,
- (2) mortality,
- (3) density, and
- (4) all of them combined, to investigate the effects on the overall disease transmission dynamics.

Several hypothetical ecological settings will be explored, differing on the relative abundance and availability of livestock and human hosts, and on the assumptions about the density-dependent regulation and carrying capacity of the adult vector population.

4.2. Methods and Results

4.2.1. Effects of livestock on the host blood index

There is compelling evidence that the proportion of vectors that feed on a given host (host blood index) may vary under the influence of host and vector related factors. Accordingly, I allow for an explicit dependence of the host blood index on the relative abundance and availability of alternative host types (livestock and humans) to the vector population.

The term *availability* is used to encompass the *accessibility* of each host to the vector, as well as the *intrinsic propensity* for the vector to feed upon that host. This includes all the factors that can influence the likelihood of the vector feeding on a given type of host, when two types of alternative hosts are present in equal numbers. The availability of humans can therefore be defined as the likelihood that a vector will bite humans, if humans and livestock are equally abundant, in an area where these two host types are the only significant blood-meal source.

As in previous zoophylaxis models (Sota and Mogi, 1989; Killeen et al., 2001; Saul, 2003), the following simple relationship can be used to model the proportion of vectors that have blood-meals from humans (human blood index, HBI, which corresponds to parameter q in our model):

$$HBI = \frac{N_h A_h}{N_h A_h + N_l A_l}, \quad \text{Eq. 4. 1}$$

which can be simplify to:

$$HBI = \frac{1}{1 + \frac{N_l A_l}{N_h A_h}}. \quad \text{Eq. 4. 2}$$

A_h and A_l are the proportional availabilities of the human and livestock hosts, respectively, and can take any value between 0 and 1, inclusive. Contrarily to the

previous models that used absolute availability values, here proportional values are used, as that overcomes the uncertainty around possible estimates of the absolute values. Hereafter, when the term “availability” is used it will refer to “proportional availability”, unless otherwise stated. A_l/A_h is the relative availability of livestock compared to humans, in an area where humans and livestock are the only significant blood sources (otherwise, the expression needs to be modified accordingly), and is equivalent to the Feeding Index defined by Kay, Borcham, and Edman (1979).

The simplified expression (Eq. 4.2) facilitates the process of fitting to data (as done in Killen et al., 2001), because the four initial parameters are reduced to two: the ratio between livestock and human numbers (N_l/N_h), and the ratio between livestock and human availabilities (A_l/A_h).

Knowing the HBI and the (absolute or relative) abundance of hosts, the relative availability can therefore be readily estimated from the derived expression:

$$\frac{A_l}{A_h} = \left(\frac{1}{HBI} - 1 \right) \frac{N_h}{N_l}. \quad \text{Eq. 4.3}$$

This equality was first presented by Sota and Mogi (1989), and later used by Killen et al. (2001) to estimate the relative availabilities of cattle and human hosts to vectors in Tanzania using field data on HBI and abundance of hosts. Parallel expressions can be derived to model the proportion of vectors that have blood-meals from livestock (livestock blood index, which for simplicity could be assumed to be $1-HBI$).

Different scenarios are illustrated in Figure 4.1. The black line corresponds to the most conservative assumption: a constant HBI. The coloured lines were obtained using the expression for HBI given by Eq. 4.2 and represent possible scenarios of host availability. When the abundance of human and animal hosts are the same, the HBI corresponds exactly to the availability of humans (A_h). When humans and livestock are equally available to the vector (diagonal dark blue line) there is a linear relationship, with HBI corresponding to the proportional abundance of humans. The relationship becomes non-linear when the availability of each host type differs. The HBI monotonically decreases with increase in the $N_l:N_h$ ratio, or with increase in the $A_l:A_h$ ratio.

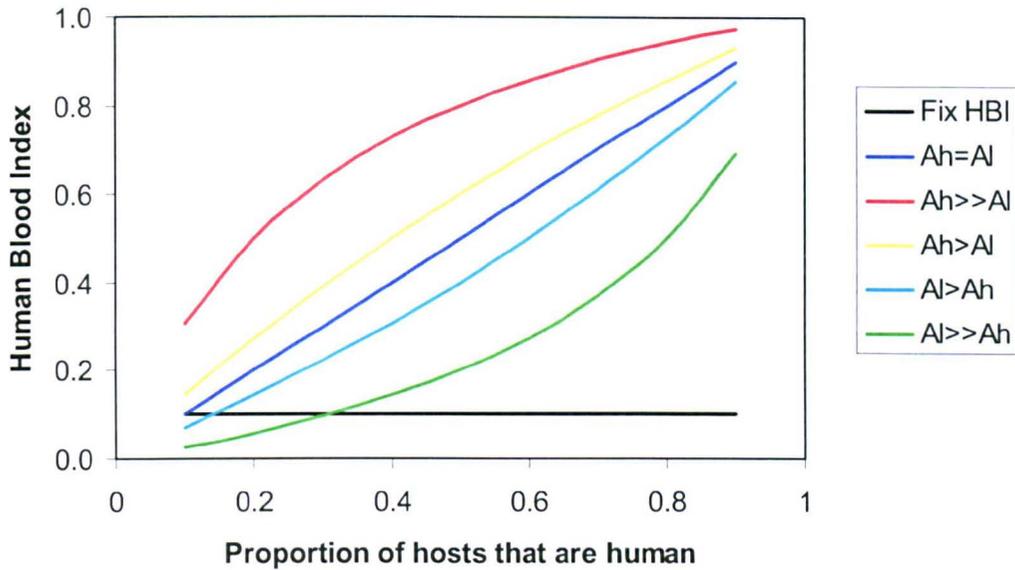


Figure 4.1. Relationship between the proportional abundance of human hosts and the human blood index.

4.2.1.1 Dissecting host availability

The availability of the human and livestock hosts depends on factors such as:

- (1) the *accessibility* (a_i) of each type i of alternative host to the host-seeking vector (which can vary with: distance from the vector breeding sites to the places where humans and/or livestock are at night; being indoor or outdoors; humans being under a bednet or not; livestock being enclosed in a shed or not); and
- (2) the *intrinsic propensity* for the vector to feed upon a given type of host (v_i) (anthropophily, v_h , vs. zoophily, v_l); and to feed in the place where the host is present (endophagy, v_{in} , vs. exophagy, v_{out}). Such intrinsic propensity for host and place preference can be conditioned by vector genetics and learning.

By modelling the availability of hosts as an explicit function of these factors it should be possible to predict the trend in the HBI for different ecological settings.

Let the above mentioned factors be denoted by the symbols described in Table 4.1.

Table 4.1. Factors that determine the availability of hosts to the malaria vector.

Symbol	Definition
N_h	Total number of humans present (indoors + outdoors)
N_l	Total number of livestock present (indoors + outdoors)
a_{iind}	Proportion of hosts of type i that is accessible to the vector indoors
a_{iout}	Proportion of hosts of type i that is accessible to the vector outdoors
v_i	Intrinsic preference of the vector for feeding on the host type i
v_{in}	Intrinsic preference of the vector for feeding indoors
v_{out}	Intrinsic preference of the vector for feeding outdoors

When allowing the availability of a given host type, A_i , to depend explicitly on the vector's favourite feeding host and on the host's accessibility at the vector's favourite feeding place, then

$$A_i = v_i [(a_{iind} v_{ind}) + (a_{iout} v_{out})]. \quad \text{Eq. 4. 4}$$

The corresponding expression for the overall HBI from mosquitoes collected indoors and outdoors is given by:

$$HBI = \frac{N_h v_h [(a_{hind} v_{ind}) + (a_{hout} v_{out})]}{N_h v_h [(a_{hind} v_{ind}) + (a_{hout} v_{out})] + N_l v_l [(a_{lind} v_{ind}) + (a_{lout} v_{out})]}, \quad \text{Eq. 4. 5}$$

which can be simplified to:

$$HBI = \frac{1}{1 + \frac{N_l v_l [(a_{lind} v_{ind}) + (a_{lout} v_{out})]}{N_h v_h [(a_{hind} v_{ind}) + (a_{hout} v_{out})]}}. \quad \text{Eq. 4. 6}$$

In a scenario where all the humans are indoors and all the livestock are outdoors during the period when the vector is seeking a host to feed upon, the above expression for HBI is further reduced to:

$$HBI = \frac{1}{1 + \frac{N_l v_l a_{lout} v_{out}}{N_h v_h a_{hind} v_{ind}}}. \quad \text{Eq. 4. 7}$$

The corresponding LBI is given by:

$$LBI = \frac{1}{1 + \frac{N_h v_h a_{hind} v_{ind}}{N_l v_l a_{lout} v_{out}}}. \quad \text{Eq. 4. 8}$$

For example, previous studies in the Konso District in Ethiopia (Tirados et al. 2006) using odour-baited entry traps (OBETs) have shown that *An. arabiensis* has a high preference for feeding on humans (high v_h). However, the vector also has a high preference for feeding outdoors (high v_{out}), where humans are less accessible than livestock ($a_{hout} < a_{lout}$), which might explain why the vector has a high number of blood-meals on livestock.

4.2.1.2. Theoretical predictions of the hosts blood index

Hereafter in this chapter all simulations were done using Berkeley Madonna™ package (Macey and Oster, 2006). Initial simulations were done to predict the HBI and LBI for various scenarios where livestock and humans are either separate (Figure 4.2) or in the same dwelling (Fig. 4.3). For simplification (and to facilitate the process of fitting to data) the following assumptions were made regarding the vector's favourite feeding host and place, and the host's accessibility to the vector:

anthropophily = 1-zoophily (i.e. $v_h = 1 - v_l$);

endophagy = 1- exophagy (i.e. $v_{in} = 1 - v_{out}$);

accessibility indoors = 1- accessibility outdoors (i.e. $a_{iin} = 1 - a_{iout}$);

and vice-versa.

Figure 4.2 shows the predictions for a scenario of separate dwellings, where most humans are accessible indoors ($a_{hin} = 0.99$), while most livestock are kept outdoors ($a_{lout} = 0.99$), and livestock are present in the same number as humans. The host blood index will then depend on the relative magnitude of anthropophily (v_h) versus exophily (v_{out}). It would be intuitive to predict that, if $v_h \approx v_{out}$, then the HBI would be likely to have an approximate value to the LBI; if $v_h > v_{out}$, then $HBI > LBI$; and conversely, if $v_h < v_{out}$, then $HBI < LBI$. However, from the results in Figure 4.2, it seems that the vector preference for a given type of host (anthropophily or zoophily) has a stronger weight on the resulting HBI than the vector preference for the place where the host is located (exophagy or endophagy). For example, in a scenario where $N_h = N_l$, $a_{hin} = 0.99$, and $a_{lout} = 0.99$, if v_{in} is high ($= 0.99$) and v_h is low ($= 0.01$), then $HBI < LBI$ (plot A3), and vice-versa (plot C1). Notwithstanding, this will also depend

on the accessibility of human and livestock outdoors, as less extreme scenarios of accessibility would produce different outputs.

Simulations were also done for a mixed dwelling (Figure 4.3), where humans and livestock are almost totally accessible to the vector indoors ($a_{hin}=0.99= a_{in}$). For a given level of anthropophily, the predicted HBI (or LBI) will always be the same, independently of the vector's preference for feeding indoors or outdoors, and will correspond to the predicted HBI (or LBI) in a scenario of separate dwellings where most humans are accessible indoors ($a_{hin}=0.99$) and livestock outdoors ($a_{lout}=0.99$) and the vector has no particular preference for feeding indoors or outdoors ($v_{in}=0.5=v_{out}$) (i.e. Figure 4.3 corresponds to the scenarios illustrated in Figure 4.2. B1, B2, and B3).

It is worth noting that in an empirical scenario, for a given residence compound, the sum of the number of humans indoors and the number of humans outdoors during the hours when vectors are seeking a blood-meal may be greater than the total number of humans present in that residence compound, because there could be persons outdoors at some point in the evening who may go indoors later in the night.

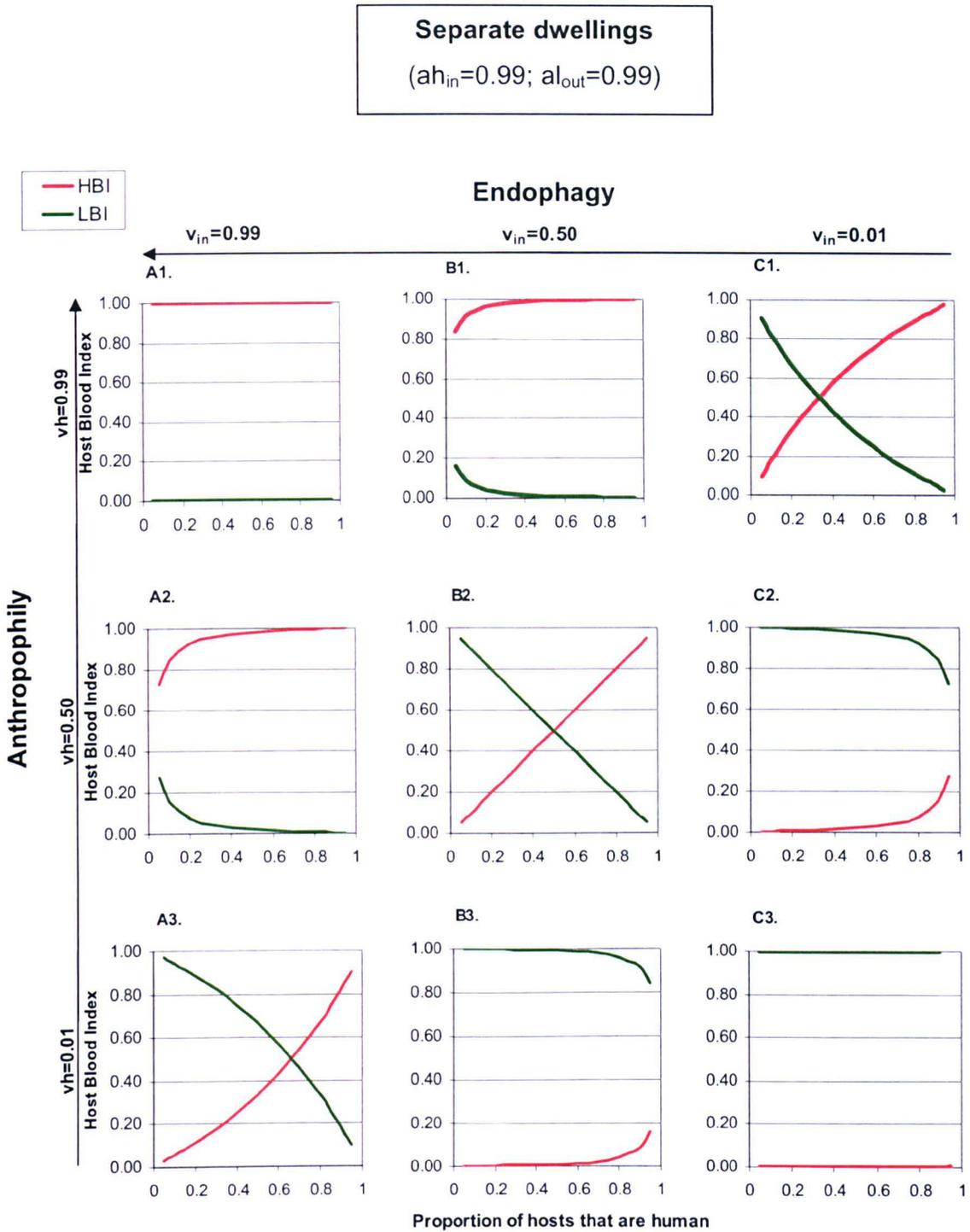


Figure 4.2. Relationship between the proportional abundance of humans and the hosts blood index, when humans and livestock are in separate dwellings.

The simulations illustrate a scenario where most humans are accessible to the vectors indoors, while most livestock are outdoors. Red=Human Blood Index; Green=Livestock Blood Index.

The plots can be grouped in three groups, with a similar behaviour being captured by the plots within each group: i) A1, A2, B1; ii) A3, B2, C1; and iii) B3, C3, C2.

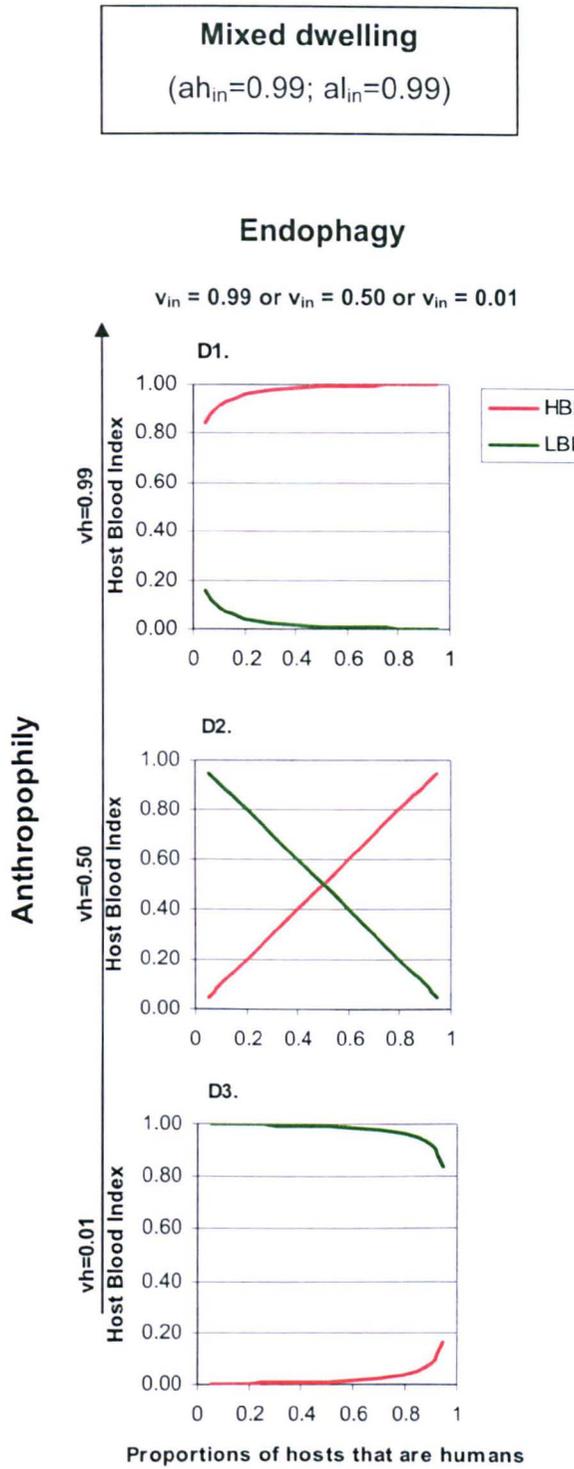


Figure 4.3. Relationship between the proportional abundance of humans and the hosts blood index, when humans and livestock are in the same dwelling.

The simulations illustrate a scenario where most humans and livestock are accessible to the vectors indoors. Red=Human Blood Index; Green=Livestock Blood Index.

4.2.1.3. Validation attempt of the extended expression for host blood index using field data for Ethiopia

An attempt was made to see whether the expression for HBI (and LBI) that explicitly accounts for the different components of host availability (Equation 4.6) could predict the HBI observed in two sites in the Konso District in Ethiopia using data from a study by Tirados et al. (2006). This was a comparative study in two settings that represent the two main types of human settlements in Konso District: an established village (Fuchucha) and a site with temporary cattle camps (Jarso), as mentioned in Chapter 1 (Section 1.8.2.1).

In Fuchucha most people slept inside huts, while cattle stayed outdoors but nearby, within the residence compound, with a ratio cattle:humans = 0.6:1. From cross-sectional mosquito collections indoors (light trap catches) and outdoors (human landing catches) Tirados et al. (2006) estimated that the *An. arabiensis* vector is 2.5 times more likely to feed outdoor than indoors, corresponding to an exophagy (v_{out}) of 71%. From resting collections indoors (spray catches) and outdoors (pit shelters), 42% of the vectors with simple blood-meals (i.e. with blood from only one host) had fed on humans. Note that this site was also one of the villages from the study area described in Chapter 2.

In Jarso, where cattle were brought to graze for months at a time, at night cattle were tethered within a "fence" of thorn bush, and humans slept outdoors near the cattle, in platforms built on trees, 5-10m above ground, with a ratio cattle:humans = 17:1 (range 10 to 30 heads of cattle per human between different camps). From resting collections outdoors (pit shelters) 15% of the simple blood-meals had human origin. Using experiments with human and cattle odour-baited entry traps (OBETs), \approx 6 times more mosquitoes were collected in the human-baited traps than in the cattle-baited ones, corresponding to a vector anthropophily (v_h) of 86%.

Table 4.2. Predicted and observed human blood index (HBI) and livestock blood-index (LBI) for two settings in Ethiopia: Jarso and Fuchucha.

Sites	Phily		Phagy		Accessibility				Abundance	Predicted		Observed*	
	Anthro	Zoo	Endo	Exo	Humans		Livestock		(In + Out)	HBI	LBI	HBI	LBI
	v_h	v_l	v_{in}	v_{out}	$a_{h_{in}}$	$a_{h_{out}}$	$a_{l_{in}}$	$a_{l_{out}}$	N_l/N_h				
Jarso	0.86	0.14	0.3	0.7	0.90	0.10	0.01	0.99	17	15%	85%	15%	85%
Fuchucha	0.86	0.14	0.3	0.7	0.99	0.01	0.01	0.99	0.6	82%	18%	42%	58%

The shaded cells contain values from published field data (Tirados et al., 2006).

$v_l=1-v_h$; $v_{out}=1-v_{in}$; $a_{h_{out}}=1-a_{h_{in}}$; $a_{l_{in}}=1-a_{l_{out}}$; predicted LBI = 1-(predicted HBI).

* The observed host blood indices refer to the percentage of blood fed *An. arabiensis* containing simple blood meals only from bovines (LBI), or only from humans (HBI), out of the total number sampled with simple blood meals. The observed host blood indices values for Jarso are from outdoor mosquito collections (no indoors collections could be made), while for Fuchucha are from indoors+outdoors collections (the HBI in mosquitoes from indoors collections was 37%, and from outdoors was 55%).

As seen in the results displayed in Table 4.2, for Jarso, when using the published vector anthro- and zoophily figures experimentally estimated in Jarso, and the same degree of endo- and exophagy estimated for Fuchucha (although using a higher accessibility of humans outdoors in Jarso than in Fuchucha), it was possible to predict exactly the same host blood indices as the ones observed in the field (HBI=15%, LBI=85%).

However, for Fuchucha, using this site's field data on endo- and exophagy and the data of anthro- and zoophily from Jarso, the model prediction (HBI=82%, LBI=18%) was far from the observed hosts blood indices (overall HBI=42%, LBI=58%). To obtain a perfect fit while keeping the Fuchucha data of the observed endo-and exophagy ($v_{in}=0.3$; $v_{out}=0.7$) and livestock:human abundance ($N_l/N_h=0.6$), and the assumed accessibility ($a_{h_{in}}=0.99$; $a_{l_{out}}=0.99$), the values of anthropo- and zoophily had to be modified such that vectors would have the same intrinsic preference for humans as for livestock ($v_h=v_l=0.5$).

Something that could be playing a role here is whether the anthropophily estimated using OBETs corresponds to the true vector intrinsic host preference. Despite OBETs are among the best known methods to estimated anthropo- vs. zoophily, since the vector needs to fly into one of the entry tubes of the trap - which could be considered as an indoors environment -, this may somehow bias the estimates of vector host preferences (Steve Torr, personal communication).

Note that the above calculations were done considering only the simple blood meals (i.e. only from livestock, LBI, or only from humans, HBI), while in the above mentioned Ethiopian study (Tirados et al., 2006), a considerable proportion of vectors were found

to have mixed blood meals (i.e. from livestock and humans). Although this is not accounted in the presented expressions for host blood index, a modification of the HBI expression to account for multiple/mixed blood meals is suggested in the discussion of this chapter.

4.2.2. Effects of livestock on vector mortality and density

The assumption that increases in livestock relative abundance and/or availability simply decrease the HBI without affecting any other parameter would, by itself, reduce the human biting rate [$HBR=(N_v/N_h)aHBI$], and consequently decrease malaria transmission. However, the opposite may happen as has been documented in some regions (e.g. Pakistan and Ethiopia; reviewed in Section 1.4.1). A possible explanation has been attributed to the impact of livestock abundance and/or availability upon vector mortality and/or density, which may vary between and even within settings (Figure 4.4).

By increasing the number of available blood-meal hosts, such as livestock, fewer attempts may be required for vectors to obtain a successful blood-meal. This may increase the probability of vectors having a successful blood-meal during each gonotrophic cycle and decrease their mortality rate. The resulting increased vector survival has two epidemiological implications. Firstly, it will increase the probability of infected vectors surviving the parasite extrinsic incubation period and becoming infectious. Secondly, since vectors can have more blood-meals during their prolonged life, more eggs can be produced and laid, potentially generating more larvae. However, this will also lead to increased larval competition in the breeding sites (as reviewed in Section 1.7.2), and therefore, the resulting outcome in the recruitment rate of emerging adult vectors will depend on the vector population density, N_v , and the system carrying capacity, K . If $N_v=K$, resources in the breeding sites will not be enough to sustain additional larvae, leading to reduction in the proportion of larvae that survive until the adult stage, with consequent no change in the recruitment rate of adult vectors, and therefore N_v remains constant. Conversely, if $N_v<K$, resources in the breeding sites will be enough to sustain the additional larvae produced, resulting in increases in the recruitment rate of adult vectors, until N_v reaches K .

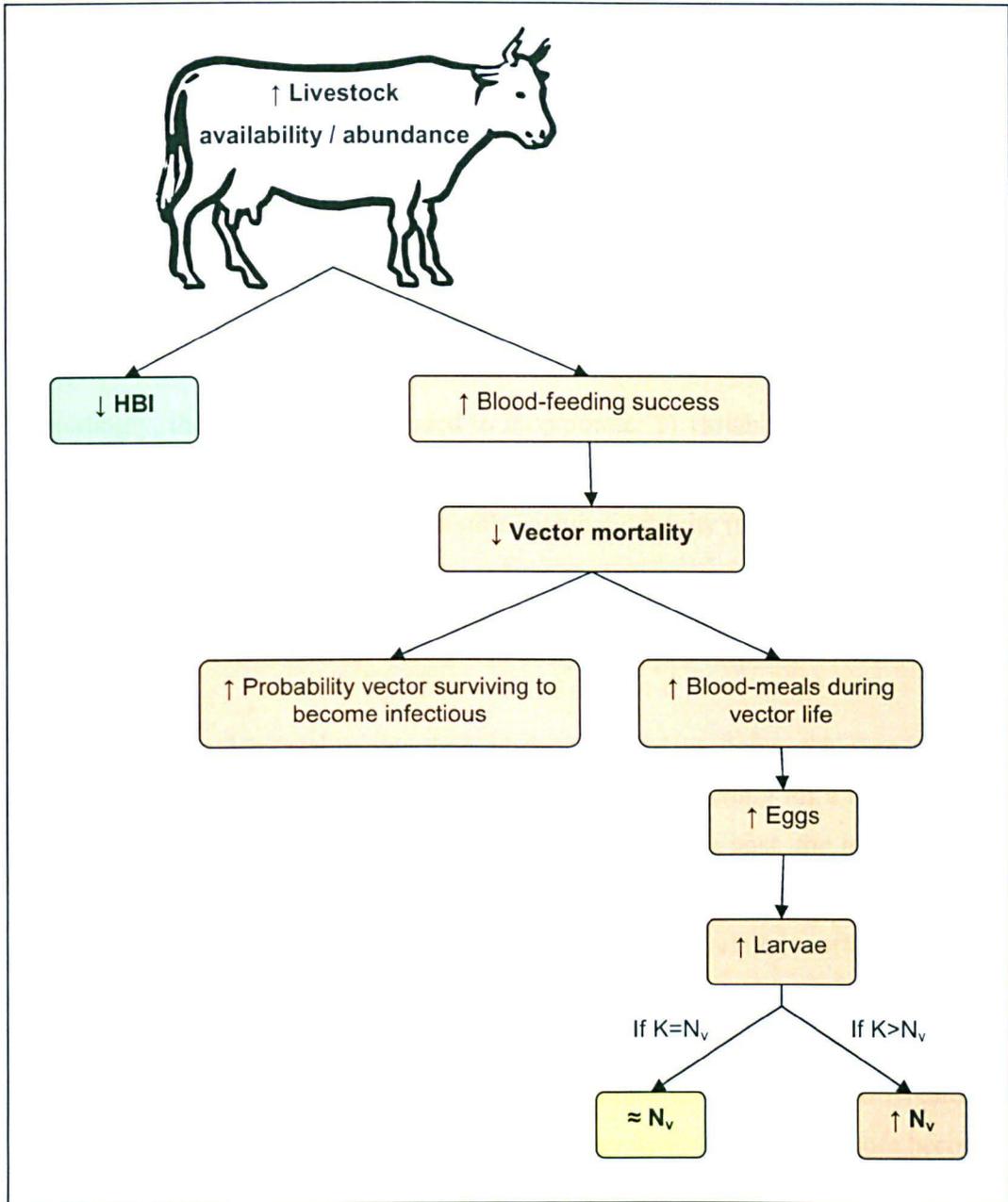


Figure 4.4. Schematic framework of the possible effects of untreated livestock on vector blood feeding behaviour, mortality and density.

HBI = human blood index; N_v = density of vector population (adult female anopheline mosquitoes); K = carrying capacity of the vector population. See explanation in the text.

Previous works have modelled the possible increase in malaria risk associated with the presence of livestock, as being due to either an increase in vector emergence rate (Sota and Mogi, 1989; Kawaguchi et al., 2004), or a decrease in vector mortality rate (Saul, 2003; Killeen and Smith, 2007). For the present model, the latter approach was chosen, as it enables exploring not only the resulting effect of increasing vector density, but also the effect of increasing the proportion of vectors that survive the parasite extrinsic incubation period and therefore become infectious.

Accordingly, the model was expanded to incorporate: 1) variable vector mortality as a function of relative host abundance and/or availability (section 4.2.2.1), and 2) variable vector density as a function of the system's carrying capacity (section 4.2.2.2).

4.2.2.1. Effects of livestock on vector mortality

The vector natural mortality rate (μ_0) is decomposed as being the sum of (1) the minimum mortality rate (μ_{min}) due to causes other than searching for a blood-meal host (i.e. mortality due to hazards during the act of feeding on a host, the gestation period, the search for oviposition sites, and the underlying aging process); and (2) the mortality due to searching for a blood-meal host (μ_{search}). The search-related mortality is assumed to be proportional to the length of the searching period, which is inversely related to the abundance and availability of possible blood-meal hosts. These assumptions follow previous models by Saul (2003) and Killeen & Smith (2007). When no livestock are treated with insecticide, the expression for the vector mortality rate therefore becomes:

$$\mu = \mu_0 = \mu_{min} + \left(\frac{1}{(N_h A_h + N_l A_l) j} \right) a, \quad \text{Eq. 4.9}$$

where the last term is the search-related mortality, hereafter also referred to as μ_{search} .

Instead of absolute values for availability, as used in the models by Saul (2003) and by Killeen & Smith (2007), the present model uses proportional availabilities, and therefore the parameter j is introduced as a scaling factor. Also, the daily biting rate, a , needs to be included in the expression, since the additional mortality due to searching for a blood-meal host is only suffered by the vector when it attempts to blood feed.

Note that, as in most previous malaria models, it is assumed that the feeding success of malaria vectors is independent of the density of vectors per available host, which has been shown to be the case where malaria vectors bite late at night, when the hosts tend to be less responsive to the bites, and where bednets are not available (Charlwood et al., 1995), although this may not be true for cattle (S. Lindsay, personal communication).

From Equation 4.9 it is clear that the higher the numbers and/or availability of blood-meal hosts, the lower will be the vector mortality rate, μ , up to the point that the minimum threshold mortality, μ_{min} , is reached. After that point, further increases in the number and/or availability of any host have no impact on μ . The lower the values of the threshold mortality rate (μ_{min}) [or the higher the values of the threshold survival rate ($surv_{max}=1/\mu_{min}$)] and/or the higher the value of j , then the stronger will be the decrease in the mortality of adult mosquitoes that feed upon livestock (or humans), per unit increase in the number of livestock (or humans) that are present with a given availability.

4.2.2.1.1. Considerations on parameters estimation

For the purpose of illustrating the model behaviour, the simulations in this chapter assume that the vector minimum mortality rate (μ_{min}) is half the natural mortality rate when no livestock are available (μ_{nolive} ; i.e. vector mortality when $N_l=0$ or $A_l=0$)¹.

The values of j were chosen to obtain $\mu_{nolive}=0.1/\text{day}$ (which is illustrative and within the limits of recorded field values – vector life expectancy of 10 days), in a village with 100 persons and where the vector feeds once every two days ($j=0.1105$, 0.2 and 1 for $A_h=0.9$, 0.5 and 0.1, respectively – which corresponds to $A_l=0.1$, 0.5 and 0.9, respectively).

When fitting the model to a particular field setting, parameter values could be estimated in the following way:

- μ_{nolive} [or $surv_{nolive}$] could be determined from a setting with a known number of humans, where no livestock are available for the vector to feed upon;
- j could be estimated, for example, after determining μ_{nolive} ;

¹ The impact of the assumptions about the relative magnitude of μ_{min} will be considered in more details in subsequent Chapters.

- Site specific values for the minimum mortality rate, μ_{min} [or maximum survival, $surv_{max}$] of adults vectors could be estimated based on (1) experimental or (2) field data, as suggested below:

(1) The longevity of mosquitoes in the laboratory could be a proxy for the longevity of mosquitoes that are only under the effect of aging, and suffer no additional mortality due to predators, or due to searching for a host to feed. The survival measured in the laboratory should however be decreased to account for the effect of predators in the field.

(2) Estimating the probability of daily survival of female vectors collected in the field (from the proportion of parous female vectors) when a virtually infinite number of hosts are available for the vector to feed on.

- a can be estimated from the inverse of the duration of the gonotrophic cycle, assuming one bite per blood-meal and one blood-meal per gonotrophic cycle.

4.2.2.2. Effects of livestock on vector population density

4.2.2.2.1. Density dependent regulation of the adult vector population due to larvae competition within the breeding sites

Different assumptions can be explored with regards to the impact that changes in vector mortality can cause on the overall vector population density. Here I consider the impact of a) decreasing vector mortality due to the increase in number and/or availability of livestock, and b) increasing vector mortality due to treatment of livestock with insecticide (although the latter will be the focus of the next two chapters, it is relevant to be mentioned also here).

The simplest assumption is to assume a worst case scenario, where changes in vector mortality have no impact at all on vector population density. Under the present model structure, this could be done by setting the recruitment rate of newly emerged female adult mosquitoes entering the susceptible class equal to the overall vector mortality rate ($\rho = \mu$). Doing so, there is always intrinsic and exact density-dependent compensation and vector population density, N_v , remains unchanged, independently of the number and/or availability of untreated or treated livestock. Even under the effects of additional mortality due to insecticide exposure, it is assumed that there is immediate replacement

of the killed vectors by newly emerged ones, so that the total vector population size does not change.

However, although this is useful for initial explorations, it is unlikely to be realistic. For example, a positive correlation has been found between the density of malaria vectors and cattle density in a study in USA Louisiana rice lands (McLaughlin and Focks, 1990). Additionally, following a community trial of ITL in Pakistan, the density of the main malaria vectors, *An. stephensi* and *An. culicifacies*, decreased by approximately 50% in treated villages (Rowland et al., 2001).

I therefore use a more realistic population growth function for N_v , based on the logistic equation

$$dN/dt = rN(1-N/K), \quad \text{Eq. 4. 10}$$

where N is the total population density, K is the system carrying capacity, and r is the net growth rate. Generically speaking, r is given by the average “birth” rate (recruitment rate, ρ , in our model) subtracted by the average mortality rate (μ).

In accordance with previous vector-borne disease modelling work, I consider the case of varying ρ and keeping μ constant, with ρ a linear function of N_v (varying both parameters is known to produce no significant changes on the dynamics of N_v) (Lord et al., 1996). Allowing density-dependent constraints to act, the vector (adult female anophelines) recruitment rate, ρ is replaced by the expression $(\rho_0 - \rho_s N_v)$. The equation describing the rate of change on the population of susceptible vectors then becomes:

$$\frac{dS_v}{dt} = (\rho_0 - \rho_s N_v)N_v - \left(aqc \frac{I_h}{N_h} + \mu \right) S_v, \quad \text{Eq. 4. 11}$$

The other model equations remain the same. The new parameters, ρ_0 and ρ_s , are the vector recruitment rate in the absence of density-dependence constraints and the strength of the density-dependence in recruitment, respectively. The vector population is therefore regulated to a constant level, given by

$$N_v^* = (\rho_0 - \mu) / \rho_s. \quad \text{Eq. 4. 12}$$

With this modelling approach, the mosquito population will return to its equilibrium level N_v^* following a perturbation, and ρ is no longer forced to be equal to μ . Examples

of such perturbations could be a decrease in mosquito mortality due to the introduction of untreated livestock, or an increase in mosquito mortality due to exposure to ITL.

In parallel with the logistic equation, ρ_0 can be interpreted as the vector recruitment rate in a scenario where its population density is at equilibrium (or carrying capacity), with vectors being recruited at the same rate as they die. Accordingly, ρ_s corresponds to the change (increase or decrease) in the baseline recruitment rate (ρ_0), per unit change (decrease or increase, respectively) on the vector population density.

The logistic equation model and the present model are equivalent when $K = \rho_0 / \rho_s$.

The implications of including explicit density dependence on the R_0 expression are in Appendix C1, as well as the derivation of an analytical expression for ρ_s :

$$\rho_s = \frac{(\rho_0 - \mu)}{N_v^*} \Leftrightarrow \rho_s = \frac{(\rho_0 - \mu)}{K}. \quad \text{Eq. 4. 13}$$

From this expression, it is easily seen that:

1) When $\rho_0 = \mu$, then $\rho_s = 0$. Since vectors are being recruited at the same rate as they die, no changes will be imposed on the baseline recruitment rate, ρ_0 . Therefore, vector population density will remain unchanged.

2) When $\rho_0 > \mu$, then $\rho_s > 0$; i.e. after the perturbation ceases (e.g. after removal of livestock that had been temporarily introduced), density dependent constraints will lead to a compensatory decrease on vector population density, until it reaches its equilibrium level (N_v^*). Since vectors are being recruited at a higher rate than they die, there will be increased competition between larvae by food resources within the breeding sites. As a result, the baseline recruitment rate ρ_0 will be subtracted by $\rho_s N_v$.

3) When $\mu > \rho_0$, then $\rho_s < 0$; i.e. after the perturbation ceases (e.g. after decay of the insecticide residual activity on treated livestock), density dependent constraints will lead to a compensatory increase on vector population density, until it achieves its equilibrium level (N_v^*). Since vectors are dying at a higher rate than they are recruited, there will be decreased competition between larvae. Consequently, the baseline recruitment rate ρ_0 will be added by $\rho_s N_{vs}$.

Overall, the largest the difference (in absolute values) between ρ_0 and μ , the largest is the (absolute) value of ρ_s , i.e. the stronger are the density-dependent constraints, and *vice versa*. In practical terms, the stronger the density-dependent constraints, the smaller is the system carrying capacity, and the shorter is the time it takes for vector population

to recover to its original density following a perturbation (e.g. after increasing vector mortality with ITL, as will be further discussed in the next two chapters).

Note that it is assumed that the number and capacity of the breeding sites remains the same independently of the hosts' abundance and availability. For instance, the potential increase in breeding sites due to livestock hoof prints is not considered here.

4.2.2.2.2. Model predictions on vector population density

The present framework allows exploring various scenarios that differ on how vector population density may be regulated: 1) constant N_v , or 2) variable N_v due to limited or 3) unlimited carrying capacity. This thesis is focused on the first two scenarios; additionally, a brief mention of the latter scenario is also made here, for the purpose of completeness. The predicted results for the three scenarios are presented below. Throughout this Chapter, the initial value of the vector population density prior to increase in numbers of livestock will be denoted by $N_{v(0)}$.

1) Constant vector population density, due to limited carrying capacity equal to $N_{v(0)}$

In a scenario where the initial vector population density, $N_{v(0)}$, is at its carrying capacity level, K , there will be perfect density-dependent compensation of the vector population. Although the introduction of livestock can result in a decrease in vector mortality, there will be exactly the same decrease in the recruitment rate of adult vectors, notably due to competition between larvae within the breeding sites, and therefore, the density of the adult vector population remains constant.

2) Variable vector population density, restricted by a limited carrying capacity higher than $N_{v(0)}$

When $N_{v(0)}$ is smaller than K , following introduction of livestock N_v will increase until reaching the K threshold. Simulations were done to explore how the vector population density was likely to vary after increasing the livestock to human density ratio from 0 to 20. The results indicate that the higher the number and/or availability of livestock, the less time it takes following the introduction of livestock for the vector population to

reach its maximum population size (K). Figure 4.5 illustrates a scenario where livestock are as available as humans to the vectors ($A_l=0.5$). The dynamics of the system would shift to the left or to the right in scenarios where livestock are more or less available than humans, respectively (simulations were performed although not shown). For example, after introduction of 1 head of livestock per 2 humans ($N_l/N_h=0.5$), the time that takes for N_v to reach K , decreases from: 19.8 years, to 3.1 years and to 1.3 year, for $A_l=0.1, 0.5$ and 0.9 respectively.

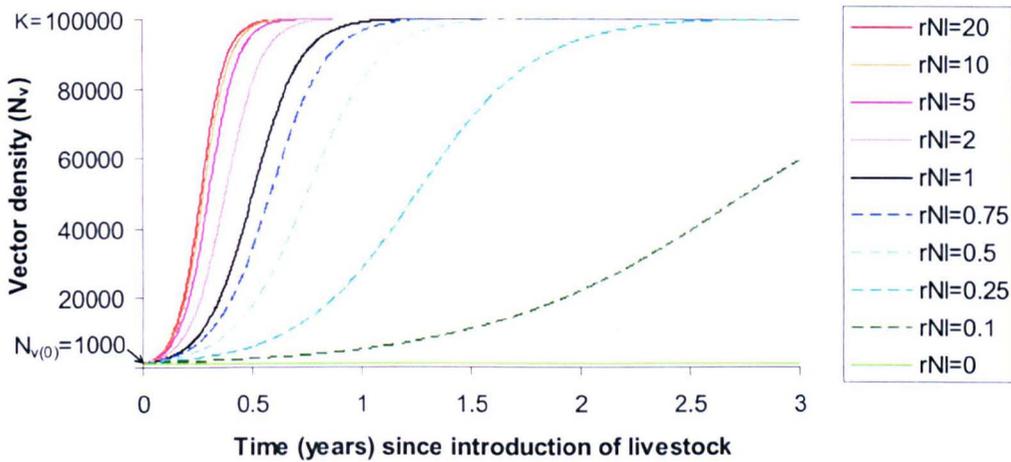


Figure 4.5. Effect of altering the number of livestock with $A_l=0.5$, on the vector population density, with limited $K > N_{v(0)}$.

Relative density of livestock:humans ($rN_l/N_l N_h$) varying from 0 (bottom green line) to 20 (top red line). $N_h=100$; $j=0.2$, to produce $\mu_{nolive}=0.1/\text{day}=\rho_0$; $\mu_{min}=0.05/\text{day}$. Since N_h was kept fix, the increase in N_l/N_h corresponds also to an increase in the absolute number of livestock. The same effects would be observed by varying the number of humans in the same proportions, while keeping N_l fix.

In a scenario where $N_{v(0)}$ is higher than K , assuming that $\rho_0 > \mu_{nolive}$, there could be two possible outcomes: a) if no livestock (or additional humans) were introduced, then N_v would remain constant and equal to $N_{v(0)}$; b) if the system was perturbed by increasing the number and/or availability of hosts, then N_v would decrease until reaching K . Like for the scenario where $N_{v(0)}$ was smaller than K , the higher the number and/or availability of hosts introduced, the quicker would N_v vary and reach the K threshold (simulations were performed although not shown).

3) Variable vector population density, unrestricted due to unlimited carrying capacity

The effect of introducing livestock on the dynamics of the vector population was also examined in a scenario where K is virtually infinite, and therefore, the density-dependent constraints at the larval stages are negligible, and the growth in vector population density is virtually unrestricted. The simulations indicate that, as in the previous scenario 2), also here the higher the number and/or availability of livestock introduced, the quicker will be the resulting increase in N_v . The difference from scenario 2) is that, when K is virtually infinite, for a given time elapsed since the introduction of certain number of livestock with a given availability, the higher will be the N_v reached (simulations were performed although not shown).

4.2.3. Putting all pieces together

As presented above, it was shown that:

- 1) to estimate the HBI it is necessary to know not only the relative abundance of hosts but also their relative availability to the vectors; and
- 2) in order to further assess the effects of livestock on malaria transmission it is also important to: a) account for variations in vector mortality, as a function of the relative host abundance and availability; and b) account for variations in vector density, as a function of the system's carrying capacity.

It is now possible to explore the overall effects of livestock abundance and availability on different outcome measures of malaria transmission. For this purpose, a range of simulations was done, focusing on scenarios of endemic malaria. Additionally, scenarios of epidemic malaria were also briefly examined. In both situations, the model was run for two density dependence scenarios: firstly, assuming that pre-introduction of livestock, the vector population density was at the carrying capacity level, therefore remaining constant, and secondly, assuming that the initial vector population density was below the carrying capacity level, therefore increasing until reaching the carrying capacity threshold (corresponding to the scenarios 1 and 2, respectively, in the previous section). Simulations were done to explore the effects of different host abundance and three availability scenarios where livestock are much less ($A_l=0.1$), equally ($A_l=0.5$), or much more ($A_l=0.9$) available to vectors than the human hosts.

All the simulations used the parameter values listed in Table 4.3, unless otherwise stated. As before, the ratio of livestock to human density was varied by changing the number of livestock while fixing the number of humans equal to 100. Therefore, the increase in ratio of livestock to human density corresponds always to an increase in the absolute number of livestock.

Table 4.3. Parameter values used for modelling the effects of untreated livestock on malaria transmission.

Symbol	Definition	Value	Ref. *
$N_{V(0)}$	Initial number of vectors, prior to change in livestock abundance / availability	10^3 - 10^5 /ha	[0]
K	Carrying capacity of the vector population	10^3 - 10^5 /ha	[0]
N_h	Human density	100 /ha	[0]
rN_l	Relative density of livestock:humans	0 - 50 /ha	[0]
A_l	Proportional availability of livestock	0.1, 0.5, 0.9	[0]
A_h	Proportional availability of humans ($A_h=1-A_l$)	0.9, 0.5, 0.1	[0]
j	Factor to scale the proportional availability values such that $\mu_{nolive}=0.1$ /day	0.1105, 0.2, 1	-
μ_{nolive}	Vector mortality rate in absence of available livestock	0.1 /day	[1]
μ_{min}	Vector minimum mortality rate when there are no hazards due to search for a blood-meal host ($\mu_{min}=1/surv_{max}$)	0.05 /day	[1]
a	Vector biting rate on any host	0.5 /day	[2]
$Dinf$	Duration of infectiousness in humans ($1/r$)	21 days	[3]
$Tlat$	Duration of latent period in surviving vectors ($1/\omega$)	14 days	[4]
b	Probability that humans become infected from the bite of an infectious vector	0.04	[5]
c	Probability that vectors become infected after biting on an infectious human	0.3	[6]

Vector=adult female anopheline mosquitoes.

* Ranges explored and references:

[0] Hypothetical scenario.

[1] Average mosquito lifespan = 10 to 25 days (Warrel and Gilles, 2002); i.e. $\mu_{min}=0.1$ to 0.04/day.

[2] Interval between blood-meals range from 2 to 4 days (Warrel and Gilles, 2002); i.e. $a=0.5$ to 0.25/day.

[3] Range explored: 14 to 180 days. The value of 21 days used in the simulations illustrates a scenario where promptly and effective malaria diagnosis and treatment are available, but could be longer otherwise. Higher values for the duration of infection were not chosen because they would result in too high prevalence of infection in the human and vector population when no livestock were present.

[4] Range explored: 9 to 37 days; corresponding to the latent period expected at temperatures ranging from 29 to 19°C, respectively (using the standard formula for *P. falciparum*: $Tlat = 111/(T-16)$, where T is the mean temperature in Celsius (Molineaux, 1988).

[5] & [6] Range explored: 0.01 to 1.

An extensive review of estimates for b , c , and $Dinf$ is given by Nedelman (1985). See also Gupta et al. (1994) and Collins and Jeffery (2003) for estimates of $Dinf$.

4.2.3.1. Endemic malaria

The outcome measures investigated for endemic malaria include: the human blood index (HBI, also designated as q in our model), daily overall vector mortality (μ), vector density (N_v), daily human-biting rate (HBR), proportion of mosquitoes that are infectious, i.e. containing sporozoites in their salivary glands (s), daily entomological inoculation rate (EIR), prevalence of infection in humans (I_h), and basic reproduction number (R_0). The HBR is the total number of mosquito bites received by a human, per day, and is calculated as the product of the number of vectors per human and the number of bites on humans per vector: $HBR=(N_v/N_h)aHBI$. The EIR is the number of infective mosquito bites received by a human per unit time, and in practice, is estimated multiplying the HBR by the number of days in the transmission season and by the proportion of mosquitoes with sporozoites in their salivary glands: $EIR=HBRs$.

Simulations were done introducing one infectious human onto a fully susceptible population of humans and mosquitoes, and running the model until the endemic equilibrium was reached. The endemic equilibrium prevalence of infection in humans and vectors were calculated using the algebraic expressions for I_h^* and I_v^* derived in Section 3.2.3, and the results were confirmed by numerical simulations.

When livestock are introduced in a scenario of endemic malaria, the endemic equilibrium of malaria transmission that existed before livestock introduction will suffer a perturbation, and a new endemic equilibrium will be established, some time after livestock introduction. Simulations were performed to investigate the impact of increasing livestock density on several outcome measures of malaria transmission at the new endemic equilibrium established (Section 4.2.3.1.1). Additional simulations also explored the temporal effects on the disease dynamics, since the earlier stages following introduction of livestock in a scenario with endemic malaria where only humans were present (Section 4.2.3.1.2).

4.2.3.1.1. At the new endemic equilibrium

4.2.3.1.1.1. Constant vector population density

Explorations were firstly done of the impact on malaria transmission due to introducing livestock in a setting where vector population density is at its carrying capacity (therefore remaining constant), for three scenarios of host availability. The modelling conditions in this section were $N_{v(0)}=K=1000$, and the fix parameter values listed in Table 4.3. These values were chosen such that, in the endemic equilibrium, when livestock are present with the same density and availability as humans there is still malaria infection ($I_h^*=1.2\%$ and $I_v^*=0.6\%$)¹. The results are displayed in Figure 4.6.

The simulations indicate that, with the present model structure, where the effect of livestock is modelled as beneficially decreasing the vector HBI while detrimentally decreasing vector mortality, and assuming a constant vector population density, the increase in the ratio of livestock:human density results always in a decrease in the equilibrium HBR, s , EIR, R_0 and prevalence of infection in humans, independently of livestock availability (as long as $A_l > 0$). For a given host abundance, the higher the availability of livestock, the stronger is the reduction in all the explored outcome measures of malaria transmission.

Despite the detrimental effect of livestock decreasing vector mortality, and consequently increasing vector survival, the predicted zooprophylactic effect on malaria transmission can be explained by the fact that the reduction in the HBI is proportionally stronger (about twice the magnitude) than the reduction in the mortality rate, for a given hosts' abundance and availability (see plots for HBI and μ in Figure 4.6 below).

¹ In order to obtain persisting levels of infection when livestock are present with the same density and availability as humans, that means that, when livestock are absent the prevalence of infection in humans and vectors is considerably high: $I_h^*=39.4\%$ and $I_v^*=15.5\%$. Although this prevalence of infection in vectors is higher than the values found in most areas of the world (usually $< 1\%$), it lies within observed ranges for the African species in the *An. gambiae* complex and *An. funestus* (normally from 1 to 5% but may approach 10-30% during certain times of the year) (Beier et al., 1990; Beier et al., 1994; Beier, 1998).

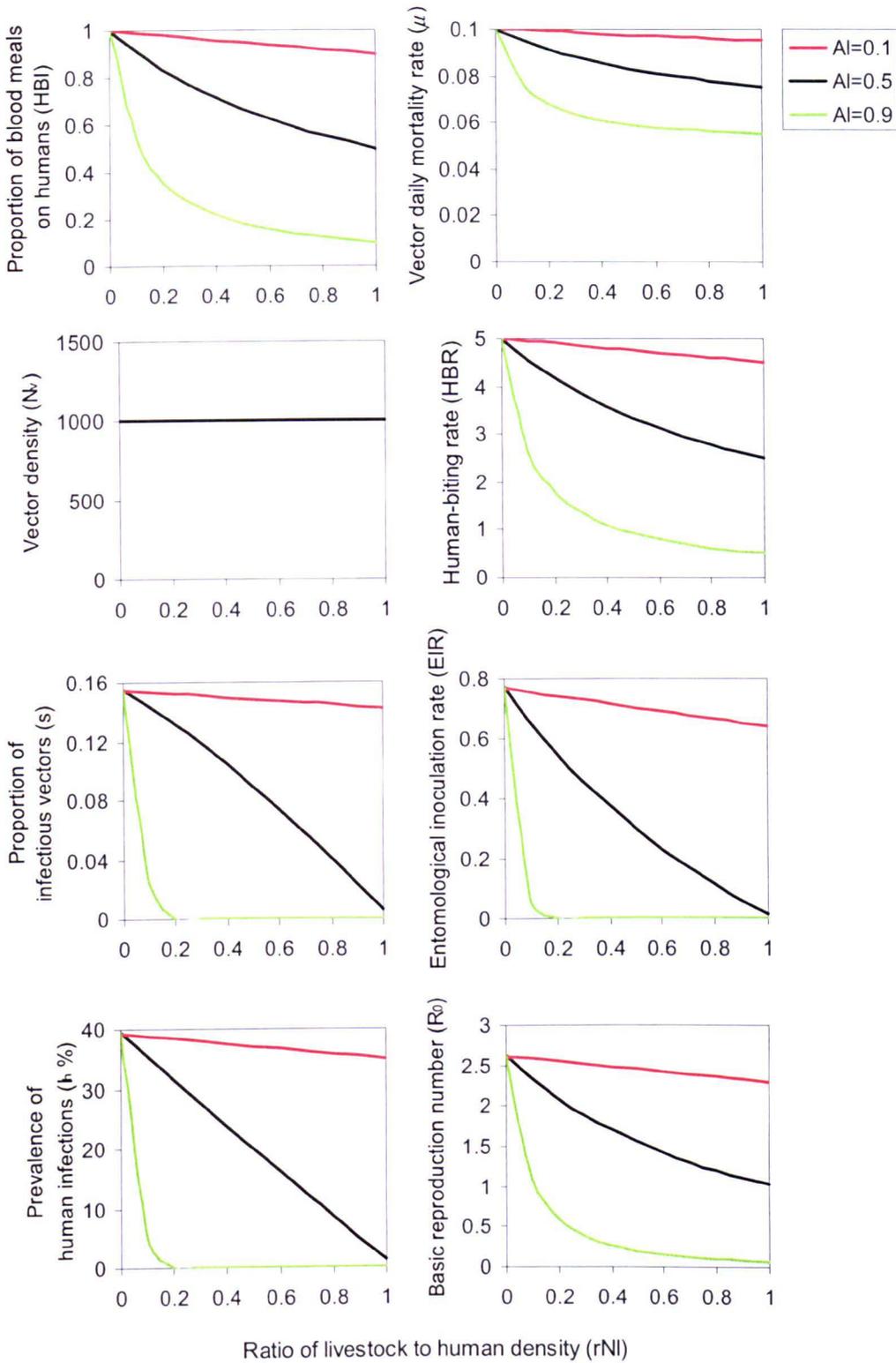


Figure 4.6. Simulation of endemic malaria with constant vector density: Effect of altering the ratio of livestock to human density from zero to 1.

Along the x axis, representing $rN_l=N_l/N_h$, the livestock density N_l is varied relative to a fixed human density $N_h=100$. $N_{v(0)}=K=1000$; vector density remained constant for the three livestock availability (A_l) scenarios. Other parameters as in Table 4.3. The outcome measures HBR and EIR are rates per day.

Under the current assumptions, it is therefore predicted that the introduction of livestock could lead to considerable reductions in malaria transmission. Notably, in scenarios where vectors have a high propensity to feed upon livestock ($A \geq 0.9$), the introduction of as little as one head of livestock per ≤ 5 persons ($rN_l \geq 0.2$) could lead to malaria extinction (see plots for I_h and R_0 in Figure 4.6). Such optimistic predictions are likely to be due to the assumption of constant vector population density. Indeed, as seen in the next section (4.2.3.1.1.2) when N_v is allowed to vary, although the introduction of livestock can still lead to malaria extinction, it is evident that the higher the K is in relation to the $N_{v(0)}$, the higher are the numbers and/or availability of livestock required to produce extinction of infection.

4.2.3.1.1.2. Variable vector population density

To explore scenarios where vector population density may increase following the introduction of livestock, the model was run for various levels of carrying capacity, K , and initial density $N_{v(0)}$ of the vector population. The other parameter values were as in the previous section 4.2.3.1.1.1.

a) Fix $N_{v(0)}$ and variable K

The model was initially run for a scenario with a fixed initial vector population density, $N_{v(0)}=1000$, and different values of the carrying capacity, ranging from 1x to 100x higher the value of $N_{v(0)}$. This corresponds to a village of 100 people where, in the equilibrium, N_v/N_h ranges from 10 to 1000, respectively. Simulations were done to predict the effect on several outcomes of malaria transmission due to increasing the ratio of livestock:human density (rN_l) from 0 to 1, for three scenarios of host availability (Figures 4.7 to 4.9).

Note that the predictions for the HBI and μ against rN_l are not presented here (Figures 4.7 to 4.9) because they are the same as in the previous section that assumed constant vector density (Figure 4.6). This is because the effects of increasing livestock density on the vector HBI and μ are independent of the assumptions about the initial vector population density and carrying capacity. Note also that, although the present parameter values produce an extremely high prevalence of malaria infection when K is high and rN_l is low, they were kept for the purpose of illustrating the sensitivity of the model outcomes to different levels of $N_{v(0)}$ and K .

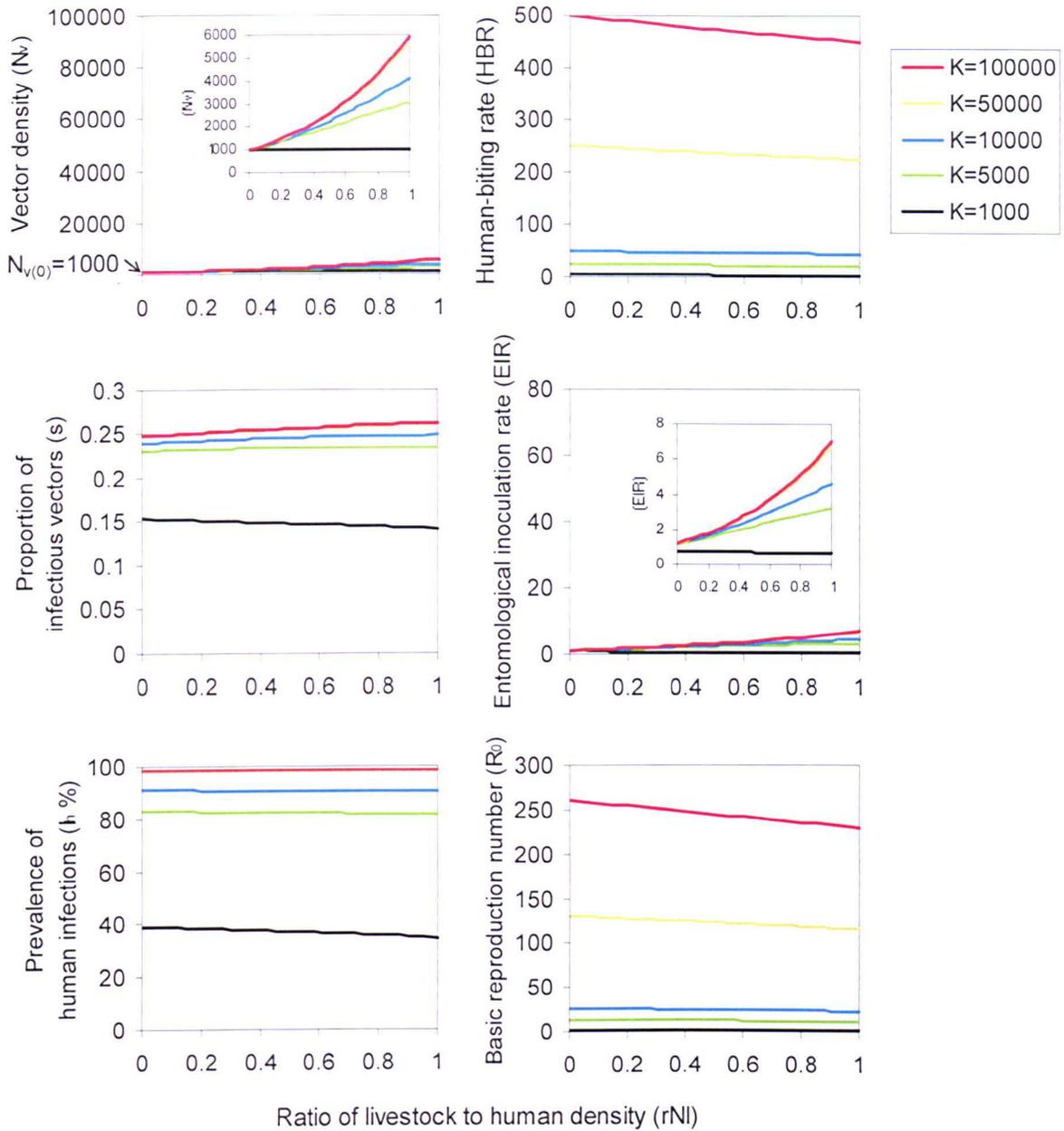


Figure 4.7. Simulation of endemic malaria: effect of altering the ratio of livestock to human density from zero to 1, for different carrying capacities (K) of the vector population, when $A_1=0.1$.

Along the x axis, representing $rN_l=N_l/N_h$, the livestock density N_l is varied relative to a fixed human density $N_h=100$. $N_{v(0)}=1000$; K increasing from 1000 (black line) to 100000 (red line).

The plot for N_v shows the effects one year after the introduction of livestock; the other plots (HBR, s , EIR, I_h , and R_0) illustrate the effects in a scenario of endemic equilibrium, i.e. when $N_v=K$. The outcome measures HBR and EIR are rates per day. The effects of altering rN_l on the human blood index and on the vector mortality rate are not shown because they are independent from K , and therefore are the same as in the previous Fig. 4.6 (see red line for $A_1=0.1$; black line for $A_1=0.5$; and green line for $A_1=0.9$). Other parameters are as in Table 4.3.

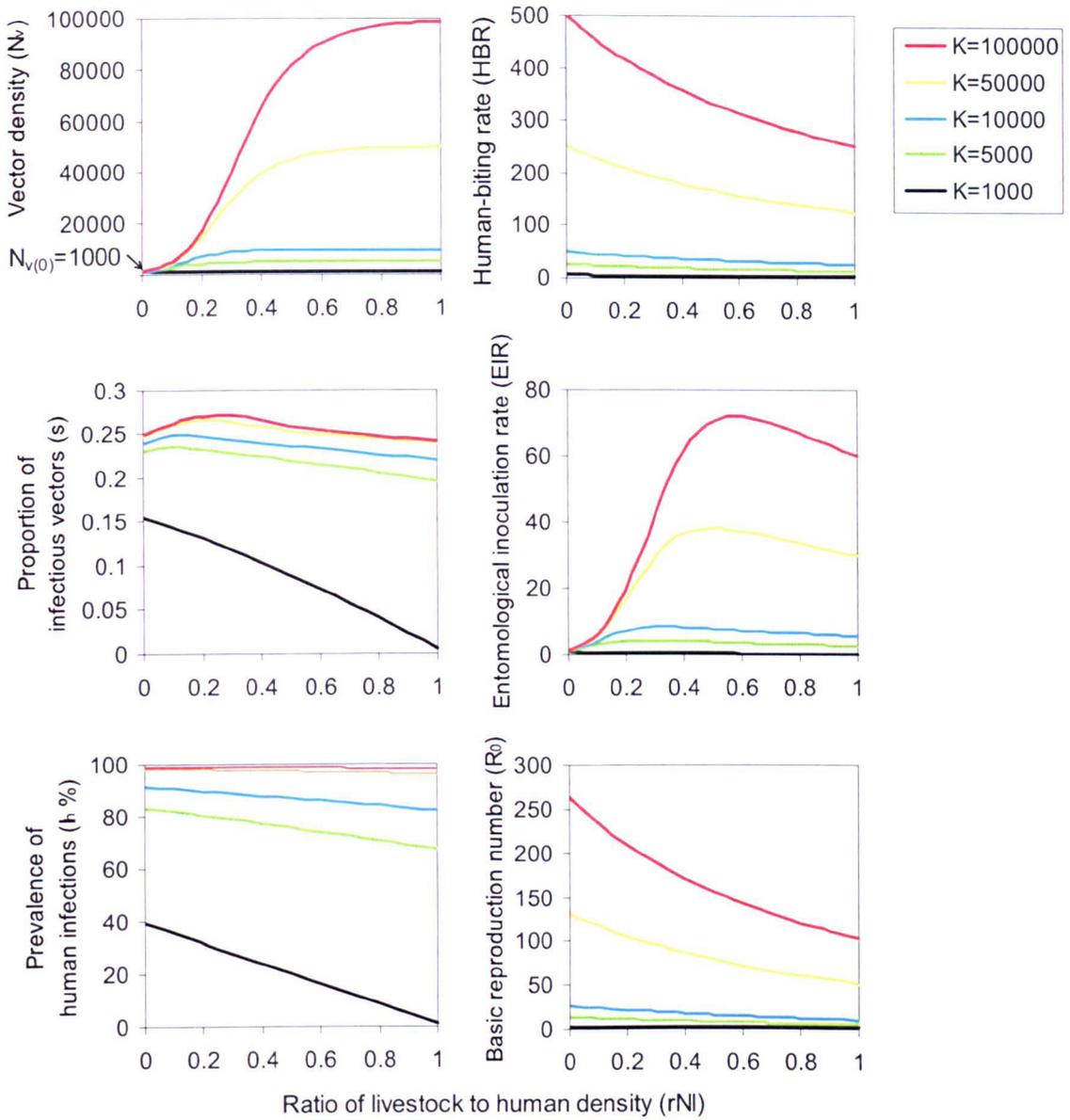


Figure 4.8. Simulation of endemic malaria: effect of altering the relative livestock to human density from zero to 1, for different carrying capacities (K) of the vector population, when $A_1=0.5$.

See notes from Figure 4.7.

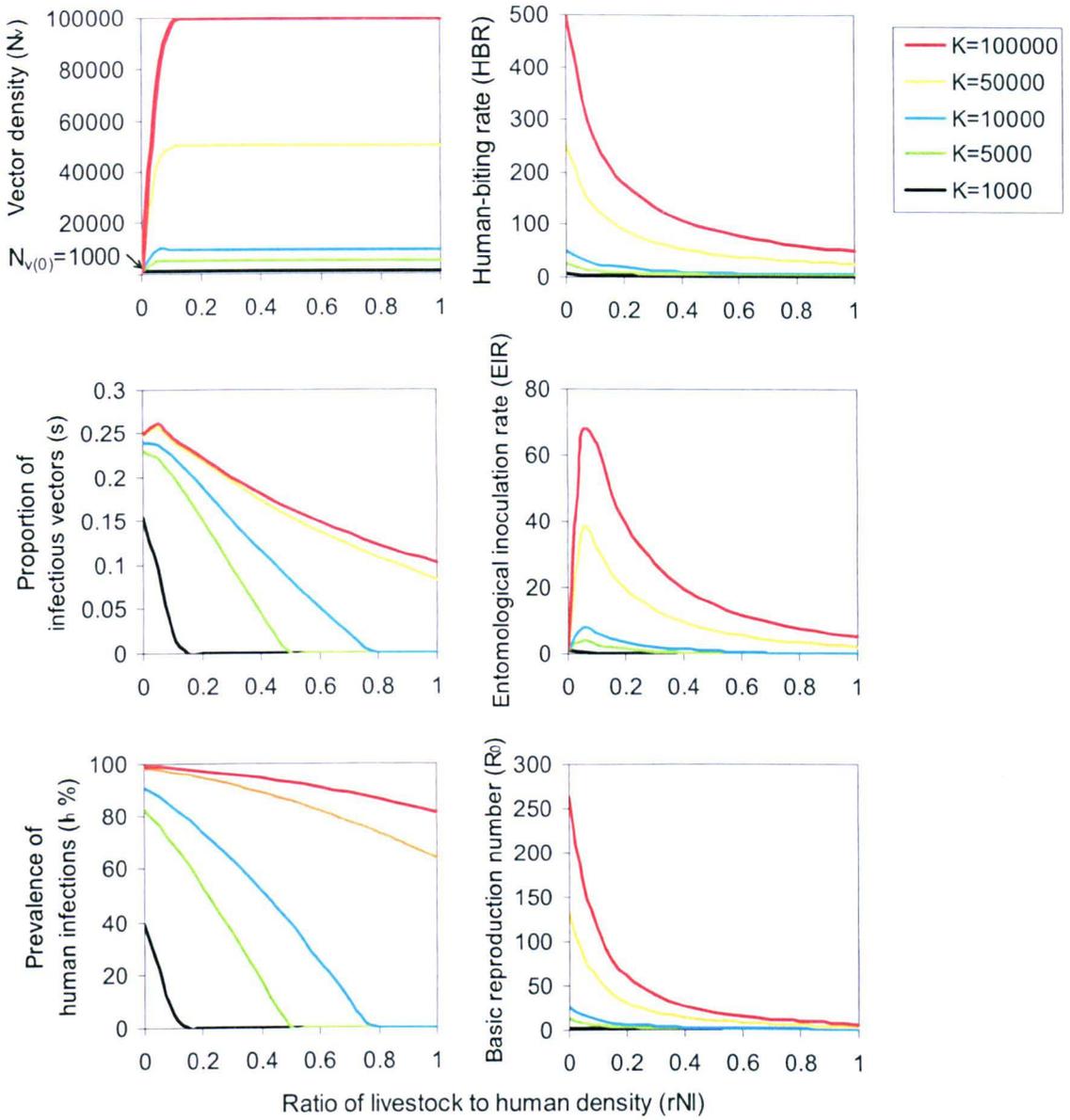


Figure 4.9. Simulation of endemic malaria: effect of altering the relative livestock to human density from zero to 1, for different carrying capacities (K) of the vector population, when $A_1=0.9$.

See notes from Figure 4.7.

The results show that for a given level of vector carrying capacity, K , higher than the initial vector population density, $N_{v(0)}$, increasing the ratio of livestock:human density, rN_l , from zero to 1, results in: decreases in the HBI and μ , increases in N_v until it reaches K , and decreases in the endemic equilibrium values of the HBR^* , I_h^* , and R_0^* , for all the livestock availability scenarios. However, the effects of increasing rN_l on the equilibrium values of s^* and EIR^* seem to vary less intuitively and depending on livestock availability. When A_l is low (e.g. $A_l=0.1$, Figure 4.7), rising rN_l from zero to 1 results in small progressive increases in s^* and in EIR^* . This contrasts with the scenario when A_l is higher, where rising rN_l above certain “threshold” values results in a decrease in s^* (e.g. when $rN_l > 0.2$ for $A_l=0.5$, Figure 4.8), and a decrease also in EIR^* (e.g. when $rN_l > 0.4$ for $A_l=0.5$, Figure 4.8). The higher the livestock availability, the lower are the “threshold” rN_l values above which the equilibrium values of s^* and EIR^* will start decreasing with increasing rN_l . These thresholds correspond to rN_l values above which, smaller increases in livestock numbers cause N_v to reach the K level.

Since the higher the livestock availability, the smaller are the increases in the rN_l required for N_v to reach K , the smaller are also the rN_l threshold values above which the equilibrium values of s^* and EIR^* will start decreasing with increasing rN_l . Accordingly, the higher the livestock availability, the stronger could be the zooprophylactic effect of livestock on malaria transmission. For example, when $A_l=0.9$ (Figure 4.9), increasing rN_l from zero to 1 could decrease malaria prevalence in humans by 20% if $K=100 \times N_{v(0)}$, or by 35% if $K=50 \times N_{v(0)}$, or even lead to extinction of infection if $K \leq 10 \times N_{v(0)}$.

Note that increasing livestock numbers may cause apparently different impacts on endemic malaria depending on the outcome indicator of malaria transmission that is being measured. For instance, both for $A_l=0.1$ and $A_l=0.5$ (Figures 4.7 and 4.8, respectively), with a ratio livestock:human density of 1 the EIR is higher while the prevalence of infection in humans is lower, than when no livestock are present.

For a given A_l , the higher the K value is in relation to the initial vector population density, the higher will be malaria transmission levels (HBR^* , s^* , EIR^* , I_h^* and R_0^*) in the endemic equilibrium. Conversely, the closer K is in relation to the initial vector population density, the stronger will be the zooprophylactic effect of livestock on malaria transmission. The outcome measures that are most sensitive to variations in K , are the HBR^* and the R_0^* . Additionally, for $A_l=0.5$ and $A_l=0.9$, varying K also has a considerable impact on the EIR.

Similar patterns are observed when the density of livestock is further increased up to 20 heads of livestock per humans (simulations not shown). There are, however, a few differences. It is then noticeable that, not only for $A_l=0.5$ and $A_l=0.9$, but also for $A_l=0.1$, there are rN_l threshold values above which s^* and EIR^* will start decreasing with increasing rN_l . As before, these thresholds correspond to rN_l values that anticipate the N_v reaching the K level. In the three availability scenarios explored, the introduction of sufficiently high numbers of livestock could potentially lead to extinction of infection, under certain levels of K . The higher the K is in relation to the $N_{v(0)}$, the higher are the numbers and/or availability of livestock required to bring down infection to extinction.

b) Variable $N_{v(0)}$ and fix K

The counter scenario to the previous, assuming different starting conditions of vector population density, $N_{v(0)}$, all converging to a single equilibrium (fix K), was also examined, although simulations are not presented here.

4.2.3.1.2. Before the new endemic equilibrium is established

4.2.3.1.2.1. Constant vector population density

Figure 4.10 shows the predicted temporal effects on the prevalence of infection in humans following the introduction of livestock in a setting where vector population density is at the carrying capacity level ($N_v=K=1000$). It compares a baseline scenario of endemic malaria (i.e. at equilibrium) where no livestock are present nor introduced and therefore the equilibrium remains unchanged (black line), with what would happen if, in a situation of endemic malaria similar to the baseline, various amounts of livestock were introduced (coloured lines), assuming that the availability of livestock to the disease vectors is the same as that of humans ($A_l=A_h=0.5$), therefore perturbing the initial equilibrium, with the consequent establishment of a new endemic equilibrium. The effects on other outcome variables are in Appendix C2 (Figure C1).

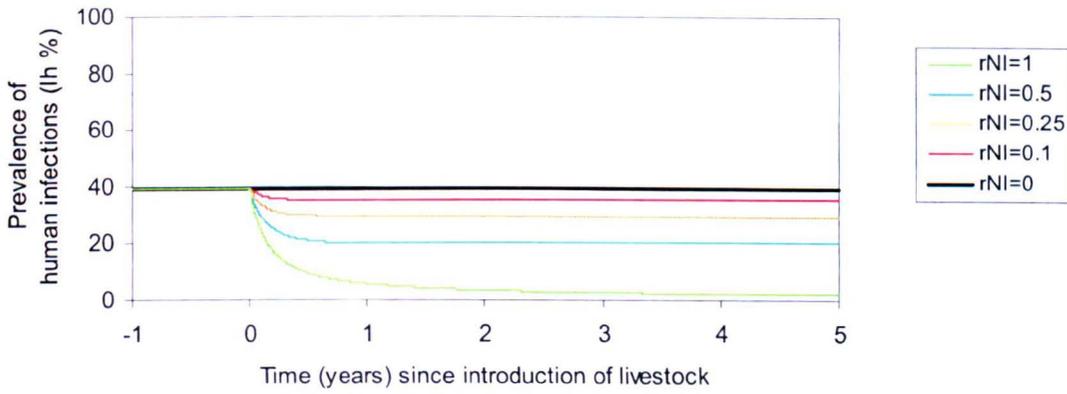


Figure 4.10. Temporal impact of introducing livestock in a setting with endemic malaria, where only humans and no livestock were present, and with constant vector density: impact of varying livestock density, when $AI=0.5$.

$rN_l = N_l/N_h$; the livestock density N_l is varied relative to a fixed human density $N_h=100$; rN_l increasing from no livestock (black line) to 1 head of livestock per person (green line). $N_{v(0)}=K=1000$. Other parameters as in Table 4.3. The effects on other outcome variables are in Appendix C2 (Figure C1).

The simulations indicate that virtually immediately after the introduction of livestock, the values of HBI, μ , HBR and R_0 will be decreased to a new value (the new endemic equilibrium value given in the legend of Figure C1 in Appendix C2), and will thereafter remain constant at that value, as long as the same numbers of livestock and human hosts remain present, with the same availability. The values of s , EIR and I_h will also change, although gradually, until the new endemic equilibrium is reached (Figure 4.10 and Figure C1 in Appendix C2). The values of I_h and EIR will monotonically decrease after livestock are introduced. Conversely, in the first few days after introduction of livestock, s will suffer a slight increase, and only after it will start decreasing.

Overall, the higher the numbers and/or availability of the introduced livestock, the stronger is the predicted zoonophylactic effect on malaria transmission; i.e., the stronger will be the decrease in all the explored outcome variables. This trend is predicted to start occurring virtually immediately after livestock abundance is increased, and to remain throughout the time, until the new endemic is reached and thereafter. The only exception is with the proportion of infectious mosquitoes, s , during the first days after introduction of livestock: during that phase, the higher the relative abundance and/or availability of the introduced livestock, the stronger will be the increase in s (Figure C1 in Appendix C2).

The higher the numbers and/or availability of the livestock introduced, the quicker will the new endemic equilibrium be established.

a) Fix vector carrying capacity and variable livestock density

Comparisons were then made of a baseline scenario of endemic malaria (i.e. at equilibrium) where no livestock are present nor introduced (black line in Figure 4.11), and therefore the equilibrium remains unchanged and N_v remains at its initial level ($N_v = N_{v(0)} = 1000$), with what would happen if, in a situation of endemic malaria similar to the baseline, various amounts of livestock were introduced (coloured lines in Figure 4.11), therefore perturbing the initial equilibrium, with the consequent establishment of a new endemic equilibrium, where $N_v = K = 100$ times higher than $N_{v(0)}$.

The effects on the prevalence of infection in humans are presented in Figure 4.11 and the effects on other outcome variables are in Appendix C3 (Figs. C2 to C4). Increasing livestock density may cause apparently different impacts on malaria transmission depending on how long following introduction of livestock the impact is measured. For the three host availability scenarios explored ($A_i = 0.1, 0.5, \text{ and } 0.9$), although in an initial phase after introduction of livestock the model predicts a decrease in the HBR, s , EIR, R_0 and I_h , all these outcome variables will subsequently start increasing. Interestingly, for $A_i = 0.1$ and $A_i = 0.5$ (Fig. 4.11 A and B, respectively, and Figs. C2 and C3 in Appendix C3) there is a non-linear time-dependent trend between the relative density of livestock introduced and its impact on some of the outcome measures explored. In the initial phase after introduction of livestock, the higher the number of animals introduced, the lower will be the HBR, s , EIR, R_0 and I_h . However, this trend will be inverted at some point later in time. Namely, approximately after I_h (or the other outcome variables above mentioned) increases above its baseline (i.e. pre-introduction of livestock) level, then, the higher the number of livestock introduced, the higher becomes the resulting I_h (or the values of the other outcome variables mentioned). Additionally, later on, once N_v approaches K , a new trend inversion occurs, such that, the higher the number of livestock introduced, the lower will be I_h (and the values of the other outcome variables mentioned).

Conversely, in the scenario with the highest livestock availability ($A_i = 0.9$, Fig. 4.11C and Fig. C4 in Appendix C3), it seems that, in general, the higher the numbers of livestock introduced the lower will be the values of all the outcome variables explored, throughout all the time: i.e., since livestock introduction until the new endemic equilibrium is reached, and thereafter.

Overall, as in the previous Section 4.2.3.1.2.1 (where $K=N_{v(0)}$), also here (where $K > N_{v(0)}$), the higher the numbers and/or availability of the livestock introduced, the quicker will the new endemic equilibrium be established, and the lower will be the values of all the explored outcome variables (HBR, I_v , EIR, R_0 , and I_h) in the new equilibrium.

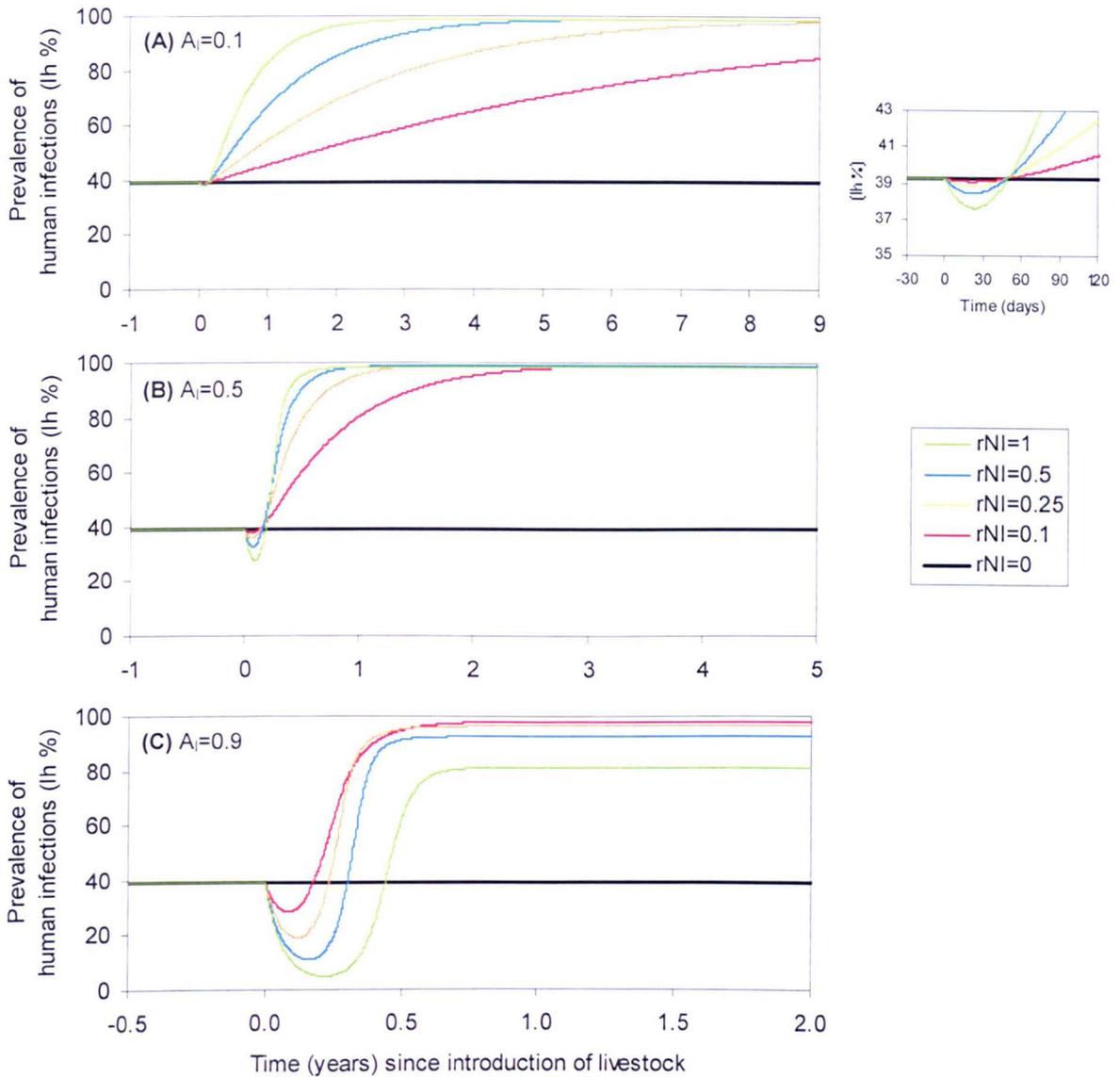


Figure 4.11. Temporal impact of introducing livestock in a setting with endemic malaria, where only humans and no livestock were present, and with variable vector density: impact of varying livestock density, for three livestock availabilities (A_1).

$rN_l = N_l/N_h$; the livestock density N_l is varied relative to a fixed human density $N_h=100$; rN_l increasing from no livestock (black line) to 1 head of livestock per person (green line). Other parameters as in Table 4.3. The effects on other variables are in Appendix C3 (Figures C2 to C4).

b) Fix livestock density and variable vector carrying capacity

Finally, simulations were performed to compare a baseline scenario of endemic malaria (i.e. at equilibrium) where no livestock are present, with what would happen if, in a similar situation of endemic malaria, a given amount of livestock were introduced (1 head of livestock per 4 persons), therefore perturbing the initial equilibrium, with the consequent establishment of a new endemic equilibrium where $N_v=K$, for various values of K , ranging from 1x to 100x higher the value of $N_{v(0)}$, with $N_{v(0)}=1000$. The effects on the prevalence of malaria in humans are displayed in Figure 4.12 and the effects on additional outcome variables are in Appendix C4 (Figure C5).

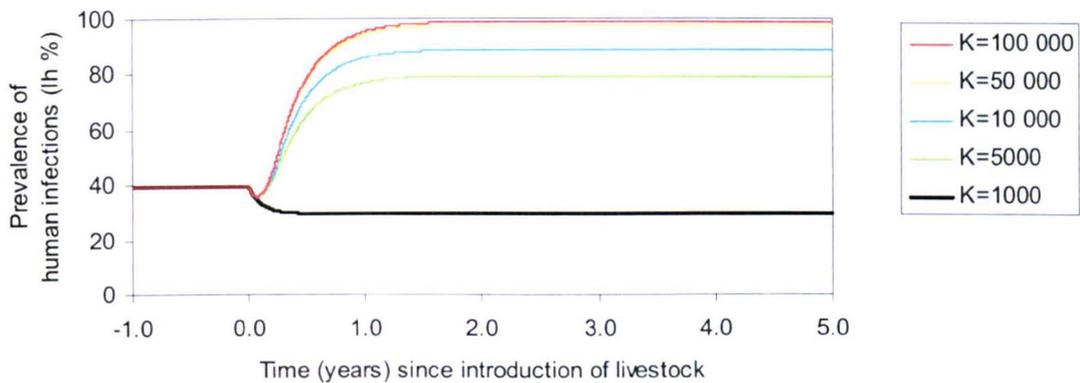


Figure 4.12. Temporal impact of introducing livestock in a setting with endemic malaria, where only humans and no livestock were present: effect of varying the carrying capacity (K) of the vector population, when $A_I=0.5$.

$N_{v(0)}=1000$; $rN_I=0.25$ (ratio of livestock:human density = 1:4); $N_I=100$. K increasing from 1000 (black line) to 100 000 (red line). Other parameters values were kept fix as in previous figure. The effects on other variables are in Appendix C4 (Figure C5).

The simulations show that, if $K=N_{v(0)}$ (black line in Fig. 4.12), N_v will remain unchanged through time, and equal to $N_{v(0)}$, resulting in a decrease in malaria transmission (HBR , s , EIR , I_h , R_0), compared to the baseline scenario without livestock. Conversely, if $K>N_{v(0)}$ (coloured lines in Fig. 4.12), N_v will increase through time until it reaches K , resulting in an increase in transmission.

Overall, for a given relative density and availability of livestock to humans, the higher the carrying capacity is in relation with the vector population density previous to the introduction of livestock, the longer will take for the new endemic equilibrium to be established, and the higher will be the resulting values of all the outcome variables (HBR , s , EIR , R_0 and I_h) in the new equilibrium (see Fig. 4.12 - and Fig. C5 in Appendix C4 - for $A_I=0.5$. Simulations were also performed for $A_I=0.1$ and $A_I=0.9$ although not shown).

4.2.3.2. Epidemic malaria

The model framework also allows exploring scenarios where malaria is introduced in a naïve setting, and understanding how livestock abundance and availability could impact on the development of an epidemic. This has particular importance for scenarios of unstable malaria transmission (Cox et al., 1999). The epidemic was modelled assuming it had originated by the introduction of one infectious malaria case in a population with 99 susceptible persons and 1000 susceptible vectors, with the parameters listed in Table 4.3. The progression rates of a malaria epidemic were compared by plotting the point prevalence of infection in humans through time. An example of the dynamic output is given in Figure 4.13 for a scenario where vector population density is kept constant ($N_v=K$).

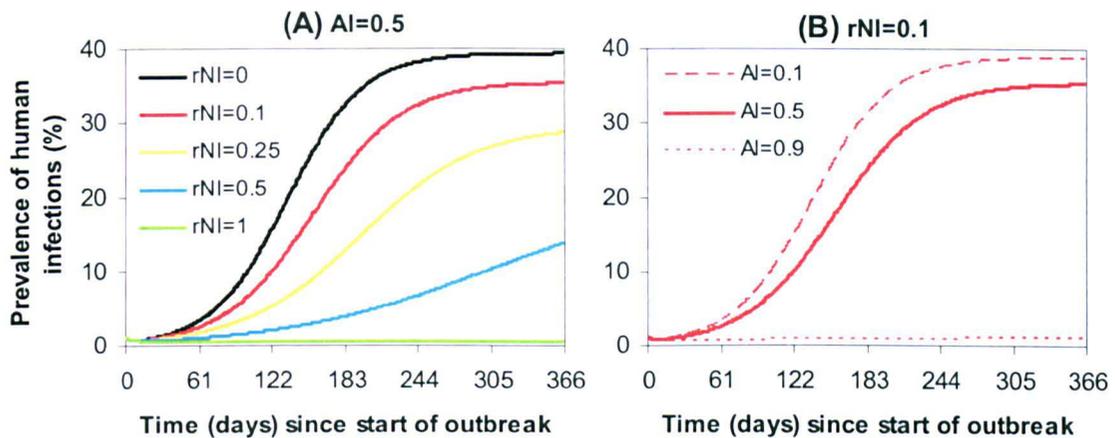


Figure 4.13. Simulation of a malaria epidemic with constant vector density: effect of varying (A) the number and (B) the availability of livestock.

$rN_I=N_I/N_h$ (A) The livestock density N_I is varied relative to a fixed human density $N_h=100$; rN_I increasing from no livestock (black line) to 1 head of livestock per person (green line). (B) Comparing the scenario where the availability of livestock to mosquitoes is the same as that of humans ($A_I=0.5$), with scenarios where the availability of livestock is 9 times smaller ($A_I=0.1$), or 9 times higher ($A_I=0.9$) than that of humans. Fix $N_{v(0)}=K=1000$. Other parameters as in Table 4.3.

When vector population density is kept constant, the higher the numbers and/or availability of livestock present the slower will the epidemic progress (Fig. 4.13). The outcomes can however become non-linear when vector population density is allowed to vary, namely during the initial phase of the outbreak, before an equilibrium has been established. Further explorations of scenarios of epidemic malaria were performed but are not presented here since the main focus of this thesis is on scenarios of endemic malaria transmission.

4.3. Discussion

4.3.1. Main findings

In this chapter a theoretical framework was built that allows exploring the potential effects of untreated livestock on malaria transmission, for different scenarios of livestock and human hosts' abundance and availability to the mosquito vectors, and different assumptions about the vector population carrying capacity. The model illustrates the effects of livestock on 1) decreasing the human blood index, while 2) decreasing vector mortality, and 3) increasing vector population density. By combining these effects, it allows us to explain situations where the presence of livestock can lead to an increase, decrease, or no impact at all on malaria transmission.

One of the features of the model is that it allows investigating situations of endemic malaria, in the equilibrium, and also investigating the temporal effects associated with that. Additionally, the model also allows exploring naïve settings where malaria is introduced for the first time.

The results show that the impact of livestock on malaria transmission will depend not only on the relative abundance and availability of alternative hosts, but also on whether vector population density was at its maximum level prior to introduction of livestock, and on the time elapsed since livestock were introduced. Following the introduction of livestock in a scenario with endemic malaria, a new endemic equilibrium will be established. In situations where sufficient time has past after introduction of livestock for a new endemic equilibrium to be established, the vector population density will then have reached its carrying capacity level, and will remain at that constant level. In these circumstances, the introduction of livestock will cause no observable change in vector density and is expected to always have a zoonophylactic effect, i.e. decrease malaria transmission, irrespectively of the relative availability of livestock to the host seeking vector (as long as the availability of livestock is >0). However, before the vector population has reached its carrying capacity, the introduction of livestock can lead to increases in vector density and malaria transmission. Noteworthy, in situations where vector density is below its carrying capacity, increasing livestock numbers could

actually cause apparently different impacts on malaria transmission depending on the outcome indicator of malaria transmission that is being measured and on how long following introduction of livestock is the impact being measured.

Overall, for a given carrying capacity of the vector population, at a level equal or higher to the initial vector population density, the higher the density and/or availability of livestock introduced, the quicker will the new endemic equilibrium be established, and the stronger will be the zooprophylactic effect of livestock on malaria transmission. Conversely, for a given density and availability of livestock, the higher the carrying capacity is in relation to the initial vector population density, the longer it will take for the new endemic equilibrium be established, and the higher will be malaria transmission levels in the endemic equilibrium.

4.3.2. Relating with previous work

The general results are in agreement with the insights from a previous zooprophylaxis model (Sota and Mogi, 1989), which also stressed that a key determinant of the effect of livestock on malaria transmission was whether the vector population had reached its maximum possible density prior to livestock introduction. It is worthwhile mentioning that this feature is captured by both the present model and the Sota & Mogi (1989) model although the two works differ in the approach used to model the potential detrimental impact of livestock on malaria transmission. The present work has explicitly modelled the effect of animal or human hosts' abundance and availability on vector mortality, with consequent impact on the dynamics and density of adult vectors. Instead, Sota & Mogi (1989) assumed a constant vector mortality rate, and modelled the effect of hosts abundance and availability on the probability of successful blood feeding of the vector, with consequent impact on the number of eggs laid and density of adult vectors in the future generations.

Aside from the work by Sota & Mogi (1989), the two other previous zooprophylaxis models that addressed the effect of untreated livestock on malaria transmission (Saul, 2003; Killeen and Smith, 2007), have also explicitly modelled the effect of animal or human hosts abundance and availability on vector mortality.

The present work is an improvement over previous zoophylaxis models (Sota and Mogi, 1989; Killeen et al., 2001; Kawaguchi et al., 2004; Killeen and Smith, 2007) since it explores, not only the static scenario of the new endemic equilibrium that has been established following introduction of livestock, but also the previous temporal effects on the disease dynamics, for different assumptions on vector population density and carrying capacity. Additionally, a feature of the present model, that was not contemplated by Saul (2003), Killeen & Smith (2007), nor Kawaguchi et al. (2004), is the incorporation of two limiting factors of the effect of livestock on the vector population and consequently on malaria transmission. The first is that increases in the abundance and/or availability of hosts will decrease vector mortality but only up to a minimum threshold value (μ_{min}); and the second is that decreases in vector mortality can lead to an increase in vector population density, but only up to a maximum threshold value that is the system carrying capacity.

The model includes regulation of the adult vector population due to density-dependent competition of the larvae stages for food resources in the breeding sites (similarly to the model by Sota & Mogi (1989), and by Kawaguchi et al. (2004)). This allows exploring the effect of different assumptions with regards to varying the carrying capacity in relation to the initial vector populations density (as done in this Chapter), and varying the strength of the density dependence (as done in the next Chapters 5 and 6).

4.3.3. Density dependent vs. independent feeding success

Similarly with most previous malaria models, it is assumed that there is no density-dependent feeding success of the adult vectors. The probability of acquiring a successful blood-meal is therefore considered to be independent of the number of vectors per host. The implications of this assumption are address below.

Laboratory and field studies have shown that density-dependent feeding success occurs with various blood-sucking arthropods such as *Culicidae* mosquitoes (e.g. Waage and Nondo (1982) for *Aedes aegypti*; Nelson et al. (1976) for *Culex tarsalis*), triatoma bugs (Schofield, 1982; Gurtler et al., 1997), tsetse flies (Vale, 1977), horse flies (Waage and Davies, 1986), sandflies (Coleman and Edman, 1987; Kelly et al., 1996), and ticks (Norval et al., 1988). Such density-dependent mechanisms have also been theoretically

suggested for black flies (Basáñez et al., 2007). For the malaria mosquito vectors, however, it has been argued that, in the absence of bednets usage, density-dependent feeding success is likely to be negligible, since the vectors tend to blood feed at night, when the hosts are likely to be asleep, and therefore less responsive against bites (Charlwood et al., 1995). Such absence of density-dependent feeding success has been shown in a field study in Tanzania (2004), although at least two other studies, one in The Gambia (Lindsay et al., 1992), and the other in Latin America (Clive Davies, unpublished observations), seemed to suggest the contrary.

Yet, even if some density-dependent feeding success of malaria vectors may occur in the real world, its inclusion in our model was not essential for the study purpose of capturing the conundrum of opposite effects that the presence of livestock can have on malaria risk. This was captured by incorporating the beneficial effect of decreasing the proportion of vector blood-meals on humans and the detrimental effect of decreasing the vector mortality with consequent rise in vector density. The epidemiological implications of density-dependent feeding success of blood-sucking insects have been theoretically addressed by Kelly & Thompson (2000) in a general model of the Ideal Free Distribution, and more recently applied to the vector of lymphatic filariasis by Basanes et al. (2007). Kelly & Thomson's model incorporates a decrease in feeding success with increasing density of vectors per available host, which under specific circumstances may result in the vectors changing to another host.

With regards to the model here presented, if density-dependent feeding success was incorporated, that could impact the effect that livestock abundance/availability has on vector population mortality rate and density, and consequently on malaria transmission, on the following way. After increasing livestock abundance/availability there will be a smaller number of vectors per host than when less livestock were available, therefore potentially improving feeding success, decreasing mortality and increasing the density of the vector population. However, as the vector population density continues to increase, at some point that may lead to either the same or even higher number of vectors per host than when less livestock were available. Such could then counteract the previous effects, resulting on either negligible or even opposite effects, with consequent decrease in feeding success, rise in mortality, and decrease in the density of the vector population.

4.3.4. Challenges and future directions

Although the model reflects the general behaviour of malaria transmission dynamics, it is important to highlight the limitations of some assumptions. The model considers complete homogeneity of the vector, parasite, human and livestock populations, as in all previous zoonophylaxis models. However, there is evidence suggesting that the transmission of *Plasmodium* within the human population can be influenced by different levels of heterogeneity, ranging from genetic to behavioural and spatial characteristics (Gillies, 1964; Coluzzi et al., 1979; Hii, 1985; Dye and Hasibeder, 1986; Burkot, 1988; Hasibeder and Dye, 1988; Woolhouse et al., 1997; Kelly and Thompson, 2000; Smith et al., 2004; Smith et al., 2007). Namely, in terms of *vector feeding behaviour*, it is assumed that each female anopheline mosquito will invariably (1) bite only once per blood-meal and per gonotrophic cycle, and (2) take a proportion q of blood-meals on humans, and the reciprocal $(1-q)$ on livestock. However, this is contrary to what has been observed in some scenarios. Accordingly, the model biological realism could be increased by incorporating heterogeneities on the feeding behaviour of the vector population, for example, as outlined below.

There are several reports of situations where anopheline mosquitoes (as well as other insects) were found with multiple blood-meals (e.g. Boreham, 1975; Briegel and Horler, 1993; Koella et al., 1998; Tirados et al., 2006). The frequency of multiple blood-meals has been documented to vary between places, and between vector species. This variation depends on the likelihood of having a partial blood-meal and on the likelihood of having a second blood-meal within the same gonotrophic (i.e. egg-production) cycle (Davies, 1990). For instance, the frequency of bloodfeeding in *An. gambiae* has been shown to increase with age (Straif and Beier, 1996) and also in the absence of sugar (Beier, 1996). When multiple blood feeding is considerably frequent, simple modifications could be made to adapt the model accordingly. Namely, the effect of multiple blood-meals per gonotrophic cycle on malaria transmission will depend on whether the partial blood-meals are being taken on humans and livestock (so-called patent mixed meals; hereafter simply referred to as 'mixed'), all on humans, or all on livestock. If vectors take more than one blood-meal per gonotrophic cycle, independently of the host species where the vector is feeding, the value of the vector biting rate, a , may need to be increased. Additionally, for mixed blood-meals on humans and livestock, the expression for HBI needs to be modified, such that the sum

of the human and livestock blood indexes still adds up to unity. A possible modification is presented below.

Let H_{only} and L_{only} denote the number of mosquitoes with a full blood-meal from humans only, and from livestock only, respectively; while $H\&L$ denotes the number of mosquitoes with a mixed blood-meal, i.e. containing human and livestock blood.

$$HBI' = \frac{H_{only} + \frac{1}{2}(H \& L)}{Total} \quad \text{and} \quad LBI' = \frac{L_{only} + \frac{1}{2}(H \& L)}{Total} ,$$

where $Total = HBI' + LBI' = H_{only} + L_{only} + (H \& L)$,

$$\text{and therefore } HBI' = \frac{H_{only} + \frac{1}{2}(H \& L)}{H_{only} + L_{only} + (H \& L)} .$$

The modified human blood index, HBI' , can be defined as the *proportion of mosquitoes containing human blood, adjusted for the size of the human blood-meal*. Likewise, the modified livestock blood index, LBI' , is the *proportion of mosquitoes containing livestock blood, adjusted for the size of the blood-meal*.

The above expressions assume that in a mixed blood-meal the mosquito will have taken half the amount of blood from humans than in a full blood-meal from humans only, thereby decreasing the HBI. For instance, assuming that 100 blood fed mosquitoes are collected, of which 50 contain only human blood, and 20 contain a mixed blood-meal, these would result in a $HBI'=60\%$ versus $HBI=70\%$. The same can be applied to the LBI' .

This work, as most of previous malaria models, assumes that there is homogeneous and complete mixing of the mosquito population. In other words, it is assumed that all mosquitoes share genetic homogeneity for host preference and are randomly dispersed (as for e.g. Sota and Mogi, 1989). This implies that, if the proportion q of mosquito bites on humans is set to e.g. 0.6, that means that, at a given point in time, 60% of the mosquito population has its blood-meals on humans and 40% on livestock. Also, the

probability of a mosquito feeding on a given type of alternative host is independent of the type of host on which the mosquito had previously fed. The extreme opposite scenario would be a high degree of heterogeneity in such way that the mosquito population would consist of two distinct sub-populations, with 60% of the vector population feeding strictly on humans, and the remaining 40 % feeding upon livestock. The real scenario is likely to lie somewhere in between these two extremes. Indeed, empirical evidence suggests there may be situations where there is non-complete mixing of the mosquito population and therefore the probability of a mosquito feeding for e.g. on a cow could be higher if the mosquito had previously fed on a cow and not on a human (Coluzzi et al., 1979; Hii, 1985; Donnelly and Townson, 2000; Petrarca et al., 2000; McCall et al., 2001; McCall and Kelly, 2002). This can be due to three reasons:

- 1) spatial determinants: if the distance between cows and humans is long (notably if this distance is greater than the mosquitoes flight range, then it is less likely that after feeding on a cow a mosquito will feed on a human);
- 2) learning and development of site and host fidelity: after feeding on a cow, the mosquito could be more likely to have its next feed on a cow (McCall et al., 2001; McCall and Kelly, 2002); and
- 3) genetic determinants, namely genetic heterogeneity and polymorphisms: variable preference for feeding on cows/humans amongst a population (or sub-population) of vectors of the same species (Coluzzi et al., 1979; Hii, 1985; Donnelly and Townson, 2000; Petrarca et al., 2000).

Experiments involving the mark-release-recapture of vector samples could be useful to clarify the degree of heterogeneous mixing in a given vector population, and to assist in the development of models that account for such heterogeneities.

Seasonality was not explicitly incorporated in the presented model, nor in any of the previous zoonophylaxis modelling approaches. This is important because of the seasonal variation of climatic factors, namely temperature, rainfall and relative humidity, which can affect the parasite, vector population and overall malaria transmission dynamics (Molineaux, 1988; Warrel and Gilles, 2002). Additionally, the environmental conditions can also affect the human sleeping habits (e.g. indoors vs. outdoors) and/or livestock management practices (e.g. animals spending the night in the village vs. in distant pastures), which may cause seasonal variation on the proportion of vector blood meals on humans (Reisen and Boreham, 1982; Lindsay et al., 1991). To address this into more detail, seasonality could be included in the model following

standard procedures for vector-borne diseases models - for *Anopheles* and malaria see, e.g. Aron and May (1982) or Wyse et al (2007); other examples include applications to *Culicoides* midges and African Horse sickness (Lord et al., 1996); triatoma bugs and Chagas disease (Cohen and Gurtler, 2001; Castanera et al., 2003); and sandflies and leishmaniasis (Bacaër and Guernaoui, 2006).

And finally, since the aim of the model was to focus on the effects of livestock on the overall malaria transmission dynamic, the details regarding the particularities of *Plasmodium falciparum* or *P. vivax* malaria were not included. Notably, as in all the previous zooprophylaxis models, the possibilities of cross immunity against re-infection by the same strain of *P. falciparum*, or the possibility of relapses of *P. vivax* were not explicitly considered.

The theoretical framework here described will form the basis for the next two Chapters, where the mathematical model will be expanded to examine the potential effects on malaria transmission of treating livestock with an insecticide that has lethal and possible excito-repellence effects on the disease vector. After initial explorations in hypothetical ecological settings (Chapter 5), the model will be applied to specific settings (Chapters 5 and 6) to assess how the ITL intervention that successfully reduced malaria transmission in the Asian scenario of Pakistan, during a community-based trial, could be best translated into an African scenario, exemplified by the Ethiopian setting described in Chapter 2.

Chapter 5

Modelling the effects of insecticide-treated livestock on malaria transmission dynamics

– Exploring different ecological settings –

Summary

Background: The dynamics involved in the effects of ITL on malaria transmission are complex and typically non-linear. My previous models have identified the importance of accounting for the density-dependent regulation of the anopheline mosquito vector population when assessing the impact that livestock can have on malaria transmission dynamics. It is also important to account for the practical considerations of the magnitude and duration of the insecticide lethal and potential excito-repellent effects upon the vector, as well as the treatment coverage, because these parameters can be modified in a control intervention, notably by improving insecticides. This Chapter addresses these complexities and incorporates them into the mathematical model presented in Chapters 3 and 4, in order to better understand the impact of ITL on malaria transmission and how that varies between ecological settings.

Methods: Explorations were done of the impact of treating livestock with an insecticide that has a lethal and possible excito-repellent effect upon the malaria vector feeding behaviour and mortality, for three hypothetical scenarios of relative availability of the livestock and human populations. The impact of the intervention with a non-excito-repellent insecticide upon the malaria transmission potential (R_0) was also explored. The threshold livestock treatment coverage required to potentially interrupt malaria transmission in a given setting was derived and analyzed as well as the threshold vector human blood index (HBI) below which transmission could, in theory, be interrupted with the intervention. The dynamics of the system was then explored, accommodating empirical estimates of the efficacy and duration of the insecticide from bioassays in Pakistan and Ethiopia. Finally, the impact of the intervention on the vector population density was explored using a density-dependent mechanism which captures the three main behaviour patterns of the vector population dynamics: monotonic dampening, oscillatory dampening, and permanent oscillations.

Findings: The results indicate that excito-repellency (defined as the probability that, when attempting to bite an insecticide-treated animal, a mosquito will be diverted to search another animal or human host) can be a key determinant of the outcome of an ITL intervention on the vector feeding behaviour and mortality, particularly in scenarios with very high availability of livestock to the vector. The higher the malaria transmission potential (R_0), e.g. due to increase in vector density, the lower the HBI range within which malaria transmission could potentially be controlled with ITL using a non-excito-repellent insecticide, and vice-versa. In scenarios where malaria vectors feeds mainly on livestock (i.e. low HBI, as in Southeast Asia), high levels of effective coverage of livestock with a non-repellent insecticide could produce a reduction of up to 60% on the pre-intervention R_0 . Interestingly, even in settings where the vector takes a higher proportion of blood-meals upon humans (as in Sub-Saharan Africa), such intervention has the potential to achieve a considerable decrease in R_0 and thereby decrease malaria transmission.

Interpretation: This work has demonstrated that it is important that a model contains possible excito-repellent effects of the insecticide, the decay rate of the insecticide residual activity, and density-dependent mechanisms regulating the vector population, in order to reliably explore the effects of an ITL intervention. To assess the robustness of the model and its application there is a need to evaluate its use in alternative transmission settings, based on empirical parameter estimates. Accordingly, the comprehensive framework here developed will next be applied to two specific settings in Pakistan and in Ethiopia, to investigate and compare the potential impact of various regimes of ITL within and between these different ecological scenarios.

5.1. Introduction

The previous chapter modelled the inherent paradoxical potential effects of livestock, minimizing or increasing malaria transmission, since animals as a whole can divert the malaria mosquito vector feeding away from humans, but can also increase vector survival and density and thereby interplay with malaria transmission.

Similarly, here this paradox is expanded into the way how insecticides work by exploring, firstly, the insecticide lethal effect upon mosquitoes, and secondly, the possible excito-repellent effects upon mosquitoes. Excito-repellency may increase vector feeding on untreated livestock and unprotected humans, although it can also indirectly decrease vector survival due to increased time spent searching for an alternative available blood-meal host.

It is known that certain insecticides, particularly DDT and pyrethroids, may exert not only (1) a toxic effect, killing mosquitoes that contact with an insecticide-impregnated surface (Lines et al., 1987; Magesa et al., 1991; Lindsay et al., 1992; Chavasse and Yap, 1997; Chandre et al., 2000), but also (2) contact-mediated irritancy, inhibiting mosquitoes from remaining on the treated surface, thereby stimulating them to exit prematurely, and (3) non-contact repellency, which acts from a distance of the treated surface inhibiting mosquitoes from entering treated areas (Lines et al., 1987; Chavasse and Yap, 1997; Rutledge et al., 1999; Chandre et al., 2000; Roberts et al., 2000; Chareonviriyaphap et al., 2004). The latter two effects are often referred together as excito-repellency. Also, a shift in host feeding from humans to domestic animals has occasionally been associated with the use of pyrethroid-treated nets (Charlwood and Graves, 1987; Magesa et al., 1991; Githeko et al., 1996; Bøgh et al., 1998; Takken, 2002). Additionally, a recent case-control study in the Pokot territory of Kenya and Uganda (Kolaczinski et al., 2008) found that people with insecticide-treated livestock had a higher risk of Visceral Leishmaniasis, suggesting that the insecticide might have repelled sandflies attempting to feed on animals and diverted them to feed on humans. However, to the best of my knowledge, such behavioural shift has not been reported for insecticide-treated livestock and anopheline mosquitoes. Nevertheless, given the evidence of excito-repellency of anopheline mosquitoes with ITNs, the possibility of it occurring with ITL should not be disregarded and it is worth being investigated.

A recent model by Killeen et al. (2007) has explored the impact of both lethal and excito-repellency effects of ITNs upon the feeding behaviour and mortality of malaria vectors, although no such work has yet been done for ITL. Additionally, despite previous works having modelled the impact of applying insecticide on animals (Saul, 2003) or on animal sheds (Kawaguchi et al., 2004) upon malaria transmission, none has accounted for the decay of insecticide residual activity or for a fluctuation in vector population density following exposure to the insecticide.

Here, these complexities are addressed and incorporated into the basic model presented in Chapters 3 and 4. Initial explorations are performed on the effects of possible excito-repellent properties of the insecticide upon the malaria vector feeding behaviour and mortality, for different host availability and livestock treatment coverage scenarios. The potential impact of ITL interventions on the malaria basic reproduction number is then investigated. Additional explorations are done on the threshold livestock treatment coverage required to potentially interrupt malaria transmission in a given setting, and how that depends on the vector blood feeding behaviour and density. Finally, the model is extended to account for the decay of the insecticide residual effect, and for its impact on the density-dependent regulation and resulting dynamical behaviour patterns of the mosquito population.

5.2. Methods and Results

5.2.1. Nomenclature

Throughout this Chapter and the next, the coverage of insecticide-treated livestock, T_l/N_l , will be referred in two contexts: *effective coverage* (ϵ or $T_{l(t)}/N_l$), which is the proportion of the livestock population that has effective insecticide at a given point in time; and *application coverage* (ϵ_0 or $T_{l(t=0)}/N_l$), which is the proportion of livestock treated with insecticide at each intervention round. For simplicity, the term excito-repellency will hereafter be referred to as repellence, encompassing diversion of vectors due to both contact-mediated irritancy and non-contact repellency.

Figure 5.1 presents a schematic framework of the possible impacts of livestock on the vector blood-feeding behaviour, mortality and density. Fig. 5.1A reminds us of the effects of untreated livestock (as presented in Chapter 4), and Figs. 5.1.B&C summarize the changes that occur when livestock are treated with insecticide without or with repellence properties upon the malaria vectors.

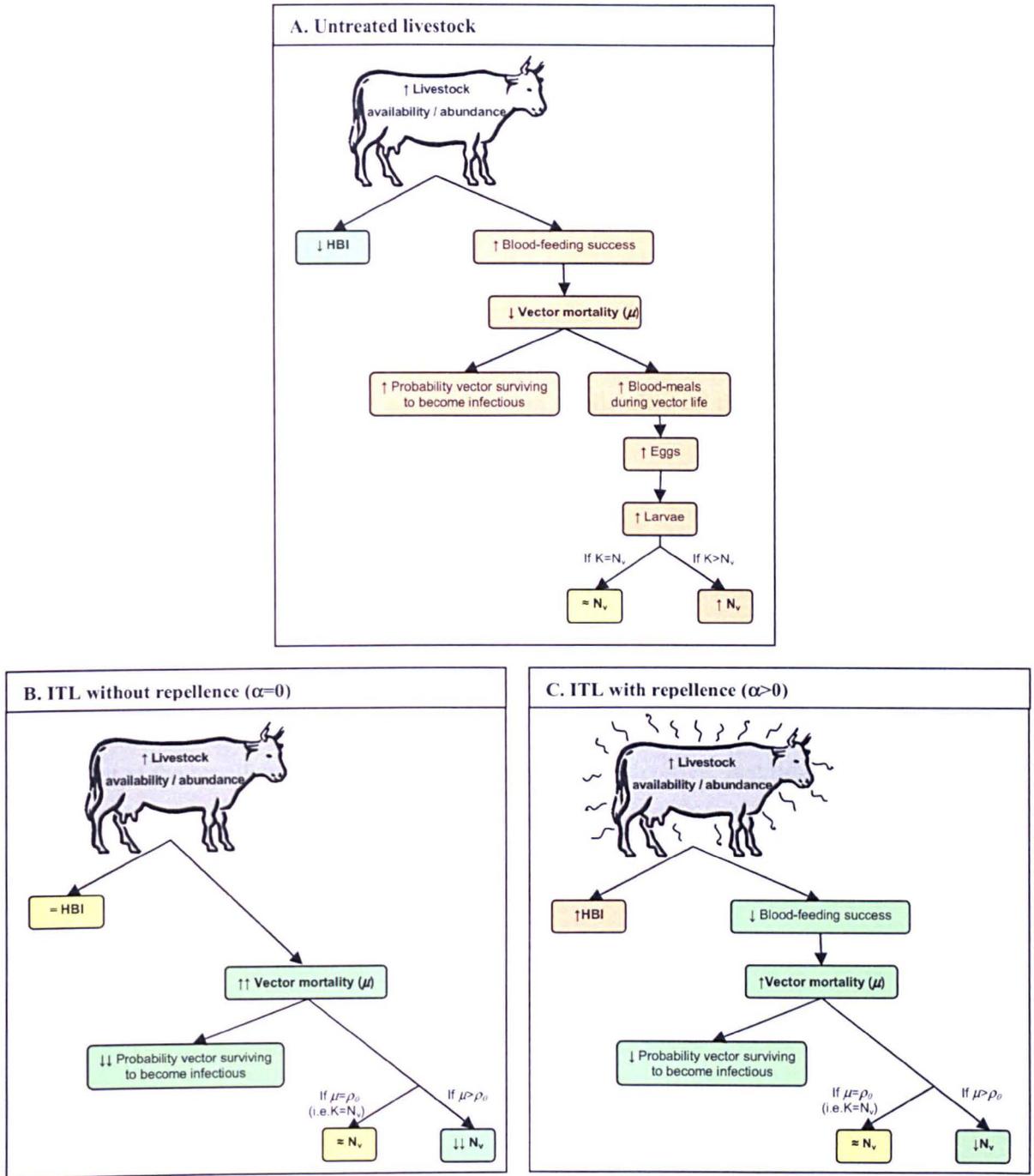


Figure 5.1. Schematic framework of the possible effects of untreated (A) and insecticide treated (B&C) livestock on vector blood feeding behaviour, mortality and density.

HBI = human blood index; N_v = density of vector population (adult female anopheline mosquitoes); K = carrying capacity of vector population; μ = overall vector mortality rate; ρ_0 = vector recruitment rate in the absence of density-dependence constraints; α = repellence probability of the insecticide. Figure A is the same as Figure 4.4. The difference between Figures B and C in the magnitude of the impact of ITL on the vector HBI, mortality (μ) and density (N_v) will depend on: 1) the relative abundance and availability of human and livestock hosts, 2) the coverage of ITL, and 3) the repellence probability of the insecticide. See further explanation in the text.

5.2.2. Effects of ITL on the host's blood index

The treatment of livestock with insecticide will only affect the host's blood index if the insecticide has some repellent effect upon the vectors. The *repellence probability* is denoted by the parameter α , and defined as the probability that, when attempting to bite an insecticide-treated animal, a mosquito will be diverted to search another animal or human host. The model assumes that the proportion of mosquitoes repelled from an insecticide-treated animal that will be diverted to other animals (untreated or treated) or humans, depends on the relative abundance and availability of these alternative hosts (See Figure 5.1 and 5.2).

Accordingly, to account for a possible repellent effect of the insecticide, the expressions for the HBI and LBI presented in Chapter 4 were expanded as follows. Out of all the available human and livestock hosts ($N_h A_h + N_l A_l$), there will be a baseline $N_h A_h$ bites on humans, irrespectively of livestock having been treated with insecticide (with repellent properties or not). Additionally, there are $\epsilon \alpha N_l A_l$ mosquito bites on livestock that, due to the repellent effect of the insecticide, will be diverted, resulting in additional bites on humans ($N_h A_h$) and/on untreated livestock ($(1-\epsilon) N_l A_l$), or on successful (i.e. non-repelled) bites on other treated livestock ($\epsilon(1-\alpha) N_l A_l$).

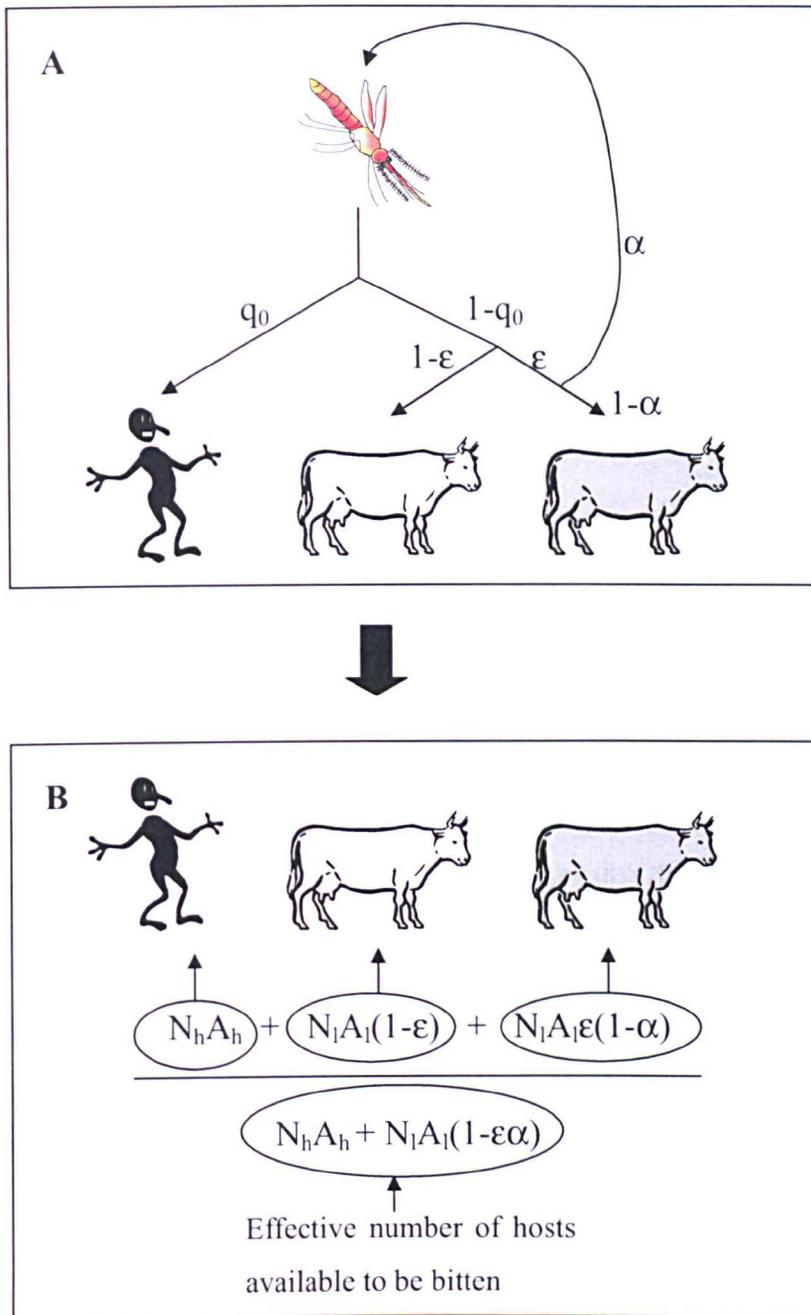


Figure 5.2. Modelling the effects of insecticide-treated livestock (ITL) and repellence on the host's blood index.

(A) A host-seeking mosquito will take a baseline proportion [$q_0 = N_h A_h / (N_h A_h + N_l A_l)$] of bites on humans. And the remainder, [$1 - q_0 = N_l A_l / (N_h A_h + N_l A_l)$], is the proportion of bites that would be potentially taken upon livestock as a whole: out of these, a proportion $(1 - \epsilon)$ will be taken upon untreated livestock, and a proportion $\epsilon(1 - \alpha)$ will be successfully taken on insecticide-treated livestock; the remaining $\epsilon\alpha$ is the proportion of bite attempts on insecticide-treated livestock that will be repelled and diverted to other hosts, depending on the hosts' relative abundance and availability.

(B) Due to repellence, the effective number of hosts available for a successful blood-meal is therefore reduced to [$N_h A_h + N_l A_l (1 - \epsilon\alpha)$], out of which there will be $N_h A_h$ bites on humans, $N_l A_l (1 - \epsilon)$ bites on untreated livestock, and $N_l A_l \epsilon (1 - \alpha)$ bites on treated livestock.

5.2.2.1. Expression for HBI accounting for repellency

The expression for HBI (q) accounting for a repellent effect of the insecticide therefore becomes:

$$HBI = \frac{N_h A_h + \epsilon \alpha N_l A_l \frac{N_h A_h}{N_h A_h + (1 - \epsilon) N_l A_l + \epsilon (1 - \alpha) N_l A_l}}{N_h A_h + N_l A_l}, \quad \text{Eq. 5.1}$$

which can algebraically be reduced to:

$$HBI = \frac{N_h A_h}{N_h A_h + N_l A_l (1 - \epsilon \alpha)} \quad \text{Eq. 5.2}$$

Note that Equation 5.2 is similar to the previous expression for HBI that did not account for repellence (Equation 4.1 in Section 4.2.1 of Chapter 4, denoted as q_0 in Figure 5.2A) which comprised only the terms above highlighted above in bold. The difference here is that, when livestock are treated with an insecticide that has repellent properties, the livestock availability is reduced by the proportion $\epsilon \alpha$ which corresponds to the proportion of bite attempts on a given animal that will be diverted to another animal or human host.

As before, by algebraic manipulation, it is possible to obtain an expression that requires only the relative abundance and availability of the livestock *versus* human populations:

$$HBI = \frac{1}{1 + \frac{N_l A_l (1 - \epsilon \alpha)}{N_h A_h}} \quad \text{Eq. 5.3}$$

5.2.2.2. Expression for LBI accounting for repellency

Likewise, the expression for the proportion of blood-meals of livestock origin, Livestock Blood Index (LBI), accounting for the repellent effect of the insecticide, is given by:

$$LBI = \frac{N_l A_l + \epsilon \alpha N_l A_l \frac{(1 - \epsilon \alpha) N_l A_l}{N_h A_h + (1 - \epsilon) N_l A_l + \epsilon (1 - \alpha) N_l A_l}}{N_h A_h + N_l A_l} \quad \text{Eq. 5.4}$$

or

$$LBI = \frac{N_I A_I (1 - \epsilon \alpha)}{N_h A_h + N_I A_I (1 - \epsilon \alpha)} \quad \text{Eq. 5.5}$$

which is equivalent to:

$$LBI = \frac{I}{I + \frac{N_h}{N_I} \frac{A_h}{A_I (1 - \epsilon \alpha)}} \quad \text{Eq. 5.6}$$

Among all the successful blood-meals of livestock origin (LBI), the proportion $\frac{(1 - \epsilon)}{(1 - \epsilon \alpha)}$ is from bites on untreated livestock (LBI_U), while the proportion $\frac{\epsilon(1 - \alpha)}{(1 - \epsilon \alpha)}$ is from successful (i.e. non-repelled) bites on treated livestock (LBI_T) (see numerical exploration in Figures 5.3 and 5.4, top and middle rows). Similarly, among all the mosquito bites attempted on livestock that are repelled ($\epsilon \alpha$), the proportion HBI are bites diverted to humans, while the proportion LBI_U are bites diverted to untreated livestock, and LBI_T are bites diverted to treated livestock that were successful (i.e. non-repelled) (Figures 5.3 and 5.4, lower rows).

The effects of extreme scenarios of effective coverage of insecticide-treated livestock and repellence probability on the HBI are described analytically in Appendix D1.

5.2.2.3. Model predictions

Simulations were done to explore the effects that increasing the effective proportion of insecticide-treated livestock could have upon the vector feeding behaviour, for two levels of repellence (total, $\alpha=1$, or partial, $\alpha=0.5$), and three scenarios of relative availability of the livestock and humans populations to the malaria vector, where livestock are much less ($A_I=0.1$), equally ($A_I=0.5$), or much more ($A_I=0.9$) available to vectors than the human host.

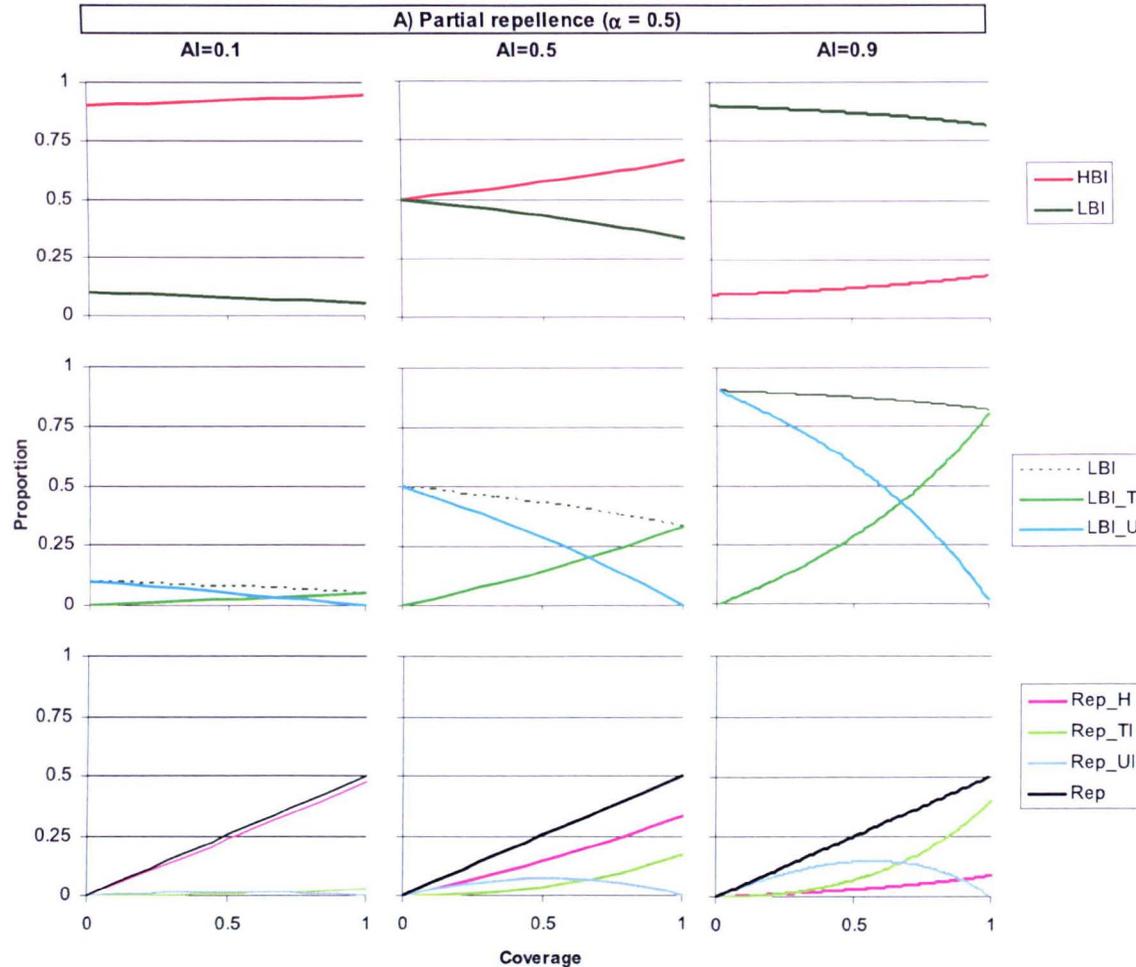


Figure 5.3. Effects of increasing the effective coverage of ITL with partial repellence on different aspects of the vector feeding behaviour. Top row: HBI (red line) and LBI (green line). Middle row: Relative contribution of the proportion of mosquito bites on untreated livestock (LBI_U, blue line) and of the proportion of successful (i.e. non-repelled) bites on treated livestock (LBI_T, green), to the overall proportion of successful bites on any livestock (LBI, slashed line). Lower row: Proportion of mosquito bites attempted on livestock that will be diverted to: any host (Rep, black), to humans (Rep_H, pink); to untreated livestock (Rep_UI, blue); and/or to treated “non-repellent” livestock (Rep_TI, green). Note: $LBI_U = [(1-\epsilon)/(1-\epsilon\alpha)] * LBI$; $LBI_T = [\epsilon(1-\alpha)/(1-\epsilon\alpha)] * LBI$; $Rep = \epsilon\alpha$; $Rep_H = Rep * HBI$; $Rep_UI = Rep * LBI_U$; $Rep_TI = Rep * LBI_T$.

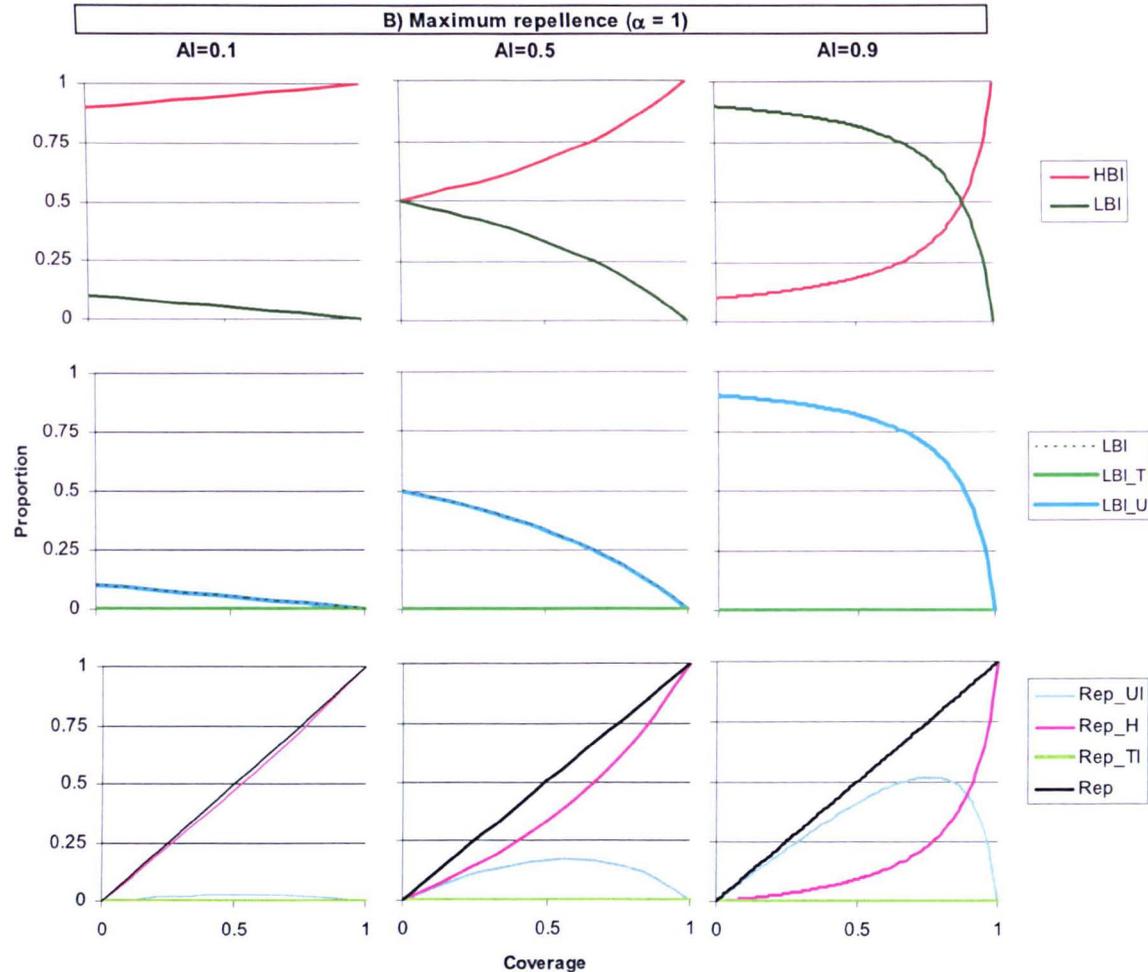


Figure 5.4. Effects of increasing the effective coverage of ITL with maximum repellence on different aspects of the vector feeding behaviour. Top row: HBI (red line) and LBI (green line). Middle row: Relative contribution of the proportion of mosquito bites on untreated livestock (LBI_U, blue line) and of the proportion of successful (i.e. non-repelled) bites on treated livestock (LBI_T, green), to the overall proportion of successful bites on any livestock (LBI, slashed line). Lower row: Proportion of mosquito bites attempted on livestock that will be diverted to: any host (Rep, black), to humans (Rep_H, pink); to untreated livestock (Rep_U, blue); and/or to treated “non-repellent” livestock (Rep_TI, green). Note: $LBI_U = [(1-\epsilon)/(1-\epsilon\alpha)] * LBI$; $LBI_T = [\epsilon(1-\alpha)/(1-\epsilon\alpha)] * LBI$; $Rep = \epsilon\alpha$; $Rep_H = Rep * HBI$; $Rep_U = Rep * LBI_U$; $Rep_TI = Rep * LBI_T$.

Independently of the relative availability of livestock to humans, when the insecticide has some repellent effect, with an increase in coverage there is an increase in the proportion of vector bites on humans (HBI) and a symmetrical decrease in the bites on livestock (LBI). When the coverage is total, if repellence is maximum, the HBI reaches unity (Figure 5.4 top row), while if repellence is only partial, the maximum HBI will always be <1 , because the remainder of vectors bites are successful (i.e. non-repelled) on treated livestock (Figure 5.3 top and middle rows). When repellence is maximum, all the bite attempts on treated livestock will be diverted, and therefore there are no successful bites on treated livestock ($LBI_T = 0$ as shown in Figure 5.4 middle row).

When comparing different availability scenarios, the outputs become increasingly non-linear with increases in the availability of livestock. Notably, the greater the proportional availability of livestock (A_l):

- the higher the effect that increasing treatment coverage will have on the relative contribution of the proportion of bites on untreated vs. successful (i.e. non-repelled) bites on treated livestock to the overall proportion of successful bites on any livestock (Figures 5.3 and 5.4, middle row); and
- the higher the proportion of bite attempts on treated livestock that will be diverted to (untreated and/or treated “non-repellent”) livestock, and the lower the proportion of bites diverted to humans (Figures 5.3 and 5.4, lower row). For example, when repellence is partial (Figure 5.3) and coverage is total, for $A_l=0.5$, the proportion of bites diverted to treated “non-repellent” livestock (16.67%) is half of those diverted to humans (33.33%), while for $A_l=0.1$ and for $A_l=0.9$ the proportion of bites diverted to treated “non-repellent” livestock is respectively much less and much more than half of those diverted to humans.

In the three scenarios of host availability explored, the ratio of livestock:human abundance was kept constant ($N_l:N_h=1$). Note that exactly the same proportional outcomes are obtained if one varies the ratio of livestock:human abundance instead of availability, since, as it can be seen from the simplified expression for HBI, Equation 5.3 (or the expression for LBI, Equation 5.6), the ratio of livestock:human availabilities (or human:livestock availabilities for LBI) appears always multiplied by the ratio of livestock:human abundances (or human:livestock abundances for LBI).

5.2.3. Effects of ITL on the vector mortality

5.2.3.1. Expression for vector mortality accounting for a lethal and possible repellent effect of the insecticide

The vector mortality rate consists of the sum of three components:

(1) the minimum mortality due to causes other than searching for a blood-meal host,

μ_{min} ;

(2) the mortality due to searching for a blood-meal host,

$$\mu_{search} = \left(\frac{1}{(N_h A_h + (1 - \varepsilon) N_l A_l + \varepsilon(1 - \alpha) N_l A_l) j} \right) a = \left(\frac{1}{(N_h A_h + (1 - \varepsilon \alpha) N_l A_l) j} \right) a$$

Eq. 5.7

and

(3) the mortality due to the lethal effect of insecticide applied on livestock

$$\mu_{txonly} = \left(\frac{\varepsilon(1 - \alpha) N_l A_l}{N_h A_h + N_l A_l} k \right) a. \quad \text{Eq. 5.8}$$

The vector mortality rate is therefore given by

$$\mu = \mu_{min} + \left(\frac{1}{(N_h A_h + (1 - \varepsilon \alpha) N_l A_l) j} + \frac{\varepsilon(1 - \alpha) N_l A_l}{N_h A_h + N_l A_l} k \right) a. \quad \text{Eq. 5.9}$$

Considerations on the vector mortality due to searching for a blood-meal host (μ_{search})

Allowing for a scenario where livestock are treated with an insecticide that can have some repellent action, the possible blood-meal hosts available to the vector include: the humans [$N_h A_h$], the untreated livestock [$(1 - \varepsilon) N_l A_l$], and the treated “non-repellent” livestock [$\varepsilon(1 - \alpha) N_l A_l$]. Overall, as seen for the host’s blood index, it is as if the availability of livestock became reduced by the proportion $\varepsilon \alpha$, which corresponds to the proportion of bites attempted on a given animal that will be diverted to another animal or human host. This will cause an increase in the time it takes for the vector to

find a blood-meal host, with consequent increase in the search related vector mortality. Conversely, increase in number and/or availability of humans, untreated livestock or treated “non-repellent” livestock would decrease the search time and the associated vector mortality. As in Chapter 4, the parameter j is kept as a scaling factor, since the model uses proportional instead of absolute values for the availability of hosts to the mosquito vectors.

Considerations on the vector mortality due to the lethal effect of insecticide applied on livestock (μ_{txonly})

When vectors try to feed on insecticide-treated livestock, they will suffer an increase in mortality that is proportional to: the proportion of successful (i.e. non-repelled) bites on treated livestock, $\frac{\epsilon(1-\alpha)N_l A_l}{N_h A_h + N_l A_l}$, and the lethal (killing) effect of the insecticide upon vectors, k .

The daily biting rate, α , needs to be included in the expressions for μ_{search} and μ_{txonly} , since the additional mortalities, either due to searching for a blood-meal host or due to insecticide-treated livestock, are only suffered by the vector when it attempts to blood feed.

Assumptions on modelling repellence

It is worth noting that repellence is modelled assuming a worst case scenario, where vectors are diverted from ITL before sufficient exposure to a knock-down or lethal (i.e. a life expectancy changing) dose of insecticide. The model is therefore not considering situations where mosquitoes may be diverted following exposure to a dose of insecticide that has either an immediate lethal effect or a knock-down effect that induces premature death of the knocked-down mosquitoes by their predators. Repellence does however increase vector mortality associated with the search for a blood-meal host, due to decreasing the availability of treated livestock and therefore increasing the time needed to find a blood-meal host.

The effects of extreme scenarios of effective coverage of ITL and repellence probability on the expression for the vector mortality rate are explored analytically in Appendix D2.

5.2.3.2. Model predictions

Numerical simulations were performed to explore the effect upon vector mortality due to treating livestock with an insecticide that has null, partial or maximum repellence probability, for various treatment coverage levels and three scenarios of relative availabilities of the livestock and human hosts, where both host types are equally abundant (Figure 5.5).

In a scenario with a highly zoophilic vector (see $A_l=0.9$ in Figure 5.5), when most livestock are effectively treated with an insecticide that causes full repellence of vectors ($\alpha=1$), the vector mortality increases considerably, because all the vectors that feed on livestock are diverted to humans whose availability is very small, thereby substantially increasing the search related vector mortality.

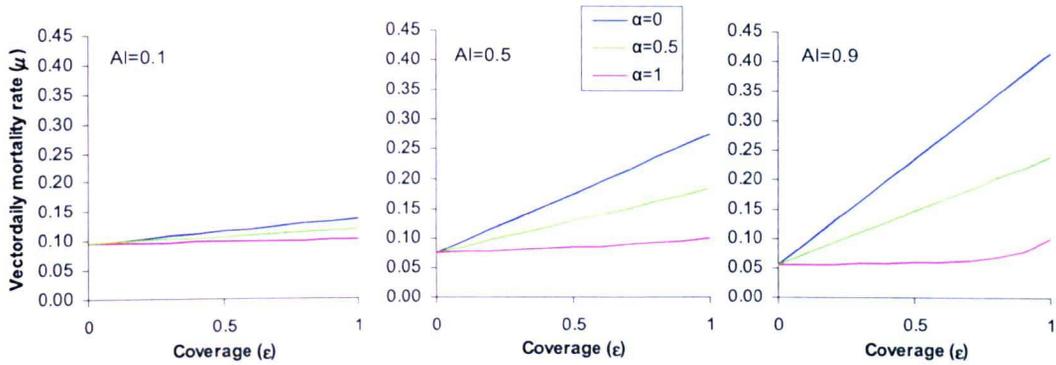


Figure 5.5. Effects of increasing the effective coverage of ITL upon the vector mortality rate, for different repellence probabilities (α) of the insecticide.

$k=0.8$; $\mu_{\min}=0.05/\text{day}$; $\alpha=0.5/\text{day}$; $N_l=N_h=100$; $j=0.1105, 0.2, \text{ and } 1$, for $A_l=0.1, 0.5 \text{ and } 0.9$ respectively (the values of j were chosen to obtain $\mu=0.1$ when there are no livestock).

5.2.4. Impact of ITL on malaria transmission potential

The impact of ITL on malaria transmission potential (R_0) was then explored for different scenarios of effective treatment coverage ($\epsilon=T_l/N_l$), vector human blood index (HBI, as a proxy for the proportion of blood feeds on humans), and vector density (N_v/N_h). All the simulations were done using the Matlab[®] 6.5 package.

5.2.4.1. Assumptions

Simulations were done assuming the best-case scenario regarding repellence, i.e. no repellent effect of the insecticide upon vectors. Conversely, the worst case-scenario was

assumed regarding insecticide impact on vector population density, i.e. no impact on the overall vector population density, N_v , which remains constant. This implies exact and intrinsic density-dependent compensation of the vector population, by setting the recruitment rate, ρ , of newly emerged female adult mosquitoes entering the susceptible class equal to the average vector mortality rate, μ . Even under the effects of additional mortality, there is immediate replacement of the insecticide killed vectors by newly emerged ones, so that the total vector population size does not change. The beneficial impact of the ITL intervention is therefore due only to the decrease on vector survival, and consequent reduction in the proportion of vectors that become infectious.

Additionally, since the simulations were done for a given effective coverage of ITL, an underlying fixed value for the average vector mortality rate was used, which was assumed to have been estimated on the day of the intervention (i.e. when the insecticide residual activity is maximum).

5.2.4.2. Impact of livestock treatment coverage and vector HBI on R_0

Initial explorations were done of the impact of ITL on the malaria transmission potential, using the expression for R_0 that was elaborated in Chapter 3 (Section 3.2.2). As illustrated in Figure 5.6, for a given effective coverage of insecticide-treated livestock (T_l/N_l), increases in HBI lead to increase in R_0 . The smaller the coverage, the steeper is the increase in R_0 , and vice-versa. Conversely, for a given HBI, increases in coverage generate a decrease in R_0 . These results are in accordance with the sensitivity analysis performed in Chapter 3 (Section 3.2.4).

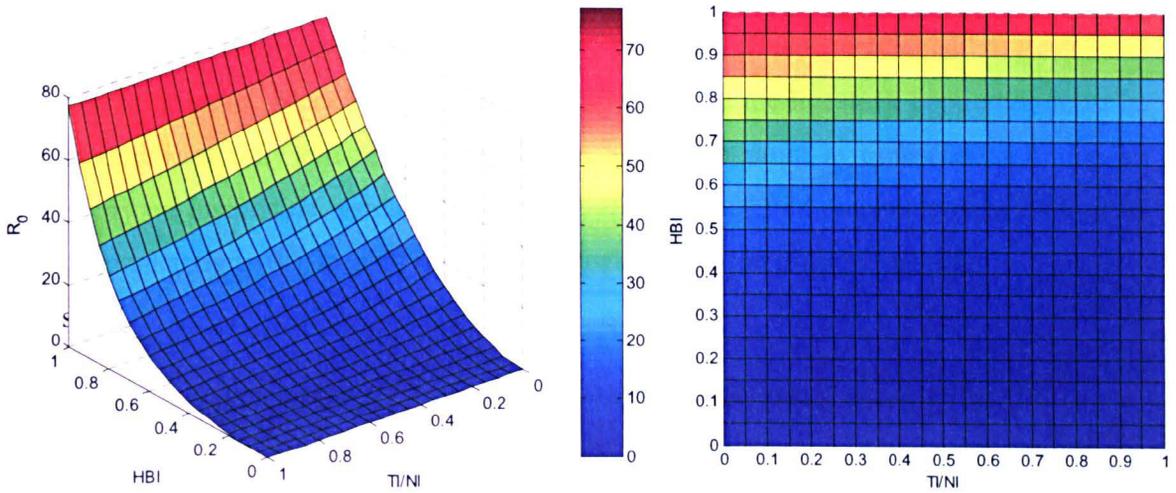


Figure 5.6. The basic reproductive number with respect to the proportion of vector feeds on humans (HBI) and effective coverage of insecticide-treated livestock (T_I/N_I).

Left: 3D view; Right: 2D view. The colour bar shows the scale of the R_0 values (y axis in the left plot). $N_v/N_h=50$, $a=1/2.5$, $b=0.5$, $c=0.95$, $r=1/21$, $\omega=1/17.5$, $\mu_0=1/4.63$, $k=0.85$; a , r , ω , μ_0 and k were estimated from Pakistan data (Chapter 6); N_v/N_h , b , c , are difficult to measure, and therefore, values were chosen to produce the observed malaria prevalence in the Pakistan villages where a trial was conducted, which is a standard procedure used in previous models (e.g. Gu et al., 2003; Reithinger et al., 2004).

5.2.4.3. Critical coverage of ITL for interruption of malaria transmission

An analysis was then performed of the critical proportion $(T_I/N_I)^*$ of livestock population that must be effectively treated with insecticide in order to interrupt malaria transmission, i.e. to decrease R_0 below 1.

5.2.4.3.1. Deriving the expression for critical coverage of ITL

The critical proportion was derived by setting $R_0=1$ and solving for T_I/N_I , through algebraic manipulation:

$$(T_I/N_I)^* = \frac{1}{2} \sqrt{\frac{r\omega \left(4 \frac{N_v a^2 q^2 bc}{N_h} + r\omega \right) - r(\omega + 2\mu_0)}{ra(1-q)k}}, \quad \text{Eq. 5.10}$$

where $q=HBI$, and μ_0 is the average vector natural mortality; i.e. in the absence of insecticide treatment of livestock ($\mu_0 = \mu_{min} + \mu_{search}$).

The considerations from the sensitivity analysis of the R_0 (Chapter 3; Section 3.2.4) have the following implications. Supposing that $(T_l/N_l)^*$ is for example 0.4, this means that in order to reduce $R_0 < 1$, then on average, 40% of the livestock population must be treated with fully effective insecticide, at any given point in time (i.e. assuming no decay on insecticide residual activity); or, if the insecticide maintains only 50% of its original activity (k), then 80% of the livestock population must be treated.

5.2.4.3.2. Impact of the HBI and vector density on the critical coverage of ITL

Numerical simulations were carried out to investigate how the critical coverage of ITL varies with the proportion of vector bloodmeals on humans (HBI) and with vector density (Figure 5.7). For a given density of vectors per human host, the lower the HBI, the higher is the proportion of vector blood-meals upon livestock, and the smaller is the effective proportion of treated livestock required to reduce R_0 (Figure 5.7A). The results presented in Figure 5.7 suggest that in areas where malaria vectors feed predominantly on non-human hosts (as in Southeast Asia), maintaining a constant coverage of the livestock population with active insecticide could potentially reduce R_0 to below unity and therefore, promote the control of this most important tropical disease. For a given HBI, the lower the density of the vector, the lower will be the transmission potential (i.e. the lower will be the R_0 pre-intervention), and therefore the smaller is the effective proportion of treated livestock required to reduce R_0 . For example, in scenarios where vectors have 20% of their blood-meals on humans, the required effective proportion of treated livestock would vary from 67% to 22% for vector densities ranging from 50 to 25 vectors: human, respectively (Figure 5.7B).

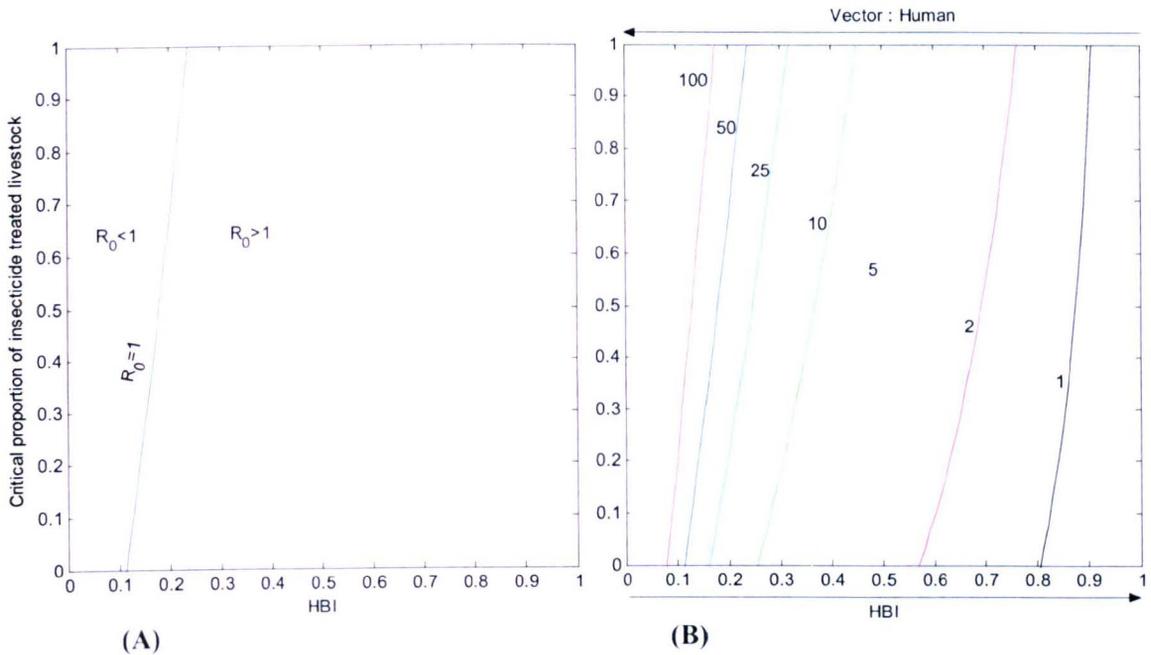


Figure 5.7. Critical effective proportion of insecticide-treated livestock (T_1/N_1)*, as a function of the vector human blood index (HBI).

(A) The full blue line depicts the effective proportion of livestock treated with insecticide, above which R_0 will be decreased below 1, for a given HBI. The dashed blue line defines the HBI value below which it could be possible to decrease R_0 below 1. (Parameter values were kept fix as in Figure 5.6; namely the ratio vector: humans is set as 50). (B) Different scenarios varying the density of vectors per human host (N_v/N_h), to illustrate the impact of the intervention under various levels of transmission potential (i.e. R_0 pre-intervention)¹. The values for the ratio of vectors per human assumed in each scenario are shown next to the corresponding line; for instance, the full black line illustrates a situation where there is 1 vector per human.

5.2.4.3.3. Threshold HBI for transmission interruption with ITL

An additional inference can be made from Figure 5.7. By tracing a vertical line from the top edge of the line defined by $R_0=1$ until the x axis, it is possible to infer the value of HBI below which ITL could bring $R_0 < 1$ and therefore potentially control malaria transmission. For example, in a setting where $N_v/N_h=50$ (and other parameters as in Figure 5.6), and $HBI < 23\%$ it could be possible to control malaria transmission with a constant proportion of

¹ In Figure 5.7B, a distinction is made between varying the HBI (bottom axis) and varying the N_v/N_h (top), for the purpose of assessing the impact of the intervention under various levels of transmission potential (i.e. R_0 pre-intervention). However, it is worthwhile mentioning that in field entomological studies N_v/N_h and HBI are usually measured together, within the measure of human landing catches per night ($(N_v/N_h)aHBI$). Knowing the value for a ($=1/\text{duration of gonotrophic cycle}$), it is possible to extract the value for $(N_v/N_h)HBI$, but it is not possible to measure N_v/N_h separately from HBI, unless a reliable measure of the HBI is determined (through analysis of mosquitoes blood meals collected from a representative sample), or unless an experimental study is done, with a controlled number of vectors per human.

effectively treated livestock above the critical threshold $(T_I/N_I)^*$ (Figure 5.7A). The higher the malaria transmission potential, e.g. due to increase in vector density, the lower the HBI range within which malaria transmission could potentially be controlled with ITL, and vice-versa (Figure 5.7B).

5.2.4.4. Impact of livestock treatment coverage and vector HBI on R_0 ratio

Finally, explorations were done on the impact of the intervention on the R_0 ratio, which is defined as the ratio between the R_0 under a given effective coverage of insecticide-treated livestock (T_I/N_I) and the R_0 pre-intervention (Figure 5.8). The proportional reduction on the pre-intervention R_0 is given by $1 - (R_0 \text{ ratio})$. In scenarios where malaria vectors feed mainly on livestock (i.e. low HBI), high levels of effective coverage could produce a reduction of up to 60% on the pre-intervention R_0 . Interestingly, even in settings where a higher proportion of blood-meals are taken upon humans (as in Africa), the intervention has the potential to achieve a considerable decrease in R_0 , and thereby decrease malaria transmission and infection.

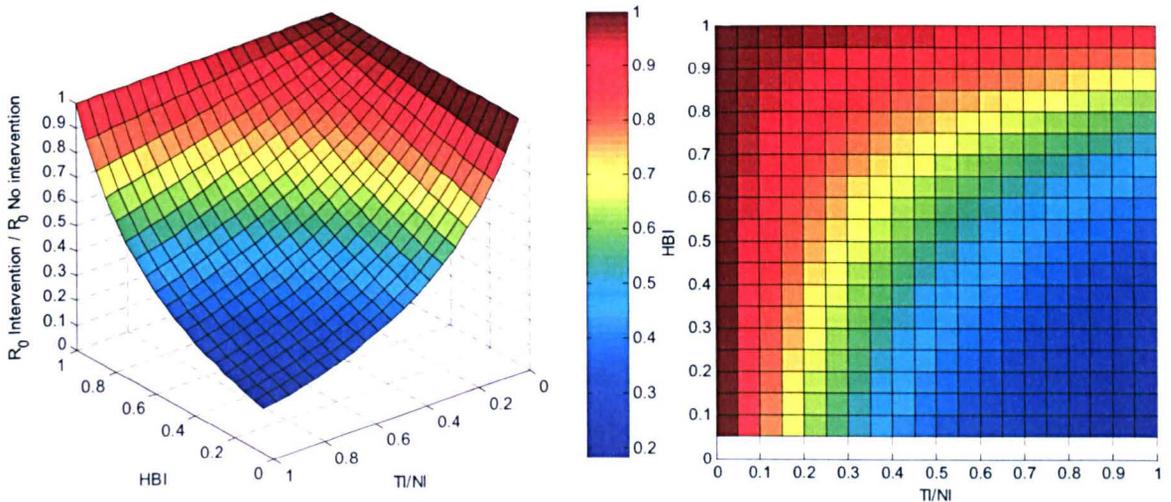


Figure 5.8. Effect of ITL on the R_0 ratio, with respect to the vector human blood index (HBI) and the effective coverage (T_I/N_I).

Left: 3D view; Right: 2D view. The colour bar shows the scale of the R_0 ratio (y axis in the left plot). (Parameter values were kept fixed as in Figure 5.6).

5.2.5. Modelling ITL as a dynamic process

The theoretical framework was then applied to investigate the effects of ITL on malaria as a dynamic process, unlike the previous sections which considered a single effective coverage, in a given point in time. The model was fit to empirical estimates of the efficacy and duration of the insecticide in order to account for the decay of the insecticide residual activity and for density-dependent fluctuations in vector population density following exposure to ITL.

5.2.5.1. Fitting the model to bioassays data

The insecticide killing effect upon vectors exposed to treated livestock and the decay rate of the insecticide residual activity were estimated based on whole-animal bioassays of deltamethrin applied to cattle in Pakistan (Hewitt and Rowland, 1999) and in Ethiopia (Habtewold, 2004; Habtewold et al., 2004), as detailed below.

5.2.5.1.1. Rational for insecticide selection

In the Pakistan bioassay three insecticides were tested: deltamethrin, permethrin, and lambda-cyhalothrin, all applied to cattle with a sponge. Deltamethrin had the stronger and longest lasting effect (Hewitt and Rowland, 1999), and was also the insecticide used in the ITL trial (Rowland et al., 2001), for which reasons the model for Pakistan was fitted to this insecticide. In the Ethiopian bioassay only the deltamethrin insecticide was tested, but in two formulations: spot-on and Decatix. The simulations for Ethiopia considered only the spot-on because it showed a longer residual effect in the bioassay (Habtewold, 2004; Habtewold et al., 2004) and was also the more commonly used formulation in the Ethiopian setting (Konso District, presented in Chapter 2), to which the model will be applied in the next Chapter.

5.2.5.1.2. Simulating a bioassay

In both the Pakistan (Hewitt and Rowland, 1999), and the Ethiopian (Habtewold, 1999, 2004; Habtewold et al., 2004) bioassays, one insecticide-treated (or untreated control)

cow was tethered inside a large closed net before sunset. During the night, host-seeking mosquitoes were collected by mouth aspirators from outside the nets and then released inside. At dawn, mosquitoes were collected from inside the net and analysed. The procedure was repeated for several days post-treatment. The mosquito mortality¹ due to exposure to the insecticide-treated cow was calculated from the observed mortality of mosquitoes exposed to a treated cow adjusted for the control mortality of mosquitoes exposed to an untreated cow using Abbot's correction (Hewitt and Rowland, 1999; Habtewold, 2004; Habtewold et al., 2004).

Accordingly, to estimate the insecticide killing effect upon vectors and the decay rate of its residual activity, a bioassay scenario was simulated, where:

- 1) there are no humans available for the vector to feed upon: $N_h A_h = 0$;
- 2) there is one head of livestock available for the vector to feed upon, $N_l A_l = 1$, which has been fully treated with a non-repellent insecticide: $\epsilon = 1$ (i.e. $T_l/N_l = 1$) and $\alpha = 0$;
- 3) all the mosquitoes collected were looking for a blood-meal: $a = 1$; and
- 4) there is negligible vector mortality due to searching for a blood-meal host, since the mosquitoes were released into a place with a fully accessible blood-meal host.

The expression for vector mortality due to insecticide treatment presented in Section 5.2.2.1:

$$\mu_{t\text{only}} = \left(\frac{\epsilon(1-\alpha)N_l A_l}{N_h A_h + N_l A_l} k \right) a \quad \text{Eq. 5. 11}$$

is therefore reduced to

$$\mu_{t\text{only}} = (T_l(t)/N_l)k. \quad \text{Eq. 5. 12}$$

5.2.5.1.2.1. Estimating the insecticide lethal effect

The probability k that vectors are killed due to exposure to insecticide-treated livestock on the day of treatment, is a proxy for insecticidal effectiveness, and can be extracted from bioassay data (graph of adjusted vector mortality due to exposure to insecticide-treated livestock, against time post-treatment). In the bioassay done in Pakistan k was

¹ Mosquito mortality included: dead or knockdown, in the Ethiopian bioassay; and dead or unfed, in the Pakistan bioassay.

~85% for anopheline mosquitoes (Hewitt and Rowland, 1999), while in Ethiopia, it was ~90% ($\pm 5\%$ standard error) for *An. arabiensis*, 90% ($\pm 8\%$) for *An. pharoensis*, and 94% ($\pm 4\%$) for *An. tenebrosus* (Hewitt and Rowland, 1999; Habtewold, 2004; Habtewold et al., 2004).

5.2.5.1.2.2. Estimating the insecticide decay rate

In a bioassay context, the Lethal Time 50 (LT_{50}) is the time following the application of insecticide to livestock when the insecticide would kill (and/or knockdown) 50% of the mosquitoes exposed to the insecticide-treated animals. In the current model, LT_{50} corresponds to the time when $\mu_{t_{only}}=0.5/day$.

From the bioassay data for Ethiopia, the insecticide residual activity appears to decay faster after a point that corresponds to the LT_{50} (Figure 5.9, closed circles). Accordingly, the insecticide residual activity was modelled to decrease exponentially with time, using two decay rates.

5.2.5.2.2.1. Deriving expressions for the decay rates

The first decay rate, d_1 , acts only until the insecticide's LT_{50} , and was derived by the expression

$$d_1 = \frac{-\ln\left(\frac{0.5 N_I}{k T_{I(0)}}\right)}{LT_{50}}, \quad \text{Eq. 5. 13}$$

where $T_{I(0)}$ = Number of animals treated with insecticide on the day of the intervention. Since in a bioassay it can be assumed that $T_{I(0)}=N_I$, then:

$$d_1 = \frac{-\ln\left(\frac{0.5}{k}\right)}{LT_{50}}. \quad \text{Eq. 5. 14}$$

The second decay rate, d_2 , acts from the day after the LT_{50} , and is faster than d_1 . The decay rate d_2 was estimated such that, at the time of the “maximum” duration of the insecticide residual effect, t_{max} , the insecticide only kills a given estimated percentage ($perc_{max}$) of the mosquitoes exposed to the treated animals, in which case at that time

$\mu_{txonly} = \text{percmax}/\text{day}$. Supposing that at a given t_{max} the percmax is 10% (i.e. $\mu_{txonly} = 0.1/\text{day}$), then:

$$d_2 = \frac{-\ln\left(\frac{0.1}{k} \frac{N_l}{T_l(LT_{50})}\right)}{t_{max}}, \quad \text{Eq. 5.15}$$

where $T_l(LT_{50})$ = Number of animals that have active residual insecticide by day LT_{50} , which can be derived as

$$T_l(LT_{50}) = T_{l(0)} \exp(-d_1 LT_{50}). \quad \text{Eq. 5.16}$$

Replacing this in Equation 5.15, and since in a bioassay it can be assumed that $T_{l(0)} = N_l$, then:

$$d_2 = \frac{-\ln\left(\frac{0.1}{k \exp(-d_1 LT_{50})}\right)}{t_{max}}. \quad \text{Eq. 5.17}$$

Input parameter values (LT_{50} , t_{max} , percmax) for the decay rates

Pakistan

According to the authors of the Pakistan bioassay (Hewitt and Rowland, 1999), a 50% decrease in the proportion of female anopheline mosquitoes alive and/or blood fed was observed, up to two weeks after treatment. Unfortunately, the published data for the proportion dead or unfed were not dissociated, and therefore there is no reference to the actual proportion of mosquitoes dead/knockdown. Also, no formal analysis was done to determine the LT_{50} . From their data (reproduced in Figure 5.9), the LT_{50} was not likely to have been longer than two weeks, although it may have been as short as 4 days. Accordingly, simulations were done for $LT_{50}=4, 9$ and 13 days, $\text{percmax}=1\%$, and $t_{max}=18, 23$, and 32 days, respectively, with values extracted from the plot of their bioassay data. Although the Pakistan bioassays were conducted for a longer period (45 days) than the Ethiopian (28 days), the former data are “noisier”: there are negative values for the proportion of mosquitoes catch dead or unfed, which probably correspond to situations where mortality/unfed was lower in mosquitoes exposed to a treated cow than in the control.

Ethiopia

In the published Ethiopian bioassay the LT_{50} was determined by probit analysis (Habtewold, 2004; Habtewold et al., 2004). When the spot-on was applied as recommended, in a continuous line along the spine of cattle hosts, the LT_{50} for *An. arabiensis* was 4 days (95%CI=1-6 days), with mortality decreasing to 5-15% by the second week of treatment. Precisely the same LT_{50} was obtained when the spot-on was applied restrictively to the legs of the animals, although with a shorter 95% CI =3-5 days. Simulations were therefore conducted using $LT_{50} = 1, 4$ and 6 days, $perc_{max}=5, 10$ and 15% respectively, and $t_{max}=14$ days.

Figure 5.9 illustrates the predicted vector mortality due to exposure to deltamethrin-treated livestock in conditions similar to the bioassays conducted in Pakistan and in Ethiopia.

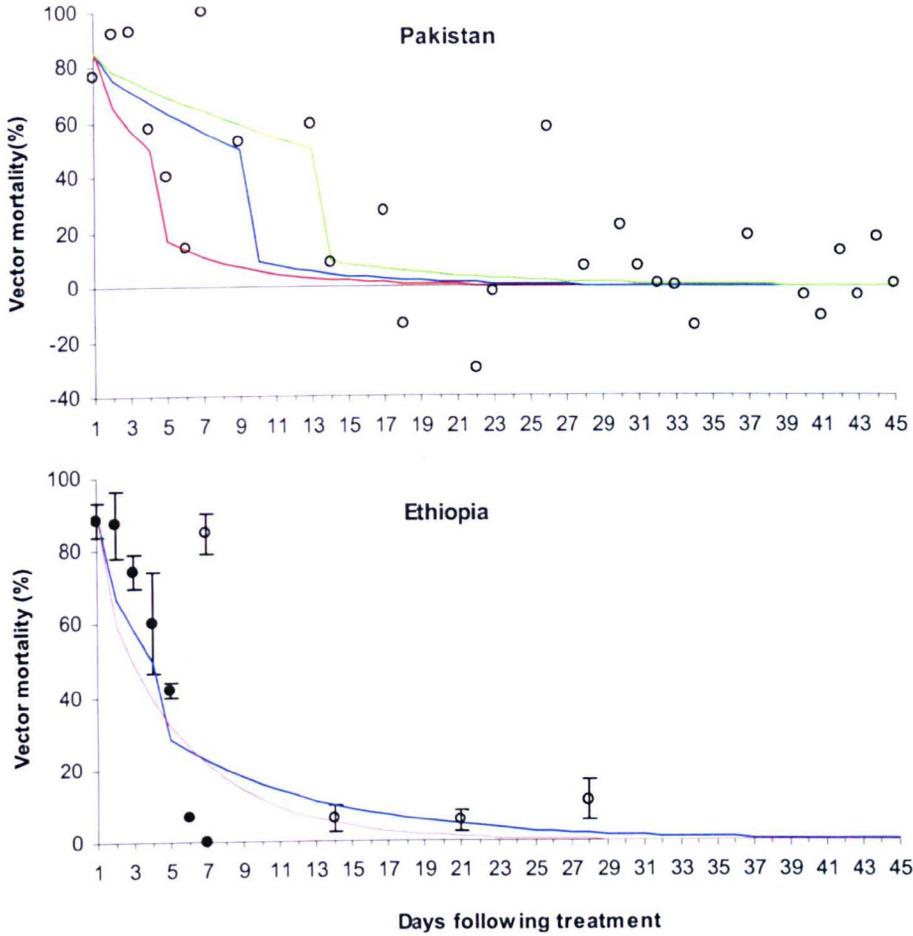


Figure 5.9. Simulating bioassays of the effect of deltamethrin-treated livestock on vector mortality.

Full lines: predicted percentage mortality of mosquitoes due to feeding on insecticide-treated animals, for three combinations of parameter values estimated from published bioassay data (Hewitt and Rowland 1999; Habtewold 2004). *Pakistan*: $k=0.85$; $LT_{50}=4, 9, 13$ days, and $LT_1=18, 23, 32$ days, for the red, blue and green line respectively. *Ethiopia*: $k=0.9$; $LT_{50}=1, 4, 6$ days, and $LT_5=14, LT_{10}=14, LT_{15}=14$ days, for the red, blue and green line respectively.

Circles: best estimates from published data of whole animal bioassays, where mosquitoes fed naturally on cattle treated with deltamethrin. *Pakistan*: mean percentage of the anophelines catch dead or unfed ($[1 - \text{proportion alive and blood fed}] \times 100$); emulsion of deltamethrin 2.5% (K-Othrine 2.5 EC, applied uniformly to the whole body of cattle using a sponge (at concentration of 0.025g/m², Hewitt and Rowland 1999). *Ethiopia*: Percentage mortality/knockdown of *An. arabiensis*; spot-on formulation of deltamethrin 1% applied in a continuous line along the spine of cattle (open circles), or applied restrictively to the legs of the animals (closed circles). Error bars indicate standard errors (Habtewold, 2004).

5.2.5.2. Effects of ITL on vector population density

5.2.5.2.1. Assumptions and expression for modelling density-dependent regulation of vector population

In Section 5.2.4 the impact of ITL on malaria transmission was investigated assuming that the intervention had no impact on vector population density. This is useful for initial explorations, but it is not realistic. For example, following the ITL trial in Pakistan, the density of the main malaria vectors, *An. stephensi* and *An. culicifacies*, decreased by approximately 50% in treated villages (Rowland et al., 2001). Accordingly, here explorations were done of possible changes on vector density imposed by density-dependent constraints following exposure to ITL.

In all the simulations of ITL in this Chapter and in the next (Chapter 6) it is assumed that, prior to intervention, an endemic equilibrium of malaria transmission had been reached and, as in most previous zooprophyllaxis models (Saul, 2003; Kawaguchi et al., 2004; Killeen and Smith, 2007), vector population density was at its carrying capacity level (K).

As in Chapter 4, the density-dependent regulation of the adult vector population due to competition within the larval stages, which depends on the abundance and extent of breeding sites, is explicitly modelled using a simple expression based on the logistic growth equation (adapted from Lord et al. 1996).

The vector recruitment rate is given by

$$\rho = (\rho_0 - \rho_s N_v) \quad \text{Eq. 5. 18}$$

and

$$\rho_s = (\rho_0 - \mu) / N_v^* \quad \text{Eq. 5. 19}$$

Where ρ_0 and ρ_s are the vector recruitment rate in the absence of density-dependence constraints and the strength of the density-dependence in recruitment, respectively; μ is the overall vector mortality rate (i.e. baseline mortality + search-related mortality + exposure to insecticide mortality); and N_v^* is the vector population density in the endemic equilibrium (assumed to equal K).

To overcome circularity when calculating ρ_s , a time-delay needs to be introduced, such that

$$\rho_{(t)} = (\rho_{0(t)} - \rho_{s(t-delay)} N_{v(t-delay)}). \quad \text{Eq. 5. 20}$$

In Chapter 4, similarly to what was presented in the paper by Lord et al. (1996), Equation 5.19 was used to illustrate the effect that increasing the number of alternative blood-meal hosts could have on vector population density, and consequently on malaria transmission dynamics. It was then assumed that the introduced perturbation (decrease in vector mortality due to increase in number of untreated livestock) would remain permanently. In this Chapter and the next, the introduced perturbation (increase in vector mortality due to exposure to insecticide-treated livestock) is temporary, lasting only as long as the insecticide residual effect is acting. Once the insecticide effect ceases, the vector population will tend to return to the pre-intervention density level.

5.2.5.2.2. Dynamical behaviour patterns of vector population density

Depending on the combination of values of the ρ_0 and the time-delay, following a perturbation of the equilibrium (in this case due to an ITL intervention), the vector population may exhibit (1) a monotonic dampening, or (2) an oscillatory dampening to the pre-intervention vector density level, or even (3) permanent oscillations (also known as “stable limit cycles”) tending to overcompensate from generation to generation without stabilising in the pre-intervention density level. The permanent oscillations are detrimental because the vector population density keeps increasing after decreasing, and never stabilizes. The oscillatory dampening is also not ideal, because during the oscillations vector density reaches levels higher than pre-intervention, before stabilizing. The desirable behaviour with an ITL intervention would be ideally, to obtain extinction of the vector population, and alternatively (more realistically), to decrease vector density for the maximum period of time possible.

Such diverse behaviour patterns are illustrated in Fig. 5.10, which simulates the effects of one round of treatment where 93% of livestock population are treated with a non-repellent insecticide, with $k=0.85$, $LT_{50}=4$ days, $LT_{10}=18$ days, upon a population of mosquitoes that feeds once every 2.5 days and survives ~ 4.63 days in the absence of treatment, in a scenario where livestock are 53.24 times more available and 0.14 times as abundant as people (parameters for *An. culicifacies* in the Pakistan ITL trial setting).

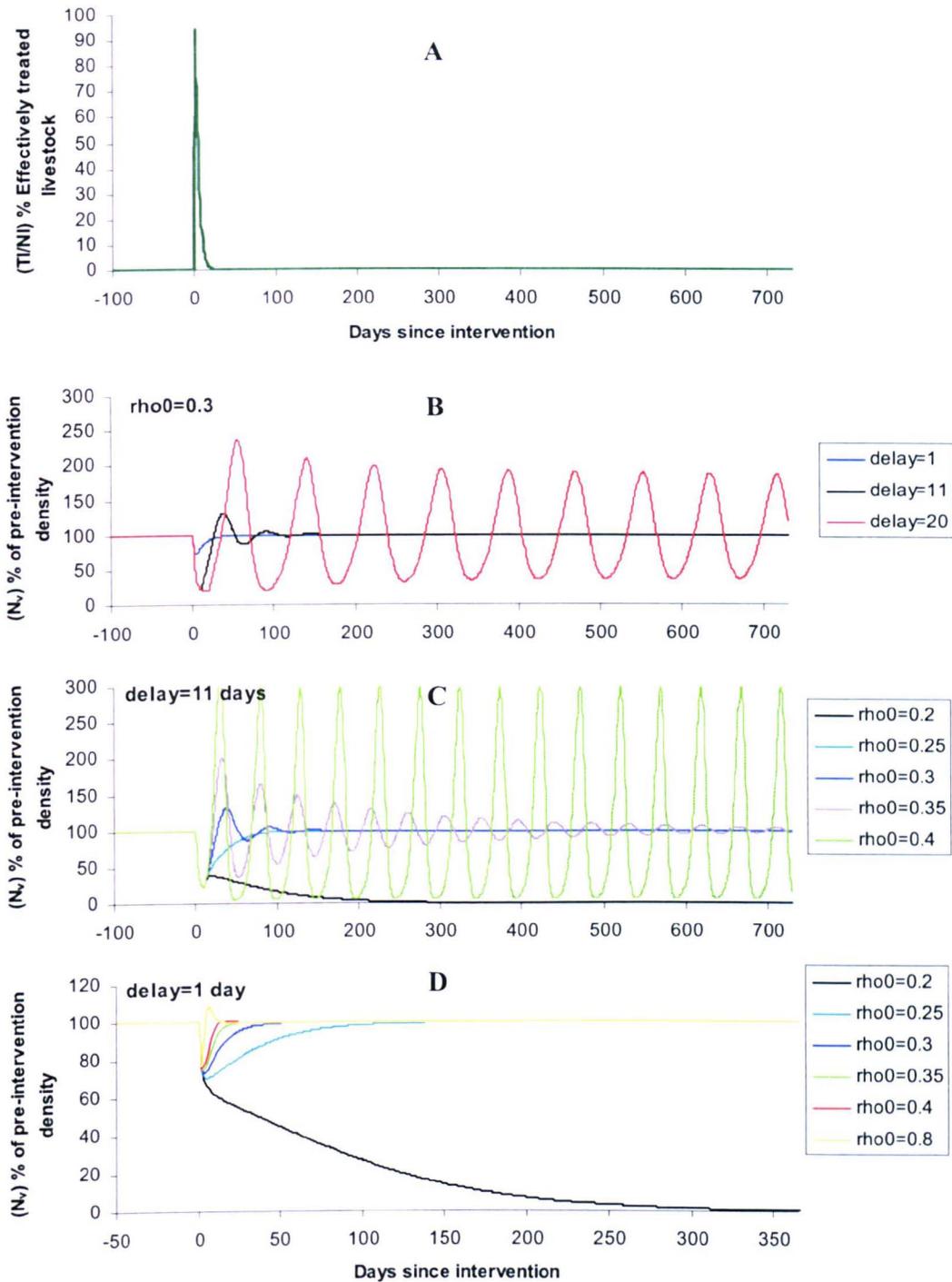


Figure 5.10. Effects of one pulse of ITL on vector population density.

Different dynamical behaviours of the vector population are shown depending on the ρ_0 (ρ_0) and time-delay values. **(B)** When $\rho_0=3$, if delay=1 day: monotonically dampening; if delay =11 days: oscillatory dampening; if delay =20 days: stable limit cycles. **(C & D)** Other combinations of ρ_0 and time-delay values can produce different behaviours. Other parameters used are as in the Pakistan ITL trial scenario: application coverage=93%; $k=0.85$; $LT_{50}=4$ days; $LT_1=18$ days; $\alpha=0$; $a=1/2.5$; $N_1/N_h=0.14$; $N_h=100$; $A_1/A_h=53.24$; $\mu_0=1/4.63$. All simulations were done using Berkeley MadonnaTM package (Macey and Oster, 2006).

For a given ρ_0 value, the longer the time-delay, the stronger is the impact of the intervention on reducing the vector population density (N_v) during the effective life of the insecticide, and the longer it will take for N_v to rebound to the pre-intervention levels. In the simulated conditions, when $\rho_0=0.3$, a time-delay from 1 to 4 days will result always in monotonic dampening; from 5 to 7 days there will be a progressively increasing hump of N_v overcompensation before stabilizing in the pre-intervention level; from 8 to 18 days, oscillatory dampening is observable; and from 19 days onwards permanent oscillations will occur (Fig. 5.10B). Different behaviours can be produced by other combinations of ρ_0 and time-delay values (Fig. 5.10 C&D). When using 1 day time-delay, the impact of varying ρ_0 on the time T that takes for N_v to rebound to the pre-intervention levels is much greater for low values of ρ_0 ($\rho_0 < 0.4$) than for higher values of ρ_0 . For example, by varying ρ_0 from 0.25 to 0.3, T is decreased from taking ~ 5 months, to taking ~ 2 months, compared with much smaller variation in T for $\rho_0 \geq 0.4$ (Fig. 5.10D).

5.2.5.2.3. *Biological context*

Biologically speaking, since the model considers density-dependent regulation of the adult vector population due to competition within the larval stages, the time-delay should correspond to the time from oviposition to the emergence of adults. For malaria vectors, this interval can take a minimum of 11 days, although it depends on rainfall and temperature (le Sueur and Sharp, 1991; Marquardt, 2005).

5.3. Discussion

The basic model presented in Chapters 3 and 4 was here extended to tackle an array of complexities inherent to the insecticide treatment of livestock for malaria control. Explorations were done of possible excito-repellent effects of the insecticide upon the malaria vector feeding behaviour and mortality. The results showed that, theoretically, excito-repellency can be a very important determinant of the outcome of an ITL intervention, particularly in scenarios with very high availability of livestock. The potential

impact of ITL interventions on the malaria transmission potential was also investigated, and thresholds for transmission interruption were assessed. Namely, the threshold livestock treatment coverage required to potentially interrupt malaria transmission in a given setting was derived, as well as the threshold vector HBI below which transmission interruption could, in theory, be achieved with ITL. Finally, the model accounted for decay of the insecticide residual effect, and for its impact on the density-dependent regulation and resulting dynamical behaviour patterns of the mosquito population.

While most previous works have overlooked these complex issues, here all these complexities were incorporated in a single framework that is succinct and robust. Notably, the complexities can be removed from the developed framework and it becomes reduced to the simpler ways of modelling that other have used.

To my knowledge, this is the first modelling approach that explores the potential effects of repellence in the context of ITL and malaria transmission. Additionally, an advantage of this model in relation to previous works is that it accounts for the decay of insecticide residual activity and for a fluctuation in vector population density following exposure to ITL. Conversely, previous models of the impact of applying insecticide on animals (Saul, 2003) or on animal sheds (Kawaguchi et al., 2004) upon malaria transmission, were limited to the simpler assumptions of a constant killing effect of the insecticide upon vectors, and a constant vector density that was unaffected by exposure to the insecticide.

5.3.1. Modelling repellence

In the present work, when modelling repellence the expression for HBI assumes that the probability of vectors being repelled to humans after attempting to bite on livestock, depends only on the proportion of livestock that is effectively treated with insecticide (effective coverage, ϵ), on the repellent effect of the insecticide (α), and on the relative number and availability of livestock or human hosts. Additionally, the expression assumes that repellence and coverage are independent.

In reality, however, the occurrence of a repellent effect may depend on factors such as characteristics of the: a) insecticide (chemical compound, formulation and concentration); b) intervention (treatment coverage – i.e. proportion of the livestock population present to which insecticide was applied –, and concentration of the

insecticide on the animal's coat, which will eventually decrease with time after application); and c) mosquito vector. Also, the insecticide concentration is likely to be heterogeneous throughout the animal's surface, and the place where the mosquitoes land on the animals can therefore be critical. With regards to ITNs, it has been hypothesized that mosquitoes strains with the *kdr* resistance gene may be less sensitive to the usual excito-repellent effect of pyrethroids, allowing them to rest longer on treated bednets and pick up a lethal dose of insecticide, which would explain why pyrethroid-treated bednets are still preventing malaria in some areas with a *kdr* resistant vector population (Chandre et al., 2000). The relative contribution of these and other factors to the occurrence of an excito-repellent effect of insecticides upon malaria vectors and its epidemiological impact upon malaria are yet to be clarified and quantified, being an area of increasing interest by the malaria scientific community (IVCC, 2007).

The model assumes that when a mosquito tries to bite on an insecticide treated animal and is repelled, it will be diverted to bite on another host. Nonetheless, it could be that the mosquito is not able to find a successful bloodmeal and does not feed in that night, ending up either feeding only on the following night, or dying earlier. The impact of repellence on vector mortality is captured by the model, since repellence reduces the availability of treated livestock, increasing the time required to find a blood-meal host and consequently increasing the host-search associated vector mortality. The impact of repellence increasing the interval between blood-meals is something that could be explored by extending the model to explicitly account for that possibility.

5.3.2. Decay of insecticide residual activity

The present model assumes that the lethal and repellent effects are maximal on the day of insecticide application and then both effects decay at a given same rate. The decay rate was estimated from bioassays data on the decay of the vector mortality due to exposure to ITL. Alternatively, one could model the lethal and repellent effects decaying at different rates. For example, repellence could be concentration dependent (i.e. depend on the concentration of active residual insecticide present on the animal). If this was case, the repellent effect could be observed only when the insecticide residual activity on the animal decreases below a given level. Alternatively, a stochastic type model could be used, where each individual animal treated with insecticide would have

attached to it a probability distribution for the likelihood of killing and another distribution for the likelihood of repelling the mosquitoes that attempt to bite on it. However, for succinctness, and for the ability of a single analytical framework, the approach used was the best approximation, particularly when, to the best of my knowledge, there is no field data to inform a more complicated approach.

5.3.3. Modelling density-dependent regulation of vector population

Density-dependent regulation of the vector population was modelled based on an expression given by Lord et al (1996), extended to accommodate the nature of a control intervention where vector mortality is temporarily increased, while the work by Lord et al (1996) explored only situations where the introduced perturbation (decrease in vector mortality due to increase in number of hosts) would remain permanently. Other approaches have been used to model density dependence. See for example, Bellows (1981) for a more extensive review, and Dye (1984) for the application of a two parameter (alpha and beta) function to explore the population dynamics of *Aedes aegypti*, upon which Atkinson et al. (2007) have recently built upon to evaluate vector-borne disease control interventions, linking the entomological component with an epidemiological outcome. Although the approach chosen in the presented model differs from these more sophisticated ones (Bellows, 1981; Dye, 1984; Atkinson et al., 2007), in all of them density dependence regulates the adult vector population due to competition within the larval stages, and is modelled with a time-delay (interval between generations). Most importantly, the expression used in the presented model, despite being simple, is able to capture diverse dynamical behaviour patterns of the mosquito population as the more complicated density-dependent regulation functions with two parameters.

5.3.4. Factors determining the effects of ITL on vector population

The impact of an ITL intervention will depend on the magnitude of the decrease in vector population density and on the time (T) that will take for the vector density to rebound to the pre-intervention levels. These are conditioned by four main factors, as follows:

(1) The magnitude of the vector mortality due to exposure to ITL: as seen in Section 5.2.3, this is determined by characteristics of the vector (biting rate on livestock and relative availability of livestock vs. humans) and intervention (treatment coverage; insecticide lethal and repellent effects, and duration of residual activity).

(2) The strength of the density-dependent constraints due to larval competition within the larval habitats: T can therefore also depend on the timing of the intervention with respect to the rainy season, with longer T likely to occur before than during the rainy season. Rain can increase the availability of breeding sites, possibly decreasing the competition for resources within the larval habitat, thereby increasing the carrying capacity and decreasing the density-dependent constraints.

(3) If there is sufficient dispersiveness of vectors and/or if the study area is not geographically isolated, there may be immigration of vectors from surrounding patches, which would reduce T.

(4) If there are vector undercrowding effects preventing population re-establishment, even despite possible immigration of dispersives, thereby increasing T: for example, this was found to be the case in a study done in three rural villages in the Pakistan Punjab Province (Reisen, 1986). Briefly, after spraying inside houses and cattle sheds with insecticides, the vector populations' density took much longer to rebound to the pre-spray levels (Malathion: 26 months for *An. stephensi*, and 20 to 12 months for *An. culicifacies*; Fenitrothion: 7 to 8 month for both species), than what would be expected given the much shorter residual toxic effect of the insecticide (ranging from 3 weeks for *An. stephensi* in Iran to 1 month in Uganda, as assessed by bioassay tests). Despite female and immature vectors being collected repeatedly in the study area during the post-spray period, these were not able to become well established. The authors therefore suggested that a critical threshold vector density needed to be reached to enable a rapid population growth and recovery to pre-spray density levels (Reisen, 1986). The limited availability of breeding sites can also contribute to undercrowding.

Additionally, it is important mentioning that mosquito dispersal does not only affect vector population density, but can also have further implications on the effectiveness of an insecticide based malaria vector-control intervention. Namely, through emigration of

mosquitoes from the areas subject to intervention and their exchange by mosquitoes immigrating from surrounding uncontrolled areas, which are more likely to be old enough to be infectious, there can be a potential decrease on the effectiveness of the intervention upon malaria transmission in the control area (Killeen et al., 2003).

Although the simulations here presented all assume a homogeneous vector population within single independent patches, vector immigration/emigration and/or undercrowding effects can occur in field scenarios, and could be incorporated in future modelling work (see Killeen et al., 2003 for a simple approach to quantify the effect of vector dispersal on the impact of a malaria vector-control intervention based on insecticide-treated bed nets).

In conclusion, I have shown here that it is important that a model accounts for possible excito-repellency effects of the insecticide, the decay rate of the insecticide residual activity, and density-dependent mechanisms regulating the vector population, in order to reliably explore the effects of an ITL intervention on malaria transmission dynamics. To assess the robustness of the model and its application there is a need to evaluate its use in alternative transmission settings, based on empirical parameter estimates. Accordingly, in the next Chapter the comprehensive framework here developed will be applied to specific settings in Pakistan and in Ethiopia, to investigate and compare the potential impact of various regimes of ITL within and between these different ecological scenarios.

Chapter 6

Modelling the effects of insecticide-treated livestock on malaria transmission dynamics

– Pakistan vs. Ethiopia –

Summary

Background: Using the theoretical framework previously developed, this Chapter has the three-fold aim of assessing: (1) whether the ITL intervention conducted in a Pakistan community-based trial could be improved; (2) the impact of applying in Ethiopia a similar ITL intervention as in the Pakistan trial; and (3) the impact of alternative ITL intervention regimens in Ethiopia.

Methods: *Parameterization* - The model was fitted to *P. falciparum* malaria transmitted by the highly zoophilic *An. culicifacies* in Pakistan and the more anthropophilic *An. arabiensis* in Ethiopia. Most of the parameterization was based on published data and additional information I gathered during a field study in Ethiopia, as described in Chapter 2. Strategies were devised to estimate parameter values when no data were available. ***Simulations*** - The fitted model was used to explore the effects that different regimens of treatment of livestock with an insecticide that has lethal and possible excito-repellent action upon malaria vectors could have on the disease transmission dynamics in both settings. The impact of ITL in Pakistan was initially modelled under the regimen of the empirical trial. The intervention effort used in the Pakistan trial was then supplanted into the Ethiopian setting, although using a different insecticide formulation specific to the African setting. The impact of different intervention efforts (livestock treatment coverage; number and interval between treatment rounds) and insecticide properties (lethal, excito-repellency, and duration of residual effect upon vectors) were explored. The effect of varying the proportion of vector mortality that is unrelated with search for a blood-meal host was also analysed. Simulations were done under three scenarios of density-dependent (DD) regulation of the adult vector population, where vector density was either kept constant (DD3), or allowed to fluctuate following exposure to ITL (DD1 and DD2).

Findings: *Parameterization* - In the Ethiopian setting, livestock were more abundant, although with a smaller availability to vectors, than in Pakistan, resulting in a higher human blood index in Ethiopia than in Pakistan. Additionally the estimated duration of the latent period in vectors was slightly shorter, while the vector life expectancy was higher in Ethiopia than in Pakistan,

contributing to the higher malaria levels in the African setting. **Simulations** - The model results suggest that the impact of the intervention on malaria incidence is highly sensitive to the duration of the insecticide residual activity, the insecticide potential excito-repellency action upon the mosquito vectors, and the coverage and frequency of treatments. In contrast, little sensitivity was shown to small changes in the insecticidal effect and in the interval between treatments. If the insecticide has excito-repellency properties, the proportion of the vector natural mortality that is unrelated with searching for a blood-meal host was also predicted to be an important factor. The different DD scenarios resulted in considerably different intervention impacts, indicating that the strength of DD acting upon the vector population is a key driver of the intervention success. Numerical simulations enabled identifying excito-repellency threshold probabilities above which the intervention could detrimentally increase malaria incidence. Only if excito-repellence probability is >60% in Pakistan, and >70% in Ethiopia would the intervention become detrimental, under the worst case scenario that assumed constant vector population density (DD3). In Ethiopia, within the ranges of excito-repellency probability for which ITL was likely to reduce malaria cases, a treatment scheme as done in the Pakistan trial (treating 93% of livestock, 3 times/year, 42 days apart) but using the Ethiopian insecticide (Deltamethrin 1% pour-on) was predicted to be the most beneficial, under the DD1 scenario of incomplete density dependence. Conversely, under scenario DD3, the greatest benefit in Ethiopia would be expected by increasing the frequency of treatments to once a month, as recommended for tsetse flies control, even if with a conservative coverage of 50%. Under scenario DD2 either of those two regimens would be the most beneficial. When comparing the impact of the ITL trial in Pakistan with the ITL regimen in use during the field study I conducted in Ethiopia, under the more realistic DD scenarios (DD1 and DD2) a greater beneficial impact was predicted in Pakistan than in Ethiopia, for any excito-repellency level; conversely, under scenario DD3 the reverse would occur.

Interpretation: The results from this study indicate that ITL is likely to be more beneficial in settings with highly zoophilic vectors as in Pakistan, than in Sub-Saharan African settings with the more opportunistic vector *An. arabiensis*. Nevertheless, the intervention is still likely to substantially decrease malaria incidence in the African settings, as illustrated here with the predictions for Ethiopia. Although this work highlights the importance of accounting for potential excito-repellency effects of the insecticide applied on livestock, the results suggest that only if the insecticide has very strong excito-repellency would ITL cause an increase in malaria cases, and thereby become prejudicial. This work also highlights that when designing and implementing an ITL intervention for malaria control it is important to have a good understanding of the DD regulation that is operating in the population(s) of malaria vectors in the study area and of the potential effects of DD upon the intervention outcome.

6.1. Introduction

The treatment of livestock with the pyrethroid insecticide deltamethrin has been shown to be an effective tool for malaria control in a community-based trial conducted in Pakistan where the disease is transmitted by the highly zoophilic vectors *An. stephensi* and *An. culicifacies* (Rowland et al., 2001). In Sub-Saharan Africa bioassays have also been conducted to test the potential of ITL upon the zoophilic malaria vector *An. arabiensis* in Ethiopia (Habtewold et al., 2004) and Tanzania (Mahande et al., 2007b). Despite the encouraging results from these bioassays, the impact of ITL on malaria transmission at the community level in Africa remains to be formally assessed.

Here, the theoretical framework developed in the previous Chapters will be applied to the Pakistan setting where the ITL community trial was conducted and to the Ethiopian setting described in Chapter 2, with the three-fold aim of assessing: (1) whether the ITL intervention conducted in the Pakistan trial could be improved; (2) the impact of applying in Ethiopia a similar ITL intervention as in the Pakistan trial; and (3) the impact of alternative ITL intervention regimens in Ethiopia.

The first section of this Chapter describes the parameterization of the model to the Pakistan and Ethiopian settings. The parameterization was based mostly on published data and additional information I gathered during a field study in Ethiopia, as described in Chapter 2; when data were unavailable, alternative strategies were devised to estimate and/or derive parameter values from the best available data. One of the key matters intrinsic to vector borne-diseases is the density-dependent (DD) regulation of the vector population and how that operates. Due to the uncertainties around the magnitude of DD acting in the study settings, the effects of ITL were explored under different density dependence scenarios, where vector density was either kept constant, or allowed to fluctuate following exposure to ITL.

The second section presents the extensive range of simulations that were performed with the fitted model. I started by modelling the empirical observations from the Pakistan ITL community trial. The Pakistan intervention was then supplanted into the Ethiopian setting. The impact of different intervention efforts and insecticide properties were explored with a sensitivity analysis. Within this, I examined one of the hypothetical problems of the intervention, which is the possibility of mosquito vectors

being diverted from treated livestock to nearby humans. This has been proposed as, if it was to occur, could in fact reduce the effectiveness of the intervention, to the extent of even potentially making it deleterious. The framework was applied to look at modifications of the intervention in order to optimize benefits and avoid deleterious effects in the African setting, given the local conditions of the scenario where the intervention was being taken into. Namely, I explored how the intervention in Ethiopia would perform under three alternative treatment regimens. Finally, I compared the predicted impact of ITL in the Pakistan and Ethiopian settings, and explicitly drew out the points of the driving differences.

6.2. Model parameterization

6.2.1. Methods

Most parameter values were either extracted or derived from empirical data from the index studies in Pakistan (ITL trial conducted by Rowland et al. 2001) and Ethiopia (Konso District described in Chapter 2 of this Thesis), or from previous studies conducted within or near the area of the index studies. When data for a parameter were available from more than one published study, the simulations used the estimates from the studies that were most recent and/or conducted in areas within or closest to the areas of the index studies.

6.2.1.1. Malaria transmission parameters

6.2.1.1.1. Rational for selection of *Plasmodium falciparum*

As in the previous chapters, I model only infection by *Plasmodium falciparum* because it is the most serious form of malaria infection, and has no relapses, unless due to treatment failure, while with *P. vivax* the more frequent relapses may obscure the effects of the intervention. Additionally, in the Ethiopian setting most cases were due to *P. falciparum*. Although in the Pakistan trial setting, *P. vivax* infections were more frequent, for comparison purposes, only the trial data for *P. falciparum* were used in the simulations.

6.2.1.1.2. Rational for selection of the species of malaria vectors

Pakistan: In the area of the Pakistan ITL trial the main malaria vectors were *An. culicifacies* and *An. stephensi* (Reisen and Borcham, 1982). The simulations considered only *An. culicifacies* because this species is more anthropophilic, has a longer life expectancy, and has been incriminated as the most important vector species in studies done in Punjab Province, near the ITL trial study area (Mahmood et al., 1984). I am therefore simulating a worse-case scenario regarding the vector species.

Despite *An. stephensi* was collected in higher densities than *An. culicifacies* in the study villages during the trial (Rowland et al., 2001), the former species is less competent as a malaria vector which precluded it from the simulations. Namely, due to the lower human blood index and life expectancy of *An. stephensi*, very high parameter values would be required to obtain persistence of infection, when using this species alone, as well as when using estimates of the availability and survival for *An. stephensi* and *An. culicifacies* weighted averaged by the proportional density of the two species¹.

Ethiopia: The simulations considered only *An. arabiensis*, as this is known to be the most important malaria vector in the study area (Habtewold, 1999; Habtewold et al., 2001; Tirados et al., 2006).

6.2.1.1.3. Estimation of parameter values

1) Duration of infectiousness in humans

The average duration of infectiousness was based on the time elapsed since start of malaria symptoms until arriving at a health facility to receive treatment. During the Pakistan ITL trial this would take around two weeks (M. Rowland, unpublished data), and was inflated to 21 days to account for situations where it may have taken longer until receiving treatment. The same baseline value was assumed for Ethiopia, to facilitate the comparison of the predicted results with the Pakistan setting. (Note, however, that infectiousness might have lasted longer in Ethiopia than in Pakistan, because health facilities were less accessible in terms of distance to villages and of cost

¹ For example, assuming other parameter values as the baseline in Table 6.5, the density of vectors per human would have to be > 1730 (for transmission by *An. culicifacies* only), or > 505 (for transmission by both *An. culicifacies* and *An. stephensi*, when both species are assumed to be equally abundant), which are values unrealistically large compared to what may normally be expected.

- diagnosis and treatment were being provide for free in Pakistan while a charge was made in Ethiopia).

2) Estimation of the duration of the latent period in vectors

The duration of the latent period (t_{lat}) was estimated, using Moshkovsky's formula (Molineaux, 1988) for *P. falciparum*: $t_{lat} = 111/(T-16)$, $18 < T < 30$, where T is the mean temperature in Celsius. The temperature data used were recorded near the Pakistan ITL trial setting at the Peshwara Meteorological Station, and in the centre of the Ethiopian setting at the Karat Meteorological Station, and were obtained by personal communication with the National Meteorological Station in each country.

3) Estimation of the infection probability of humans (b)

In two separate studies, 5 out of 10 volunteers have developed parasitaemia after being bitten by one or two *An. stephensi* infected with *P. falciparum* (Rickman et al., 1990; Verhage et al., 2005). No data were found for *An. culicifacies*, and therefore the *An. stephensi* data were assumed. Similar patterns of sporozoite transmission between *An. stephensi* and *An. gambiae* have been proposed (Beier et al., 1991). Accordingly, b was set to 0.5 for both the Pakistan and Ethiopian simulations.

4) Infection probability of vectors (c) and relative density of vectors to humans

As in previous vector borne diseases models, the values for the *infection probability of vectors (c)* (e.g. Gu et al., 2003) , and the *relative density of vectors to humans* (N_v/N_h) (e.g. Reithinger et al., 2004) were chosen to produce malaria prevalence levels similar to the observed in the study areas. Also, it was assumed that the density of vectors pre-intervention was at the system's carrying capacity.

5) Malaria prevalence in the study areas

Pakistan: The prevalence of *P. falciparum* infection in people in non-intervention villages during the ITL trial, ranged from 0.3% (village 6, year 1) to 7.8% (village 3, year 2) (M. Rowland, unpublished data). The simulations were conducted using baseline infection prevalence in people of 6%. This higher end range was chosen for three reasons: (1) it fell within the observed prevalence range; (2) however, as the villages chosen for the ITL trial had been subject to recurrent indoor spraying campaigns

(Rowland et al., 2001)¹, the observed prevalence range would on average be lower than expected in the majority of communities not experiencing regular vector control interventions and so, to understand the potential effects of the ITL intervention it is important to examine the higher prevalence settings as that enables quantifying the full potential public health benefits of targeting livestock; (3) from a theoretical perspective, the chosen starting condition allows examining a greater range of prevalence dynamics than starting at the lower end of the observed prevalence range.

Ethiopia: To the best of my knowledge at the date of this study, no data exists on the precise prevalence of *Plasmodium spp.* infection in the Ethiopian setting. However, since the figures were likely to be higher in Ethiopia than in Pakistan, simulations were done with a conservative baseline prevalence of infection in people of 10%. The corresponding predicted prevalence of sporozoite infection in mosquitoes was 0.38 %. This is consistent with field estimates in a village (Fuchucha) of the Ethiopian setting, where Tirados et al. (2006) found that the *P. falciparum* sporozoite prevalence in samples of *An. arabiensis* was 0.38% and 0.18%, for mosquitoes attracted only to human or only to cattle baits, respectively (the overall *P. falciparum* sporozoite prevalence was 0.33%).²

6) Estimation of the duration of gonotrophic cycle (g)

Pakistan: The mean duration of gonotrophic cycle, *g*, for *An. culicifacies* (and *An. stephensi*), was based on a study conducted in the Pakistan Punjab Province near the ITL area, that determined that after the first gonotrophic cycle which may take 4 days, the other cycles took 2 days in Summer (from Aug -Oct), and 3 days in Winter (Nov-Dec) (Mahmood and Reisen, 1981).

Ethiopia: Findings from a study done in Gambella, an Administrative Region west bordering the Region of my study, suggested a 2-days interval between feeding and oviposition for *An. arabiensis* (Krafsur, 1977). Studies in North-Eastern Tanzania demonstrated a 3-days interval for the closely-related *An. gambiae s.s.* (Gillies and

¹ "Only villages whose Annual Parasite Index (incidence per year) had fallen below the threshold for indoor spraying were included in the study by permission of the United Nations High Commissioner for Refugees (UNHCR). In these villages malaria incidence had fallen to its lowest level for 5 years as a result of recurrent indoor spraying campaigns." (Rowland et al., 2001).

² Samples were taken in 2003 (year previous to the field study described in Chapter 2) from the June and July catches, which corresponds to the second half of the peak in density, when mosquitoes might be expected to be older generally and therefore more likely to be infected (Tirados et al. 2006).

Wilkes, 1965) and *An. funestus* (Gillies and Wilkes, 1963). In a later study, also in Gambella, hypothetical sporozoite prevalences have been estimated using a 2 to 3-days interval (Krafsur and Armstrong, 1982).

Accordingly, for both Pakistan and Ethiopia simulations, g was set to 2.5 days.

7) Estimation of the vector natural life expectancy

The average life expectancy of the malaria vectors was derived using the formula (after Davidson, 1954, and Garret-Jones, 1964):

$$\text{average life expectancy} = -1/\ln(p) = -1/\ln(Pr^{1/g})$$

where p is the probability of daily survival, Pr is the proportion of parous female mosquitoes, and g is the mean duration of the gonotrophic cycle.

It is valid to use the proportion parous to determine vector survival providing the following are observed: the population has reached a stationary age distribution; the survival rate is the same for all age groups; different age groups are sampled with similar efficiency; and the gonotrophic cycle duration is known and is constant (Dye, 1992). Although in reality these conditions are rarely met, they are assumed to have been the case, as done in previous zooprophyllaxis models.

8) Density of blood-meal hosts

Since for the purpose of the simulations it is sufficient to use relative density of hosts, a density of 100 persons/hectare was considered, for illustrative purposes. Additionally, for simplicity, it was assumed that one head of cattle was equivalent to one head of sheep/goats or donkey. The implications of assuming that two heads of sheep/goats equals one head of cattle or donkey, or of assuming only cattle (cows+oxen+bulls+calves), were also explored (see Appendix E2 for the resulting values of N_l/N_h , HBI, A_l/A_h , and j).

9) Estimation of the availability of livestock and human hosts

9.1) The *relative availability of livestock to humans* (A_l/A_h) in a given setting can be estimated from the relative abundance of hosts and the HBI, using the formula:

$$\frac{A_l}{A_h} = \frac{N_h}{N_l} \left(\frac{1}{HBI} - 1 \right) \quad \text{Eq. 6. 1}^*$$

Since there were no data on the HBI for the Pakistan ITL trial area, nor for the whole of the Ethiopian study area, the A_l/A_h was estimated using the relative abundance of hosts and HBI from previous studies conducted in the Pakistan Punjab Province near the ITL area (study in 1978 by Reisen and Boreham, 1982), and in the Ethiopian Fuchucha village, within my study area (study in 2003, by Tirados et al., 2006).

9.2) Having the value for A_l/A_h allows also to easily derive the *proportional availability* of each type of host (A_l and A_h) as follows.

$$\text{Let } rA_l = A_l/A_h, \quad \text{Eq. 6. 2}$$

by replacing $A_h = 1 - A_l$ in the above equation for rA_l , and doing algebraic manipulation, then

$$A_l = \frac{rA_l}{1 + rA_l}. \quad \text{Eq. 6. 3}$$

9.3) To transform the proportional availabilities into the *absolute availability* values required for estimating μ_{search} , a *scaling factor* was used (j), as in Chapters 4 and 5. The novelty here is that an analytical expression was derived for j that allows this parameter to be estimated for any setting with known abundance of hosts (N_l and N_h), HBI (which enables estimating the proportional availability of hosts, A_l and A_h), vector biting rate (a), and proportion parous (which enables estimating the natural vector mortality rate, μ_{notx}), and an hypothetical or estimated μ_{min} .

Knowing that

$$\mu_{notx} = \mu_{min} + \mu_{search}, \text{ where } \mu_{search} = \left(\frac{1}{(N_h A_h + N_l A_l) j} \right) a, \quad \text{Eq. 6. 4}$$

by solving for j it is possible to derive the following expression:

$$j = \frac{a}{(N_h A_h + N_l A_l) (\mu_{notx} - \mu_{min})}. \quad \text{Eq. 6. 5}$$

Letting $\mu_{min} = x\mu_{notx}$, the expression for j becomes equivalent to:

* This is the same as Eq. 4.3 in Chapter 4, Section 4.2.1, after Sota & Mogi (1989).

$$j = \frac{a}{(N_h A_h + N_l A_l)(\mu_{notx}(1-x))} \quad \text{Eq. 6. 6}$$

The model used the latter expression (Eq. 6.6), where x is the proportion of the vector natural mortality (μ_{notx}) that is unrelated with searching for a blood-meal host.

10) Vector minimum mortality rate (μ_{min})

The vector mortality rate when there are no hazards due to searching for a blood-meal host (μ_{min}) was conservatively assumed to be half of the average vector natural mortality observed in Pakistan and also in Ethiopia: $x=0.5$, which results in a maximum longevity ($\text{surv}_{\max}=1/\mu_{min}$) of 9.3 days and 16.0 days for *An. culicifacies* and *An. arabiensis*, respectively. A sensitivity analysis was done to assess the impact that alternative μ_{min} could have on the predicted outcomes, by exploring a conservative wider range than the one that is likely to occur in many natural settings: parameter x was varied from 0.1 to 0.99, which corresponds to a maximum longevity ($\text{surv}_{\max}=1/\mu_{min}$) varying from 46.3 to 4.7 days for *An. culicifacies*, and 80 to 8.1 days for *An. arabiensis* (if using the baseline values for μ_{notx} , a , N_h , N_l , A_h and A_l as in Table 6.5 for Pakistan and Table 6.7 for Ethiopia).

11) Estimation of the vector HBI

The HBI for the vector(s) in each setting was predicted with the A_l/A_h above estimated from previous studies (Reisen and Boreham, 1982; Tirados et al., 2006) and the N_l/N_h from the index studies (Rowland et al. 2001; Chapter 2 in this thesis), using the standard formula:

$$HBI = \frac{1}{1 + \frac{N_l A_l}{N_h A_h}} \quad \text{Eq. 6. 7}$$

12) Density-dependent regulation of the adult vector population

An important factor intrinsic to vector borne-diseases is the density-dependent (DD) regulation of the vector population and how that operates. Given the uncertainty about the magnitude of DD constraints acting upon the adult vector population (specifically due to competition for resources at the larval stages) in the Pakistan and Ethiopian settings, the impact of ITL was investigated under three DD scenarios. Simulations were done firstly under a scenario with perfect and instantaneous DD compensation, where N_v remains constant (DD3: achieved by fixing $\rho=\mu$). Secondly, two scenarios of incomplete DD compensation were explored, where N_v was allowed to fluctuate following exposure to ITL (DD1 and DD2: achieved by allowing ρ to differ from μ , using the formula: $\rho=\rho_0-\rho_5N_v$).

Estimation of the density-dependent strength

Pakistan

As an initial attempt to assess the strength of DD effects acting on the vector population following exposure to the ITL, the vector density and parous rates as well as the incidence data from the Pakistan trial were plotted through time (Appendix E1). Unfortunately, the data were collected only on a monthly basis which, being at too low resolution, did not allow inferring the DD strength acting during the trial.

An alternative method was therefore devised, where ρ_0 values were chosen to produce a decrease on vector density and on malaria incidence as close as possible to the observed during the ITL trial, assuming a time delay of 11 days between oviposition and emergence of adult vectors (Marquardt, 2005). These 11 days are the minimum interval between two successive generations of adult mosquito vectors, although this depends on temperature and rainfall (le Sueur and Sharp, 1991; Marquardt, 2005).

To determine the required ρ_0 values a manual fitting was done, with a parameter plot varying ρ_0 (from 0.01 to 0.61) against the outputs of cumulative incidence ratio and vector density ratio calculated during the same time periods as in the trial¹ (Figure E4 in Appendix E3, and Table 6.3).

¹ Incidence ratio was calculated from day 46 to day 168 post start of intervention, corresponding to beginning of September to end of December; vector density ratio was calculated from day 15 to day 137 post start of intervention, corresponding to beginning of August to end of November.

Ethiopia

Since no ITL trial has yet been done in Ethiopia, there is no empirical data against which to fit the model to estimate the DD strength acting on the vector population in a similar way as done for Pakistan. For this reason, and for comparison with the Pakistan simulations, in Ethiopia the ρ_0 for the two DD scenarios with variable N_v was calculated assuming the same time-delay and pre-intervention density-dependent strength (ρ_s) as in the corresponding Pakistan scenarios, but using the vector carrying capacity (K) and pre-intervention mortality ($\mu=\mu_0$) values of the Ethiopian scenario. The values of the Pakistan ρ_s and of the Ethiopia ρ_0 were derived with the formula: $\rho_s=(\rho_0-\mu)/K \Leftrightarrow \rho_0=\rho_s K+\mu$ (Table 6.4).

A sensitivity analysis was done to explore the impact of other ρ_0 values on the model predictions in the Ethiopian setting (Figure E4 in Appendix E3).

6.2.1.2. ITL intervention parameters*1) Insecticide properties*

The probability that vectors are killed due to feeding on ITL on the day of treatment, k , and the duration of the insecticide residual effect were estimated from published bioassays data from Pakistan (Hewitt and Rowland, 1999) and Ethiopia (Habtewold, 2004; Habtewold et al., 2004), as described in Chapter 5. The insecticide used in both settings was deltamethrin. In Pakistan, a solution of deltamethrin was applied uniformly to the whole body of the animals using sponges; in Ethiopia, deltamethrin 1% pour-on with an oil base was applied along the spine of the animals.

2) Intervention effort

The baseline simulations for Pakistan and Ethiopia were done assuming the same intervention effort as in the Pakistan trial: three rounds of treatment were conducted (between mid July and mid October), with 6 weeks interval between rounds, with overall treatment coverage of 93% of all livestock population (cattle, goats, and sheep) (Rowland et al., 2001).

6.2.2. Results

6.2.2.1. Estimation of the duration of the latent period in vectors

Pakistan: The average of the monthly mean temperatures recorded in Peshwara during the three years of the ITL (1995-97) was 22.3 °C, which predicts a latency period of 17.5 days (range: 6.6 days in June to 18.9 days in April).

Ethiopia: The average of the monthly mean temperatures recorded in Karat during the year of my field study (2004) was 23.1 °C, corresponding to a latency period of 15.6 days (range: 11.9 days in March to 19.0 days in June).

6.2.2.2. Estimation of the vector natural life expectancy

Pakistan: The proportion parous was 0.58 (*An. culicifacies*) and 0.51 (*An. stephensi*), as determined by Rowland et al. (2001, complemented with unpublished data), from indoors resting mosquitoes collected in one of the untreated villages, from July to December in the second year of the ITL trial¹. The estimated life expectancies are therefore 4.6 days for *An. culicifacies*, and 3.7 days for *An. stephensi*.

Ethiopia: The value of proportion parous used was 0.732, which is the average of the proportion parous estimated from October 2001 to August 2002, by Taye et al (2006), from indoors and outdoor human landing collections of *An. arabiensis* female mosquitoes in Sille town, near the Konso District. The corresponding life expectancy was 8.0 days.

In both the Pakistan and Ethiopian settings the proportion parous was determined by the authors (Rowland et al. 2001, and Taye et al. 2006, respectively) using the ovarioles tracheolar method of Deltinova (1962).

6.2.2.3. Estimation of the availability of livestock and human hosts

Pakistan: The estimated A_i/A_h was 53.24 for *An. culicifacies* and 295.56 for *An. stephensi* (Table 6.1).

¹ Only the second year of the trial was considered, because in this year a higher number of mosquitoes was dissected than in the first year of the trial when parous rates were also determined.

Ethiopia: From the estimated A_l/A_h of 0.94 it is noticeable that, despite in Fuchucha livestock were ~50% more abundant than humans, the proportional availability of livestock ($A_l=0.48$) is slightly lower than that of humans ($A_h=0.52$) (Table 6.2). This is consistent with the anthropophilic behaviour that has been demonstrated for *An. arabiensis* in the study area (Tirados et al., 2006), and is therefore evidence in favour of using Eq. 6.7 instead of just calculating HBI as a function of N_l/N_h .

6.2.2.4. Estimation of the vector HBI

Pakistan: The predicted HBI for the ITL trial setting (11.8% for *An. culicifacies*, and 2.4% for *An. stephensi*) was more than twice the HBI that had been estimated near the trial study area in the past (4.8% and 0.9%, respectively (Reisen and Boreham, 1982)) (Table 6.1). Such difference may be due to the higher relative density of humans:livestock during the ITL trial setting than during the previous study by Reisen and Boreham (1982).

Ethiopia: The predicted overall HBI for *An. arabiensis* in the study area (49%; 95% CI= 39%-64%; Table 6.2) is concordant with previous estimates of HBI for Fuchucha (~49% in Habtewold, 1999) and for other areas in Ethiopia (~40% in Hadis et al., 1997).

Table 6.1. Estimating the relative and proportional availabilities of livestock and human hosts, vector HBI and natural mortality rate, and availability scaling factor, for *An. culicifacies* and *An. stephensi* in Pakistan.

Parameter	Punjab Province		ITL trial area	
	A.c.	A.s.	A.c.	A.s.
<i>N_i/N_h</i>	0.37^a		0.14^c	
<i>HBI</i>	0.048^a	0.009^a	0.118	0.024
<i>Al/Ah</i>	53.24	295.56	→ 53.24	→ 295.56
<i>Al</i>	0.982	0.997	→ 0.982	→ 0.997
<i>Ah</i>	0.018	0.003	→ 0.018	→ 0.003
<i>g</i>	2.5^b		2.5^b	
<i>Pr</i>	-	-	0.583^c	0.510^c
<i>surv_{notx}</i>	-	-	4.63	3.71
<i>x</i>	-	-	0.5	
<i>j</i>	-	-	0.238	0.220

Figures in bold are from observed data: ^a Reisen & Boreham 1982; ^b Mahmood & Reisen 1981; ^c Rowland et al. 2001. The remaining values were derived as illustrated with the slashed arrows and as explained in the text (section 6.2.1.1.3, Points 7, 9 and 11), except *x* which was hypothetically set to 0.5. The full arrows denote parameters values that were assumed to be the same in the ITL trial area as in the nearby Punjab Province. A.c. = *An. culicifacies*; A.s.=*An. stephensi*.

Table 6.2. Estimating the relative and proportional availabilities of livestock and human hosts, vector HBI and natural mortality rate, and availability scaling factor, for *An. arabiensis* in Ethiopia.

Parameter	Previous studies		Field study	
	nearby/within		setting	
<i>N_i/N_h</i>	1.49^a		1.13 (0.61-1.64)^d	
<i>HBI</i>	0.417^a		0.485 (0.636-0.394)	
<i>Al/Ah</i>	0.938		→ 0.938	
<i>Al</i>	0.484		→ 0.484	
<i>Ah</i>	0.516		→ 0.516	
<i>g</i>	2.5^b		2.5^b	
<i>Pr</i>	0.732^c		0.732^c	
<i>surv_{notx}</i>	8.01		→ 8.01	
<i>x</i>	-		0.5	
<i>j</i>	-		0.060 (0.079-0.049)	

Figures in bold are from observed data: ^a Tirados et al. 2006; ^b Krafstur & Armstrong 1982; Krafstur 1977; ^c Taye et al. 2006; ^d Chapter 2, Table 2.2. The remaining values were derived as illustrated with the slashed arrows and as explained in the text (section 6.2.1.1.3, Points 7, 9 and 11), except *x* which was hypothetically set to 0.5. The full arrows denote parameters values that were assumed to be the same in my field study area as in previous studies in within or nearby areas. *N_i/N_h* is the mean ratio of the number of animals per person calculated from the number of animals/person in each individual household. In the study by Tirados et al. 2006 (a) all the households of the kebele were sampled; while in my field study (d) only some houses in each kebele were sampled, and therefore the sampling weighted mean *N_i/N_h* is presented (the weight of each kebele was calculated dividing the total number of households in a kebele by the number of households interviewed in that kebele). Inside brackets are 95% CI.

6.2.2.5. Estimation of the density-dependent (DD) strength

Table 6.3. Impact of ITL on the malaria incidence ratio and on the vector density ratio for three density-dependent (DD) scenarios, in Pakistan.

DD scenario	Incidence ratio (Sept-Dec)	Vector density ratio (Aug-Nov)	
	0.44 (95%CI 0.22-0.86 p=0.02)	0.54 (0.26-0.97)	Obs.
DD1) $\rho_0=0.2572$	0.05	0.54*	Pred.
DD2) $\rho_0=0.3260$	0.17*	0.94	
DD3) $\rho_0=\mu$	0.43	1.00	

Obs.= Observed data from the Pakistan trial (as published in Rowland et al. 2001): Incidence ratio was calculated from the overall incidence of *P. falciparum* cases per 1000 person-year¹, within each group of treated and untreated villages, adjusted for village and year effects; Vector density ratio is for *An. culicifacies* and was estimated from the monthly catches of resting mosquitoes, comparing density in treated vs. untreated villages².

Pred.= Predicted. The * identifies the variable (incidence ratio or vector density ratio) for which the model was fitted to estimate the ρ_0 value.

DD scenarios - **DD1**: fitted to entomological data from the Pakistan trial; **DD2**: fitted to incidence data from the Pakistan trial; **DD3**: perfect and instantaneous DD compensation of vector density which remains constant.

Intervention consisting of 3 rounds of livestock treatment with insecticide, with 42 days interval, from mid July to mid October, 93% livestock treated in each round; Insecticide with $k=0.85$; $LT_{50}=4$ days and $LT_1=18$ days.

Table 6.4. Density-dependent (DD) scenarios and parameter values for Pakistan vs. Ethiopian simulations.

DD scenario	Pakistan		Ethiopia	
	DD1	DD2	DD1	DD2
μ	0.2160		0.1248	
K	5000		1500	
ρ_0	0.2572	0.3260	0.1372	0.1579
ρ_s	8.25×10^{-6}	2.20×10^{-5}	8.25×10^{-6}	2.20×10^{-5}

All parameters refer to the adult vector population: $\mu=\mu_0$ =overall mortality pre-intervention; K= carrying capacity; ρ_0 =density independent recruitment rate; ρ_s =density-dependent strength pre-intervention. The DD scenarios (DD1 and DD2) are defined in legend of Table 6.3. The impact of the intervention on the malaria incidence ratio and on the vector density ratio for the three density dependence scenarios in Ethiopia is summarized in Table E3 in Appendix E3.

¹ Incidence rate was measured by passive surveillance from records of the village clinics, and consists of the number of all malaria episodes (even if they were recurrent or secondary infections, rather than the number of new infections only) diagnosed during the specified time period.

² Vector collections were done by spraying living rooms and animal sheds of 15 sentinel compounds in each village. Density calculations used the geometric mean density per 10 compounds per village, of the collections done during the first 2 years of the ITL trial.

6.2.2.6. Malaria transmission in Pakistan vs. Ethiopian simulated settings

All the model parameters are listed in Tables 6.5 to 6.8, together with the baseline values and ranges explored in the simulations for Pakistan and Ethiopia. The main differences in the malaria transmission parameters between the Asian and the African simulated settings are as follows. In Ethiopia, livestock were 8.1 times more abundant, although with an estimated 56.8 times lower availability to the main malaria vector, than in Pakistan, resulting in a predicted HBI over 4 times higher in the former than in the latter setting. Additionally, the estimated duration of the latent period in vectors was slightly shorter, while the vector life expectancy was 75% higher in Ethiopia than in Pakistan. The initial density of vectors per human and the probability of infection in vectors were set to be, respectively, 3.3 and 13.6 times higher in Pakistan than in Ethiopia.

Table 6.5. Malaria transmission parameters for the Pakistan ITL setting.

Parameter	Definition	Baseline value	[Range explored]	Unit	Reference
D_{inf}	Average duration of infection in humans	21	[14 to 28]	days	(M. Rowland unpublished data; Gupta et al.1994)
r	Human recovery rate from infection (1/average duration of infection)	1/21	[1/14 to 1/28]	/day	–
T_{lat}	Duration of latent period in vectors (time from ingestion of gametocytes to presence of sporozoites in salivary glands)	17.5	–	days	Derived from temperature data
ω	Rate at which infected mosquitoes become infectious (1/latent period)	1/17.5	–	/day	–
b	Probability that humans become infected from the bite of an infectious vector	0.5	–	–	(Rickman et al. 1990; Verhage et al., 2005)
c	Probability that vectors become infected after biting on an infectious human	0.95	[0.01 to 1]	–	–
g	Duration of vector gonotrophic cycle	2.5	[2-3]	days	(Mahmood & Reisen 1981)
a	Vector biting rate on any host (1/gonotrophic cycle)	1/2.5	[1/2 to 1/3]	/day	–
$surv_{notx}$	Vector natural average life expectancy	4.63	–	days	Derived from data on gonotrophic cycle (Mahmood & Reisen 1981) and proportion parous (Rowland et al. 2001)
μ_{notx}	Vector natural mortality rate (1/natural life expectancy)	1/4.63	–	/day	–
x	Proportion of the vector natural mortality that is unrelated with searching for a bloodmeal host	0.5	[0.10 to 0.99]	–	–
$surv_{max}$	Vector maximum average life expectancy when there are no hazards due to search for a bloodmeal host ($surv_{notx}/x$)	9.27	[46.30-4.68]	days	–
μ_{min}	Vector minimum mortality rate when there are no hazards due to search for a bloodmeal host ($1/surv_{max}$ or $x^*\mu_{notx}$)	1/9.27	[0.03-0.21]	/day	–

Vectors = adult female *An. culicifacies* mosquitoes. Figures in bold are the same for the Pakistan and Ethiopian simulations.

(Continued...)

For D_{inf} , c , g , and n , the specified ranges were explored during the initial fitting of the model, although not shown. For x (also μ_{min} and $surv_{max}$) and ρ_0 (also ρ_s and ρ) the specified ranges were explored in a sensitivity analysis, presented in the main text and Appendix E3, respectively.

Table 6.5. Malaria transmission parameters for the Pakistan ITL setting. (Continued)

Parameter	Definition	Baseline value	[Range explored]	Unit	Reference
ρ	Vector daily average recruitment rate [#] (DD3: $\rho = \mu$; DD1 and DD2: $\rho = \rho_0 - \rho_s \cdot N_v$)	0.2160	–	/day	–
ρ_0	Vector recruitment rate in the absence of density-dependence constraints	0.2160 (DD3) 0.3260 (DD2) 0.2572 (DD1)	[0.01 to 0.61]	/day	–
ρ_s	Strength of the density-dependence in recruitment [#] ($\rho_s = (\rho_0 - \mu) / K$)	0 (DD3) 2.20×10^{-5} (DD2) 8.25×10^{-6} (DD1)	$[-4.12 \times 10^{-5}$ to $7.88 \times 10^{-5}]$	/day	–
n	Initial relative density of vectors:humans (N_v/N_h)	50	[5 to 50]	/hectar	–
K	Carrying capacity of the vector population ($n \cdot N_h$)	5000	[500 to 5000]	/hectar	–
N_h	Human density	100	–	/hectar	–
rN_l	Relative density of livestock:humans ($rN_l = N_l/N_h$)	0.14	–	–	(Rowland et al. 2001)
rA_l	Relative availability of livestock:humans [#] ($rA_l = A_l/A_h$)	53.24	–	–	Derived using data on N_l , N_h , and HBI (from Reisen & Boreham 1982)
A_l	Proportional availability of livestock [#] ($A_l = rA_l / (1 + rA_l)$)	0.980	–	–	Derived from AI
A_h	Proportional availability of humans [#] ($A_h = 1 - A_l$)	0.02	–	–	Derived from AI
j	Factor to scale the proportional availability (into absolute availability) values	0.238	–	–	Derived
q	Human Blood Index (HBI, Proportion of vector bloodmeals with human origin) [#] ($q = 1 / (1 + rN_l rA_l)$)	11.8	–	%	Derived from the rA_l (derived from Mahmood & Reisen 1981) and the rN_l from ITL trial setting (Rowland et al. 2001)

[#] Parameter values pre-intervention that will be affected if livestock are treated with an insecticide with possible repellent properties (except ρ_s , which will be affected by the ITL intervention independently of the repellence probability). **DD= Density dependence scenarios:** **DD1)** fitted to entomological data from the Pakistan trial; **DD2)** fitted to incidence data from the Pakistan trial; **DD3)** perfect and instantaneous DD compensation of vector density which remains constant ($\rho_0 = \mu$).

Table 6.6. Intervention parameters for the Pakistan ITL setting.

Parameter	Definition	Baseline value	[Range explored]	Unit	Reference
Intervention effort					
<i>frequency</i>	Number of treatment rounds	3	[1 to 3]	pulses	(Rowland et al. 2001)
<i>interval</i>	Interval between rounds	42	[30 to 60]	days	
<i>application coverage</i>	Livestock population treated with insecticide in each intervention round	93	[30, 50, 80, 93]	%	
Insecticide properties					
<i>k</i>	Probability that vectors are killed due to exposure to ITL on the day of treatment	0.85	[0.8 to 0.9] [0.1 to 0.9]	–	(Hewitt & Rowland 1999)
<i>LT50</i>	Time from treatment until there is only 50% vector mortality due to exposure to ITL (on bioassay)	4	[4, 9, 13]	days	(Hewitt & Rowland 1999, complemented by M. Rowland's personal communication)
<i>LT1</i>	Time from treatment until there is only 1% vector mortality due to exposure to ITL (on bioassay)	18	[18, 23, 32]	days	
<i>d1</i>	Decay rate of the insecticide residual activity acting until LT50	0.133	[0.133, 0.059, 0.041]	/day	(Derived from Hewitt & Rowland 1999 - see Chapter 5, Section 5.2.5)
<i>d2</i>	Decay rate of the insecticide residual activity acting after LT50	0.279	[0.279, 0.279, 0.206]	/day	
<i>α</i>	Repellence probability: probability that, when attempting to bite an insecticide-treated animal, a vector will be diverted to search another animal or human host	0	[0 to 1]	–	–

Insecticide: Deltamethrin emulsion 2.5% applied uniformly to the whole body of animals using sponges.

Table 6.7. Malaria transmission parameters for the Ethiopian setting.

Parameter	Definition	Baseline value	[Range explored]	Unit	Reference
D_{inf}	Average duration of infection in humans	21	[21 to 35]	days	–
r	Human recovery rate from infection (1/average duration of infection)	1/21	[1/21 to 1/35]	/day	–
T_{lat}	Duration of latent period in vectors (time from ingestion of gametocytes to presence of sporozoites in salivary glands)	15.6	–	days	Derived from temperature data
ω	Rate at which infected mosquitoes become infectious (1/latent period)	1/15.6	–	/day	–
b	Probability that humans become infected from the bite of an infectious vector	0.5	–	–	(Rickman et al. 1990; Verhage et al., 2005)
c	Probability that vectors become infected after biting on an infectious human	0.07	[0.01 to 1]	–	–
g	Duration of vector gonotrophic cycle	2.5	[2-3]	days	(Krafsur 1977; Krafsur & Armstrong 1982)
a	Vector biting rate on any host (1/gonotrophic cycle)	1/2.5	[1/2 to 1/3]	/day	–
$surv_{notx}$	Vector natural average life expectancy	8.01	–	days	Derived from data on gonotrophic cycle (Krafsur 1977; Krafsur & Armstrong 1982) and proportion parous (Taye et al. 2006)
μ_{notx}	Vector natural mortality rate (1/natural life expectancy)	1/8.01	–	/day	–
x	Proportion of the vector natural mortality that is unrelated with searching for a bloodmeal host	0.5	[0.10 to 0.99]	–	–
$surv_{max}$	Vector maximum average life expectancy when there are no hazards due to search for a bloodmeal host ($surv_{notx}/x$)	16.02	[80.10-8.09]	days	–
μ_{min}	Vector minimum mortality rate when there are no hazards due to search for a bloodmeal host ($1/surv_{max}$ or $x^*\mu_{notx}$)	1/16.02	[0.02-0.12]	/day	–

Vectors = adult female *An. arabiensis* mosquitoes. Figures in bold are the same for the Pakistan and Ethiopian simulations.

(Continued...)

For D_{inf} , c , g , and n , the specified ranges were explored during the initial fitting of the model, although not shown. For x (also μ_{min} and $surv_{max}$) and ρ_0 (also ρ_s and ρ) the specified ranges were explored in a sensitivity analysis, presented in the main text and Appendix E3, respectively.

Table 6.7. Malaria transmission parameters for the Ethiopian setting. (Continued)

Parameter	Definition	Baseline value	[Range explored]	Unit	Reference
ρ	Vector daily average recruitment rate [#] (DD3: $\rho = \mu$; DD1 and DD2: $\rho = \rho_0 - \rho_s * N_v$)	0.1248	–	/day	–
ρ_0	Vector recruitment rate in the absence of density-dependence constraints	0.1248 (DD3) 0.1579 (DD2) 0.1372 (DD1)	[0.01 to 0.61]	/day	–
ρ_s	Strength of the density-dependence in recruitment [#] ($\rho_s = (\rho_0 - \mu) / K$)	0 (DD3) 2.20×10^{-5} (DD2) 8.25×10^{-6} (DD1)	$[-7.66 \times 10^{-5} \text{ to } 3.23 \times 10^{-4}]$	/day	–
n	Initial relative density of vectors:humans (N_v / N_h)	15	[5 to 50]	/hectar	–
K	Carrying capacity of the vector population ($n * N_h$)	1500	–	/hectar	–
N_h	Human density	100	–	/hectar	–
rN_l	Relative density of livestock:humans ($rN_l = N_l / N_h$)	1.13	–	–	(Chapter 2)
rA_l	Relative availability of livestock:humans [#] ($rA_l = A_l / A_h$)	0.938	–	–	Derived using data on N_l , N_h , and HBI (from Tirados et al. 2006)
A_l	Proportional availability of livestock [#] ($A_l = rA_l / (1 + rA_l)$)	0.484	–	–	Derived from rA_l
A_h	Proportional availability of humans [#] ($A_h = 1 - A_l$)	0.516	–	–	Derived from A_l
j	Factor to scale the proportional availability (into absolute availability) values	0.060	–	–	Derived
q	Human Blood Index (HBI, Proportion of vector bloodmeals with human origin) [#] ($q = 1 / (1 + rN_l * rA_l)$)	48.5	–	%	Derived from the rA_l (from Tirados et al. 2006), and the rN_l from the field study setting (Chapter 2)

[#] Parameter values pre-intervention, that will be affected if livestock are treated with an insecticide with possible repellent properties (except ρ_s , which will be affected by the ITL intervention independently of the repellence probability). **DD = Density-dependent scenarios: DD1)** fitted to entomological data from the Pakistan trial; **DD2)** fitted to incidence data from the Pakistan trial; **DD3)** perfect and instantaneous DD compensation of vector density which remains constant ($\rho_0 = \mu$).

Table 6.8. Intervention parameters for the Ethiopian setting.

Parameter	Definition	Baseline value	[Range explored]	Unit	Reference
Intervention effort					
<i>frequency</i>	Number of treatment rounds	3	[1 to 3; 6; 12]	pulses	(Rowland et al. 2001)
<i>interval</i>	Interval between rounds	42	[30 to 60]	days	
<i>application coverage</i>	Livestock population treated with insecticide in each intervention round	93	[30, 50, 80, 93]	%	
Insecticide properties					
<i>k</i>	Probability that vectors are killed due to exposure to ITL on the day of treatment	0.9	[0.85 to 0.95] [0.1 to 0.95]	–	(Habtewold 2004)
<i>LT50</i>	Time from treatment until there is only 50% vector mortality due to exposure to ITL (on bioassay)	4	[1, 4, 6]	days	
<i>percm_{ax}</i> (at <i>t_{max}</i> = 14 days)	Vector mortality due to exposure to ITL by day 14 after treatment (on bioassay)	10	[5, 10, 15]	%	
<i>d1</i>	Decay rate of the insecticide residual activity acting until LT50	0.147	[0.588, 0.147, 0.098]	/day	(Derived from Habtewold 2004; see Chapter 5, Section 5.2.5)
<i>d2</i>	Decay rate of the insecticide residual activity acting after LT50	0.161	[0.177, 0.161, 0.150]	/day	
<i>α</i>	Repellence probability: probability that, when attempting to bite an insecticide-treated animal, a vector will be diverted to search another animal or human host	0	[0 to 1]	–	–

Insecticide: Deltamethrin 1% pour-on with an oil base applied along the spine of animals.

6.3. Simulations

6.3.1. Methods

The fitted model was initially applied to simulate the impact of a baseline ITL intervention scenario on malaria in the Pakistan and Ethiopian settings. In the baseline scenario in both settings livestock were treated with a non-repellent insecticide under the same intervention regimen as that implemented in the Pakistan community trial, except that in the Ethiopian baseline scenario a different insecticide formulation was used, specific to the African setting. Additionally, the baseline scenario assumed that vector population density remained constant.

Secondly, a comprehensive sensitivity analysis was performed for both Pakistan and Ethiopia to explore the impact of varying intervention effort (livestock treatment coverage; number and interval between treatment rounds) and insecticide properties (killing, repellence, and duration of residual effect upon vectors). The effect of varying the proportion of vector mortality that is unrelated with search for a blood-meal host was also analysed. To assess the impact of varying the DD strength acting on the vector population the scenario of constant vector density was compared with two other scenarios where vector density varied following exposure to ITL.

Thirdly, to assess whether the impact of ITL on malaria in the Ethiopian setting could be improved, the baseline intervention regimen was compared with three other plausible regimens, under each of the three DD scenarios. Two sets of simulations were done: firstly, using a non-repellent insecticide, and secondly, using an insecticide with possible repellent properties.

Finally, comparisons were made between the predicted impact of ITL in the Pakistan and in the Ethiopian settings, for different repellence probability, under the three DD scenarios.

The model equations were solved by numerical integration using the Berkeley Madonna™ package (Macey and Oster, 2006), with the built-in method *fourth order Runge-Kutta*. Simulations were done introducing one infectious human onto a fully susceptible

population of humans and mosquitoes, and running until the endemic equilibrium. Once the endemic equilibrium was reached, the impact of different regimens of ITL in several outcomes of malaria transmission was explored. All simulations used the baseline parameter values from Tables 6.5 to 6.8, unless otherwise stated.

The main outcome explored was the variation in incidence of *P. falciparum* malaria cases per 1000 persons, expressed as cumulative incidence ratio,¹ which is defined as the incidence rate predicted under a given intervention regimen divided by the incidence rate predicted under no intervention. Additionally, simulations were done for the temporal effects of the intervention on the point incidence and prevalence of malaria cases, as well as on a range of entomological parameters: adult vector population survival, recruitment, density (N_v), biting rate on humans (HBR), sporozoite prevalence and entomological inoculation rate (EIR= number of potentially infectious bites received per human, per unit of time).

In all the simulations where comparisons were made between the impact of ITL in Pakistan and in Ethiopia (Sections 6.3.2.1, 6.3.2.2. and 6.3.2.4), the outcome cumulative incidence ratio was calculated over the same time period as in the published trial in Pakistan (from 1.5 to 5.5 month after the intervention start, corresponding to the beginning of September to end of December), to allow comparison with the published trial results. Also, this time period reflects the seasonal transmission of malaria in the Pakistan trial setting. Conversely, in the simulations that compare different ITL regimens in Ethiopia only (Section 6.3.2.3), the outcome cumulative incidence ratio was calculated over one year after the intervention start, to reflect the perennial transmission of malaria in the Ethiopian study setting.

¹ Both in the published Pakistan ITL trial (Rowland et al. 2001) and in the simulations in this thesis, incidence rates were calculated dividing the total number of malaria cases occurring during a given period by the total human population. This therefore corresponds to what in epidemiology is designated as *crude incidence*, as opposed to *cumulative incidence rate*, where the denominator is the number of persons initially disease free. The main outcome measure presented here is designated as cumulative incidence ratio, to highlight the fact that it was calculated from the sum of the number of all cases of malaria occurring during a given period. Note, however, that the incidence ratio is the same, irrespectively of using crude or cumulative incidence rates on its calculation, because the denominators of the incidence rates (total population, for crude rates; or number of persons initially disease free, for cumulative rates) will cancel out each other.

6.3.2. Results

6.3.2.1. Baseline simulations of the ITL intervention in Pakistan and Ethiopia

The predicted impact of the baseline ITL intervention in the Pakistan and Ethiopian settings is presented in Figures 6.1 and 6.2, respectively. The baseline simulations assume that the intervention effort was the same in both settings: three rounds of livestock treatment with deltamethrin, separated by 42 days, with 93% of all livestock population treated in each round (application coverage). Due to differences in the deltamethrin concentration and formulation between the settings, the insecticide effect differed slightly: in Pakistan, vector mortality due to exposure to ITL was 85% on treatment day, decreasing to 50% after 4 days, and 1% after 18 days; while in Ethiopia vector mortality due to exposure to ITL was 90% on treatment day, decreasing to 50% after 4 days, and 10% after 14 days. Additionally, it was assumed that the insecticide has no repellent effect upon vectors, and that vector population density remains constant.

Note that the proportional impact of the intervention on the EIR and incidence of human malaria cases is the same if the plots of these outcome measures are scaled accordingly, since the expressions that define these outcomes are a proportion of each other:

$$\text{EIR} = (N_v/N_h) a q s; \text{ Incidence of human malaria cases} = \text{EIR} b S_h.$$

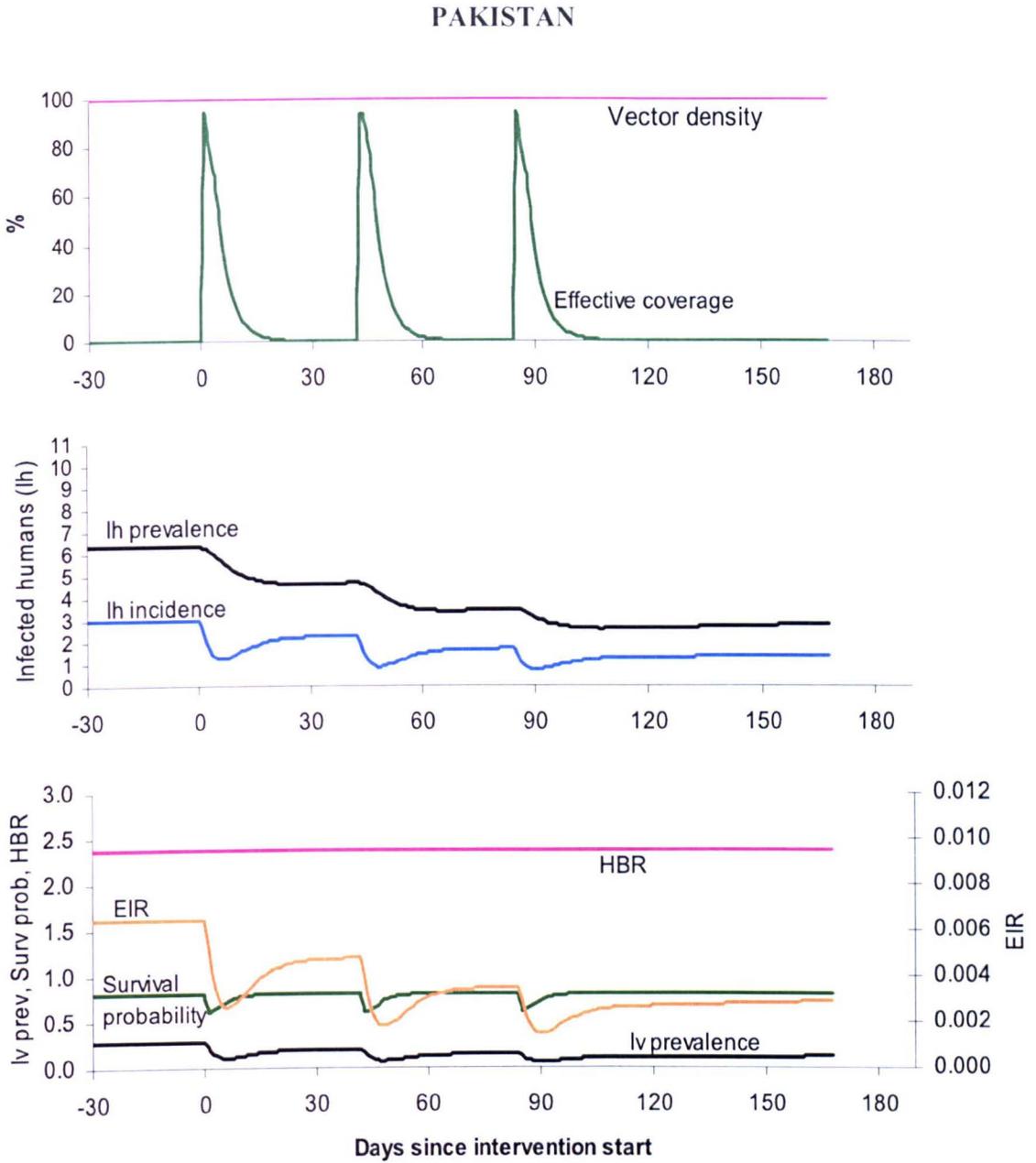


Figure 6.1. Impact of the baseline ITL intervention in Pakistan with a non-repellent insecticide and constant vector population density.

Effective coverage = percentage of the total livestock population with active insecticide at a given point in time. **Epidemiological outcomes:** I_h prevalence = point prevalence of malaria infections in humans (%); I_h incidence= incidence of malaria infections per 1000 persons/day. **Entomological outcomes** all respective to adult *Anopheles culicifacies* mosquitoes: Vector density expressed as a % of the pre-intervention density; HBR= human biting rate/day; Surv. prob = probability of daily survival; I_v prevalence = point prevalence of sporozoite infection (%); EIR=entomological inoculation rate (infectious bites per person per day).

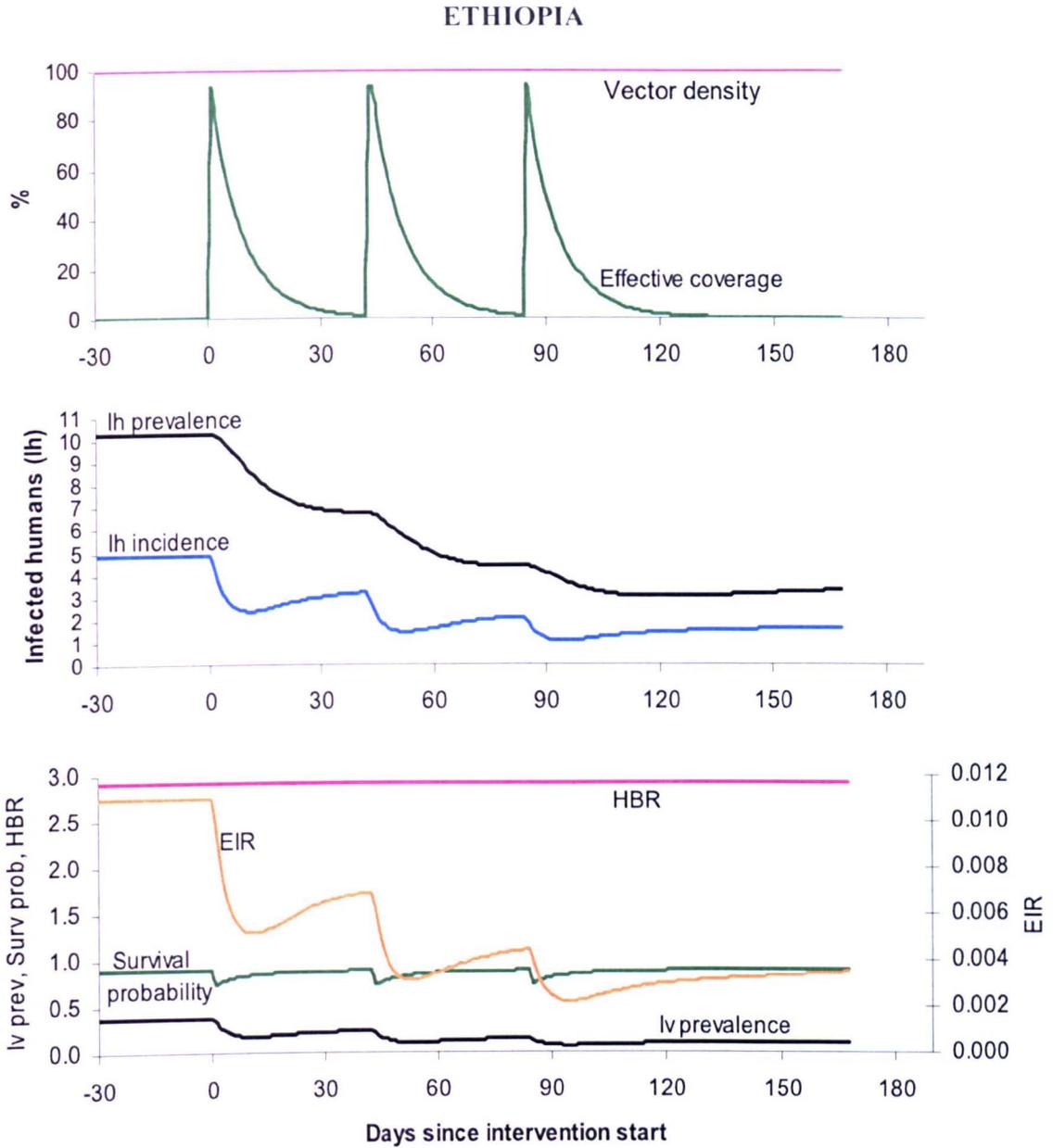


Figure 6.2. Impact of the baseline ITL intervention in Ethiopia, with a non-repellent insecticide and constant vector population density.

Effective coverage = percentage of the total livestock population with active insecticide at a given point in time. **Epidemiological outcomes:** I_h prevalence = point prevalence of malaria infections in humans (%); I_h incidence = incidence of malaria infections per 1000 persons/day. **Entomological outcomes** all respective to adult *Anopheles arabiensis* mosquitoes: Vector density expressed as a % of the pre-intervention density; HBR = human biting rate/day; Surv. prob = probability of daily survival; I_v prevalence = point prevalence of sporozoite infection (%); EIR = entomological inoculation rate (infectious bites per person per day).

6.3.2.2. Sensitivity analysis for Pakistan and Ethiopia

Based on the baseline simulations, manual sensitivity analysis was done for several intervention parameters and also for the density-dependent strength acting on the vector population, by varying the parameter values and assessing the impact on the predicted incidence ratio. The impact on the EIR ratio was also simulated, but it is not presented because it was only slightly stronger than the impact on the incidence ratio.

The sensitivity analysis was initially done for the intervention parameters values that were uncertain - insecticide *killing* effect upon vectors and *duration of residual activity* -, by varying them within the likely¹ ranges of the insecticide used in the Pakistan trial and in Ethiopia. The impact of varying the insecticide killing effect within a wider range was also explored. Additionally, the *intervention effort* was varied, namely: the percentage of the livestock population treated with insecticide in each round (*application coverage* = 93%, 80%, 50% and 30%); the *interval* between consecutive rounds of treatment (varied from 1 month to 2 months); and the *number of treatment rounds* in a year (decreased from 3 to 2 or 1 round). The implications of treating livestock with an insecticide that has a potential *repellent* action upon vectors were also extensively investigated, as well as the effect of varying the proportion of vector mortality that is unrelated with search for a blood-meal host.

Finally, I looked at the effects of *vector density dependence* on the impact of the ITL intervention, by comparing the scenario where vector density remains unchanged with two other scenarios where vector density will vary following exposure to treated livestock.

6.3.2.2.1. Insecticide killing effect and duration

From the available bioassay data, there is a greater uncertainty about the confidence interval of the duration of the insecticide residual effect used in Pakistan than in Ethiopia, and this is reflected in Figure 6.3. In the Pakistan simulated scenario the incidence ratio is considerably sensitive to the duration of the insecticide effect, but only marginally sensitive to the insecticidal effect (k) on the day of treatment, when these

¹ Ranges estimated from published bioassays, as detailed in Chapter 5, Section 5.2.5.1.

parameters are varied within the likely ranges of the insecticide used in the Pakistan trial (Figure 6.3A). Conversely, in Ethiopia, the incidence ratio shows a small sensitivity to variations in both the killing effect and duration of the insecticide within the likely ranges of the insecticide used in the Ethiopian setting (Figure 6.3B).

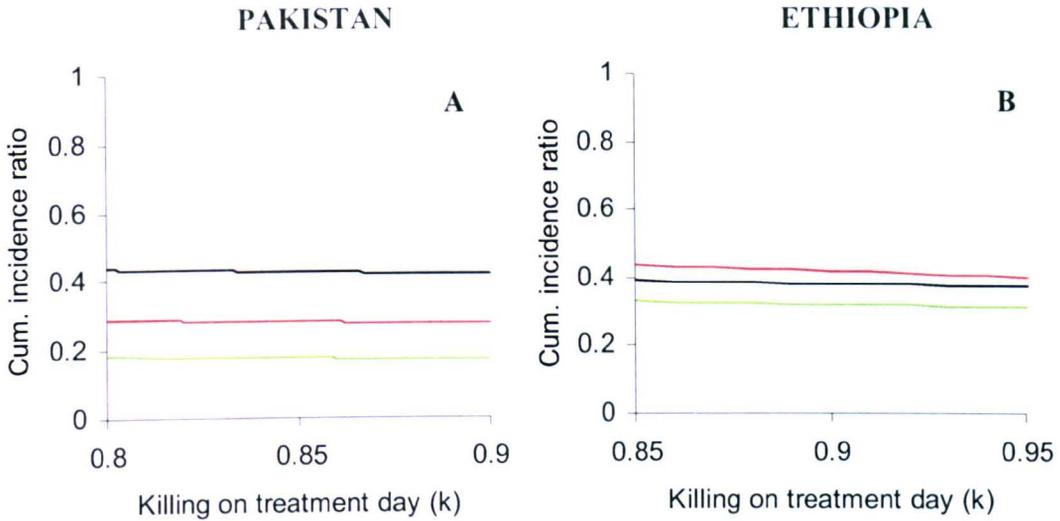


Figure 6.3. Sensitivity analysis for the effects of the insecticide killing effect (k) and duration of residual activity on the malaria cumulative incidence ratio.

Pakistan: black: LT50=4; LT1=18 (baseline); red: LT50=9; LT1=23; green: LT50=13; LT1=32;
 Ethiopia: black: LT50=4; LT10=1 (baseline); red: LT50=1; LT5=14; green: LT50=6; LT15=14
 LTz=Lethal time; i.e. days from insecticide application until there is only z % vector mortality due to exposure to insecticide-treated livestock (on bioassay).

6.3.2.2.2. *Insecticide killing effect, interval between treatments, and coverage*

In both Pakistan and Ethiopia, when using a non-repellent insecticide, for a given value of the insecticide killing effect or for a given interval between treatment rounds, the higher the treatment coverage the lower is the predicted incidence ratio. Additionally, the higher the treatment coverage, the stronger is the sensitivity of the incidence ratio to changes in the insecticide killing effect and interval between treatments.

The sensitivity of the incidence ratio to the insecticide killing effect becomes noticeable when k varies in a wider range than in Figure 6.3. The higher the insecticide killing effect the lower the incidence ratio.

Irrespective of the treatment coverage, reducing the interval between the three rounds of ITL treatments from 42 to 30 days would cause virtually no benefit in the number of malaria cases prevented. On the other hand, increasing the interval from 42 to 60 days would result in a slight reduction in impact (simulations not shown).

6.3.2.2.3. *Insecticide repellence probability*

For any treatment coverage, the stronger the repellence, the higher is the incidence ratio (Figure 6.4). Also, the higher the treatment coverage, the stronger is the sensitivity of the incidence ratio to variations in the repellence probability. Importantly, the impact of repellence on malaria incidence varies non-linearly with increase in coverage. Specifically, there seems to be a threshold repellence level above which treating livestock with insecticide results in a higher number of malaria cases (incidence ratio > 1) than when no livestock are treated.

Under the conditions modelled, up to repellence ~ 0.6 in Pakistan and ~ 0.75 in Ethiopia, the higher the treatment coverage, the higher the number of cases prevented with ITL, while the opposite is predicted to occur for repellence above that level - the higher the treatment coverage, the higher the number of cases occurring with ITL. For high (e.g. 93%) treatment coverage of livestock with an insecticide with very strong repellent properties (e.g. 95%), the incidence of malaria cases following ITL could double in Pakistan and be up to 1.3 times higher in Ethiopia compared to no intervention.

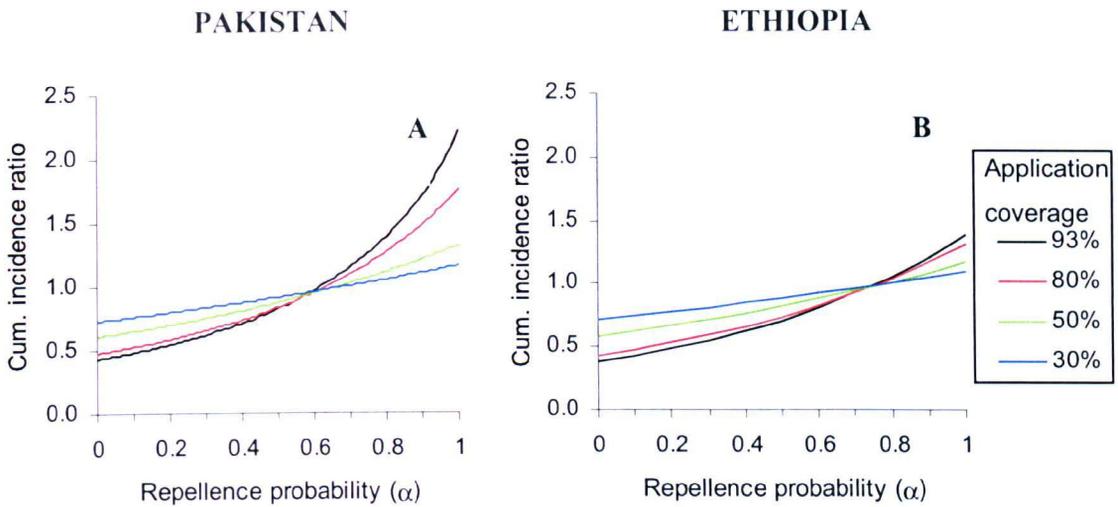


Figure 6.4. Impact of varying the insecticide repellence probability on the malaria cumulative incidence ratio for various treatment coverage levels.

Other parameters as in baseline simulations: 3 treatment rounds separated by 42 days; Pakistan: $k=0.85$; $LT_{50}=4$; $LT_1=18$; Ethiopia: $k=0.90$; $LT_{50}=4$; $LT_{10}=14$. LT_z =Lethal time; i.e. days from insecticide application until there is only z % vector mortality due to exposure to insecticide-treated livestock (on bioassay).

6.3.2.2.4. Repellence vs. Number of treatment rounds

The fewer the treatment rounds the lower is the sensitivity of the incidence ratio to changes in the insecticide repellence probability (as well as to changes in the insecticide killing effect). Only a slight reduction in impact was predicted when comparing 3 vs. 2 rounds, or 2 vs. 1 round of treatment. Interestingly, the threshold repellence probabilities remained approximately the same for an intervention with 3, 2 or 1 rounds of treatment (simulations not shown).

6.3.2.2.5. Repellence vs. Duration of insecticide

The same threshold repellence probability was predicted when varying the duration of the insecticide residual activity (within the likely ranges of the insecticide used in Pakistan and Ethiopia, assuming the baseline initial treatment coverage of 93%), and also when varying the initial coverage (from 30% to 93%) assuming the baseline duration of the insecticide residual activity (simulations not shown).

6.3.2.2.6. Repellence vs. Vector minimum mortality rate

One of the most uncertain parameters used in the model is the *vector minimum mortality rate* ($1/\text{vector maximum life expectancy}$), which was assumed to be a proportion $x=0.5$ of the observed average mortality rate in the absence of insecticide-treated livestock ($\mu_{min}=x\mu_{notx}$). Despite there being uncertainty about the true value of the parameter x , its magnitude only affects the impact of the intervention if the insecticide has repellent properties. Accordingly, a sensitivity analysis was also performed (Figures 6.5 and 6.6) to assess the effect of varying x on the impact of an ITL intervention with an insecticide with possible repellent properties.

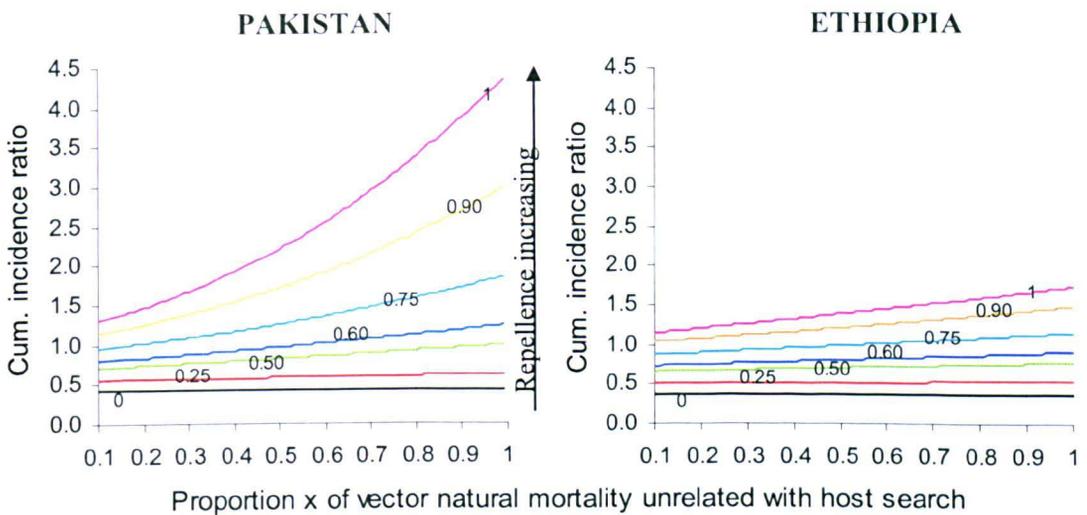


Figure 6.5. Impact of varying the vector minimum mortality rate (determined by the parameter x) on the malaria incidence ratio, for various levels of insecticide repellence.

Repellence probability (α) increasing from bottom to top: the values assumed in each scenario are shown next to the corresponding line; for instance, the horizontal black line illustrates a non-repellent insecticide ($\alpha=0$ as in the baseline simulations). Note that ($\mu_{min}=x\mu_{notx}$). The baseline simulations assume $x=0.5$. Other parameters were kept as in baseline simulations, namely: application coverage=93%; 3 rounds of treatment with 42 days interval.

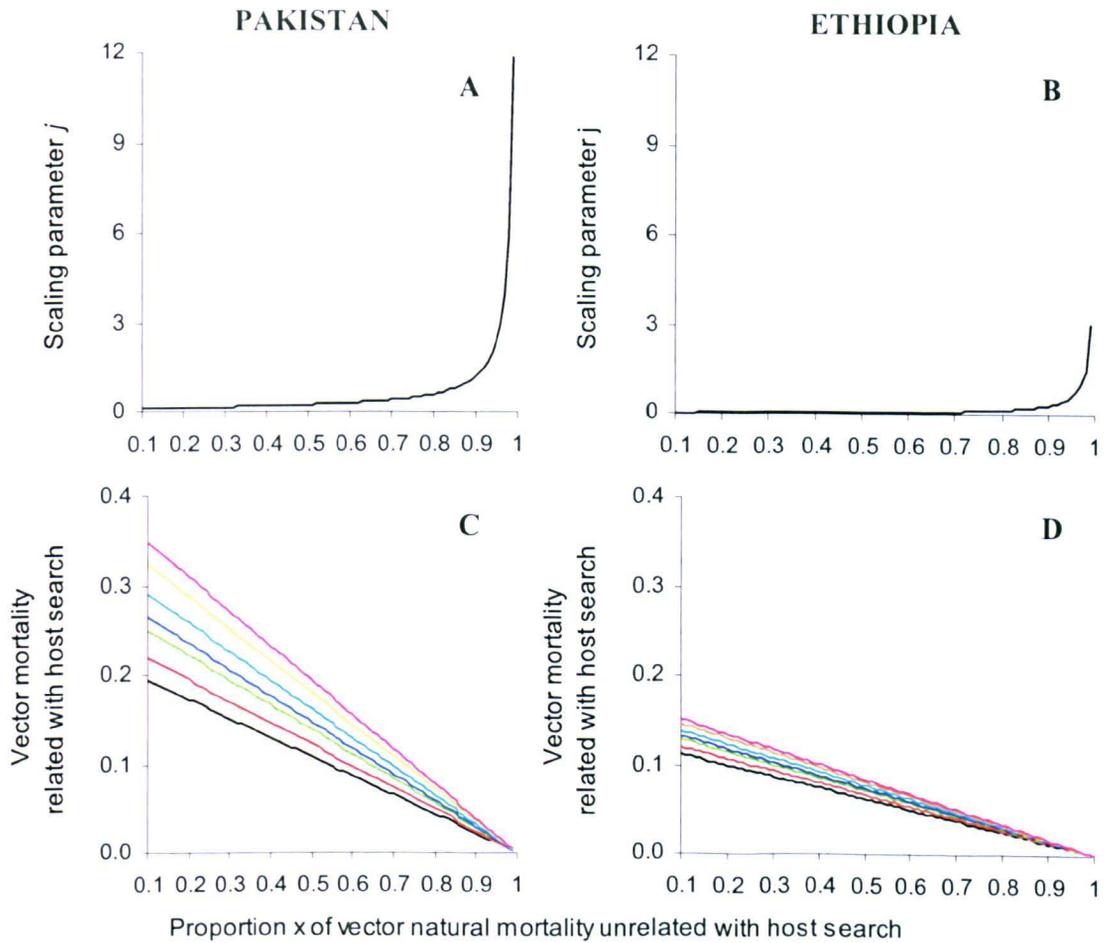


Figure 6.6. Impact of varying the vector minimum mortality rate (determined by the parameter x) on the parameter j and on the search-related vector mortality for various levels of the insecticide repellence probability.

(A&B) Note that the value of j is independent of the percentage of effectively treated livestock or insecticide repellence [$j = a / ((N_h A_h + N_v A_v) (\mu_{natv} (1-x)))$]. (C&D) Assumes 50% of effectively treated livestock. Repellence probability (α) increasing from bottom to top - Black: 0; Red: 0.25; Green: 0.5; Dark blue: 0.6; Light blue: 0.75; Orange: 0.90; Pink: 1.

For a given maximum survival of the vector (or minimum mortality rate, determined by the parameter x), the stronger the repellence probability, the smaller will be the beneficial impact of the intervention (i.e. the higher will be the incidence ratio). When repellence is above 50% in Pakistan or above 70% in Ethiopia, the intervention could produce a considerable increase in the number of cases, depending on the x value. When repellence is above 75% in Pakistan or 90% in Ethiopia, the model predicts that the number of cases post intervention would always be higher than before, independently of the x value (Figure 6.5). Additionally, the stronger the repellence, the stronger will be the sensitivity of the intervention impact to changes in the value of the parameter x (i.e. to changes in the vector maximum survival, or minimum mortality rate) (Figure 6.5).

This can be explained by looking closer at the expression for vector mortality derived in section 5.2.3.1:

$$\mu = \underbrace{\mu_{\min} + \frac{\overbrace{a}^{\mu_{\text{search}}}}{(N_h A_h + (1 - \epsilon \alpha) N_l A_l) j}}_{\mu_{\text{notx}}} + \underbrace{\frac{\epsilon(1 - \alpha) N_l A_l}{N_h A_h + N_l A_l} ka}_{\mu_{\text{txonly}}}$$

and the considerations given in section 6.2.1.1.3 (point 9.3):

Letting $\mu_{\min} = x \mu_{\text{notx}}$, the expression for j becomes equivalent to:

$$j = \frac{a}{(N_h A_h + N_l A_l) (\mu_{\text{notx}} (1 - x))}$$

where x is the proportion of the vector natural mortality (μ_{notx}) that is unrelated with searching for a blood-meal host.

The higher the x value, the higher the vector minimum mortality rate (μ_{\min}) and the higher the value of j required to obtain a given observed μ_{notx} (Figure 6.6A&B). Higher j values result in smaller search-associated vector mortality (Figure 6.6C&D). Thereby, increases in the x value will counteract the only benefit of repellence (which was an increase on the search-associated vector mortality), and consequently decrease the predicted beneficial impact of an ITL intervention, as shown in Figure 6.5 and Figure 6.6C&D. Note that repellence has about half the impact on the incidence ratio in Ethiopia than in Pakistan (Figure 6.5), which can be explained by the fact that

repellence has about half the impact on μ_{search} in Ethiopia than in Pakistan (Figure 6.6C&D).

6.3.2.2.7. Density-dependent (DD) regulation of the vector population

6.3.2.2.7.1. Effects of DD on the baseline simulations of the ITL intervention

Simulations were performed to assess how the impact of the baseline ITL intervention in two scenarios of incomplete density-dependent compensation of the adult vector population (DD1 and DD2, where N_v will vary following intervention) would differ from the previous scenario that assumed perfect and instantaneous density-dependent compensation (DD3, where N_v remains unchanged). The results for Pakistan and Ethiopia are presented in Figures 6.7 and 6.8, respectively.

Note that the same overall vector mortality is acting in the three DD scenarios, and consequently the impact of the intervention on vector survival probability and life expectancy is the same for the three DD scenarios. However, the scenarios differ in the recruitment response of adult vectors, ρ , which causes different effects on vector population density, N_v , and consequently different impact on the incidence of malaria cases. In scenario DD3, following each intervention round, ρ increases to immediately and perfectly compensate the decrease in the life expectancy, $1/\mu$, and thereby N_v remains unchanged. Conversely, in scenarios DD1 and DD2, N_v will vary because only 11 days after each intervention round will ρ increase to compensate the decrease in life expectancy.

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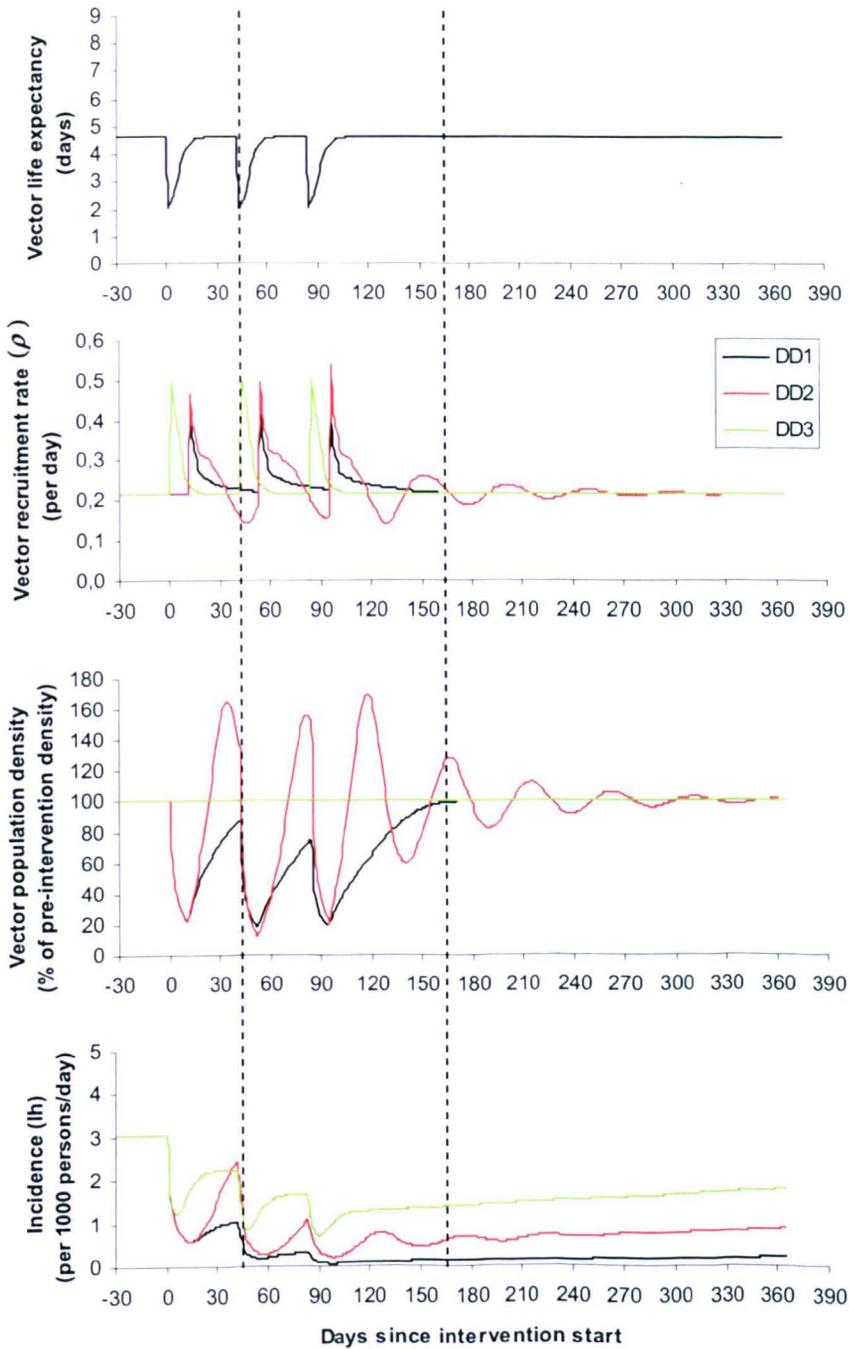


Figure 6.7. Comparing the impact of the baseline intervention with a non-repellent insecticide on malaria transmission through time under three density-dependent (DD) scenarios in Pakistan.

Vector = adult female *An. culicifacies* mosquitoes.

DD1: fitted to entomological data from the Pakistan trial; **DD2:** fitted to incidence data from the Pakistan trial; **DD3:** perfect and instantaneous DD compensation of vector density which remains constant ($\rho_0 = \mu$ as in the baseline simulations). The slashed vertical lines delimit the four month interval over which the cumulative incidence ratio was calculated (days 46 to 168 post start of intervention) in the published ITL trial conducted in Pakistan (Rowland et al. 2001).

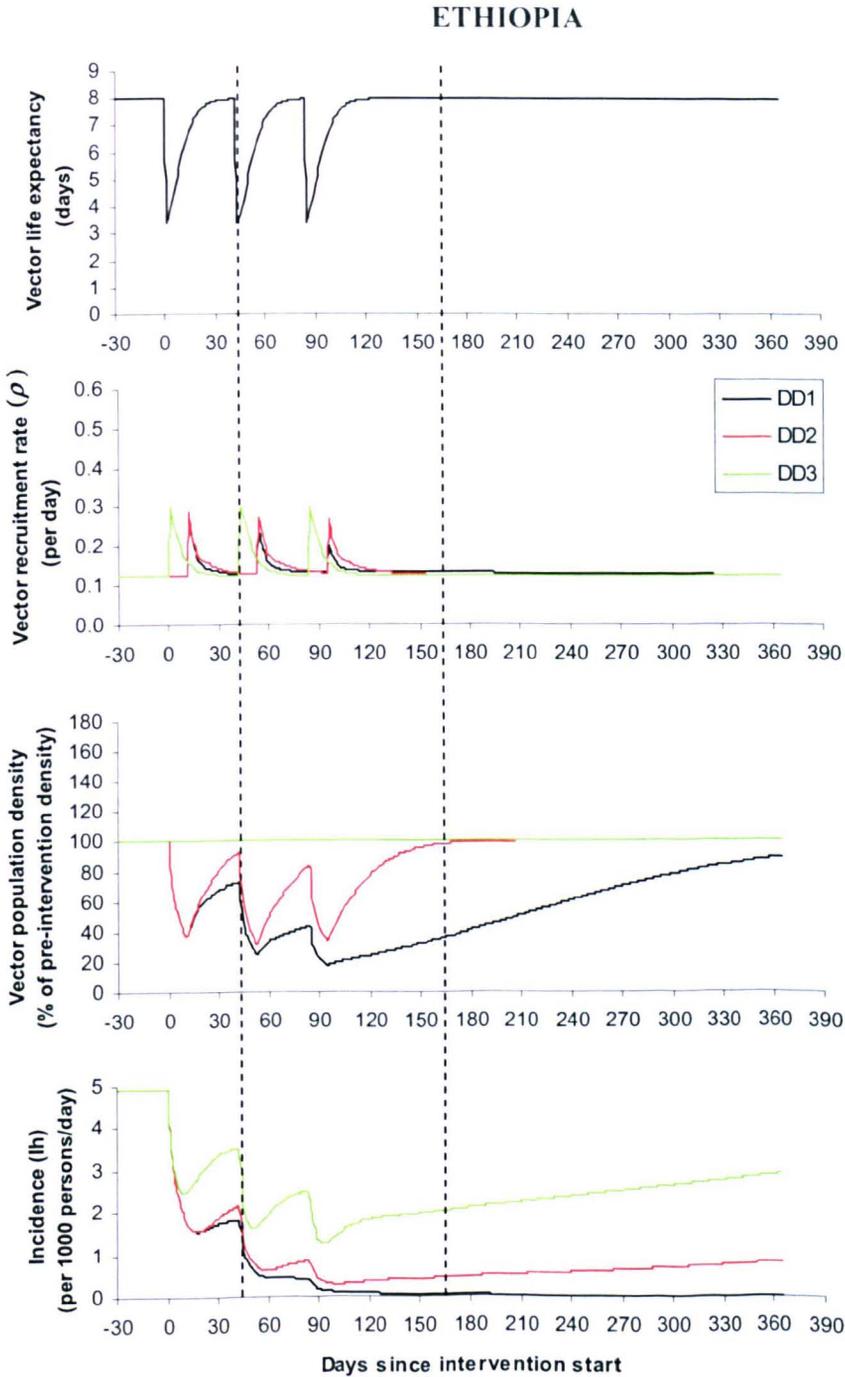


Figure 6.8. Comparing the impact of the baseline intervention with a non-repellent insecticide on malaria transmission through time under three density-dependent (DD) scenarios in Ethiopia.

Vector = adult female *An. arabiensis* mosquitoes.

See footnote of Figure 6.7 for definition of DD scenarios and vertical slashed lines.

6.3.2.2.7.2. Effect of DD on the sensitivity of the intervention to the insecticide repellence probability

Exploring repellence thresholds

I then assessed whether the repellence thresholds observed under DD3 (Figure 6.4) remained under scenarios DD1 and DD2 (Figure 6.9).

In the Pakistan scenarios DD1 and DD2, where N_v is allowed to vary following the intervention, the threshold repellence is no longer observed as in the scenario DD3, where N_v remained constant. There is however, another non-linear behaviour (Figure 6.9A&C). While in scenario DD3 increasing repellence resulted always in a decrease in the beneficial impact of ITL on incidence independently of treatment coverage (Figure 6.4A), in scenarios DD1 and DD2 for high treatment coverage there is a repellence level above which that trend is inverted, and the beneficial impact of ITL starts increasing. The treatment coverage and repellence levels above which that inversion occurs are lower under scenario DD1 (where the average N_v is almost halved by the intervention), than under DD2 (where the average N_v is only marginally affected by the intervention). On the other hand, in the Ethiopian scenarios with variable N_v , there are threshold repellence probabilities above which, the higher the treatment coverage, the higher is the number of cases occurring due to ITL, although these thresholds occur at a higher repellence level (DD1: $\alpha \approx 0.95$; DD2: $\alpha \approx 0.90$, Figure 6.9B&D) than in the scenario with constant N_v (DD3: $\alpha \approx 0.75$, Figure 6.4B).

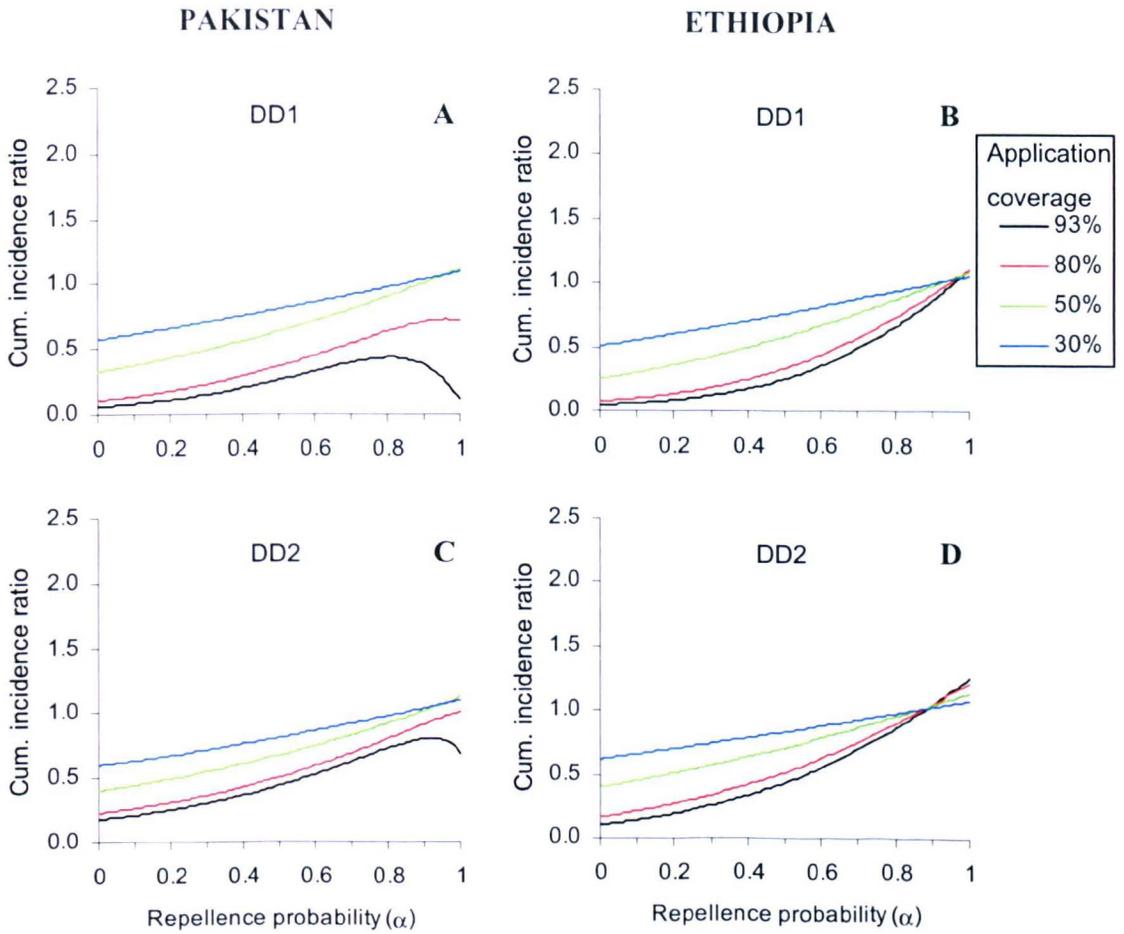


Figure 6.9. Impact of varying the insecticide repellence probability on the malaria incidence ratio for various treatment coverage levels and two density-dependence (DD) scenarios, in Pakistan (A&C) and in Ethiopia (B&D).

DD1: fitted to entomological data from the Pakistan trial; **DD2:** fitted to incidence data from the Pakistan trial. Other parameters as in baseline simulations: application coverage =93%; 3 rounds of treatment with 42 days interval; Pakistan: $k=0.85$; $LT_{50}=4$; $LT_{10}=18$; Ethiopia: $k=0.90$; $LT_{50}=4$; $LT_{10}=14$.

6.3.2.2.7.3. Effect of DD on the interaction between repellence probability and vector minimum mortality rate

Simulations were also performed to assess the effects of varying the vector minimum mortality rate (determined by parameter x) upon malaria incidence ratio for a range of repellence probability under the three DD scenarios. In general, both in Pakistan and Ethiopia, the benefits of ITL will always be lower in scenario DD3 than in scenarios DD1 and DD2, irrespectively of the insecticide's repellence probability and of the x value. Similarly to the predictions for scenario DD3, also in scenarios DD1 and DD2 the intervention could potentially considerably increase the number of malaria cases, depending on the x value, but only for $\alpha > 60\%$ in Pakistan or $\alpha > 80\%$ in Ethiopia (simulations not shown), while in DD3 it was for $\alpha > 50\%$ in Pakistan and $\alpha > 60\%$ in Ethiopia (Figure 6.5). In all of the three DD scenarios, the higher the repellence probability, the higher is the sensitivity of the intervention outcome to changes in the minimum mortality rate of malaria vectors.

6.3.2.3. Comparing the outcomes of the baseline ITL intervention regimen in Ethiopia with three alternative regimens

This section examines whether the impact of ITL on malaria in the Ethiopian setting could be improved, by comparing the baseline intervention regimen explored in the previous section with three other plausible regimens, under each of the three DD scenarios.

The parameter values used in each intervention regimen are given in Table 6.9. While the baseline (**Regimen 0**) consists of applying the intervention effort used in the Pakistan trial (treating 93% of livestock, 3 times, with 42 days interval) and the insecticide used during the Ethiopian field study presented in Chapter 2 (deltamethrin 1% pour-on), the alternative regimens consist of the following:

Regimen 1: mimicking not only the intervention effort but also the insecticide used in the Pakistan trial (deltamethrin emulsion 2.5% applied with sponging), which had a slightly smaller killing effect and duration than the Ethiopian insecticide;

Regimen 2: mimicking the intervention effort and the insecticide that were being applied during the Ethiopian field study¹ – i.e. increasing the annual treatment frequency from 3 times with 42 days interval to 6 times with 30 days interval, and decreasing the application coverage from 93% to 50%; and

Regimen 3: using the Ethiopian insecticide, but increasing the annual treatment frequency to 12 times with 30 days interval, as recommended for control of tsetse flies and animal trypanosomiasis (Vale et al., 1999), with a conservative application coverage of 50%.

The different intervention regimens were compared based on the number of malaria cases predicted over one year after the intervention start.

¹ In the Ethiopian setting, the most reported frequency of insecticide treatment of livestock was once a month, therefore a *30 days interval* was used. This frequency tended to be the case only during a government program (part of the Southern Regions Rift Valley tsetse eradication project) that was providing free insecticide and lasted 6 months (February to July 2006; source: interview to the Konso Veterinary officers). After the end of the government program, some livestock owners might still have been able to individually purchase the insecticide, and therefore continue treating their livestock. However, since these further treatments would have occurred on a less systematic way, they were not considered for the purpose of the simulations, which considered only the *6 pulses of monthly treatment*.

6.3.2.3.1. *Insecticide with no repellence*

Figure 6.10 shows the predicted impact of treating livestock with a non-repellent insecticide on the incidence of malaria cases one year following the start of treatment, for the baseline and three alternative intervention regimens, under the three DD scenarios explored in Ethiopia. The cumulative percentage of prevented malaria cases during the same time period is summarized in Table 6.9.

The results suggest that the impact of treating livestock with a non-repellent insecticide in the Ethiopian setting could be more beneficial if the treatment frequency was increased to 12 times per year (regimen 3), under scenario DD3 (24%, 14% and 12% more prevented cases than in regimens 1, 0 and 2, respectively). Interestingly, the baseline regimen 0 remained the best intervention scheme under scenarios DD2 (15%, 13% and 4% more prevented cases than in regimens 1, 2 and 3, respectively), and DD1 (5% more prevented cases than in regimens 1, 2 and 3). Under DD3, regimen 2 performed similarly (2% more prevented cases) to the baseline regimen. Additionally, under DD3 and DD2, regimen 3 performed considerably better (12% and 9% more prevented cases, respectively) than regimen 2, while regimen 1 was the intervention that would prevent the smallest number of cases. Under DD1, regimens 1, 2, and 3 were predicted to have precisely the same impact.

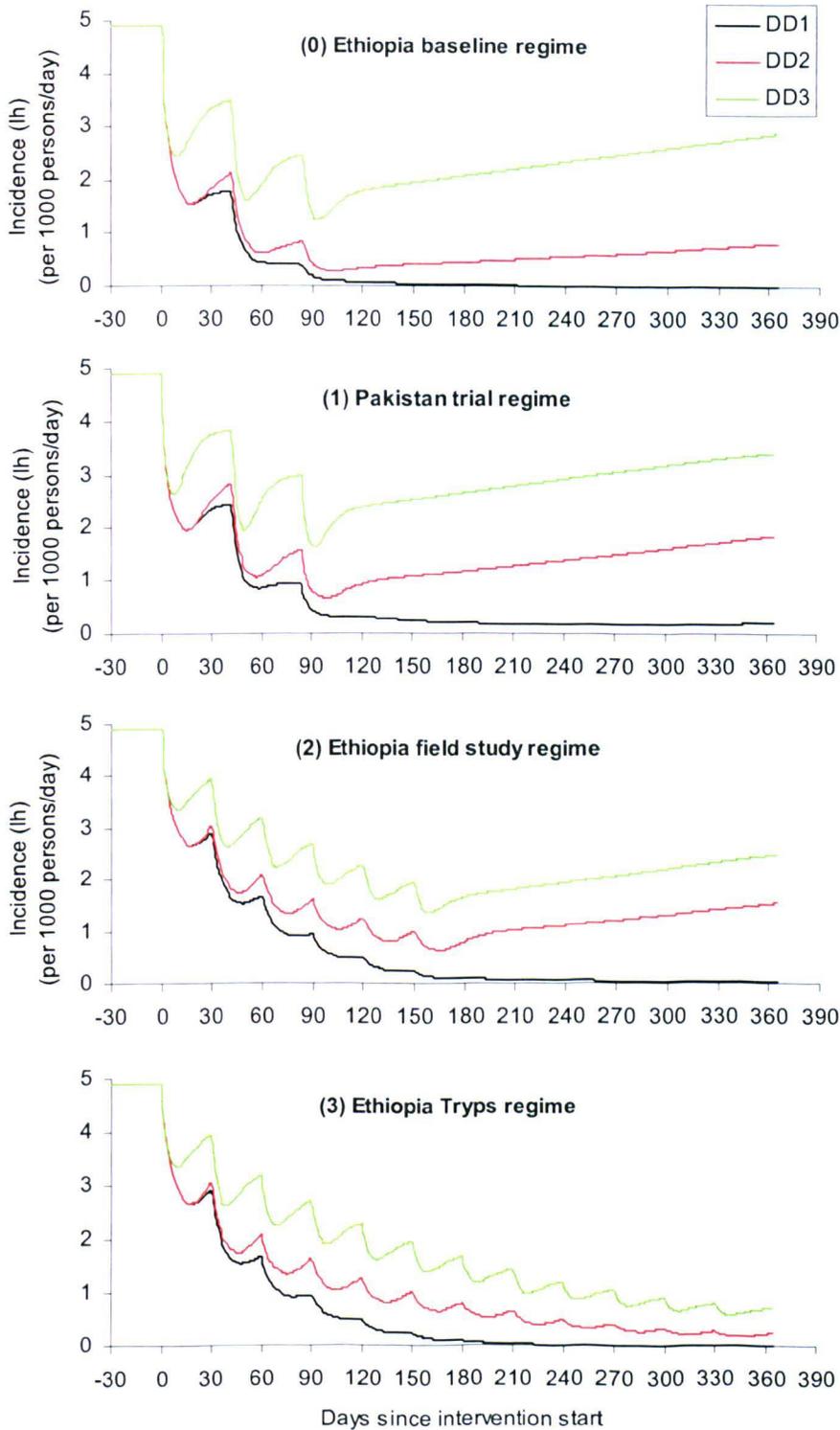


Figure 6.10. Impact of treating livestock with a non-repellent insecticide on the incidence of malaria cases in Ethiopia, for several intervention regimens and three density-dependent (DD) scenarios.

DD1: black - fitted to entomological data from the Pakistan trial; **DD2:** red - fitted to incidence data from the Pakistan trial; **DD3:** green - perfect and instantaneous DD compensation of vector density which remains constant. See Table 6.9 for parameters values used in each intervention regimen.

Table 6.9. Impact of treating livestock with a non-repellent insecticide on the percentage of prevented malaria cases in Ethiopia, for several intervention regimens and three density-dependent (DD) scenarios.

Intervention regimes		(0) Ethiopia baseline	(1) Pakistan trial						(2) Ethiopia field study	(3) Ethiopia trys
Effort	Frequency	3 pulses	3 pulses	6 pulses	*	*	*	*	6 pulses	12 pulses
	Interval	42 days	42 days	*	30 days	*	*	*	30days	30days
	Coverage	93%	93%	*	*	50%	*	*	50%	50%
Insecticide	Killing probability (k)	0.9	0.85	*	*	*	0.9	*	0.9	0.9
	Duration	14 days (LT10)	18days (LT1)	*	*	*	*	14 days (LT10)	14 days (LT10)	14 days (LT10)
Prevented Cases	DD scenarios									
	DD3	52%	42%	61%	42%	26%	43%	51%	54%	66%
	DD2	85%	70%	85%	72%	38%	72%	83%	72%	81%
	DD1	93%	88%	91%	91%	55%	89%	93%	88%	88%

k= Probability that vectors are killed due to exposure to ITL on the day of treatment; LT= Lethal time; LT50, LT10, LT1 = time from treatment until there is only 50%, 10%, and 1%, respectively, vector mortality due to exposure to insecticide. LT50=4 days in all the intervention scenarios. * Intervention parameter values kept as in the Pakistan trial (regimen 1).

Percentage of prevented cases = (1-Annual Cumulative Incidence Ratio)×100.

DD scenarios - DD1: fitted to entomological data from the Pakistan trial; **DD2:** fitted to incidence data from the Pakistan trial; **DD3:** perfect and instantaneous DD compensation of vector density which remains constant.

6.3.2.3.2. *Insecticide with possible repellent properties*

Figure 6.11 shows the predicted impact of the four regimens of livestock treatment in the Ethiopian setting accounting for possible repellence of the insecticide. The baseline intervention (regimen 0) was predicted to be the best under DD1 irrespectively of the repellence probability (α). Under DD2 the baseline regimen performed similarly to regimen 3, with either of these two regimens being the best, up to $\alpha < 80\%$. Under both DD1 and DD2 regimen 3 performed better than regimen 2 (or regimen 1), up to $\alpha < 80\%$. For DD1, regimen 1 would also provide greater benefit than regimen 2 for $\alpha > 60\%$, and even greater benefit than regimen 3 if $\alpha > 80\%$. Under scenario DD3 regimen 3 would be the best intervention for α varying from null to 70%.

Overall, the effects of ITL on the annual cumulative malaria incidence in the Ethiopian setting would only become null or deleterious (incidence ratio ≥ 1) if the insecticide has repellence probability $> 73\%$ (DD3), $> 82\%$ (DD2), or $> 89\%$ (DD1) (Figure 6.11 and Table 6.10). In these circumstances, the intervention with least deleterious effects would be either regimen 1 or 0 (in DD1 and DD2), or regimen 1 or 2 (in DD3) (Figure 6.11).

The repellence probability above which ITL starts having a deleterious impact is approximately the same for the intervention regimens 1 and 0, and for regimens 2 and 3. In the two latter regimens, the threshold repellence probability is slightly lower than in the former two, under scenarios DD1 and DD2; on the other hand, the reverse occurs under DD3 (Figure 6.11, summarized in Table 6.10).

Note that these threshold repellence probabilities have values similar to the threshold repellence above which an inversion was observed in the relation between intervention coverage and the effects of treatment on malaria incidence. As seen previously, for repellence $\sim > 0.75$ (DD3, Figure 6.4B), > 0.90 (DD2; Figure 6.9D), or > 0.95 (DD1; Figure 6.9B), the higher the intervention coverage the higher became malaria incidence (calculated over a 4 month period). This indicates that there is a threshold repellence level above which, increasing the intervention effort (application coverage and/or frequency and/or insecticide killing effect and duration) results in a potential increase in malaria incidence.

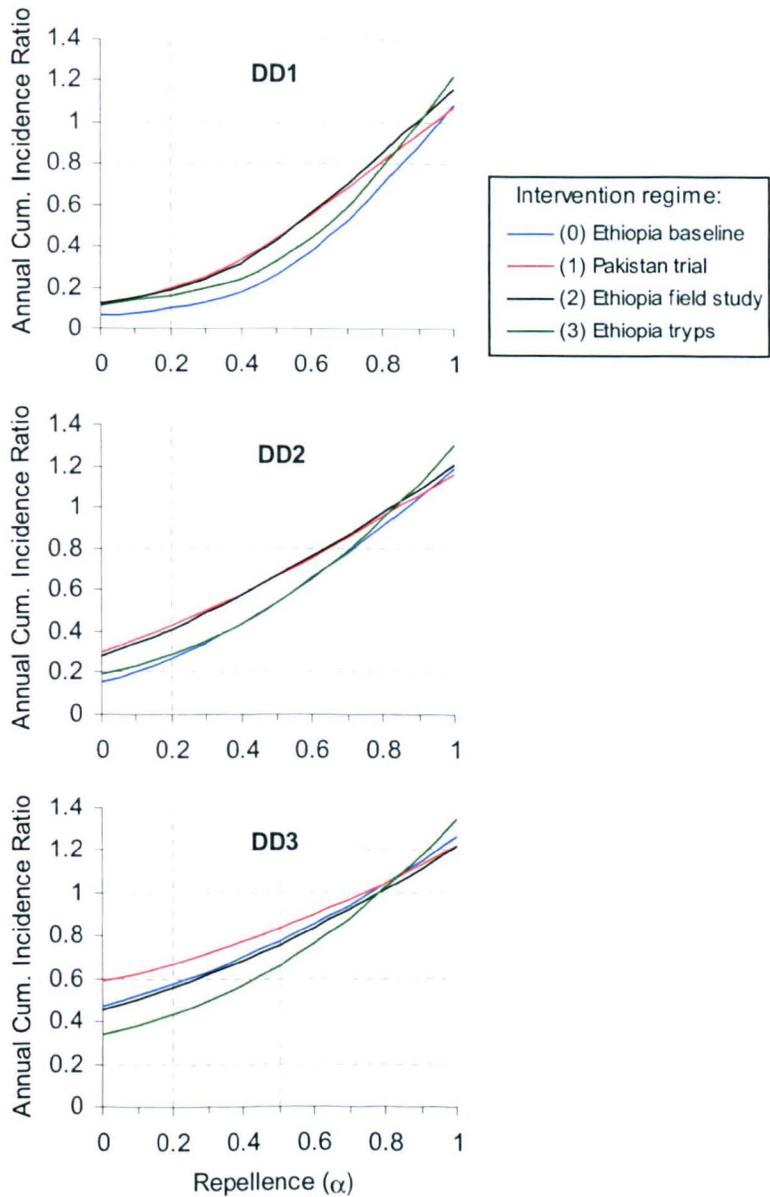


Figure 6.11. Comparing the outcomes of the baseline livestock treatment regimen with three alternative intervention regimens, for different repellence probabilities of the insecticide under three density-dependent (DD) scenarios in Ethiopia.

DD scenarios - DD1: fitted to entomological data from the Pakistan trial; **DD2:** fitted to incidence data from the Pakistan trial; **DD3:** perfect and instantaneous DD compensation of vector density which remains constant. See Table 6.9 for parameters values used in each intervention regimen.

Table 6.10. Repellence probability above which ITL starts having a negative impact on the annual cumulative malaria incidence in Ethiopia.

Transmission scenario	Ethiopia			
	(0) Ethiopia baseline	(1) Pakistan trial	(2) Ethiopia field study	(3) Ethiopia tryps
DD scenario				
DD1	>0.95	>0.94	>0.89	>0.90
DD2	>0.86	>0.84	>0.82	>0.83
DD3	>0.75	>0.73	>0.77	>0.78

Density dependence scenarios - **DD1**: fitted to entomological data from the Pakistan trial; **DD2**: fitted to incidence data from the Pakistan trial; **DD3**: perfect and instantaneous DD compensation of vector density which remains constant. See Table 6.9 for parameters values used in each intervention regimen.

6.3.2.4. Comparing the effects of ITL on malaria in Pakistan and Ethiopia (Regimen 2 and 1)

Comparisons were made between the predicted impact of ITL in the Pakistan trial and in the Ethiopian setting (regimen 2), for different repellence probability under the three DD scenarios (Figure 6.12).

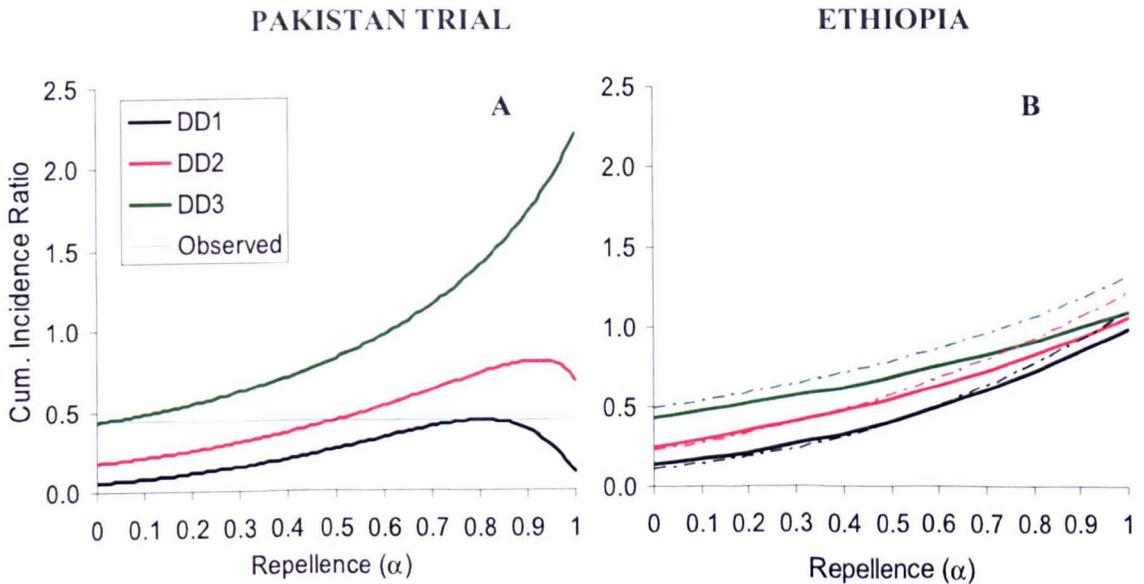


Figure 6.12. Impact of varying the repellence probability of the insecticide, on the malaria incidence ratio*, for three density-dependent (DD) scenarios, in Pakistan (A) and Ethiopia (B).

(A) Transmission parameters, intervention effort and insecticide as in the Pakistan trial (Rowland et al. 2001). The horizontal blue line marks the incidence ratio = 0.44 observed in the Pakistan trial.

(B) Transmission parameters as in the Ethiopian setting; intervention effort and insecticide as in the Ethiopian setting (Regimen 2 - full lines), vs. as in the Pakistan trial (Regimen 1 - dashed lines). See Table 6.9 for parameters values used in each intervention regimen.

DD scenarios - **DD1**: fitted to entomological data from the Pakistan trial; **DD2**: fitted to incidence data from the Pakistan trial; **DD3**: perfect and instantaneous DD compensation of vector density which remains constant.

* Cumulative incidence ratio calculated over a 4 month period, from 1.5 to 5.5 months post intervention start.

Table 6.11. Repellence probability above which ITL starts having a negative impact on the cumulative malaria incidence*, for three density-dependent (DD) scenarios, in Pakistan and Ethiopia.

Transmission scenario	Pakistan	Ethiopia
Intervention regime	(1) Pakistan trial	(2) Ethiopia field study
DD scenario		
DD1	NA	NA
DD2	NA	>0.95
DD3	>0.60	>0.90

See Table 6.9 for parameters values used in each intervention regimen.

DD scenarios - DD1: fitted to entomological data from the Pakistan trial; **DD2:** fitted to incidence data from the Pakistan trial; **DD3:** perfect and instantaneous DD compensation of vector density which remains constant.

NA = Non applicable because incidence ratio is always ≤ 1 for any repellence probability.

* Cumulative incidence ratio calculated over a 4 month period, from 1.5 to 5.5 months post intervention start.

6.3.2.4.1. Pakistan

The simulations for Pakistan summarized in Figure 6.12A suggest that, only under a scenario of constant vector density (DD3 – green line) would treating livestock with a non-repellent insecticide have resulted in the cumulative malaria incidence ratio observed in the Pakistan trial (0.44). When the model was fitted with entomological data (DD2 – red line), a repellence probability of 50% was required to reproduce the observed incidence ratio; the required repellence probability increased to 80% when the model was fitted with incidence data (DD1 – black line). Therefore, according with the model predictions, *the likely repellence probability of the insecticide used in Pakistan would have been between 50% and 80%*, depending on the fitting that best reflects reality.

6.3.2.4.2. Pakistan vs. Ethiopia

Irrespectively of the repellence probability, under *scenarios DD1 and DD2 a stronger reduction in incidence was predicted in Pakistan*, for an ITL intervention similar to the published trial, *than in Ethiopia*, for an intervention similar to the one in practice during the field study (regimen 2). On the contrary, under scenario DD3 the reverse was predicted (Figure 6.12).

The results further suggest that, *in Pakistan*, under scenarios DD1 and DD2 *the intervention is predicted to have always a positive effect, i.e. reduce malaria incidence, independently of the repellence probability*. Conversely, under scenario DD3 the intervention could produce a negative effect, increasing malaria incidence, but only if repellence probability is above 60% (Figure 6.12A, and Table 6.11).

By contrast, *in Ethiopia*, an intervention similar to the one in practice during the field study *was predicted to have a negative effect on incidence for very high repellence probabilities, under scenario DD2 ($\alpha > 90\%$) or DD3 ($\alpha > 95\%$)* (Figure 6.12B and Table 6.11). Note that in Ethiopia, if the cumulative incidence ratio is measured over one year after intervention start (instead of over a 4 months period as in Figure 6.12), then the predicted threshold repellence levels would be decreased to $\alpha = 89\%$ (DD1), 82% (DD2), and 77% (DD3) (Figure 6.11 and Table 6.10).

I also accessed how much of the predicted differences in the impact of ITL in the Ethiopian setting (regimen 2) vs. in the Pakistan trial could be assumed to be simply due to differences in the underlying transmission scenarios, namely due to differences in: livestock abundance and availability, vector density and survival, and parasite extrinsic incubation period (listed in Tables 6.5 and 6.7, and summarized in Section 6.2.2.6). For that, I looked at the predicted impact of a scenario in the Ethiopian setting where an ITL intervention was conducted with the same effort and insecticide as in the Pakistan trial (regimen 1), for various repellence probabilities. The results are shown in Figure 6.12B, dashed lines).

Finally, I explored the relative contribution of each intervention parameter to the predicted differences in the impact of treating livestock in Ethiopia with the effort and insecticide as in the Pakistan trial (regimen 1) vs. as in the Ethiopian field study (regimen 2). For that, the intervention parameter values of the Pakistan trial were varied, one by one, to match the values of the intervention undergoing during the Ethiopian field study, assuming a non-repellent insecticide (see shaded columns in Table 6.9).

In all the DD scenarios, the impact of the intervention on the annual cumulative malaria incidence showed little sensitivity to small changes in the insecticide killing effect and in the interval between treatments, while it was highly sensitive to the duration of the insecticide residual effect, and to the coverage and frequency of treatments. Scenario DD1 showed, however, relatively less sensitivity to changes in insecticide duration and frequency of treatments than the other two DD scenarios.

Under the DD3 scenario, the parameters that are most likely to have driven the higher beneficial impact of ITL in Ethiopia predicted for an intervention with the effort and insecticide as in the Ethiopian field setting (54% prevent cases in regimen 2) compared to an intervention as in the Pakistan trial (42% in regimen 1) are, therefore, the increased duration of the insecticide residual effect (vector mortality due to exposure to ITL increased from 1% by day 18 to 10% by day 14), and doubling the frequency of treatment rounds. In the other two DD scenarios the difference in impact in regimen 2 compared to regimen 1 was marginal (72% vs. 70% in DD2) or null (DD1).

6.4. Discussion

The aims of the present study were to assess: (1) whether the ITL intervention conducted in Pakistan could be improved; (2) the impact of applying in Ethiopia a similar ITL intervention as in Pakistan; and (3) the impact of alternative ITL intervention regimens in Ethiopia. For this purpose, the model developed in the previous Chapters was fitted to *P. falciparum* malaria transmitted by *An. culicifacies* and *An. arabiensis* in the Pakistan and Ethiopian settings, respectively. The fitted model was then used to explore the effects that different regimens of treatment of livestock with an insecticide with a lethal and possible repellent action upon malaria vectors could have on the disease transmission dynamics in each of the settings, for three scenarios of density-dependent regulation of the adult vector population.

When assuming that livestock were treated with a *non-repellent insecticide*, only under the DD scenario where vector density remains constant (DD3) was it possible to reproduce the reduction in malaria incidence observed in the Pakistan trial (Rowland et al., 2001). This has been the DD scenario assumed in most of previous zooprophylaxis models (except in Sota and Mogi, 1989; and in Kawaguchi et al., 2004), and also in other general malaria models. It is the simplest assumption, thereby facilitating the modelling process and being useful for initial explorations. However, this scenario is unrealistic because it assumes that there is always immediate and precise density-dependent compensation of the adult vector population, while evidence is to the contrary. For example, in Pakistan, a 46% reduction in vector density was estimated following the ITL trial (Rowland et al., 2001).

Accordingly, I made the model more realistic by decreasing the DD constraints (i.e. by setting ρ_0 different than μ), and therefore allowing vector density to decrease following

the intervention. As a consequence, a stronger reduction in malaria incidence was predicted than in the previous scenario of constant vector density, and than the observed in the Pakistan trial (when assuming a non-repellent insecticide). Thus, in scenario DD2, where a ρ_0 value was chosen to produce a decrease in malaria incidence as similar as possible to the observed in the Pakistan trial, the model still predicts a stronger decrease in incidence (83%) than observed (56% [95% CI 14%-78% $p=0.02$]). This does not seem to be due to a reduction in vector density on its own, because although vector density does vary in this scenario, the average effect on vector density is minimum: N_v ratio=0.94 which is approximately the same as in pre-intervention and as following the intervention under DD3 where N_v ratio=1.00. Despite the predicted average effect on vector density appears to be far from the point estimate of the observed effect (N_v ratio=0.54), the prediction still falls within the confidence interval for the observed effect (95% CI=0.26-0.97). On the other hand, in scenario DD1, where the ρ_0 value was chosen to fit the model to vector density, precisely the same reduction on density was predicted as the observed, although this resulted on an even stronger reduction in malaria incidence (95%) than the observed.

Therefore, assuming that the insecticide used in the Pakistan trial had no repellent properties, scenarios DD2 and DD1 overestimated the observed effect of ITL on malaria incidence, and scenario DD2 also seems to underestimate the observed effect on vector density.

The differences between the observed and predicted results for the effect of ITL on malaria incidence and vector density in Pakistan are not due to increased vector mortality in DD1 or DD2 compared to DD3, because mortality is the same in the three scenarios. Moreover, it is not because scenarios DD1 and DD2 are less correct than scenario DD3; on the contrary, the former two scenarios, which allow for a decrease in vector density due to the exposure to ITL, are more realistic than the latter scenario, which assumes that vector density remains unchanged. On the other hand, since vector density is one of the most difficult parameters to accurately estimate in the field, one cannot rule out the possibility that the true effect of ITL on vector density in the Pakistan field trial might have been smaller than estimated. Additionally, note that the above considerations on the predicted results assume that the insecticide used in the Pakistan trial had no repellent properties. However, the insecticide used might have had some repellence action that was only evident under the trial conditions, while having been undetected in the experimental bioassay (Hewitt and Rowland, 1999). In the

bioassay the possibility of diversion of host-seeking vectors from ITL to humans was assessed by mosquito landing catches in men seating 2 meters from a cow outdoors, from dusk until midnight. Although the bioassay showed no evidence of increased diversion of host-seeking mosquitoes from insecticide-treated cattle to nearby men at any stage after treatment, it is possible that mosquitoes might be repelled and feed on men further away after some recovery from sub-lethal poisoning. Indeed, when assuming an *insecticide with possible repellent* properties, the repellence probability required to reproduce the reduction in malaria incidence observed in the Pakistan trial was estimated to range between 50% (for DD2) and 80% (DD1), depending on the fitting that best reflects reality.

Considerations should also be given on certain features that were not included in the present model but that could be incorporated in futures extensions of this work, in particular: (a) potential heterogeneities in the vector feeding behaviour; (b) seasonality; (c) acquired immunity; and (d) livestock demography.

a) The model assumes *perfect and complete mixing of the vector population*. However, in reality, there may be only partial mixing of the vector population, since there may be a subpopulation of vectors that feeds preferentially on livestock and other subpopulation feeding preferentially on humans, as discussed in Chapter 4 (Section 4.3.4). If this was the case, then applying insecticide on livestock would kill the vectors that feed mostly on livestock, while having a smaller impact on the vectors that feed more on humans. Accordingly, the model could be extended to look at the potential impact of ITL in scenarios of heterogeneous vector feeding behaviour (e.g. see: Dye and Hasibeder, 1986; Burkot, 1988; Hasibeder and Dye, 1988; Woolhouse et al., 1997; Kelly and Thompson, 2000; Smith et al., 2004; Smith et al., 2007).

b) It has been hypothesized that by targeting vector control interventions to timings when mosquito density is low may facilitate lowering even more the already low mosquito densities (Smith et al., 1995). Similarly, this could be explored by introducing *seasonality* in the model, using standard procedures for vector-borne diseases models, as mentioned in Chapter 4 (Section 4.3.4). For example, this would enable investigating whether treatment of livestock during a four week round in the season when mosquito densities are lower would be enough to produce a longer lasting decrease in malaria transmission that would persist along the transmission season. Therefore, the

incorporation of seasonality into the malaria model could also provide useful insights into the best timing of the intervention, with respect to the vector breeding and malaria transmission season.

c) *Acquired immunity* was not included in the model since the main focus of the work was to investigate the effect of livestock and its treatment with insecticide on malaria, and not investigate the complex effects of acquired immunity on malaria transmission and the outcomes of infection. Additionally, in the Pakistan ITL trial area, the low transmission levels might not have been sufficient for the development of significant acquired immunity to malaria infections. Similarly, in the Ethiopian setting, although malaria transmission was higher than in Pakistan, a very high proportion (58%) of the clinical malaria cases occurred in the over 15 years old age-group (source: Karat Health Centre, Konso), suggesting that the adult population was not immune to infection.

As acquired immunity was not accounted for, the present model is likely to be more applicable to scenarios with low malaria transmission. Future work could consider incorporating the effects of acquired immunity in the theoretical framework here presented, which could allow a more accurate investigation of the effect of the intervention in settings of medium-high malaria transmission, where acquired immunity has been shown to play a stronger role in determining the outcomes of a malaria infection (Doolan et al., 2009).

d) For simplicity, the numerical simulations undertaken have assumed a constant and closed population of both humans and livestock. However, *livestock demography* (birth, death or sold) does impact the proportion of animal population with residual insecticide at a given point in time. Therefore, future simulations could account for variability in livestock demography, in accordance with different agro-pastoralist scenarios.

Contrary to previous zoonophylaxis models which considered only cattle, the present work had additionally accounted for other types of livestock (sheep/goats and donkeys/horses) since these may also act as alternative sources of bloodmeal for malaria vectors, and the model was fitted to empirical studies done in Pakistan and Ethiopia where other animals besides cattle were also treated with insecticide. In Pakistan all of the above mentioned animal types were treated, while in Ethiopia, mostly cattle but also some sheep and goats were treated.

For simplicity, it was assumed that one head of cattle was equivalent to one sheep or goat or donkey. The relative availability of each of these animal types may however be different, not only because different animal types may be kept in different locations (at different distances from people's sleeping room and from vector breeding sites), but also because the vector's feeding preference and even feeding success may differ between animal types. For example, a study in Pakistan has found that, despite malaria vectors preferred to feed on goats than on cattle, feeding success was smaller on the former than on the latter hosts (Buykx, 2000). Such differences could be accounted by explicitly decomposing the availability term to equal the sum of the availabilities of each of these animal kinds, weighted averaged by their proportional abundance. The proportional availability of each animal type could be estimated if knowing the proportion of blood-meals taken upon each animal type. Alternatively, one could roughly assume that one head of cattle is equivalent to two sheep/goats, as done in experimental studies (Hewitt et al., 1994). In the context of the present model, changing the assumptions about the relative contribution of each animal type to the overall abundance of livestock hosts will change: the estimated host availabilities, the HBI, and the availability scaling factor j , consequently affecting also the predicted vector mortality due to host search. For the Pakistan simulations the implications would be minimal because most livestock were cattle. For Ethiopia, however, the impact would be greater, as the density of sheep/goats was more than twice than cattle (see Appendix E2 for comparison of the values of N_i/N_h , HBI, A_i/A_h , and j , when considering all types of livestock assuming that (a) one head of cattle is equivalent to one sheep/goat, or (b) one head of cattle is equivalent to two sheep/goats, or (c) when considering only cattle).

The present work is an improvement in relation to previous malaria models of the impact of applying insecticide on animals (Saul, 2003), on animal sheds (Kawaguchi et al., 2004), or on bednets (Killeen and Smith, 2007), as those have considered only a constant intervention effort and insecticide activity. Additionally, all previous works have assumed that the intervention had no effect upon vector density, which remained constant; i.e. they were limited to scenario DD3 in the present model, which is likely to be unrealistic. Also, none of the former two models (Saul, 2003; Kawaguchi et al., 2004), have explored a repellent effect of the insecticide, and Saul's model (Saul, 2003) was not applied to any particular ecological setting. Although the recent work by Killeen and Smith (2007) has looked at repellence and livestock applied to a Tanzanian setting, it did so in the context of insecticide-treated bednets and diversion of malaria

vectors to humans and/or untreated cattle, without referring to treatment of cattle. Furthermore, despite its findings suggesting that repellence would be beneficial, it did not explore possible repellence thresholds above which the intervention might become deleterious by increasing malaria risk.

The comprehensive range of simulations here presented illustrates the flexibility and robustness of the theoretical framework developed. Still, only a glimpse of its potential was explored. The framework could also be applied to other ecological settings, and even more intervention regimens of ITL could be assessed. Moreover, the model could be adapted to evaluate other malaria vector-control interventions based on insecticide, such as insecticide-treated bednets, and other insecticide-treated materials.

In conclusion, the results from this study indicate that ITL is likely to be more beneficial in settings with highly zoophilic vectors as in Pakistan and other areas of Southeast Asia, than in Sub-Saharan African settings with the more opportunistic vector *An. arabiensis*. Nevertheless, the intervention is still likely to substantially decrease malaria incidence in the African settings, as illustrated here with the predictions for Ethiopia. Although this work highlights the importance of accounting for a potential excito-repellent effect of the insecticide applied on livestock, the results suggest that only if the insecticide has a very strong excito-repellent effect would ITL cause an increase in malaria cases, and thereby become prejudicial. Namely, only if repellence probability is >60% in Pakistan, and >70% in Ethiopia would the intervention become detrimental, under the worst case scenario that assumed constant vector population density (DD3). For repellence probability below those thresholds any vector diversion to humans was predicted to be overcompensated by the insecticide induced vector mortality or reduction of longevity. Interestingly, these threshold repellence values are independent of the search-related vector mortality. Finally, this work also highlights that when designing and implementing an ITL intervention for malaria control it is important to have a good understanding of the DD regulation that is operating in the population(s) of malaria vectors in the study area, given the strong influence that DD can have upon the intervention outcome and the relative impact of different intervention regimens.

Chapter 7. Discussion

7.1. Major findings

This work aimed to clarify the different effects that livestock can have on human malaria in areas where the disease is transmitted by zoophilic vectors, in order to understand under which circumstances livestock-based interventions could play a role in malaria control programmes. In particular, I have looked at the impact of livestock presence, abundance, and management practices, with a focus on the insecticide treatment of livestock (ITL). This was accomplished through the development of a robust theoretical framework and its integration with empirical data from Pakistan, where an ITL trial for malaria control has been performed (Rowland et al., 2001), and from Ethiopia, where I conducted a field study to parameterize the model.

My study was conducted in the Konso District of Southwest Ethiopia, an area of typical malaria transmission in Africa, where people's subsistence depends largely on livestock which are highly abundant (1.13 animals/person in the villages; 95%CI=0.61-1.64) and have occasionally been treated with pyrethroid insecticides to control animal trypanosomiasis transmitted by tsetse flies and tick-borne diseases (Chapter 2). Entomological studies previously conducted nearby, suggested that, under some circumstances, ITL might be effective against the main local malaria vector, *An. arabiensis*, and therefore reduce malaria transmission (Habtewold et al., 2001; Habtewold, 2004; Habtewold et al., 2004; Tirados et al., 2006), although the effects of the intervention the actual disease in human remained to be assessed.

A comprehensive deterministic mathematic model was developed, by extending the classic Ross-Macdonald malaria model to 1) discriminate the feeding behaviour of the mosquito vector on its alternative hosts: livestock and human populations, and 2) to incorporate the treatment of livestock with insecticide that has lethal and possible excito-repellent effects on the vectors, accounting for the decay of the insecticide residual effect. In initial analyses, the threshold dynamics of the system was investigated, and the basic reproduction number was analytically derived, as well as its sensitivity to parameter values (Chapter 3).

The model allows us to explain situations where the presence of livestock by itself can lead to an increase, decrease or no impact at all on malaria transmission to humans, by combining the effects of livestock on decreasing the human blood index, while decreasing vector mortality and increasing vector population density. The key factors affecting the effects of untreated livestock on malaria transmission were shown to be the relative density and availability of the livestock and human hosts, the vector population density in relation to the carrying capacity of the ecological system previous to introduction of livestock, and the time elapsed since livestock introduction (Chapter 4).

The results from initial explorations of ITL in hypothetical ecological settings indicate that excito-repellency (defined as the probability that, when attempting to bite an insecticide-treated animal, a mosquito will be diverted to search another animal or human host) can be a key determinant of the outcome of an ITL intervention on the vector feeding behaviour and mortality, particularly in scenarios with very high availability of livestock. The higher the malaria transmission potential (R_0), e.g. due to increase in vector density, the lower the HBI range within which malaria transmission could potentially be controlled with ITL using a non-excito-repellent insecticide, and vice-versa. In scenarios where the malaria vector feeds mainly on livestock (i.e. low HBI, as in certain areas of Asia), high levels of effective coverage of livestock with a non-repellent insecticide could produce a reduction of up to 60% on the pre-intervention R_0 . Interestingly, even in settings where the vector takes a higher proportion of blood-meals upon humans (as in Africa), such intervention has the potential to achieve a considerable decrease in R_0 and thereby decrease malaria transmission (Chapter 5).

The model was then fitted to two specific ecological settings (Chapters 5 and 6) in Asia, the Pakistan area of the ITL trial (Rowland et al., 2001), and in Sub-Saharan Africa, the Ethiopia area of my field study (described in Chapter 2). Simulations were done under three scenarios of density-dependent regulation of the adult vector population, where vector density was either kept constant (DD3), or allowed to fluctuate following exposure to ITL (DD1 and DD2). The underlying density-dependent strength was indirectly estimated by fitting the model to incidence (DD2) or vector density (DD1) data from the Pakistan trial (Chapter 6).

The different DD scenarios resulted in considerably different intervention impacts, indicating that the strength of DD acting upon the vector population is a key driver of the intervention success. If the insecticide has repellence properties, the proportion of the

vector natural mortality that is unrelated with searching for a bloodmeal host was another important factor. Thresholds of repellence probability were identified above which the intervention could detrimentally increase malaria incidence. Only if repellence probability is >60% in Pakistan, and >70% in Ethiopia would the intervention become detrimental, under the worst case scenario that assumed constant vector population density (DD3). For repellence probability below those thresholds any vector diversion to humans was predicted to be overcompensated by the insecticide induced vector mortality or reduction of longevity. Interestingly, these threshold repellence values were independent of the search-related vector mortality.

Simulations were also done to assess how the intervention conducted in Pakistan could best be translated into the African setting. Within the ranges of repellence probability for which ITL was likely to reduce malaria cases in Ethiopia, a treatment scheme as done in the Pakistan trial (treating 93% of livestock, 3 times/year, 42 days apart) but using the Ethiopian insecticide (Deltamethrin 1% pour-on) was predicted to be the most beneficial, under the DD1 scenario of incomplete density dependence. Conversely, under scenario DD3, the greatest benefit in Ethiopia would be expected by increasing the frequency of treatments to once a month, as recommended for tsetse flies control (Vale et al., 1999), even if with a conservative coverage of 50%. Under scenario DD2 either of those two regimens would be the most beneficial. When comparing the impact of the ITL trial in Pakistan with the ITL regimen used during the field study in Ethiopia, under the more realistic DD scenarios (DD1 and DD2) a greater beneficial impact was predicted in Pakistan than in Ethiopia, for any repellence level; conversely, under scenario DD3 the reverse would occur (Chapter 6).

This is the first modelling approach that has explored the effects of ITL on malaria transmission accounting for potential excito-repellency effects, as well as for the decay of insecticide residual activity and for a fluctuation in vector population density following exposure to ITL. Overall, the findings from this work indicate that, even though ITL is likely to be more beneficial for malaria control in settings with highly zoophilic vectors as in Pakistan, than in Sub-Saharan African settings with the more opportunistic vector *An. arabiensis*, the intervention is still likely to substantially decrease malaria incidence in the latter settings, as illustrated here with the predictions for Ethiopia. Also, although this work highlights the importance of accounting for a potential excito-repellent effect of the insecticide applied on livestock, the results suggest that only if the insecticide has a very strong excito-repellent effect would ITL cause an increase in malaria cases, and

thereby become prejudicial. Another insight from this work results is that, prior to implementing a large scale ITL intervention, it is important to have a good understanding of the DD regulation that is operating in the population(s) of malaria vectors in the study area, given the determinant effects that DD can have upon the intervention outcome.

7.2. Additional challenges and future directions

The model framework could be extended to incorporate additional *heterogeneities* in the epidemiology and ecology of malaria, such as: heterogeneities in the vector feeding behaviour, seasonality, and immunity.

Regarding heterogeneities in the vector feeding behaviour, a factor that could impact the outcome of ITL interventions is the possibility of vector sub-populations with distinct zoophilic behaviour, being differentially affected by the insecticide applied on livestock. This possibility should not be disregarded, and more studies are recommended to test this hypothesis. For instance, this could be explored theoretically, by including in the presented model a second population of vectors (e.g. *An. pharoensis* in Ethiopia), and accordingly parameterizing the model for it. Parameter values for this vector that should be different from those for the population of *An. arabiensis* would include: relative availability of livestock/humans to vectors (A_v/A_h); relative density of vectors/humans pre-intervention (N_v/N_h); proportional carrying capacity (K') of the two vector populations, assuming that the density of each of these species is dependent on the density of the other species ($K'_{An. pharoensis} = 1 - K'_{An. arabiensis}$); and, eventually, the vector mortality (μ), and the probabilities of transmission from vectors to humans (b), and from humans to vectors (c).

Additionally, further insights could be obtained by linking the mathematical model into a *Geographic Information Systems (GIS) platform*. Namely, such could allow identifying the African regions where ITL is likely to produce the greatest reduction in malaria transmission, in order to inform where to conduct a community-based intervention trial (see Appendix F for preliminary work I have done towards this aim in

collaboration with others, as part of a project for the UK Department for International Development (DFID) (Franco et al., In preparation).

It is important to highlight once more that, although I explored the impact that treating livestock with insecticides could have on malaria transmission, this intervention has been traditionally used with a veterinary purpose, to control tsetse flies, ticks and other ectoparasites, and the diseases they transmit to animals. Therefore, when evaluating the *cost-effectiveness* of the intervention, both the animal health benefits and the public health benefits need to be captured. Given the potential double side benefits of veterinary interventions like this, and given the central role of livestock in poor tropical settings, to control human disease and improve livestock health will have disproportionate economic impact that needs to be captured. Here lies a challenge to the Public Health community, which will require strengthened collaboration with the Animal Health community.

The model presented in this thesis could be used as a platform for a cost-effectiveness analysis. The annual cost of the intervention could be estimated from: (number of treatment pulses per year) × (number of animals to be treated in each pulse) × (cost of treating one animal). The gain from the intervention would be the human health benefits (number of malaria cases prevented per year, converted into Disability Adjusted Life-Years – DALYs- prevented), added to the animal health benefits, such as reduction of trypanosomiasis and/or tick-borne diseases, with consequent increase in livestock productivity, such as milk and meat yield, and decrease in the expenses to treat the animals from these diseases.

In order to optimize this double benefit, care should be taken to prevent possible disruption of enzootic stability of tick-borne diseases in cattle (Van den Bossche and Mudenge, 1999; Habtewold, 2004; Bourn et al., 2005; Peter et al., 2005; Torr et al., 2007) and eventual collateral effects in the soil flora responsible for decomposing the cattle dung in mixed crop-livestock systems (Wardhaugh et al., 1998; Vale et al., 1999; Vale et al., 2004; Bourn et al., 2005). Additionally, the insecticide should be present at high concentration in the areas of the animal's body where both tsetse flies and anopheline mosquitoes tend to bite most. By identifying key target areas in the animal's body to be selectively treated, the same protective effect could be achieved using a smaller dose of insecticide than when the entire animal's body was treated. Such will

minimize the cost of application, enabling to treat more animals and with a higher frequency, achieving higher treatment coverage. Furthermore, it will decrease dung contamination with insecticide residues, minimizing its impact on the invertebrate dung flora that has an important role in keeping soil fertility in mixed crop-livestock farming (Wardhaugh et al., 1998; Vale et al., 1999; Vale et al., 2004; Bourn et al., 2005).

It has been shown that both tsetse flies (in Zimbabwe: Thomson, 1987*; Torr and Mangwiro, 2000**; Torr et al., 2001**) and *Anopheles arabiensis* (in Zimbabwe: Prior and Torr, 2002*; and in Ethiopia: Habtewold, 2004***) prefer to feed on the legs and on older and/or larger cattle. Additionally, tsetse flies also feed considerably on the belly (Thomson 1987), while the attachment sites of ticks tend to differ from the tsetse feeding sites (Torr et al. 2002 in Bourn et al. 2005). Restricting the application of insecticide to the legs and belly of older animals at more frequent intervals than the monthly interval recommended for whole body treatment could improve the cost-effectiveness of ITL for both tsetse flies (Bourn et al., 2005; Torr et al., 2007) and *An. arabiensis* mosquitoes control (Habtewold, 2004), without disrupting enzootic stability. Similar studies should be conducted in other locations where ITL is to be used for the integrated control of animal trypanosomiasis and human malaria.

A concern inherent to any vector control intervention based on *insecticides* is the potential development of *resistance*. Namely, pyrethroid resistance is becoming increasingly spread across mosquitoes (Hodjati and Curtis, 1997; Curtis et al., 1998; Casimiro et al., 2006; N'Guessan et al., 2007) and other arthropods that feed on livestock, such as ticks (Beugnet and Chardonnet, 1995; Rodriguez-Vivas et al., 2006) and horn flies (Byford et al., 1999). It has been argued that the treatment of livestock with pyrethroids is not likely to induce stronger selection pressure for resistance in malaria vector than insecticide-treated nets or indoor residual spraying of houses and cattle sheds (Hewitt et al., 1994; Hewitt and Rowland, 1999; Rowland et al., 2001). However, since the insecticide dosage applied in livestock is lower than in the other methods (Hodjati and Curtis, 1997; Curtis et al., 1998; Rowland et al., 2001; Habtewold, 2004), that may make resistance more likely to develop with ITL. Accordingly, appropriate monitoring of the vector populations is required if wide scale and long term ITL interventions are implemented.

* Preference for feeding on the legs of cattle; ** Preference for feeding on older and/or larger cattle; *** Preference for feeding on the legs of older and/or larger cattle.

The theoretical framework here presented could also be used to explore insecticide resistance, either by explicitly incorporating in it a model of the population genetics of the mosquito vectors or by using a simpler approach as in the zooprophyllaxis model of Kawaguchi *et al.* (2004), reviewed in Chapter 1, Section 1.6). Such could allow examination of resistance pressure as a function of treatment coverage, and capture the non-linear effects of coverage having a beneficial impact on public health, but potentially driving insecticide resistance.

The bioassays that have been conducted to test the effects of ITL on malaria vectors have all focused on the application of single insecticides, mostly pyrethroids (McLaughlin *et al.*, 1989; Nasci *et al.*, 1990; Vythilingam *et al.*, 1995; Hewitt and Rowland, 1999; Habtewold, 2004; Habtewold *et al.*, 2004; Mahande *et al.*, 2007), except one bioassay that tested DDT (Lysenko *et al.*, 1957). Lessons can be taken from the current policy for treatment of human malaria where a combination of antimalarials has started being used to improve treatment efficacy and counteract the development of drug resistance by malaria parasites. Likewise, a combination of insecticides could be applied on livestock to prevent the emergence of insecticide resistance by the mosquito vectors. Cocktails of non-pyrethroid and pyrethroid insecticides have started being tested for bednets (e.g. Curtis *et al.*, 1998; Guillet *et al.*, 2001; Hougard *et al.*, 2003; Asidi *et al.*, 2005), raising optimistic prospects in the fight against insecticide resistance. Similar research efforts are needed for the insecticide treatment of livestock. It's important to note however that, despite the potential advantages of combining two different insecticide classes, this strategy also has its pitfalls. Namely, as with the combination of antimalarials, it is necessary to do toxicology studies of the combination of insecticides, which are highly expensive studies, on the top of the high cost of the toxicology studies of each of the separate insecticides.

The model developed here could be *adapted to zoonotic vector-borne diseases* where there is a non-human host for the vector and/or for the infectious agent, to investigate the impact of similar veterinary interventions on reducing the burden of the human and/or animal diseases. Examples of such diseases where animals act as a host both for the vector and for the infectious agent include: African trypanosomiasis caused by *Trypanosoma brucei rhodesiense*, where insecticide-treated livestock could protect both humans and livestock; and Visceral Leishmaniasis, where the application of deltamethrin on dogs as lotions in China (Xiong *et al.*, 1995), and as collars in Iran

(Gavvani et al., 2002), Italy (Maroli et al., 2001; Ferroglia et al., 2008) and Brazil (Reithinger et al., 2004), were shown to reduce the incidence of the disease in dogs, with a decrease in the disease in humans also observed in the studies in China and Iran. Similarly, recent experimental trials have shown that applying insecticide on dogs (Fipronil pour-on: Gentile et al., 2004; deltamethrin-treated collars: Reithinger et al., 2005; Reithinger et al., 2006) could also be effective against the triatomine vectors of Chagas disease in Argentina.

It is hoped that the work presented in this thesis may lead to increasing awareness about the non-linear effects of livestock on malaria transmission, and encourage further investigations, paving the way for the implementation of a large scale field trial to assess the impact of ITL in a region of Africa where the zoophilic malaria vector *An. arabiensis* predominates and where this strategy could potentially contribute to the integrated control of malaria and livestock diseases. A possible design could be a randomized control trial, assigning to different villages a different coverage of treatment of livestock with insecticide, and assessing the impact on the entomological and clinical malaria infection rates, in children previously cleared of the infection.

7.3. From science into practice

The use of any animal-based intervention for malaria control (active zooprophylaxis or ITL) will only be a component of the broader integrated malaria control approach, and will have to work alongside case detection, treatment and prevention. The relative importance of animal-based interventions within the broader approach will vary between settings. Also, the adoption of any recommended intervention is intimately related to the socio-economics of the setting and it is therefore vital to understand the drivers for adoption by the target population. The best malaria prevention strategy, particularly in areas where vectors are endophagic, is increasingly regarded as insecticide-treated bednets (Lengeler, 2004); however, their use by some populations at risk is still far from the desirable. By understanding why bednets have not been successfully adopted in the field, lessons can be learned for how to better implement other interventions, such as ITL, in a more effective and sustainable way.

Notably, in the study conducted in the Konso District of Ethiopia (Chapter 2) the vast majority of persons interviewed did not know any method of protection against mosquito bites. Amongst the few who knew a protection method, several mentioned covering with a blanket when sleeping outdoors; and only about one third were aware of the existence of bednets. Yet, of these, only about half actually used the net. The main reason was that people could not afford it. Additionally, several persons mentioned not knowing where they could buy a bednet. And perhaps the most worrying, some of the persons who bought a bednet did not use it because they could not set it up or did not know how to set it up.

These findings, despite secondary to the main study aim, reinforce the importance of social awareness to the strategies that people can use to prevent malaria and the need to promote such awareness (Jones and Williams, 2002; Williams et al., 2002; Heggenhougen et al., 2003; Panter-Brick et al., 2006). At the time of my field study, the distribution of bednets had only recently been introduced in the area and was limited to only a few villages, which may partially explain the lack of bednet knowledge and usage. Still, the findings highlight an urgent need to: firstly, increase local knowledge about the existence and importance of bednets, and where to obtain them; secondly, decrease the cost of bednets so that they may be more widely accessible; and last but not the least, assure that whenever a bednet is acquired, adequate care is given to explain and demonstrate how to set it up.

To contribute towards filling this need, subsequently to these findings recommendations to improve the knowledge and usage of bednets in the area were given to the Konso population, incorporated in the dissemination of findings from a previous project conducted in the area by researchers from the Natural Resources Institute (NRI - University of Greenwich) and FARM-Africa (UK based NGO in Ethiopia) (Tirados, 2005).

Evidence from other studies suggests that people may be more willing to use bednets once they hear that treated nets eradicate bedbugs, in addition to protecting from the bites of malaria vectors and other nuisance insects (Professor Chris Curtis, LSHTM, personal communication). Previous research has also shown that untreated nets are only useful so long as they are not torn (Clarke et al., 2001; Mwangi et al., 2003). However, standard nets are usually torn after a few months of use. Therefore, in addition to the initial insecticide treatment of nets at the time of purchase, it is also very important to

assure that standard nets are re-treated at least once a year. In particular, the nets should be properly impregnated with insecticide when the transmission is higher, i.e. end of the rainy seasons. An effective way to achieve this could be to organize a system for a trained person on a motorcycle to go to each village at least once a year to re-treat all the nets, ideally free of charge. This system has been adopted in other malaria endemic countries, namely Tanzania, where it has proven to be easily organised (Professor Chris Curtis, LSHTM, personal communication).

In summary, it is not enough to discover means for malaria prevention. As for other diseases, it is also necessary to ensure that those means are made available in the whole sense of the word: that people at risk are aware of their existence and usefulness, that people can acquire them, are explained how to use them, and are willing to use them.

APPENDIX 
(CHAPTER 2)

A1. Human population in each kebele of the Konso District of Ethiopia.

S.N	Kebele	H/I	Family size			Age group	
			Male	Female	Total	<18 yrs	>18 yrs
1	Karat town	1781	2541	2742	5283	2863	2420
2	Mechelo	447	1167	1245	2412	1241	1170
3	Sorobo	771	1964	2092	4056	2356	1700
4	Buso	528	1379	1442	2821	1497	1324
5	Nalaya Segen	713	1564	1741	3305	1813	1492
6	Abaroba	1109	3209	3360	6569	3720	2849
7	Duraite	854	1906	2033	3939	2157	1782
8	Dera	551	1383	1479	2862	1573	1289
9	Dokatu	1136	2561	2898	5459	2955	2504
10	Jarso	1808	4297	4629	8926	4774	4152
11	Gamole	447	1082	1139	2221	1192	1029
12	Gocha	415	1148	1197	2345	1305	1040
13	Baide&Fuchucha	870	2278	2219	4497	2430	2067
14	Meka (Gato+Mecheka)	983	2879	2950	5829	3284	2545
15	Sewgeme	446	1434	1543	2977	1619	1358
16	Kamala	1379	4641	2992	8633	4601	4032
17	Debana or Tebana	949	3469	2728	6197	3502	2695
18	Gesergio	696	2434	2504	4938	2744	2194
19	Toha	1015	3885	4167	8052	4484	3568
20	Gera	409	1382	1394	2776	1508	1268
21	Fasha	1106	3594	3763	7357	4541	2816
22	Kashele	807	2640	2777	5417	2976	2441
23	Madder&Gizaba	1298	3778	3989	7767	4130	3637
24	Borkora	878	2615	2669	5284	2744	2540
25	Wayto or Masoy	172	600	373	973	343	630
26	Gelgele & kolmele	1524	4299	4420	8719	4399	4320
27	Kugnera	674	1927	2096	4023	2269	1754
28	Tebela &kuchale	1411	4684	4795	9479	4540	4939
29	Karkarte	423	1677	1675	3352	1792	1560
30	Turuba	231	609	610	1219	593	626
31	Eyana	323	904	989	1893	973	920
32	Wolango or Golango	551	1431	1541	2972	1544	1428
33	Gergema	477	1162	1280	2442	1180	1262
34	Gewada	780	2806	2862	5668	3062	2606
35	Gama	552	1473	1592	3065	1593	1472
36	Lehayte	671	1752	1772	3524	1919	1605
37	Teshmale	626	1595	1720	3315	1850	1465
38	Arfayde	524	1403	1577	2980	1630	1350
39	Gelabo	1047	2505	2532	5037	2789	2248
40	Segen town	753	1657	1864	3521	1799	1722
41	Aylota dokatu	696	2752	2792	5544	3563	1981
42	Lultu	854	2992	3029	6021	3315	2706
43	Birbirsa	515	2480	3468	5948	3327	2621
44	Becho or Addis Gebere	565	1610	1456	3066	1707	1359
45	Melese	564	1340	1406	2746	1562	1184
46	Gerche	165	564	614	1178	670	508
TOTAL		35494	101452	104155	206607	112428	94178

In yellow are the kebeles selected for the study. Source: Census for the year of 2004, estimated according with projections from a previous census done in 1994. Provided by the Agriculture Department of the Konso Rural Development Office.

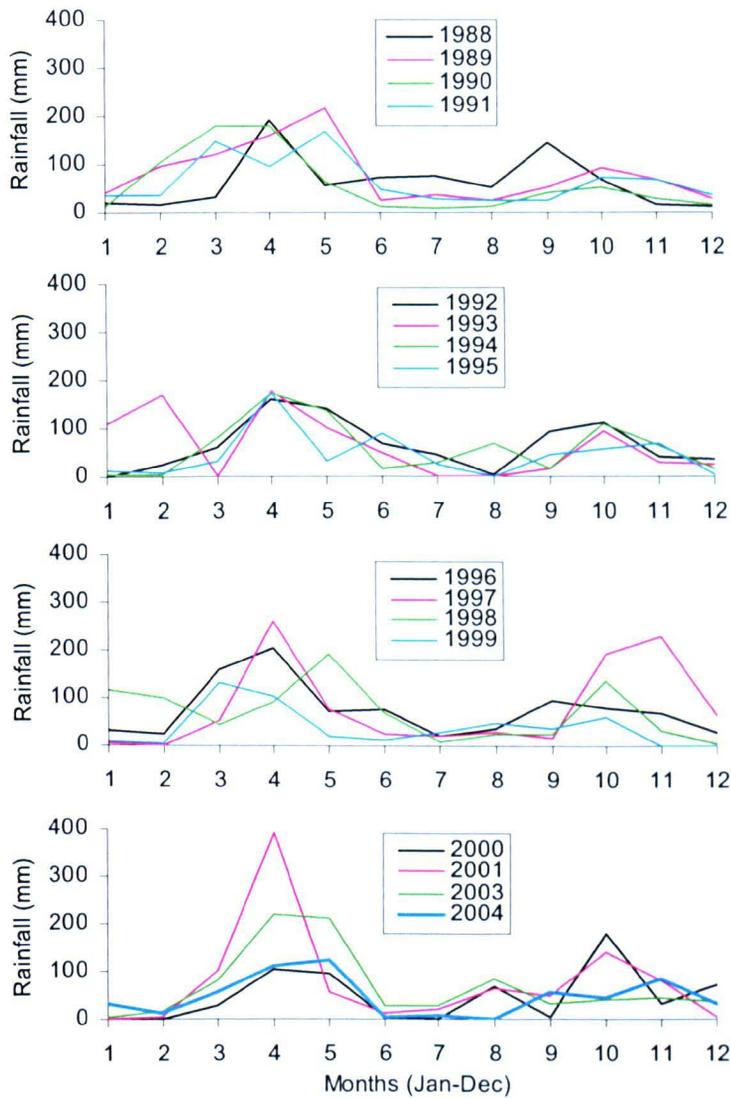
A2. Livestock population in each kebele of the Konso District of Ethiopia.

SN	Kebele	Ox (> 3 years)	Cow (> 3years)	Heifer (2-3years)	Calf (<2 years)	Sheep	Goat	Horse	Mule/ Donkey	Chicken	All livestock
1	Karat town	1284	321	213	109	238	1270	0	2	816	4253
2	Mechelo	2354	2617	1301	255	892	2101	0	29	534	10083
3	* Sorobo	2165	2364	1020	249	580	2997	0	45	1258	10678
4	Buso	2054	1892	1032	800	1200	3420	0	37	1301	11736
5	* Nalaya Segen	2160	2300	1097	274	254	2358	0	4	1342	9789
6	Abaroba	6894	7034	3425	1946	1899	12840	0	286	2364	36688
7	* Duraite	3893	3278	1428	988	564	9428	0	97	1028	20704
8	* Dera	1327	1425	799	205	304	2470	0	2	521	7053
9	* Dokatu	2472	3201	1008	523	639	6293	0	18	624	14778
10	* Jarso	7498	7425	3656	2005	2363	12468	0	198	1465	37078
11	Gamole	165	208	124	97	189	898	0	14	243	1938
12	Gocha	127	134	96	63	203	868	0	16	214	1721
13	* Baide	4632	3975	2352	1241	204	3642	1	86	368	16501
	* Fuchucha	3548	2792	230	81	65	763	0	23	216	7718
	Meka (Gato + Mecheka)	2165	2423	164	87	2680	3125	0	35	324	11003
14	Sewgeme	2141	2322	186	96	324	2689	0	24	326	8108
15	Kamala	1867	388	78	42	248	269	0	35	276	3203
16	Debana or Tebana	285	169	29	25	326	295	0	40	268	1437
17	Gesergio	279	315	165	76	218	325	0	28	219	1625
18	Toha	268	289	216	109	325	678	0	19	320	2224
19	Gera	108	109	96	68	168	467	0	8	241	1265
20	Fasha	106	118	76	66	279	416	0	54	324	1439
21	Kashele	78	82	41	40	68	204	0	5	219	737
22	* Madder&Gizaba	548	469	214	189	467	3686	0	23	426	6022
23	* Borkora	267	198	104	96	267	2675	0	16	278	3901
24	* Wayto or Masoy	476	464	219	196	627	22169	0	217	468	24836
25		364	278	174	102	523	6785	0	18	279	8523
26	* Gelgele & Kolmele										
27	Kugnera	275	263	171	104	328	4279	0	29	306	5755
28	* Tebela & kuchale	245	233	103	93	234	3264	0	23	203	4398
29	* Karkarte	363	236	193	69	205	2695	0	105	464	4330
30	Turuba	276	263	207	93	207	1695	0	98	305	3144
31	Eyana	324	363	275	198	309	4653	0	78	294	6494
32	Wolango or Golango	297	304	195	108	218	2603	0	77	249	4051
33	Gergema	267	303	168	94	204	2563	0	54	252	3905
34	Gewada	305	312	124	103	197	2641	0	28	219	3929
35	Gama	273	197	118	92	165	342	2	49	245	1483
36	Lehayte	242	368	108	97	241	234	0	29	419	1738
37	* Teshmale	346	364	204	103	196	1996	0	45	343	3597
38	Arfayde	236	246	198	94	89	896	0	84	363	2206
39	Gelabo	564	235	168	69	196	293	0	75	368	1968
40	* Segen town	343	236	181	105	204	2364	0	64	403	3900
41	* Aylota dokatu	373	294	149	101	109	2146	0	52	342	3566
42	* Lultu	297	265	156	98	213	2364	0	43	224	3660
43	* Birbirs	273	313	199	88	181	2818	0	24	242	4138
44	* Becho or Addis Gebere	237	243	98	78	168	1968	0	36	234	3062
45	* Melese	248	238	196	108	186	2464	0	43	343	3826
46	* Gerche	284	245	166	103	167	2644	0	56	365	4030
	TOTAL	55593	52111	22920	12026	20131	150521	3	2471	22445	338221

In yellow are the kebeles selected for the study.

* Kebeles where livestock were being treated with insecticide as part of a government program for trypanosomiasis control. Source: Census for the year of 2003/04 done by the Konso Rural Development Office.

A3. Graphs with changes in the total monthly precipitation through time from 1988 to 2004, as recorded by the Meteorological Station in Karat, Konso District.



Source: Ethiopian National Meteorological Services Agency. The data from 1988 to 2001 were kindly provided by Inaki Tirados and Steve Torr, who had previously obtained it. No data were available for 2002. The detailed data are provided in Appendix A4.

A4. Tabled data for changes in the total monthly precipitation from 1988 to 2004, as recorded by the Meteorological Station in Karat, Konso District.

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
1988	18.80	17.00	32.10	191.80	54.80	72.50	77.30	50.40	142.20	68.60	14.10	11.60	751.20
1989	38.60	94.60	121.10	158.90	218.00	22.70	34.60	25.19	53.80	92.78	67.59	26.26	954.12
1990	11.50	105.90	178.40	179.90	64.80	10.40	10.00	11.90	41.10	50.80	28.20	16.90	709.80
1991	35.50	37.40	148.60	95.10	166.60	46.30	28.62	25.19	24.30	72.30	67.90	37.40	785.21
1992	0.20	23.30	59.70	159.50	138.30	67.40	42.60	4.90	92.40	112.10	39.00	34.40	773.80
1993	107.50	169.90	1.80	176.90	98.50	46.90	1.00	1.60	14.90	90.10	29.60	24.70	763.40
1994	2.50	4.20	81.80	171.10	137.00	16.70	29.70	69.30	15.70	109.30	64.60	16.70	718.60
1995	10.20	7.10	31.10	171.70	31.80	87.20	22.80	1.40	44.10	57.80	67.59	3.20	535.99
1996	32.70	24.00	161.10	205.40	74.00	76.70	19.80	35.80	94.90	80.60	67.59	26.26	898.85
1997	5.80	0.00	52.60	259.10	74.80	22.30	19.30	26.20	15.00	193.40	229.70	64.20	962.40
1998	117.90	100.40	44.80	91.40	192.60	67.20	8.20	23.00	23.40	137.90	33.90	5.60	846.30
1999	9.80	4.50	131.30	102.10	18.30	13.10	26.50	48.60	37.30	58.30	1.60	0.00	451.40
2000	1.60	0.00	28.30	104.50	95.20	5.00	0.00	69.50	3.70	181.30	34.00	72.00	595.10
2001	1.70	4.10	98.90	391.70	57.80	11.00	19.80	63.90	49.40	140.50	81.10	2.40	922.30
2002													
2003	3.80	14.20	80.20	221.60	210.40	26.70	27.20	85.00	30.10	41.50	44.00	35.30	820.00
2004	33.40	13.00	54.20	112.60	123.30	4.90	6.20	0.30	56.30	42.10	83.50	33.20	563.00
MEAN	26.97	38.73	81.63	174.58	109.76	37.31	23.35	33.89	46.16	95.59	59.62	25.63	762.03
SD	36.06	50.60	53.01	75.50	62.58	28.66	18.78	27.58	36.47	47.14	51.35	20.75	154.28

Source: Ethiopian National Meteorological Services Agency. The data from 1988 to 2002 were kindly provided by Iñaki Tirados and Steve Torr, who had previously obtained it. No data were available for 2002. SD=Standard deviation.

A4. Tabled data for changes in the total monthly precipitation from 1988 to 2004, as recorded by the Meteorological Station in Karat, Konso District.

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	TOTAL
1988	18.80	17.00	32.10	191.80	54.80	72.50	77.30	50.40	142.20	68.60	14.10	11.60	751.20
1989	38.60	94.60	121.10	158.90	218.00	22.70	34.60	25.19	53.80	92.78	67.59	26.26	954.12
1990	11.50	105.90	178.40	179.90	64.80	10.40	10.00	11.90	41.10	50.80	28.20	16.90	709.80
1991	35.50	37.40	148.60	95.10	166.60	46.30	28.62	25.19	24.30	72.30	67.90	37.40	785.21
1992	0.20	23.30	59.70	159.50	138.30	67.40	42.60	4.90	92.40	112.10	39.00	34.40	773.80
1993	107.50	169.90	1.80	176.90	98.50	46.90	1.00	1.60	14.90	90.10	29.60	24.70	763.40
1994	2.50	4.20	81.80	171.10	137.00	16.70	29.70	69.30	15.70	109.30	64.60	16.70	718.60
1995	10.20	7.10	31.10	171.70	31.80	87.20	22.80	1.40	44.10	57.80	67.59	3.20	535.99
1996	32.70	24.00	161.10	205.40	74.00	76.70	19.80	35.80	94.90	80.60	67.59	26.26	898.85
1997	5.80	0.00	52.60	259.10	74.80	22.30	19.30	26.20	15.00	193.40	229.70	64.20	962.40
1998	117.90	100.40	44.80	91.40	192.60	67.20	8.20	23.00	23.40	137.90	33.90	5.60	846.30
1999	9.80	4.50	131.30	102.10	18.30	13.10	26.50	48.60	37.30	58.30	1.60	0.00	451.40
2000	1.60	0.00	28.30	104.50	95.20	5.00	0.00	69.50	3.70	181.30	34.00	72.00	595.10
2001	1.70	4.10	98.90	391.70	57.80	11.00	19.80	63.90	49.40	140.50	81.10	2.40	922.30
2002													
2003	3.80	14.20	80.20	221.60	210.40	26.70	27.20	85.00	30.10	41.50	44.00	35.30	820.00
2004	33.40	13.00	54.20	112.60	123.30	4.90	6.20	0.30	56.30	42.10	83.50	33.20	563.00
MEAN	26.97	38.73	81.63	174.58	109.76	37.31	23.35	33.89	46.16	95.59	59.62	25.63	762.03
SD	36.06	50.60	53.01	75.50	62.56	28.66	18.78	27.58	36.47	47.14	51.35	20.75	154.28

Source: Ethiopian National Meteorological Services Agency. The data from 1988 to 2002 were kindly provided by Iñaki Tirados and Steve Torr, who had previously obtained it. No data were available for 2002. SD=Standard deviation.

A5. Quality control of the parasitological diagnosis of malaria cases in the Konso District of Ethiopia

Methods

The reliability of the parasitological diagnosis of malaria cases in Konso was assessed through quality control of a sub-sample of 105 blood slides. The sub-sample consisted of all the blood slides (thick smears, stained with Giemsa) diagnosed as positive or negative for *P. falciparum* spp. at the main health facility in the study area (Karat Health Centre - KHC) during the last week of the study (29th November to 4th December 2004). The validity of the slide readings at the KHC was estimated by calculating the sensitivity and specificity (and positive and negative predictive values) to identify *P. falciparum* spp. infection, considering as gold standard a second reading done at the LSHTM Malaria Reference Laboratory.

Results

The results from the 105 slides that were read at the Karat Health Centre (KHC), in Konso, Ethiopia, and at the LSHTM are summarized and compared in the Tables A1 to A3. The readings at the KHC laboratory showed a higher specificity than sensitivity to detect infection by *Plasmodium* spp. (Table A2). It was estimated that they correctly identified 90% (74/82) of the true negative slides and 70% (16/23) of the true positive slides. Likewise, the negative predictive value (91% - 16/24) was higher than the positive predictive value (67%) (74/81). The accuracy of the diagnosis was 86% (90/105 slides were correctly identified as positive or negative). Out of the 16 slides correctly identified as positive, 12 were correctly identified as *P. falciparum* (PF), 2 were correctly identified as *P. vivax* (PV), and 2 other slides of PV were incorrectly identified as PF (Table A3).

Table A1. Contingency table comparing the diagnosis from the Karat Health Centre (KHC) Laboratory with the LSHTM Malaria Reference Laboratory.

KHC	LSHTM		Total
	Positive	Negative	
Positive	16	8	24
Negative	7	74	81
Total	23	82	105

Table A2. Assessing reliability of the KHC parasitological diagnosis: sensitivity and specificity, positive and negative predictive values, calculated considering as gold standard the LSHTM parasitological diagnosis.

Total number of slides read	105
	% 95% CI
Sensitivity	70 (61.23 - 78.77)
Specificity	90 (84.26 - 95.74)
Positive predictive value	67 (58.01 - 75.99)
Negative predictive value	91 (85.53 - 96.47)

Table A3. Accuracy of the KHC parasitological diagnosis, considering as gold standard the LSHTM parasitological diagnosis.

	n	(% of +ves)	(% of total)
Correctly identified as positive	16	(100)	(15)
Correctly identified as PF	12	(75)	(11)
Correctly identified as PV	2	(13)	(2)
PV incorrectly identified as PF	2	(13)	(2)
Correctly identified as negative	74	-	(70)

APPENDIX B
(CHAPTER 3)

B1. Deriving the malaria R_0 with the Next Generation Operator

As presented in Chapter 3, the dynamics of infection in the human population is given by:

$$\frac{dS_h}{dt} = -\left(aqb \frac{I_v}{N_h}\right)S_h + rI_h, \quad \text{Eq. B1}$$

$$\frac{dI_h}{dt} = \left(aqb \frac{I_v}{N_h}\right)S_h - rI_h, \quad \text{Eq. B2}$$

where $N_h = S_h + I_h$ (total human population size).

And the dynamics of infection in the vector population is given by:

$$\frac{dS_v}{dt} = \rho N_v - \left(aqc \frac{I_h}{N_h} + \mu\right)S_v, \quad \text{Eq. B3}$$

$$\frac{dL_v}{dt} = \left(aqc \frac{I_h}{N_h}\right)S_v - (\omega + \mu)L_v, \quad \text{Eq. B4}$$

$$\frac{dI_v}{dt} = \omega L_v - \mu I_v, \quad \text{Eq. B5}$$

where $N_v = S_v + L_v + I_v$ (total vector population size),

$$\text{and } \mu = \mu_0 + a(1-q)k \frac{I_l}{N_l}.$$

To derive the basic reproduction number (R_0) of malaria implementing the next-generation operator approach (Diekmann et al., 1990; van den Driessche and Watmough, 2002) we need to linearize the system at the disease-free equilibrium (DFE), which is a state where there is no disease and therefore $S_h^* = N_h^*$ and $S_v^* = N_v^*$.

Firstly, we need to find L_v^* in the DFE, by setting $\frac{dL_v}{dt} = 0$ and replacing $S_v = N_v$ in the

expression for $\frac{dL_v}{dt}$ (Eq. B4):

$$\frac{dL_v}{dt} = 0 \Leftrightarrow \left(aqc \frac{I_h}{N_h} \right) S_v - (\omega + \mu) L_v = 0 \quad \text{Eq. B6}$$

$$\Leftrightarrow \left(aqc N_v \frac{I_h}{N_h} \right) - (\omega + \mu) L_v = 0 \quad \text{Eq. B7}$$

$$\Leftrightarrow L_v^* = \frac{aqc N_v \frac{I_h}{N_h}}{\omega + \mu} \quad \text{Eq. B8}$$

Secondly, replace $S_h = N_h$ in the expression for $\frac{dI_h}{dt}$ (Eq. B2):

$$\frac{dI_h}{dt} = \left(aqb \frac{I_v}{N_h} \right) N_h - r I_h \quad \text{Eq. B9}$$

$$\Leftrightarrow \frac{dI_h}{dt} = aqb I_v - r I_h \quad \text{Eq. B10}$$

Thirdly, replace $L_v = L_v^*$ in the expression for $\frac{dI_v}{dt}$ (Eq. B5):

$$\frac{dI_v}{dt} = \omega \frac{aqc N_v \frac{I_h}{N_h}}{\omega + \mu} - \mu I_v \quad \text{Eq. B11}$$

$$\Leftrightarrow \frac{dI_v}{dt} = \frac{N_v}{N_h} \frac{aqc \omega I_h}{\omega + \mu} - \mu I_v \quad \text{Eq. B12}$$

We then consider only the equations for the infectious human host (Eq. B10) and mosquito vector (Eq. B12). The Jacobian for this reduced system at the DFE is:

$$J(DFE) = \begin{bmatrix} -r & aqb \\ \frac{N_v}{N_h} \frac{aqc \omega}{\omega + \mu} & -\mu \end{bmatrix} \quad \text{Eq. B143}$$

Let $J(DFE) = M-D$, where M is the non-negative matrix

$$M = \begin{bmatrix} 0 & aqb \\ \frac{N_v}{N_h} \frac{aqc\omega}{(\omega + \mu)} & 0 \end{bmatrix} \quad \text{Eq. B14}$$

and D is the positive diagonal matrix

$$D = \begin{bmatrix} r & 0 \\ 0 & \mu \end{bmatrix}. \quad \text{Eq. B15}$$

The next-generation operator is given by the matrix MD^{-1} :

$$MD^{-1} = \begin{bmatrix} 0 & \frac{aqb}{\mu} \\ \frac{N_v}{N_h} \frac{aqc\omega}{r(\omega + \mu)} & 0 \end{bmatrix}, \quad \text{Eq. B16}$$

and the basic reproduction number is given by the dominant eigenvalue λ of the matrix MD^{-1} , i.e., the eigenvalue that is larger in absolute value than all other eigenvalues of MD^{-1} . The eigenvalues of MD^{-1} are given by the solutions of $|MD^{-1} - \lambda I| = 0$, and we therefore obtain

$$R_0 = \sqrt{\frac{N_v (aq)^2 bc}{N_h r \mu} \frac{\omega}{(\omega + \mu)}} \quad \text{Eq. B17}$$

Replacing back $\mu = \mu_0 + a(1-q)k \frac{T_l}{N_l}$ in the above Eq. B17 gives the final expression

for the malaria R_0 :

$$R_0 = \sqrt{\frac{N_v (aq)^2 bc}{N_h r} \frac{\omega}{\left(\mu_0 + a(1-q)k \frac{T_l}{N_l} \right) \left((\omega + \mu_0) + a(1-q)k \frac{T_l}{N_l} \right)}} \quad \text{Eq. B18}$$

Note that all the terms that characterize R_0 are ≥ 0 , and consequently, $R_0 \geq 0$.

Let us interpret in more detail the derived R_0 , based on the simplified expression given by Eq. B17. Near the DFE, each infected human is expected to generate $\frac{N_v}{N_h} \frac{aqc\omega}{r(\omega + \mu)}$ new infected vectors during its infectious period, and each infected mosquito vector is expected to generate $\frac{aqb}{\mu}$ new infected humans during its infectious period (van den Driessche and Watmough, 2002). The square-root reflects the biological requisite in the vector-human host system for the parasite to pass through two types of individuals to complete its life cycle (Lord et al., 1996). Starting from, for example a human host, the parasite needs to pass through a vector before it can be transmitted to a new human host (Diekmann and Heesterbeek, 2000). Therefore, it takes two “generations” for an infected vector or host to “reproduce” itself, i.e. for infection to be transmitted from human to human or from vector to vector (van den Driessche and Watmough, 2002). And in every two “generations” the numbers of infections in humans and vectors are multiplied by

$$\frac{N_v}{N_h} \frac{aqc\omega}{r(\omega + \mu)} \times \frac{aqb}{\mu} = \frac{N_v}{N_h} \frac{(aq)^2 bc}{r\mu} \frac{\omega}{(\omega + \mu)}.$$

Thereby, the geometric average of secondary cases per generation of infection is

$$R_0 = \sqrt{\frac{N_v}{N_h} \frac{(aq)^2 bc}{r\mu} \frac{\omega}{(\omega + \mu)}}.$$

Note that the majority of previous malaria models present a formula for R_0 that does not include a square root, while using the next-generation operator approach we obtain a squared root expression. This is because traditionally, R_0 is defined as the average number of secondary infections in humans caused by one infectious human introduced in a population of fully susceptible individuals (Macdonald, 1952; Anderson and May, 1991). However, according to Diekmann et al (1990), the traditional R_0 definition amounts to looking two generations of infection ahead, and instead, with the next-generation operator approach, R_0 is defined as the average number of secondary infections caused by one infectious individual (be it human or vector); i.e. the number of secondary infections by generation of infection. Therefore, for host-vector diseases one needs to take the square root of the classical R_0 .

In practice, the relevance of the square root in the R_0 expression depends on the question being addressed. When investigating the threshold conditions for disease persistence ($R_0 \geq 1$) or eradication ($R_0 < 1$), the square root could be ignored, since for any number $\lambda > 1$ if and only if $\lambda^2 > 1$, and therefore, the same threshold is obtained using the R_0 derived with the traditional way (i.e. without square root) or with the next-generation operator (i.e. with square root). However, when estimating the level of disease control efforts necessary to decrease disease transmission sufficiently to obtain a specified goal, the insights can be significantly different depending on the omission or inclusion of the square root (Dietz, 1993). For instance, a higher effort is required to control disease in a scenario where $R_0 = 16$, than in a scenario where $R_0 = \sqrt{16} = 4$. Therefore, when evaluating control efforts, if defining R_0 like Diekmann et al. (1990), one should consider $R_0 = \lambda^2 = (\text{the dominant eigenvalue of } MD^{-1})$ squared:

$$R_0 = \frac{N_v (aq)^2 bc}{N_h r \mu} \frac{\omega}{(\omega + \mu)}, \quad \text{Eq. B19}$$

which allows comparison with most previous malaria models.

Replacing back $\mu = \mu_0 + a(1-q)k \frac{T_l}{N_l}$ in the above Eq. B19 gives:

$$R_0 = \frac{N_v (aq)^2 bc}{N_h r} \frac{\omega}{\left(\mu_0 + a(1-q)k \frac{T_l}{N_l} \right) \left((\omega + \mu_0) + a(1-q)k \frac{T_l}{N_l} \right)}, \quad \text{Eq. B20}$$

The analyses and simulations of R_0 throughout the thesis used this latter expression (Eq. B20).

APPENDIX 
(CHAPTER 4)

C1. Impact of vector density dependence on the malaria R_0

An analytical expression for ρ_s can be obtained by setting $dN_v/dt = 0$, and simple algebraic manipulation as described below.

Previous to a control intervention, the total vector population is governed by:

$$\frac{dN_v}{dt} = (\rho_0 - \rho_s N_v) N_v - \mu N_v.$$

At equilibrium, $dN_v/dt = 0$, and therefore:

$$N_v^* ((\rho_0 - \rho_s N_v^*) - \mu) = 0.$$

The non-trivial solution to the equilibrium, is given by:

$$(\rho_0 - \rho_s N_v^*) = \mu$$

$$\rho_s N_v^* = \rho_0 - \mu$$

$$N_v^* = \frac{(\rho_0 - \mu)}{\rho_s}$$

or

$$\rho_s = \frac{(\rho_0 - \mu)}{N_v^*}.$$

Incorporating explicit density dependence on vector recruitment induces the following modification on R_0 expression, obtained from simply replacing N_v by its equilibrium level, $N_v^* = (\rho_0 - \mu) / \rho_s$:

$$R_0 = \frac{(\rho_0 - \mu_0 + a(1-q)kT_l / N_l) (aq)^2 bc}{\rho_s N_h} \frac{\omega}{\left(\mu_0 + a(1-q)k \frac{T_l}{N_l} \right) \left((\omega + \mu_0) + a(1-q)k \frac{T_l}{N_l} \right)},$$

where $\mu = \mu_0 + a(1-q)k \frac{T_l}{N_l}$,

and μ_0 is the average vector natural mortality ($\mu_0 = \mu_{min} + \mu_{search}$).

C2. Endemic malaria: Constant vector population density with variable livestock density

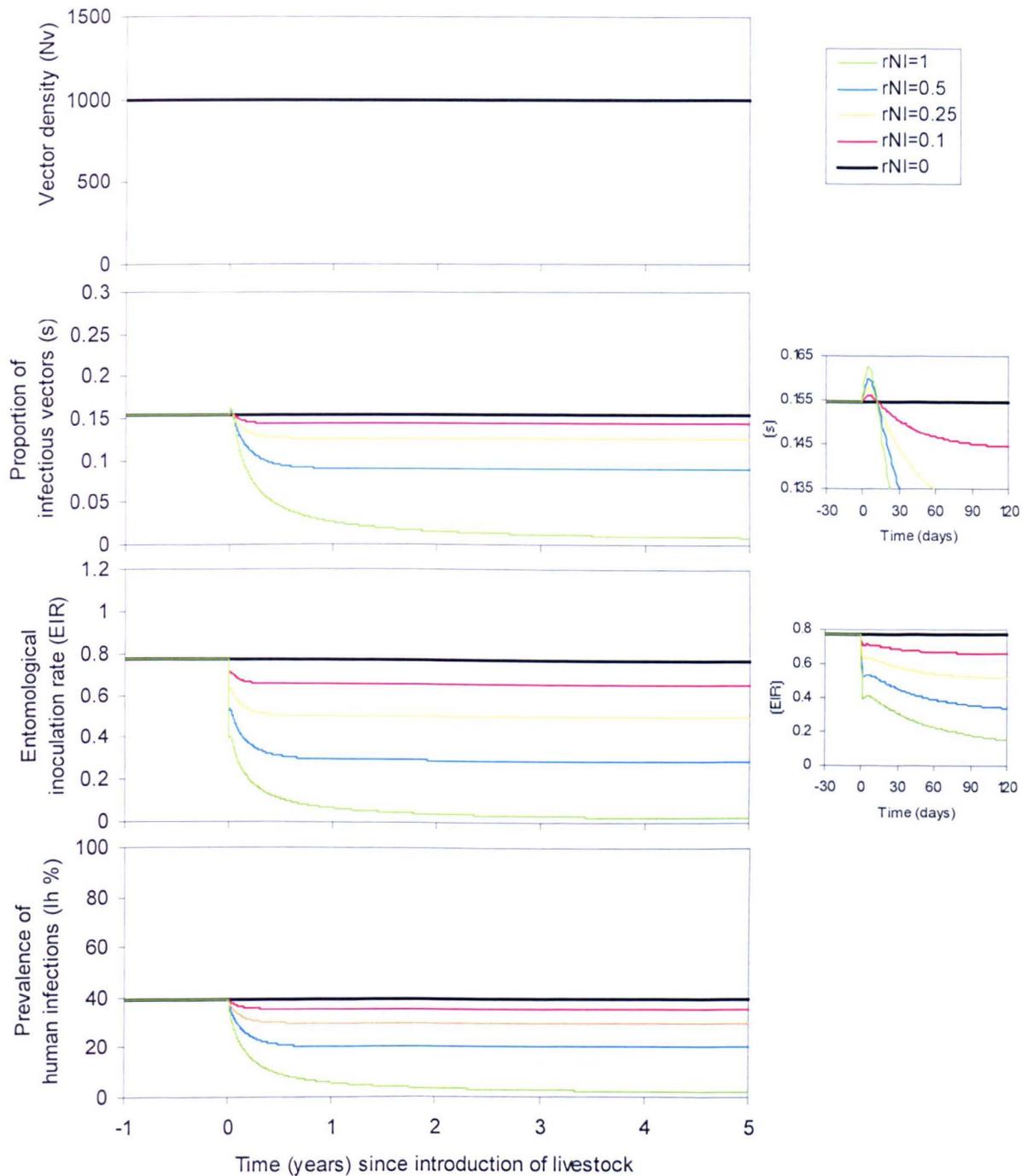


Figure C1. Temporal impact of introducing livestock in a setting with endemic malaria, where only humans and no livestock were present, and with constant vector density: impact of varying livestock density, when $A_1=0.5$.

$rN_l = N_l/N_h$; the livestock density N_l is varied relative to a human density $N_h=100$; rN_l increasing from no livestock (black line) to 1 head of livestock per person (green line). $N_{v(0)}=K=1000$. Other parameters as in Table 4.3. EIR = infectious bites per human per day. Additional outcome variables explored but not shown in the figure: virtually immediately after the introduction of livestock, the values of the human blood index (HBI), vector mortality rate (μ), human-biting rate (HBR) and basic reproduction number (R_0) will be decreased to a new value (the new endemic equilibrium value), and will thereafter remain constant at that value, as long as the same numbers of livestock and human hosts remain present, with the same availability. For $rN_l=0.1, 0.25, 0.5, 1$, respectively, HBI is reduced from 1 to 0.91, 0.80, 0.67, 0.50; μ is reduced from 0.100/day to 0.095, 0.090, 0.083, 0.075/day; HBR is reduced from 5 bites/day to 4.5, 4.0, 3.3, 2.5 bites/day; and R_0 is reduced from 2.63 to 2.33, 1.98, 1.55, 1.02.

C3. Endemic malaria: Variable vector population density – with fix vector carrying capacity and variable livestock density

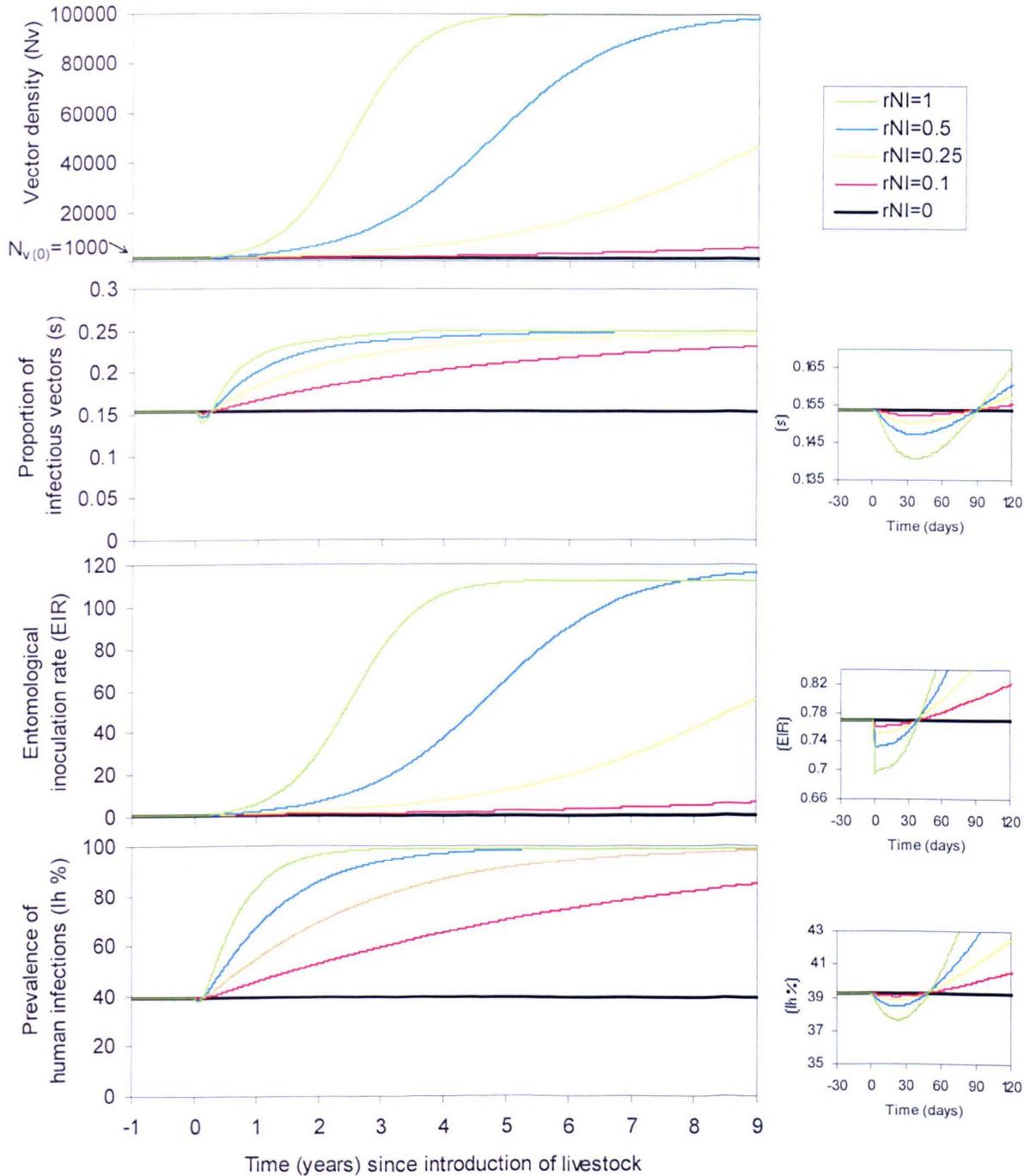


Figure C2. Temporal impact of introducing livestock in a setting with endemic malaria, where only humans and no livestock were present, and with variable vector density: impact of varying livestock density, when $A_1=0.1$.

$rN_I=N_I/N_h$; the livestock density N_I is varied relative to a fixed human density $N_h=100$; rN_I increasing from no livestock (black line) to 1 head of livestock per person (green line).

Other parameters as in Table 4.3. EIR = infectious bites per human per day. Additional outcome variables explored: Virtually immediately after the introduction of livestock, the values of the human blood index (HBI), and vector mortality rate (μ) will be decreased to a new value (the new endemic equilibrium value), and will thereafter remain constant at that value, as long as the same numbers of livestock and human hosts remain present, with the same availability. For $rN_I=0.1, 0.25, 0.5, 1$, respectively, HBI is reduced from 1 to 0.99, 0.97, 0.95, 0.90; and μ is reduced from 0.100/day to 0.0997, 0.0989, 0.0976, 0.0952/day. Conversely, the human-biting rate (HBR) and the basic reproduction number (R_0) will change gradually through time following introduction of livestock, in a similar pattern as that shown for the EIR although with a different scale.

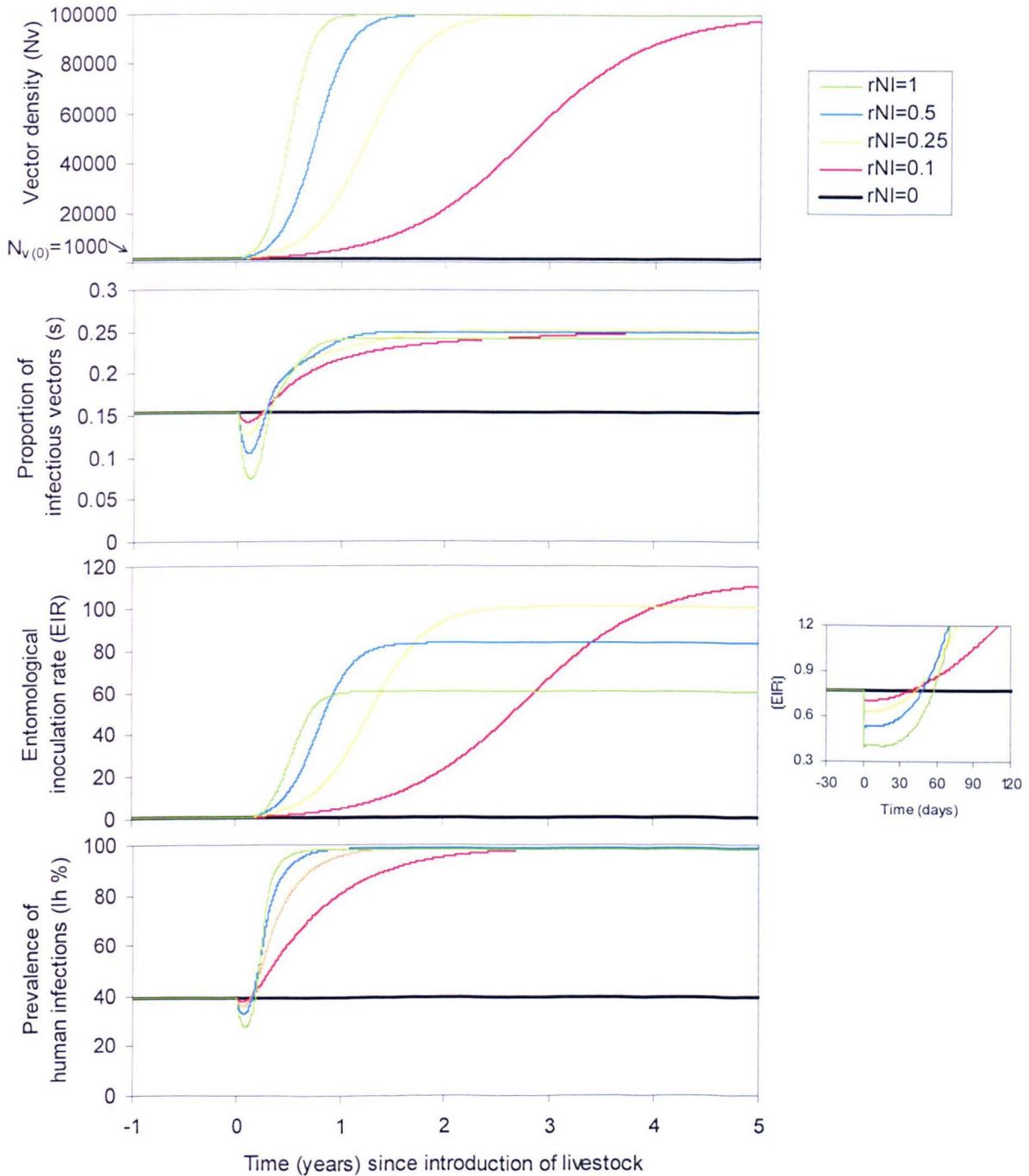


Figure C3. Temporal impact of introducing livestock in a setting with endemic malaria, where only humans and no livestock were present, and with variable vector density: impact of varying livestock density, when $A_1=0.5$.

See notes from Figure C2. Additional outcome variables explored: for $rN_1=0.1, 0.25, 0.5, 1$, respectively, HBI is reduced from 1 to 0.91, 0.80, 0.67, 0.50; and μ is reduced from 0.100/day to 0.095, 0.090, 0.083, 0.075/day (Note that these new endemic equilibrium values for HBI and μ are the same as in the simulations with constant vector density, mentioned in the notes of Figure 4.10).

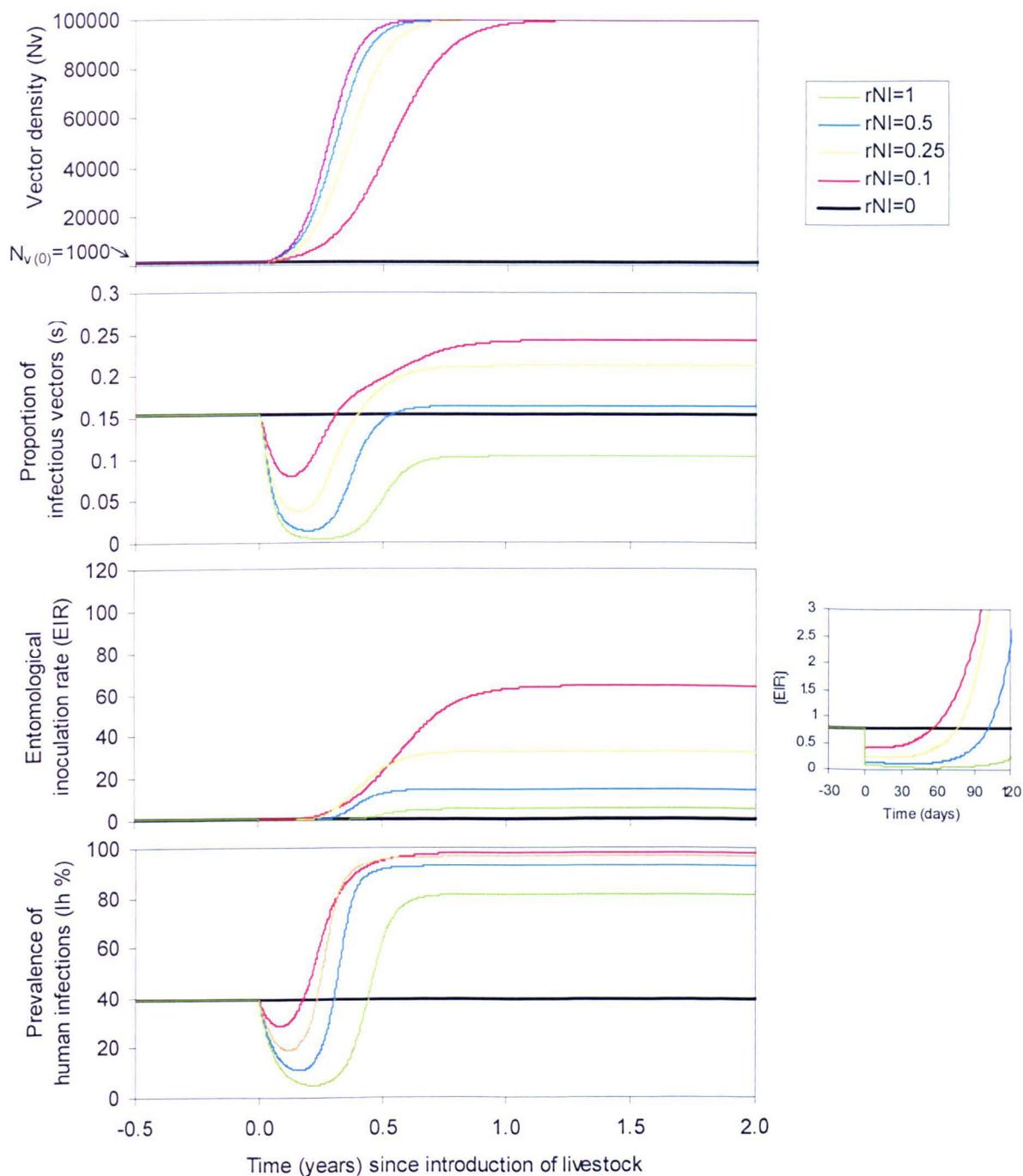


Figure C4. Temporal impact of introducing livestock in a setting with endemic malaria, where only humans and no livestock were present, and with variable vector density: impact of varying livestock density, when $A_1=0.9$.

See notes from Figure C2. Additional outcome variables explored: for $rN_I=0.1, 0.25, 0.5, 1$, respectively, HBI is reduced from 1 to 0.53, 0.31, 0.18, 0.10; and μ is reduced from 0.100/day to 0.076, 0.065, 0.059, 0.055/day.

C4. Endemic malaria: Variable vector population density – with fix livestock abundance and variable vector carrying capacity

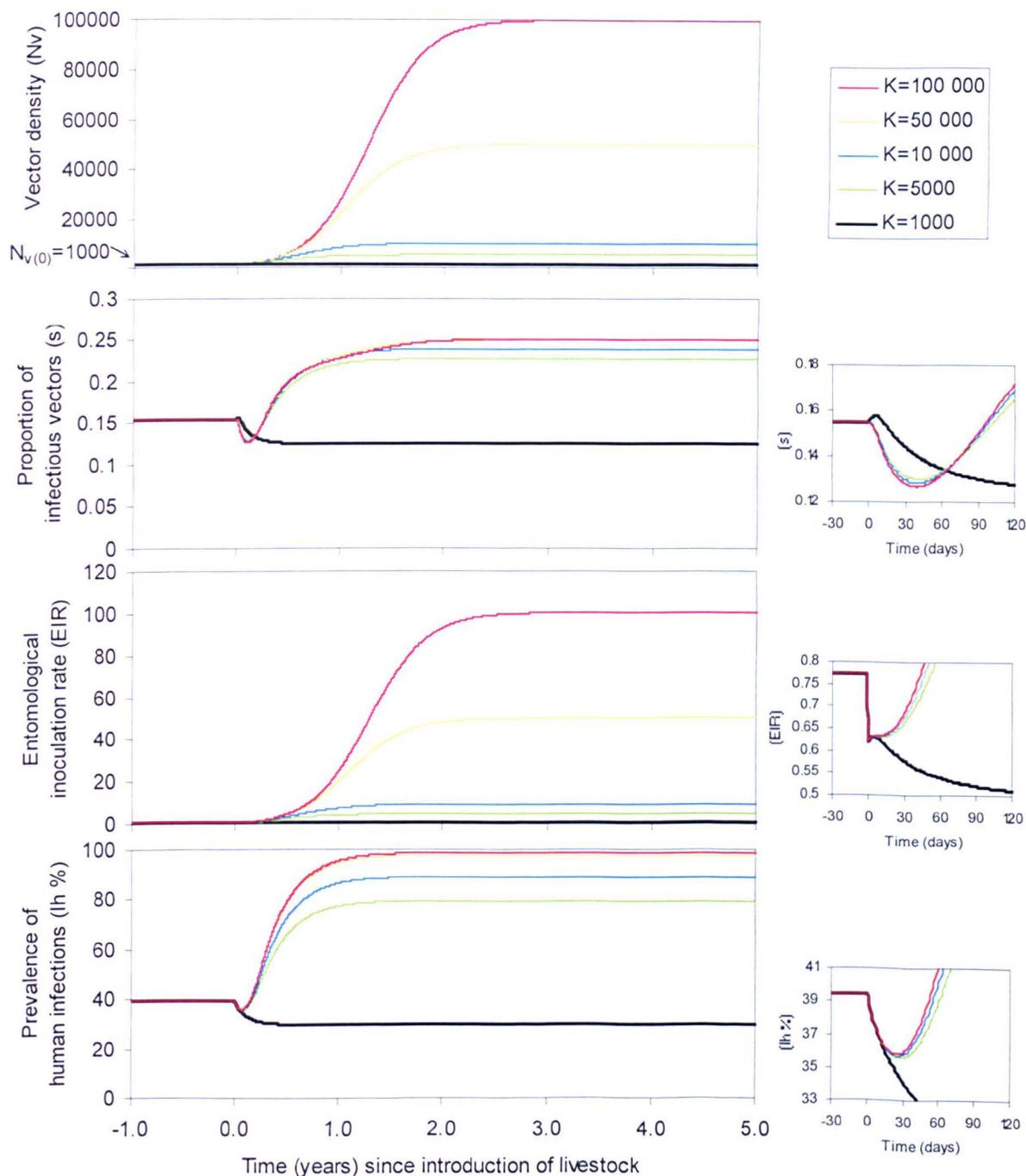


Figure C5. Temporal impact of introducing livestock in a setting with endemic malaria, where only humans and no livestock were present: effect of varying the carrying capacity (K) of the vector population, when $A_1=0.5$.

$N_{v(0)}=1000$; $rN_1=0.25$ (ratio of livestock:human density = 1:4); $N_h=100$. Other parameters values were kept fix as in previous figure. Additional outcome variables explored: Virtually immediately after the introduction of livestock, the values of the human blood index (HBI), and vector mortality rate (μ) will be decreased to a new value (the new endemic equilibrium value – which is the same value as that for the simulations with constant vector density), and will thereafter remain constant at that value, as long as the same numbers of livestock and human hosts remain present, with the same availability. The HBI is decreased from 1.00 to 0.80, and μ is decreased from 0.100 to 0.090, irrespectively of the carrying capacity level. Conversely, the human-biting rate (HBR) and the basic reproduction number (R_0) will change gradually through time following introduction of livestock, depending on the carrying capacity and in a similar pattern as that shown for the EIR although with a different scale.

APPENDIX D
(CHAPTER 5)

D1. Exploring the effects of extreme scenarios of ITL on the HBI

In extreme scenarios of effective coverage of insecticide-treated livestock, ε , and/or repellence probability, α , the expression for the proportion of blood-meals of human origin (Equation 5.2):

$$HBI = \frac{N_h A_h}{N_h A_h + N_l A_l (1 - \varepsilon \alpha)}$$

is reduced to the following:

1) If coverage is null ($\varepsilon=0$), or if repellence is null ($\alpha=0$) independently of the coverage, then

$$HBI = \frac{N_h A_h}{N_h A_h + N_l A_l},$$

which is the same as the initial expression for the human blood index that does not account for repellence (Equation 4.1).

2) If coverage is partial ($0 < \varepsilon < 1$) with maximum repellence ($\alpha=1$), then all the vectors that try to bite on treated livestock will be diverted to untreated livestock and/or to humans.

$$HBI = \frac{N_h A_h + \varepsilon N_l A_l \frac{N_h A_h}{N_h A_h + (1 - \varepsilon) N_l A_l}}{N_h A_h + N_l A_l} = \frac{N_h A_h}{N_h A_h + N_l A_l (1 - \varepsilon)}.$$

3) If coverage is total ($\varepsilon=1$) with maximum repellence ($\alpha=1$), then all the vectors that try to bite on livestock will be diverted to the humans available.

$$HBI = 1$$

4) If coverage is total ($\varepsilon=1$) with partial repellence ($0 < \alpha < 1$), then the vectors that try to bite on livestock may be diverted to other (“non-repellent”) livestock and/or human hosts.

$$HBI = \frac{N_h A_h + \varepsilon N_l A_l \frac{N_h A_h}{N_h A_h + (1 - \alpha) N_l A_l}}{N_h A_h + N_l A_l} = \frac{N_h A_h}{N_h A_h + N_l A_l (1 - \alpha)}.$$

D2. Exploring the effects of extreme scenarios of ITL on vector mortality

In extreme scenarios of effective coverage of ITL, ε , and/or repellence probability, α , the expression for the vector mortality rate (Equation 5.9):

$$\mu = \mu_{\min} + \left(\frac{1}{(N_h A_h + (1 - \varepsilon \alpha) N_l A_l) j} + \frac{\varepsilon (1 - \alpha) N_l A_l}{N_h A_h + N_l A_l} k \right) a$$

is reduced to the following:

1) If coverage is null ($\varepsilon = 0$),

$$\mu = \mu_{\min} + \left(\frac{1}{(N_h A_h + N_l A_l) j} \right) a$$

there is obviously no vector mortality due to insecticide, and vectors feed on humans and on livestock, as in Chapter 4.

2) If coverage is total ($\varepsilon = 1$) with no repellence ($\alpha = 0$),

$$\mu = \mu_{\min} + \left(\frac{1}{(N_h A_h + N_l A_l) j} + \frac{N_l A_l}{N_h A_h + N_l A_l} k \right) a$$

the vector mortality due to insecticide is maximum, and vectors feed on humans and on livestock.

3) If coverage is total ($\varepsilon = 1$) with maximum repellence ($\alpha = 1$),

$$\mu = \mu_{\min} + \left(\frac{1}{(N_h A_h) j} \right) a$$

there is no direct vector mortality due to insecticide. Instead, there is indirect mortality due to increased time searching for a host. It is as if no livestock were available for the vector to feed upon, since all the vectors are diverted to humans.

4) If coverage is partial ($0 < \varepsilon < 1$), with maximum repellence ($\alpha = 1$),

$$\mu = \mu_{\min} + \left(\frac{1}{(N_h A_h + (1 - \varepsilon) N_l A_l) j} \right) a$$

there is no direct vector mortality due to insecticide; rather there is only indirect mortality due to a longer search time, although less than in the previous scenario 3). With increasing coverage there is a decrease in the number of livestock available for the vector to feed upon, with a consequent increase in search time and in the associated vector mortality.

5) If coverage is total ($\varepsilon=1$), with partial repellence ($0 < \alpha < 1$),

$$\mu = \mu_{\min} + \left(\frac{1}{(N_h A_h + (1 - \alpha) N_l A_l) j} + \frac{(1 - \alpha) N_l A_l}{N_h A_h + N_l A_l} k \right) a$$

mosquito vectors will suffer both direct mortality due to the insecticide lethal effect, and indirect mortality due to longer search time. The higher the repellence probability, the lower is the magnitude of the former (μ_{txonly}) and the higher is the latter (μ_{search}) form of mortality.

APPENDIX E
(CHAPTER 6)

E1. Observed impact of treating livestock with insecticide on: 1) malaria incidence, 2) proportion of vectors parous; and 3) vector density, in Afghan refugee villages in Pakistan. (Source: Rowland et al. 2001, complemented with unpublished data courtesy of M. Rowland).

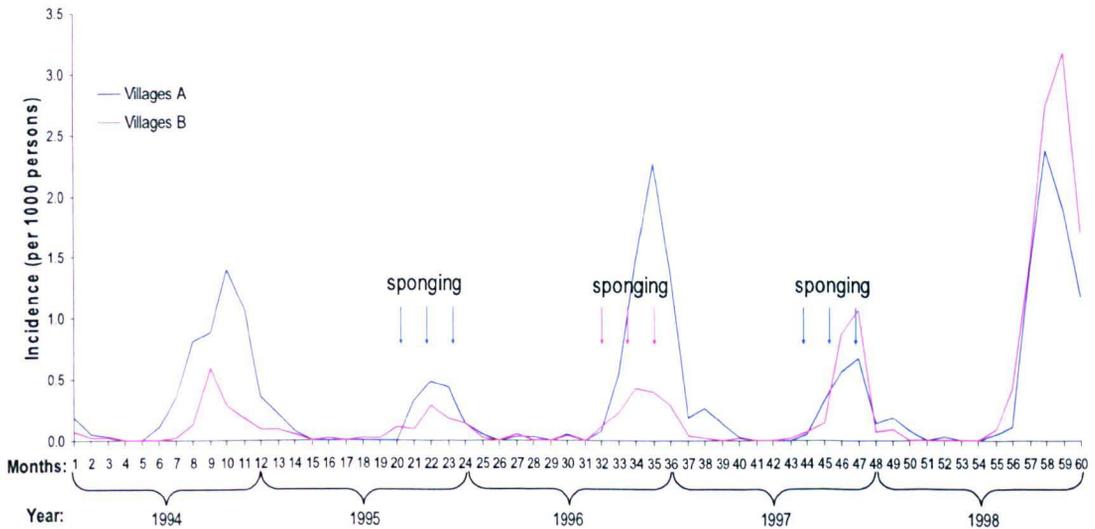


Figure E1. Impact of treating livestock with insecticide on incidence of *P. falciparum* malaria cases (per 1000 persons within each group of villages).

Villages A = 3 villages treated in years 1995 and 1997; total population = 37 206; Villages B = 3 villages treated in year 1996; total population = 56 329. The arrows represent the treatment rounds. Incidence was measured by passive surveillance from records of the village clinics, and consists of the number of all malaria episodes (even if they were recurrent or secondary infections, rather than the number of new infections only).

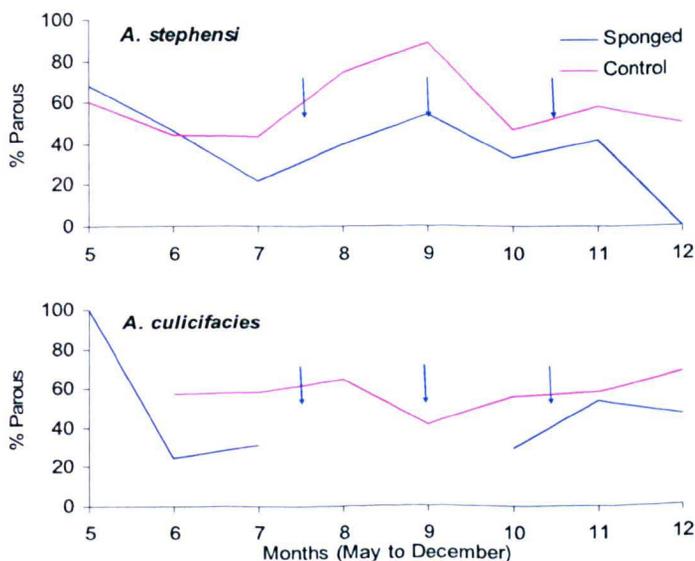


Figure E2. Parous rates during 1996 (year 2 of the trial) in one sponged village and one control village. The arrows represent the treatment rounds.

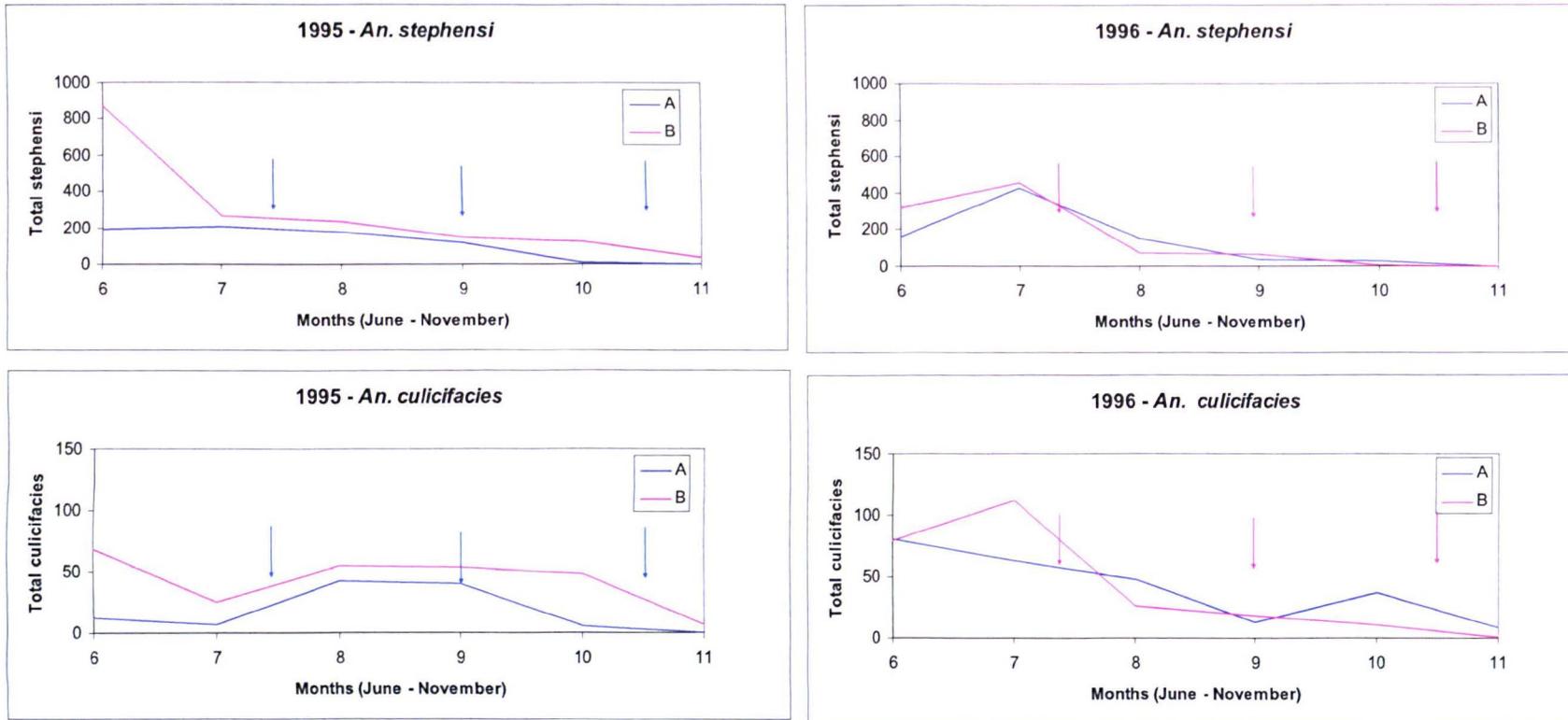


Figure E3. Impact of treating livestock with insecticide on vector density within each group of villages.

Villages A = 3 villages treated in years 1995 and 1997; total population = 37 206; Villages B = 3 villages treated in year 1996; total population = 56 329. The arrows represent the treatment rounds. In the analysis published (Rowland et al., 2001), only the post treatment data were considered: i.e. months 8 to 11 (August to November). The effect is more apparent in the second year of the trial (1996), where pre-treatment vector density was higher in villages B than in villages A, and post-treatment vector density became lower in villages B than in villages A.

E2. Model parameterization: Considering difference types of livestock

Table E1. Estimated A_l/A_h and predicted HBI and j for *An. culicifacies*, based on the observed HBI and relative abundance of difference groups livestock vs. humans, in Pakistan.

	A) Punjab Province			B) ITL trial area		
	All livestock	All livestock 2	Only Cattle	All livestock	All livestock 2	Only Cattle
N_l/N_h	0.370	0.368	0.363	0.140	0.115	0.090
HBI	0.05	0.05	0.05	0.12	0.14	0.17
A_l/A_h	53.24	53.95	54.68	53.24	53.95	54.68
j	-	-	-	0.238	0.262	0.347

All livestock=cattle+sheep+goats+donkeys; All livestock_2=cattle+(sheep+goats)/2+donkeys; Only Cattle=cows+ox+bulls+calves.

Source: Green - observed data - A) Reisen & Boreham 1982; B) Rowland et al. 2001; Blue - derived from observed data; White -predicted values. In bold are the values used in the Pakistan simulations. Additional parameters used for j calculation: $N_h=100$; $g=2.5$ (Mahmood & Reisen 1981); $surv_{noix}=4.63$ (derived from Rowland et al. 2001); $x=0.5$.

Table E2. Estimated A_l/A_h and predicted HBI and j for *An. arabiensis*, based on the observed HBI and relative abundance of difference groups of livestock vs. humans, in Ethiopia.

	A) Fuchucha village			B) Fuchucha+ 8 other kebelles (field study area)		
	All livestock	All livestock 2	Only Cattle	All livestock	All livestock 2	Only Cattle
N_l/N_h	1.49	0.98	0.46	1.13 (0.61-1.64)	0.70 (0.41-0.98)	0.26 (0.20-0.32)
HBI	0.42	0.42	0.42	0.49 (0.64-0.39)	0.5 (0.63-0.42)	0.56 (0.62-0.51)
A_l/A_h	0.94	1.43	3.04	0.94	1.43	3.04
j	-	-	-	0.060 (0.079-0.049)	0.078 (0.098-0.065)	0.145 (0.161-0.131)

All livestock=cattle+sheep+goats+donkeys; All livestock_2=cattle+(sheep+goats)/2+donkeys; Only Cattle=cows+ox+bulls+calves.

Source: Green - observed data - A) Tirados et al. 2006; B) Interviews to the villagers in the field study described in Chapter 2); Blue - derived from observed data; White -predicted values.

In bold are the values used in the Ethiopian simulations. Additional parameters used for j calculation: $N_h=100$; $g=2.5$ (Krafsur & Armstrong 1982); $surv_{noix}=8.01$ (derived from Taye et al. 2006); $x=0.5$. N_l/N_h =mean ratio of the number of animals per person calculated from the number of animals/person in each individual household. In A) all the households of the kebele were sampled; while in B) only some houses in each kebele were sampled, and therefore the sampling weighted mean is presented (the weight of each kebele was calculated dividing the total number of households in a kebele by the number of households interviewed in that kebele). Inside brackets are 95% CI.

Note: In A) the ratio of cattle to humans (0.46) differs from the value in the published paper (0.57). This is because the former is the mean of the N_l/N_h calculated for all the individual households of the Fuchucha village (from the raw data kindly provided by Tirados et al.), while the latter is the ratio of the total number of cattle vs. the total number of persons in all the households of the village.

APPENDIX F
(CHAPTER 7)

F1. Predicting the impact of ITL on the EIR of *P. falciparum* across Africa (DFID Project)

This Appendix describes preliminary work I have done in collaboration with others (colleagues from the LSHTM and the Natural Resources Institute, as part of a project for the UK Department for International Development - DFID), towards identifying the African regions where ITL is likely to produce the greatest reduction in malaria transmission, in order to inform where to conduct a community-based intervention trial. Since this was collaborative work, it was not presented as a chapter of this thesis and will be published elsewhere (Franco et al., In preparation).

Briefly, geographical data on the density of human (SEDAC, Columbia University, USA) and cattle (FAO) populations, and the distribution of *Anopheles arabiensis* and *An. gambiae s.s.* (Universities of Oxford and Durham, UK; MARA, South Africa) were assembled using GIS (see Table F1 for data sources details). These data were linked with a simplified version of the model developed in this thesis, and an EIR surface for *P. falciparum* malaria in Africa was generated on which the impact of insecticide-treated cattle was explored. Studies were made of the effect of varying underlying assumptions such as the proportion of blood meals from cattle and the proportion of cattle treated with insecticide, as illustrated in Figure F1.

The observed EIR dataset is small and unevenly spread in comparison to the extent of the spatial analysis and thus the outputs must be treated with caution. Nevertheless, in general the analysis indicates that treating cattle with a non-repellent insecticide would have a significant impact on malaria in the Sahel, East Africa and the savannah regions of southern Africa. Future work could account for the possibility of repellence and focus at a finer resolution country scale.

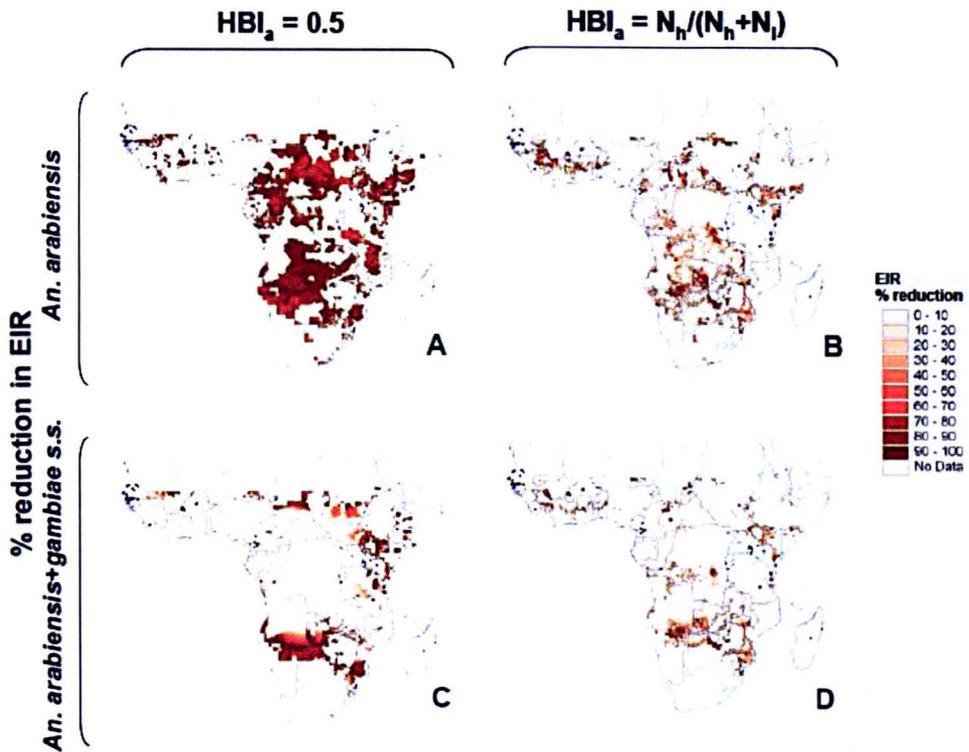


Figure F1. Predicted impact of insecticide-treated cattle on the Entomological Inoculation Rate (EIR) of *P. falciparum* across Africa.

The maps show the percentage reduction in the EIR for *An. arabiensis* only (A&B) and in the weighted averaged EIR for *An. arabiensis* and *An. gambiae s.s.* (C&D), assuming that the proportion of *An. arabiensis* blood meals on humans (HBI_a) is either fix (A&C) or spatially variable with the density of humans (N_h) and cattle (N_c) (B&D). Additional assumptions: fixed proportion of *An. gambiae s.s.* blood meals on humans: $HBI_g=0.94$; The availability of humans and cattle to vectors is the same: $A_h=A_c=0.5$; effective proportion of cattle treated with a non-repellent insecticide =100%. HBI = human blood index.

Table F1. Data sources for the GIS livestock and malaria DFID Project.

Name	Description	Date	Source*
Distribution of <i>An. gambiae s.s.</i>	Predicted environmental suitability distributions based on Fourier analysis of satellite data and digital elevation modelling	2002	1
Distribution of <i>An. arabiensis</i>	Predicted environmental suitability distributions based on Fourier analysis of satellite data and digital elevation modelling	2002	1
Distribution of <i>An. gambiae s.s.</i> (Boolean)	Extent of <i>An. gambiae s.s.</i> distribution	1998	2
Distribution of <i>An. arabiensis s.s.</i> (Boolean)	Extent of <i>An. arabiensis</i> distribution	1998	2
Estimated proportion of <i>An. gambiae s.s.</i> to <i>An. arabiensis</i> - in <i>An. arabiensis</i> areas	Proportion of <i>An. gambiae s.s.</i> to <i>An. arabiensis</i> within the boundaries of <i>An. arabiensis</i>	1998	2
Entomological Inoculation Rate (EIR) Georeferenced	Collected Observed EIR rates from various dates 1980s and 1990s (1981-1996)		3
Human Population Density	Density of human population per km ²	2000	4
Cattle observed reports 2002 (density)	Density of Observed cattle reports per km ²	2002	5
Mask of unsuitable areas for ruminants		2000	5
Country Boundaries/Countries	Administrative national level boundaries	1998	6

*

- 1 David Rogers, University of Oxford.
- 2 Chris Thomas - Durham University; GIS products from Lindsay et al. (1998).
- 3 Mapping malaria risk in Africa/Atlas du risque de la malaria en Afrique (MARA/ARMA URL; <http://www.mara.org.za>); collated source, from Hay et al. (2000). Original data from multiple sources collected in the 1960's and 1970's by Ross Institute.
- 4 Global Rural Urban Mapping project (GRUMP) - Center for International Earth Science Information Network (CIESIN), Columbia University; International Food Policy Research Institute (IPFRI); the World Bank; and Centro Internacional de Agricultura Tropical (CIAT); 2004. Global Rural-Urban Mapping Project (GRUMP): Urban/Rural Population grids Palisades, NY: CIESIN, Columbia University. Available at <http://sedac.ciesin.columbia.edu/gpw/>
- 5 Tim Robinson - data produced and made available by the Food and Agricultural Organisation Animal Production and Health Division (FAO-AGA), in collaboration with ERGO and the TALA research group, University of Oxford, UK.
- 6 ESRI Digital Chart of the World.

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