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Epidemiology of Traumatic Myiasis due to *Chrysomya bezziana* in Indonesia

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ABSTRAK

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Epidemiologi myiasis traumatika di Indonesia dipelajari melalui larva lalat yang dikoleksi dari ternak di berbagai daerah dengan melakukan surveilan pasif yang melibatkan Pos Kesehatan Hewan (Poskeswan). Data bulanan kasus myiasis di Kediri – Jawa Timur pada tahun 2006-2009 juga dianalisis untuk memperoleh gambaran kejadian musiman myiasis di daerah tersebut. Larva yang dikoleksi dari 260 kasus myiasis di berbagai daerah dan 341 kasus di Kediri diidentifikasi. Berdasarkan hasil identifikasi, seluruh penyebab myiasis traumatika di Indonesia adalah lalat *Old World Screwworm* (OWS), *Chrysomya bezziana* (Diptera : Calliphoridae) kecuali lima kasus pada ayam yang disebabkan oleh spesies lalat *Musca sp*. Jumlah kasus myiasis traumatika setiap bulan di Kediri sangat bervariasi, namun demikian peningkatan kasus secara nyata terjadi pada bulan Januari dan Desember pada saat musim hujan. Jumlah kasus infestasi larva OWS tertinggi pada studi ini adalah sapi dan kambing. Hasil ini membuktikan bahwa ternak sapi memiliki resiko terserang myiasis traumatika lebih tinggi dibandingkan ternak yang lain. Vulva dan umbilikus (tali pusar) adalah bagian tubuh yang paling sering terserang myiasis dan memiliki korelasi positif dengan proses beranak. Analisis DNA mitokondria terhadap 176 sampel membuktikan bahwa marka ini mampu digunakan untuk mendeteksi adanya multi-infestasi, namun tidak dapat menunjukkan korelasi positif antara garis keturunan tertentu dengan inangnya. Kombinasi iklim Indonesia yang terletak di garis katulistiwa dan sistem peternakan yang masih traditional menjadi faktor utama berkembangnya lalat OWS sepanjang tahun. Apabila myiasis tidak mendapat perhatian, maka akan menjadi ancaman peternakan di Indonesia termasuk menjadi masalah terhadap kesejahteraan para peternak. Data epidemiologi pada studi ini merupakan data myiasis traumatika yang cukup lengkap dan menjadi studi yang penting dalam mendukung program manajemen hama yang terintegrasi.

Kata Kunci: Myiasis, Mitokondria, *Chrysomya bezziana*, Epidemilogi

ABSTRACT

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Epidemiology of traumatic myiasis in Indonesia was studied by the widespread collection of fly larvae from infested livestock in passive case detection surveys involving veterinary clinics. In addition, monthly data from Kediri regency in Eastern Java were analysed from 2006-2009 to explore the seasonality of myiasis. Larvae from a total of 260 cases from the nationwide survey and 341 cases from Kediri were identified. Except for 5 cases of chicken infestation due to *Musca species* in the nationwide survey, all other cases were exclusively caused by the Old World screwworm (OWS) fly, *Chrysomya bezziana* (Diptera: Calliphoridae). The monthly numbers of cases at Kediri were very variable, with cases in all months, but there was statistical evidence for an increase in cases in January and December, during the rainy season. The greatest numbers of infestations recorded were from cattle and goats. The most frequently infested sites nationwide and in Kediri were the vulva and umbilicus, associated with calving, which is a major risk period for traumatic myiasis. Mitochondrial DNA typing of 176 specimens was useful for detecting multiple infestations, but no association was found between genetic lineage and host. The equatorial climate of Indonesia, combined with poor husbandry systems are factors that help to support OWS fly development year round. Even if not considered a disease of strategic importance, screwworm myiasis remains a threat to livestock production in Indonesia and a major welfare issue that requires constant interventions by farmers. The new and collated epidemiological data presented represent the most extensive survey of traumatic myiasis in Indonesia to date and provide a valuable baseline to support integrated pest management programmes.

Key Words: Myiasis, Mitochondria, *Chrysomya bezziana*, Epidemiology

INTRODUCTION

The larvae of the Old World screwworm (OWS) fly, *Chrysomya bezziana* (Diptera: Calliphoridae), cause traumatic myiasis and remain a major problem in sub-Saharan Africa and Asia (Hall et al. 2016). The disease is considered to be a serious animal health and welfare problem in the central livestock regions of Indonesia, particularly in the East where livestock are raised freely in the field for their entire life, a practice called the extensive husbandry system (Partoutomo 2000). The disease is found in both traditional and commercial farms and cases occur throughout the year (Wardhana et al. 2014).

Since the first report in 1926, in Minahasa, North Sulawesi (Kraneveld & Schaaf 1937), myiasis has continued to be a livestock problem in Indonesia, and since 1948 the fly causing most infestations has been identified as the OWS fly (Wardhana et al. 2014). However, this disease is not considered to be of strategic national importance and therefore livestock owners rather than veterinarians apply treatments that are highly diverse in manner and outcome. In particular, because effective insecticides, such as Dichlorfenthion (Gusanex®) favoured by veterinarians, are expensive and difficult to find in the markets, less affluent rural farmers depend instead on herbal medicines to kill the larvae in the wound, such as liquid of tobacco extract and others (Sukarsih et al. 1989; Wardhana & Diana 2014; Mustika et al. 2016; Wientarsih et al. 2017). They also traditionally used kerosene, battery carbon with motor oil, gasoline and petrol to treat myiasis (Sukarsih et al. 1989). These methods might kill the larvae, but lead to skin irritation rather than wound healing. In East Sumba, many farmers additionally used insecticides developed for control of plant pests such as Isoprocarb 50% WP (MIPCIN 50 WP®), which is potentially dangerous for livestock and can cause poisoning, even death (Wardhana 2006).

Partoutomo (2000) reported three main reasons why acute outbreaks of traumatic myiasis still occur in Indonesia. Firstly, the primary myiasis agent, the OWS fly, is distributed throughout the Indonesian archipelago; secondly, the environment and warm tropical weather with high humidity are suitable for development of *C. bezziana* almost throughout the year; and thirdly, a large supply of livestock hosts are available year-round, many of which are raised in an extensive livestock management system where they are not always inspected daily and so not treated in a timely manner if infested. Based on observations on commercial farms, it was concluded that imported livestock and those raised under an extensive grazing system are at high risk for myiasis infestation (Sigit & Partoutomo 1991; Wardhana 2006). The World Organisation for Animal Health (OIE) provides country and disease data related to animal health that is held in the World Animal Health Information Database (WAHID) interface. According to the WAHID interface, myiasis cases remain a threat in many tropical countries. For example, Malaysia provided annual reports to WAHID between 2005-2014 which indicate that myiasis due to the OWS fly persists there. However, unlike its northerly neighbour, Indonesia has not submitted information on myiasis to WAHID. Clearly, this lack of reporting gives a wrong impression of the situation, because the disease is endemic and widely distributed throughout Indonesia (Wardhana et al. 2003; Wardhana et al. 2014).

The Sterile Insect Technique (SIT) has been successfully applied to eradicate a primary myiasis agent, *Cochliomya hominivorax*, from the North American continent and more recently from Libya and from most countries of Central America (Welch & Hall 2013). One of the reasons for successful eradication of *C. hominivorax* in Libya using the SIT was due to a good understanding of interrelated components such as epidemiology and surveillance of the disease, treatment techniques, rigorous animal inspection and quarantine, and a good communication between farmers, veterinarians and other stakeholders (Lindquist et al. 1992). Robinson et al. (2009) stated that the collection of epidemiological data is the central source of management information for SIT programmes, because the data are used to determine the status and severity of the infestations and the economic magnitude of the problem. In addition, epidemiological data are correlated with the release rate of sterile insects to maximise the effect of the programme in eradicating *C. hominivorax* (Cox & Vreysen 2005). Trials in Papua New Guinea revealed that the SIT should also be effective against *C. bezziana* (Spradbery et al. 1989; Mahon 2002).

However, before the SIT is applied in any country, the essential first steps are to collect comprehensive baseline data, including the distribution of myiasis, assessment of ecological and genetic data and also the infestation dynamics (Hall et al. 2001; Vargas-Terán et al. 2005). Moreover, Cox & Vreysen (2005) proposed that mapping a pest's distribution on a regional and national scale is a fundamental phase to assess the feasibility and spatial targeting of the SIT. Modelling of the distribution of the OWS fly in Indonesia was undertaken by Wardhana et al. (2014), but this included only records that could be geo-referenced. Therefore, the present study reports all known presence records by island, province and regency. It focuses on the infestation dynamics and epidemiology of OWS myiasis in Indonesia, including the seasonal dynamics in Kediri regency and potential behavioural differences between OWS flies characterised by different mitochondrial DNA lineages (Wardhana et al. 2012) in Banten and Kediri regencies, all on Java Island.

MATERIALS AND METHODS

Surveillance for myiasis of livestock and collection of infesting fly larvae

Three kinds of surveillance were co-ordinated by the Indonesian Research Center for Veterinary Science (IRCVS), Bogor, Java Island. 'Collaborative sampling' was performed by the IRCVS and a regional team during field trips of 1-2 weeks, after which the regional team was encouraged to carry out 'Continuing sampling' following the same protocols. 'Oriented sampling' was performed solely by a regional team, based on a protocol communicated by IRCVS. All three approaches were effectively passive case detection, because livestock owners were only asked to bring infested animals to a sampling point, not to carefully screen all body parts of each of their animals for any wounds.

'Collaborative sampling' was carried out by visiting a total of 170 farms, both traditional and commercial, during three years, 2005, 2007 and 2009 on the islands of Borneo, Sumatra, Java, Lombok, Sumbawa, Sumba and Sulawesi (Figure 1). 'Oriented sampling' was undertaken on these islands and others (Madura, Timor and New Guinea) 2002-2009 by contacts in local veterinary clinics, where the staff collected larvae from cases of myiasis without any selection based on species identification. Fly larvae were sampled from wounds and preserved according to a protocol (see below) that was sent to local veterinarians, livestock agencies and Disease Investigation Centres (DICs), and all samples were then sent to the IRCVS in Bogor.

Preservation and identification of fly larvae from myiasis wounds

Larvae were collected from wounds using forceps, killed by immersion in boiling water for *c.* 15 seconds (not always for 'Oriented sampling'), soaked in 80% ethanol for 15 minutes and then transferred into a tube of fresh 80% ethanol for preservation (Hall et al. 2001). The tubes were labelled with date, collector, host species, stages of larvae, wound location and capture location (village, district, regency, and province) and kept at -20°C. All samples were identified using a published suite of diagnostic morphological characters (Hall 2008).

Seasonal dynamics of myiasis cases in Kediri regency, East Java

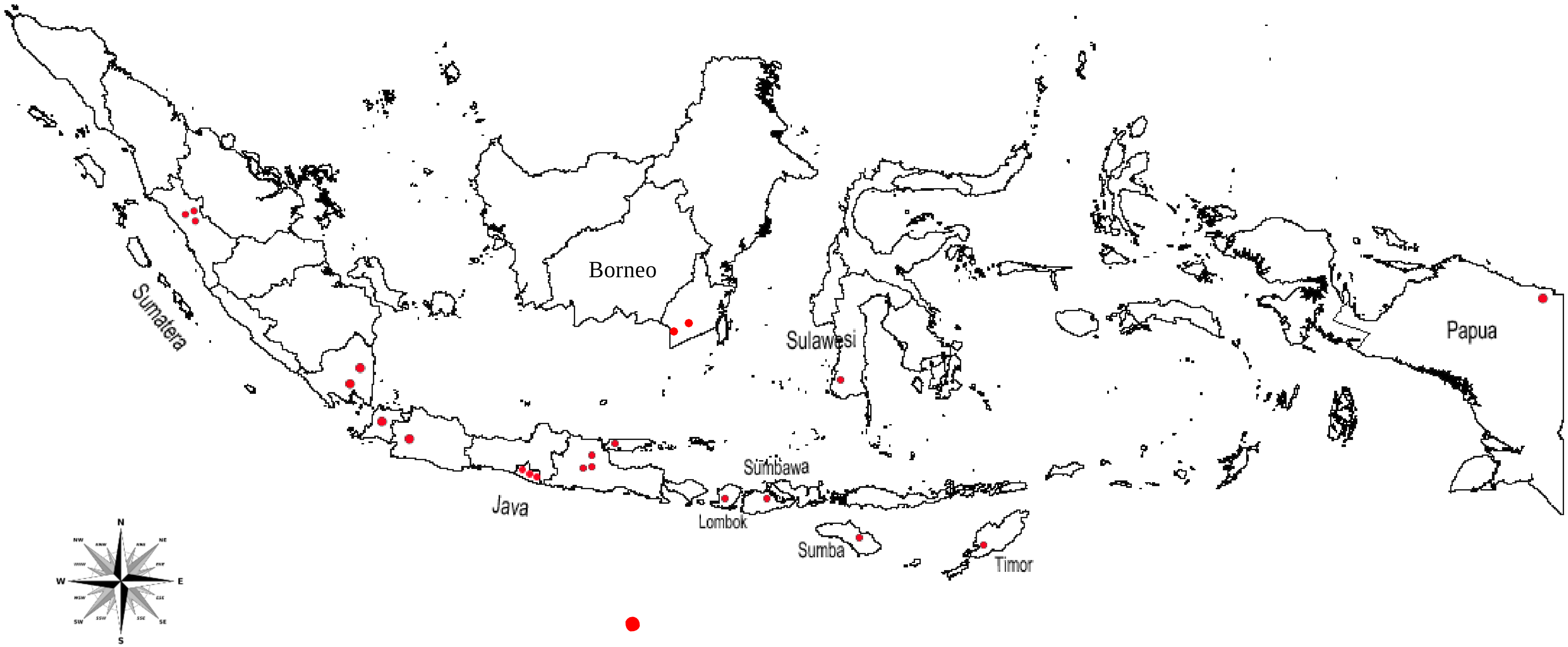
Kediri regency was chosen for a seasonal study because it is in an endemic area for traumatic myiasis, with a semi-intensive husbandry system, and the local veterinary clinic had a veterinarian who was prepared to collect data throughout a four-year period. The veterinarian and his two paramedic assistants monitored and controlled animal diseases across five districts (Kandat, Ngadiluwih, Ringinrejo, Kras, and Wates), and they recorded traumatic myiasis cases on their animal health reports to the regional livestock agency each calendar month, January 2006-December 2009.

Kediri regency (Figure 1) is located near the centre of East Java province, with 26 districts and 344 villages. Its agricultural system is mixed crop-livestock farming, typically with traditional semi-intensive livestock husbandry, for which animals are released from a stable (beside the farmer's house) c. 9 am and then returned to the stable c. 4 pm. No commercial farms, typically with >100 livestock and involved in short-term fattening programmes, were included in our sample. In the period 2006 - 2009 the maximum and minimum annual temperatures were 33°C and 22°C, respectively, and the area received an average rainfall of 1000-2000 mm per annum (BMKG 2017).

Mitochondrial DNA typing

The DNA extraction technique was performed according to (Chomczynski et al. 1997) using the DNAzol® kit (Invitrogen Corp., Carlsbad, CA, U.S.A.). Successfully extracted DNA samples were dissolved in 1x Tris-EDTA solution and stored at -20ºC until analysis.

The primers used in this study were synthesized by MWG Biotech (UK) Ltd (Milton Keynes, U.K.). The 3' terminal end of the mitochondrial gene *cytochrome b* (*cyt b*) (761 base pairs (bp) of the CB fragment plus primers) was amplified using the primer pair CB1-SE (5' TATGTACTACCATGAGGACAAATATC 3') and PDR-WR04 (5' ATTTCACGCTCATTAACT 3').


Sumatera
Borneo
Sulawesi
Papua
Java
Sumbawa
Lombok
Sumba
Timor
N
E
S
W

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PCR conditions in the present study were optimized for dried specimens and those preserved in 80% ethanol (Ready et al. 2009; Wardhana et al. 2012), so that all could be properly analyzed (Hall et al. 2009a; 2009b). For this *cyt b* fragment (CB), the PCR conditions were commenced by a 'hot start' at 80°C, followed by an initial 3 min. denaturation step at 94°C, then 5 cycles of denaturation at 94°C for 30 seconds, annealing at 40°C for 30 seconds, ending with an extension step at 72°C for 90 second. This was followed by 35 cycles of denaturation, annealing and extension, where running parameters were those above except for a substituted annealing temperature of 44°C. The PCR reaction was finished by a final extension step of 72°C for 10 minutes and subsequent holding at 4°C. Sanger sequencing of each DNA strand used the primers CB1 and PDR-WR04, following the protocol of Ready et al. (2009).

Statistical analysis

All data were entered into a Microsoft Excel 2008 spreadsheet by date, location, host species, sex, age, and wound site. Categorical data were analysed by a Yates corrected χ^2 (Chi Square) test in Epi Info version 6.04d (Dean 1996) to test the effect of host species, host gender and wound site on the number of infestations. Continuous data, such as number of cases in different districts, were analysed with Unistat v. 6.0 and ANOVA in Stat-View v. 5.0. The results were considered significantly different at the 95% probability level ($P<0.05$).

RESULTS AND DISCUSSION

Predominance of OWS in traumatic myiasis wounds of livestock in Indonesia

The OWS fly was found to be the predominant species responsible for traumatic myiasis (255/260; 98.1%) in all collections from 2002-2009 (Table 1). *Musca* species (Muscidae) accounted for the other cases (5/260; 1.9%), with all found in rectum wounds of chickens as part of the 'oriented sampling'. These rare cases were not considered in any further analysis.

Pre-2002 cases were discussed by Wardhana et al. (2014), and again the OWS fly predominated from the first time this species was definitively recorded (Kraneveld & Pettinga 1949). The earliest case from 1926, reported by Kraneveld & Schaaf (1937), is now believed to have been caused by *Booponus intonsus* (Calliphoridae). Only OWS cases are considered further in the current report.

Geographical distribution of OWS in Indonesia

Historical records of OWS in Indonesia were reported and discussed by Wardhana et al. (2014), but they did not include a comprehensive table of all distribution records by province and regency because their report focused on ecological niche modelling based only on presence records that could be geo-referenced. The three kinds of surveillance carried out from 2002-2009 produced a total of 255 traumatic myiasis cases caused by the OWS fly in livestock from the western end to the eastern end of Indonesia. Table 1 includes these 255 cases from 22 regencies on 9 islands, which consist of records from one regency on Kalimantan (Tanah Laut), 5 regencies on Sumatra (Agam, Mungka, Payakumbuh, Lampung, Seputih Rahman), 9 regencies on Java (Banten, Sukabumi, Gunung Kidul, Klaten, Sleman, Blitar, Jember, Lamongan, Kediri), one regency on Madura (Bangkalan), one regency on Lombok (Mataram), one regency on Sumbawa (Sumbawa Besar), one regency on Sumba (East Sumba), one regency on Timor (Kupang), one regency on Sulawesi (Makassar) and one regency on New Guinea (Jayapura).

Larvae of *C. bezziana* were successfully collected from 12 regencies by 'collaborative sampling' and 'continuing sampling', which yielded 76 of the 255 cases (Table 2). The total livestock numbers on the 170 farms visited were recorded, allowing for an estimate of minimum prevalence rates of OWS on the two major hosts: cattle 0.3% (54/17359) and goats 3.2% (22/696).

'Oriented sampling' generated 179 cases. However, on the larger islands, veterinary clinics recorded no OWS cases in two out of two locations on Borneo (compared with cases discovered in one location investigated more actively by 'collaborative sampling' and 'continuing sampling'), no OWS cases in three out of three locations on Sumatra (compared with cases discovered in all 5 locations investigated more actively), and some OWS cases in one out of three locations on New Guinea (no locations investigated more actively) and, in marked contrast, many OWS cases in 8 out of 10 locations on Java. Table 1, based on 255 cases from widespread locations throughout the Indonesian archipelago, together with Table 3, based on 341 cases from Kediri, represent the most comprehensive record of myiasis cases from Indonesia to date.

Seasonal distribution of OWS in Kediri regency

A total of 341 traumatic myiasis cases was recorded in the five districts of Kediri province during ‘collaborative sampling’ and ‘continuing sampling’ 2006–2009 (Table 3). These cases were additional to the 255 mentioned previously (Table 1). The annual total was highest in 2007 (29.6% of the overall total) and lowest in 2008 (16.1%). The highest number of myiasis cases was found in Kandat district (66.6% of the overall total) followed by Ngadiluwih (27.6%). The other districts (Kras, Ringinrejo, Wates) had very few cases, with none reported in 2008.

The mean monthly abundance of myiasis cases in the five districts (based on the mean percentage of the annual total in each month, to enable comparison of years) was relatively stable, fluctuating between 5-13% of the annual total each month (equivalent to a mean of 5-11 cases per month) (Figure 2). There was variation from year to year, as shown by the standard error (SE) bars, but cases were found in all months with a peak occurring in December-January. If data from December and January were excluded, then in each year except 2006, and also for the combined data (2006-2009), the monthly percentages for February to November did not differ significantly from an expectation of an equal number of cases in each month (χ^2 = 10.5-14.5, df = 9; P = 0.10-0.31).

Table 1. Number of OWS cases found by ‘collaborative sampling’, ‘continuing sampling’ and ‘oriented’ sampling in different regions of Indonesia

Province	Island	Regency/City	Species		Total
			C. bezziana	*Musca sp*	
South Borneo	Borneo	Tanah Laut	2	-	2
West Sumatra	Sumatra	Agam	28	-	28
		Payakumbuh	1	-	1
		Mungka	1	-	1
Southeast Sumatra		Seputih raman	1	-	1
		Lampung	11	2	13
Banten	Java	Banten	28	-	28
West Java		Sukabumi	2	-	2
Central Java		Klaten	5	-	5
		Sleman	2	-	2
		Gunung Kidul	41	-	41
East Java		Kediri	69	-	69
		Blitar	4	-	4
		Lamongan	1	-	1
		Jember	17	1	18
	Madura	Bangkalan	1	-	1
West Nusa Tenggara	Lombok	Mataram	10	-	10
	Sumbawa	Sumbawa Besar	14	-	14
East Nusa Tenggara	Sumba	East Sumba	4	-	4
	Timor	Kupang	1	2	3
South Sulawesi	Sulawesi	Makassar	8	-	8
West PNG	PNG	Jayapura	4	-	4
Total			255	5	260

Table 2 Seventy-six OWS cases discovered by collaborative sampling and continuing sampling in Indonesia and livestock numbers on the affected farms

Year	Province	Island	Regency	Total Farm	Total population		Number of cases	
					Cattle	Goat	Cattle	Goat
2005	East Java	Java	Kediri	32	105	15	2	1
	West Nusa Tenggara	Sumbawa	Sumbawa Besar	16	41	67	0	9
		Lombok	Mataram	23	34	103	4	8
	East Nusa Tenggara	Sumba	East Sumba	7	20	0	0	0
	South Sulawesi	Sulawesi	Sidrap	2	7003	0	8	0
	West Sumatra	Sumatra	Agam	12	40	0	4	0
2007	Banten	Java	Banten	10	3107	300*	25	1*
	Lampung	Sumatra	Seputih rahman	2	130	3	0	1
			Lampung	25	498	56	0	0
	South Borneo	Borneo	Banjar	25	737	0	0	0
2009	West Sumatra	Sumatra	50 Kota	1	0	2	0	1
			Payakumbuh	3	38	150	0	1
			Agam	9	31	0	7	0
	Banten	Java	Banten	3	5575	0	4	0
Total				170	17359	696	54	22

*=sheep

Hosts and wound sites of OWS

Based on the 255 cases discovered by all three types of sampling throughout Indonesia, myiasis occurred both in livestock and dogs (Table 4). The two most commonly infested hosts were cattle, 65.5% (167/255) followed by goats, 22.34% (57/255). The data also showed that the umbilicus and vulva were the most common wound sites among the hosts, in 23.1% (59/255) and 16.5% (42/255) of cases, respectively. As expected, the majority of umbilical myiasis cases was found in calves 79.7% (47/59), while vulval myiasis was found mostly in adult cattle, 71.4% (30/42). Other common wound sites were legs (12.6%, 32/255) and hooves (10.6%, 27/255). Of the cases specifying a gender, the number in female hosts (55.4%, 124/224) was significantly higher than in males (44.6%, 100/224) (χ^2 = 4.43, df = 1, P = 0.1088).

Similarly to the 255 more widespread cases, the two most frequently recorded wound sites in Kediri during the investigation of seasonality (Table 3) were the vulva (25.8%, 88/341), mainly of cows after calving, and umbilicus of newborn calves (25.2%, 86/341). Unlike in the more widespread survey, where legs were found to be a major wound site (associated especially with poor stabling in Banten), in Kediri leg infestations were grouped in the bottom three (0.6%). However, as in the widespread survey, hoof infestations were common, being the only other site with more than 10% of records (14.4%, 49/341).

Mitochondrial typing of OWS flies from wounds

Of the 520 specimens from Indonesia typed for *cyt b* haplotype (Wardhana et al. 2012), 69 (13.3%), and 107 (20.6%) larvae came from wounds sampled for epidemiological investigations (Tables 3 and 4) in the regencies of Banten and Kediri, respectively (Table 5). Each larva was characterized by only one *cyt b* haplotype, which is consistent with maternal inheritance of the mitochondrial genome in flies. All haplotypes contained just one long open reading frame (ORF) with the expected stop codon, and so none was identified as a pseudogene that might be inherited chromosomally. The predominant haplotypes in each regency were haplotype CB_bezz06 of sub-lineage 2.2 and haplotype CB_bezz14 of sub-lineage 2.3, which together characterized 89.8% and 86.9% of the specimens from Banten (26 wounds) and Kediri (107 wounds), respectively (Table 5).

Most of the wounds in Banten (Table 6) and Kediri (Table 7) contained only one larval instar (73.1% and 55.8%, respectively), but some wounds contained different combinations of two larval instars (15.4% and 32.6%, respectively) or all three instars (11.5% and 11.6%, respectively). The proportion of these three types of wounds was not significantly different (Chi-square P>0.05) between the Banten sample with larvae typed for *cyt b* and the larger sample of 255 wounds from widespread Indonesian locations (Table 8), which comprised wounds with single instars (76.5%), two instars (18.4%) or three instars (5.1%). However, the proportion of these three types of wounds was significantly different (Chi-square P<0.05) between the *cyt b*-typed sample from Kediri and the larger sample of 255 wounds. This difference between Banten and Kediri remained if the analysis included only the wounds on cattle (25 and 26 wounds from Banten and Kediri, respectively) and not those on other hosts (just one wound on a sheep in Banten; but 15 wounds on goats and two wounds on dogs in Kediri) (Table 6 and 7). In Kediri, the ratio of wounds on different hosts was 26 cattle:15 goats:2 others, which was significantly different (Chi-square P < 0.05) to the ratio for the more general results, namely 167 cattle:57 goats: 31 others (Table 4). Any associations between *cyt b* haplotypes (or sub-lineages) and hosts were not analysed statistically, because the samples were temporally mixed. However, it can be noted that the two predominant haplotypes from sub-lineage 2.2 (CB_bezz06) and sub-lineage 2.3 (CB_bezz14) were found on each of the main hosts (cattle and goats). These hosts were also infested by each of the predominant haplotypes from sub-lineage 2.1 (CB_bezz02, CB_bezz12) on Sumatra.

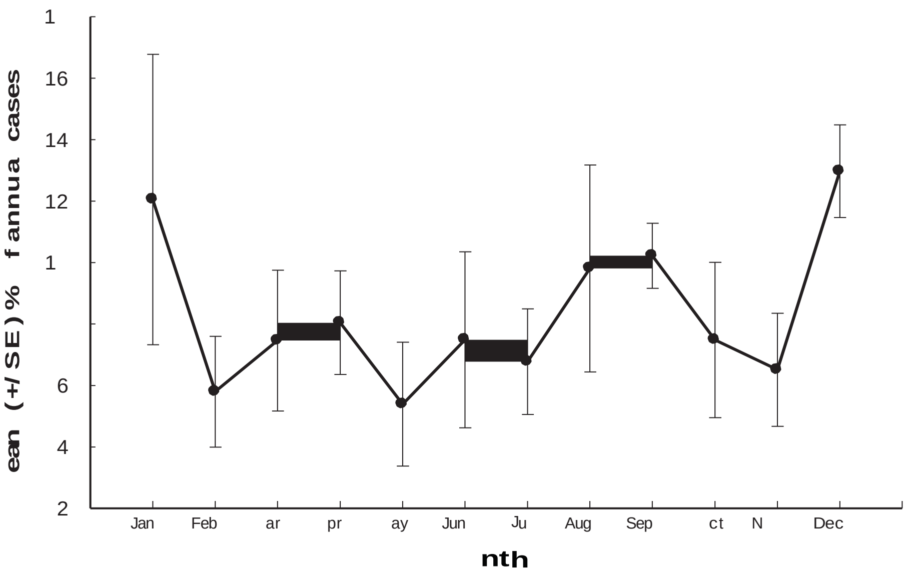

Figure 2. Mean monthly OWS cases as a percentage of the annual total (r SE) in the Kediri regency, 2006-2009.

Table 3. Number of OWS cases recorded by collaborative sampling and continuing sampling in five districts of Kediri regency

Districts	Years				Total
	2006	2007	2008	2009	
Kandat	59	66	33	69	227
Ngadiluwih	18	29	22	25	94
Wates	2	3	-	2	7
Ringinrejo	2	2	-	1	5
Kras	5	1	-	2	8
Total	86	101	55	99	341

Table 4. Distribution of myiasis wounds by livestock host and wound location among the 255 OWS cases discovered by 'collaborative sampling', 'continuing sampling' and 'oriented sampling' in Indonesia

Wound location	Gender of host			Host species											Total
				Cattle		Sheep		Goat							
	F	M	NR	Cattle	Calf	Sheep	Lamb	Goat	Kid	Buffalo	Horse	Dog	Pig	NR	
Umbilicus	20	28	11	4	47	-	3	3	2						59
Vulva	42	-	-	30	2	1	1	7	1	-	-	-	-	-	42
Leg	6	15	11	26	-	-	1	-	1	1	3	-	-	-	32
Hoof	18	8	1	17	-	-	-	9	-	-	-	-	1	-	27
Tail	7	3	-	-	-	5	-	2	3	-	-	-	-	-	10
Udder	9	-	-	8	-	1	-	-	-	-	-	-	-	-	9
Neck	3	4	2	5	1	-	-	-	-	-	2	1	-	-	9
Prepuce	-	8	-	5	-	-	-	2	1	-	-	-	-	-	8
Muzzle	2	6	-	6	2	-	-	-	-	-	-	-	-	-	8
Rectum	4	3	-	-	1	3	-	3	-	-	-	-	-	-	7
Mouth	2	5	-	-	3	-	-	4	-	-	-	-	-	-	7
Ear	4	1	-	2	-	-	-	2	-	-	-	1	-	-	5
Abdomen	-	7	1	-	-	-	-	5	2	-	-	1	-	-	8
Horn	2	2	-	1	-	-	-	3	-	-	-	-	-	-	4
Hip	1	3	-	-	1	-	-	3	-	-	-	-	-	-	4
Back	-	3	1	-	-	-	-	1	-	-	-	3	-	-	4
Eye	2	1	-	2	1	-	-	-	-	-	-	-	-	-	3
Upper Head	-	2	-	1	-	-	-	-	-	-	-	1	-	-	2
Thorax	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Scapula	-	1	-	1	-	-	-	-	-	-	-	-	-	-	1
N R	1	-	4	1	-	-	-	2	-	-	-	-	-	2	5
Total	124	100	31	109	58	10	5	47	10	1	5	7	1	2	255

Comment: NR : Not Recorded

Table 5. Frequencies of mitochondrial cyt b haplotype in larvae of the OWS fly sampled from livestock wounds in Banten and Kediri regencies

Mitochondrial *cyt b* typing		Banten sample		Kediri sample	
Haplotype CB_bezz	Sub-lineage	Number of larvae	Proportion, or haplotype frequency	Number of larvae	Proportion, or haplotype frequency
06	2.2	35	0.507	66	0.617
25	2.2	2	0.029	0	-
26	2.2	2	0.029	0	-
27	2.2	2	0.029	0	-
37	2.2	0	-	3	0.028
14	2.3	27	0.391	27	0.252
15	2.3	0	0.000	2	0.019
22	2.3	1	0.015	0	-
36	2.3	0	-	9	0.084
Total larvae		69		107	
Total wounds		26 [a]		43 [b]	

[a] See Table 6, [b] see Table 7

Table 6 Different complements of mitochondrial cyt E haplotypes (CB_Bezznn) in larvae D of the OWS fly from single wounds on 26 livestock in Banten regency

Haplotype complement	Larval instars with haplotype complement in each wound			Numbers of each host with such wounds			Total wounds
	L1	L2	L3	Cattle	Sheep (goats)	Dogs	All hosts
Single larval instar in each wound							
HC 2	[b]	06	-	1	0	0	1
HC 3	-	-	06	10	1	0	11
HC 4	-	-	14	5	0	0	5
HC 6	-	-	06, 14	1	0	0	1
HC 8	-	-	14, 22	1	0	0	1
Sub-totals				18	1	0	19
Multiple larval instars in each wound							
HC 9	06, 14	06	-	1	0	0	1
HC 13	-	06	25	1	0	0	1
HC 16	-	14	14	2	0	0	2
HC 20	06	06	06	1	0	0	1
HC 22	14	14	06, 14	1	0	0	1
HC 23	27	06, 14	26	1	0	0	1
Sub-totals				7	0	0	7

[a] Two larvae of each instar were individually typed from each wound; b Not present
L1 1st instar; L2 2nd instar, L3 3rd instar

Table 7. Different complements of mitochondrial *cyt b* haplotypes (CB_bezzNN) in larvae [a] of the OWS fly from single wounds on 43 livestock in Kediri regency

Haplotype complement	Larval instars with haplotype complement in each wound			Numbers of each host with such wounds			Total wounds
	L1	L2	L3	Cattle	Goats	Dogs	All hosts
				Single larval instar in each wound			
HC 1	06	- [b]	-	1	0	0	1
HC 2	-	06	-	3	0	0	3
HC 3	-	-	06	8	6	0	14
HC 4	-	-	14	1	3	0	4
HC 5	-	-	36	1	0	0	1
HC 7	-	-	06, 36	0	1	0	1
Sub-totals				14	10	0	24
				Multiple larval instars in each wound			
HC 10	14	06	-	1	0	0	1
HC 11	06	-	06	1	0	0	1
HC 12	-	06	06	1	0	0	1
HC 13	-	06	14	2	0	0	2
HC 14	-	06	14, 36	1	1	0	2
HC 15	-	06	36	0	1	0	1
HC 17	-	14	06	2	0	0	2
HC 18	-	15	06	1	0	0	1
HC 19	-	36	36	1	0	0	1
HC -	-	37	NR [c]	0	0	1	1
HC -	-	NR	06	0	1	0	1
HC 21	06	14	14	0	1	0	1
HC 24	37	06	06	0	0	1	1
HC -	NR	NR	14	1	0	0	1
HC -	NR	06	14	0	1	0	1
HC -	NR	06	06, 14	1	0	0	1
Sub-totals				12	5	2	19

a Two larvae of each instar were individually typed from each wound; b Not present ; c No result
L1 1st instar; L2 2nd instar, L3 3rd instar

Multiple OWS infestations of the same wound

Multiple infestations of a wound were detected by the presence of more than one larval instar and/or more than one *cyt b* haplotype of the OWS fly. Each larva contained only one identified *cyt b* haplotype, and only two larvae of each instar were usually typed from each wound. Therefore the detection of all haplotypes in a wound was not guaranteed, and the results provide a minimum estimate of multiple infestations.

Considering only the presence of single or multiple larval instars, not of any *cyt b* haplotype diversity, infestations with third instars alone formed the most common type of myiasis recorded among 255 wounds from widespread locations (65.5%), followed by multiple infestations with both second and third instar larvae (15.7%) (Table 8). When comparing the proportions of occurrence of single- and multi-instar infestations, there were no significant differences (Chi-square P>0.05) between the six regencies with large numbers of OWS infestations (Agam and Lampung on Sumatra; Banten, Gunung Kidul, Kediri and Jember on Java; Table 1), between the predominant hosts (cattle, goats and sheep) (Table 8), or between the five most infested sites on hosts (umbilicus, vulva, leg, hoof and tail) (Table 4). However, there were insufficient data to perform multivariate analyses to identify any co-variation between geographical region, host species, wound location and season.

Furthermore, multiple infestations are likely to be underestimated because, in well sampled locations, *cyt b* typing indicates that secondary infestations can occur over periods short enough to produce wounds occupied by only a single instar, i.e. with two or more haplotypes present in the same instar. This was the case for three wounds with only third instar larvae but two *cyt b* haplotypes (two in Banten, one in Kediri) and five multi-instar wounds with two *cyt b* haplotypes found in larvae of the same instar (three in Banten, two in Kediri) (Table 6 and 7).

There was no evidence for any association of a *cyt b* haplotype or sub-lineage with primary or secondary infestation. Based on 23 fully characterized multi-instar wounds (Table 6 and 7), only haplotypes of one sub-lineage were found in all the instars from 8 wounds; 6 wounds contained the oldest instars with haplotype CB_bezz06 (or another haplotype of sub-lineage 2.2) in the absence of haplotype CB_bezz14 (or another haplotype of sub-lineage 2.3); the reverse was true for 7 wounds; and, both sub-lineages were present in the oldest instars from two wounds.

Discussion

Geographical distributions of myiasis caused by fly species and their genetic lineages

The OWS fly was the primary cause of traumatic myiasis of livestock in Indonesia, and no larvae of the facultative parasite *Chrysomya megacephala*, the sister species, were found in any of the wounds, despite this species being widespread in the country (Wardhana et al. 2012). Only a very few cases of chicken myiasis caused by *Musca* species were recorded. Myiasis of poultry by species of Calliphoridae is generally rare, reported for *Lucilia sericata* [on turkeys in Iraq (Al-Khalidi & Shareef 1985) and on geese in Hungary (Farkas et al. 2001)] and for *Cochliomyia macellaria* and *Phaenicia* species [on chickens in Panama (Bermúdez et al. 2007)] and the OWS fly [on chickens in India (Jeyathilakan et al. 2011)].

In the present study, more larvae and myiasis cases were found in Kediri, East Java (27.1% of 255) than in any other location. However, we believe that the results were correlated with sampling intensity and local veterinarians' interest in myiasis and their enthusiasm for surveillance, rather than with case prevalence or the OWS fly's population density. Most infestations discovered 2002-2009 came from local veterinary clinic services participating in the 'collaborative sampling' and 'continuing sampling' schemes, such as in Kediri, rather than our 'oriented sampling' scheme, which was ineffective outside Java. All three sampling schemes

Table 8. Numbers of myiasis wounds on different livestock hosts that contained single and multiple larval instars among the 255 OWS cases discovered by 'collaborative collections', 'continuing collections' and 'oriented collections' in Indonesia

Host species	Larval instars [a]							Total
	L1	L2	L3	L1 + L2	L1 + L3	L2 + L3	L1 + L2 + L3	
Cattle	6	13	105	2	3	30	8	167
Sheep	1	1	12	-	-	1	-	15
Goat	3	4	38	2	-	6	4	57
Horse	-	-	5	-	-	-	-	5
Buffalo	-	-	1	-	-	-	-	1
Dog	-	-	3	-	-	3	1	7
Pig	-	-	1	-	-	-	-	1
NR [b]	-	-	2	-	-	-	-	2
Total	10	18	167	4	3	40	13	255

[a] L1 1st instar; L2 2nd instar, L3 3rd instar
[b] NR Not recorded

were variants of passive case detection, which cannot be relied upon (Vreysen 2005) to demonstrate changes in the population density of screwworm in an area. It is more likely to be a reflection of reporting efficiency and the level of farmer cooperation.

The OWS fly in Indonesia is characterised by mitochondrial DNA haplotypes grouped into four sub-lineages, with the Asian mainland sub-lineage 2.1 predominating on Sumatra in the west, sub-lineages 2.2 and 2.3 predominating on Java, sub-lineage 2.2 predominating on the smaller islands of Wallacea (Nusa Tenggara and Maluku), and sub-lineage 2.4 predominating on New Guinea in the east (Wardhana et al. 2012). The samples analysed in the current report reflected this pattern.

Seasonal and environmental distribution of OWS myiasis infestations

The surveillance data from 2006-2009 showed numbers of reported myiasis cases in the range of 55-101 per year in five districts of Kediri and demonstrated that traumatic myiasis is a major problem for small farmers in this regency of East Java. Kandat and Ngadiluwih districts showed a higher number of cases of myiasis than other districts (Table 3). These two districts are only about 2 km from the animal clinic where the data was collected, making it more likely that traditional farmers would report their livestock problems to the veterinarians. Wates, Ringinrejo and Kras were approximately 10 km from the clinic and the larger farmers in those districts reported their livestock problems to private veterinarians who do not maintain an accurate record of myiasis cases. Therefore the myiasis problem is likely to be significantly greater than suggested by the reported data.

Even though cases could be found in every month during the four-year study period, there was a slight seasonal trend for myiasis cases to peak in Kediri in December-January (Figure 1), during the rainy season (http://www.worldweatheronline.com). This finding is consistent with what is known about the seasonality of OWS in other Asian regions. Although the peak of myiasis cases might occur in different months in different geographical regions, the peak months everywhere experience similar climatic conditions, i.e. moderately high temperature and humidity (Sutherst et al. 1989). In this way, the highest number of OWS cases in Iraq and Oman occurred in the cooler months of December-March (Al-Taweel et al. 2000; Spradbery 1992; Siddig et al. 2005), and cases in Saudi Arabia usually peak in March-May when there is higher rainfall (Alahmed et al. 2006). However, even if temperature and humidity are major determinants of the seasonality of the OWS fly, many other environmental factors can directly or indirectly influence its distribution, such as human population density, cattle density, elevation and tree cover (Wardhana et al. 2014).

Seasonality is unlikely to have significantly affected our investigations into host preferences and wound locations of OWS (see the next section), because most of the widespread cases came from the larger islands, where seasonal differences in climate are small and similar to those in Kediri on Java (Wardhana et al. 2014).

Hosts of traumatic myiasis and wound sites

The predominant host animals attacked by *C. bezziana* in Indonesia in this study were cattle (65.5%) followed by goats (22.4%) (Table 2 and 4). However, this in itself does not necessarily imply that cattle are more susceptible to myiasis or are the preferred host because the numbers of livestock at risk of infestation in the same herds/flocks need to be taken into account. Where available, these data suggest that goats are at much greater risk of OWS infestation than cattle, with 5.3% (21/396) of goats infested compared to 0.31% (54/17,359) of cattle in the same geographical areas (Table 2). The goat data were skewed by the relatively high level of infestation of goats in West Nusa Tenggara (17/170 = 10%; Table 2). Clearly more extensive surveys of OWS myiasis prevalence are required in Indonesia to definitively report on OWS host preferences and the risks of myiasis infestation by OWS to different hosts in different regions of the country.

The major wound sites recorded in this study were the umbilicus of calves and the vulva of cattle, associated with calving. Calving is clearly a major risk period for infestation by the OWS fly and a period in which farmers should regularly check their livestock. The same is true for NWS fly, *Cochliomyia hominivorax*, but wound sites are also clearly related to environmental factors. Hence in the Yucatan Peninsula of Mexico, the most frequently observed sites of NWS infestations on cattle were related to vampire bat bite sites on the neck and shoulders, umbilicus sites being third numerous (Thomas 1987). Other common sites of myiasis in our study were the leg and hoof, possibly attributed to the poor condition of the stables: those with slippery and dirty floors caused some livestock to be wounded in their limbs or to develop foot-rot (Muharsini et al. 2010; personal observations in this study).

Hall et al. (2009a) noted that identification of wound sites is important in the treatment of myiasis because it indicates the best sites for the potential application of topical prophylactics for control of myiasis. Giving treatment preferentially to the hind quarters of the host would assist the protection of organs such as the genitalia, anus, udder and umbilicus.

Multiple infestations

Mature, third instar larvae were those most frequently removed from wounds, suggesting that farmers generally neglected to check their livestock as regularly as they should or simply overlooked smaller wounds with immature larvae during the first 5-6 days after egg laying. In the current study, the proportions of livestock wounds with multiple larval instars were somewhat similar in cattle (42/167 = 25.7%) and goats (12/57 = 21.1%). A substantial number of wounds were found with two larval instars (18.4%, 47/255) and a smaller number with all three instars (5.1%, 13/255) in sites as varied as hoof, prepuce, vulva, umbilicus, muzzle, thigh, ear and head (Table 8). Infestations in host sites that are difficult to handle and inspect would encourage the occurrence of multiple infestations. For example, although only a few prepuce infestations were recorded, a high proportion had multiple-instar infestations (75%, 6/8). Multiple infestations are underestimated if based only on the presence of multiple larval instars, because mitochondrial DNA typing demonstrated that larvae of the same instar often originate from two or more mothers, which must lay their eggs within 1-36 hours of each other.

Overlooked economic importance of OWS

This study clearly demonstrated that cases of traumatic myiasis caused by the OWS fly are widespread in Indonesia and occur frequently, despite the relatively small number of cases reported in the literature and by veterinary services. One reason for this might be the low mortality associated with myiasis if treatment is timely. Myiasis is not classified nationally among the diseases of strategic importance, such as anthrax, brucellosis, rabies, foot and mouth disease and avian influenza. Therefore, myiasis treatments are left to livestock owners to apply. Nevertheless, if infested livestock are not treated appropriately they can die or suffer permanent disability, and so the economic effects on small farms, with small numbers of livestock, can be relatively severe. Although our survey was completed in 2009, the dangers of screwworm myiasis still persist in Indonesia, not only to wild and domesticated animals, but also to humans (Hidayat et al. 2016).

The Indonesian government has yet to analyse the economic impact of OWS infestations on the livestock industry, but the impact will be significant if case prevalences are similar to those in adjacent Malaysia. Grindle et al. (2001) showed that the total annual loss of livestock production due to myiasis infestations in Malaysia was around RM 18 million (US $4.7 million). Similarly, Nor (2002) estimated that the annual losses to the Malaysian livestock industry due to myiasis infestation were about RM 23 million (US $ 6 million). Taking into account the plans of the Malaysian government to expand animal production, the economic impact was projected to reach RM 32 million (US $ 8.5 million) in 2005 and RM 50 million (US $ 13.1 million) in 2010 (Grindle et al. 2001). These estimates are similar to those from an economic analysis of the impact of *Cochliomyia hominivorax* in Cuba (Grindle et al. 2001).

A cost-benefit analysis of OWS myiasis prevention and control is required, in order to assess the economic effects on national and international trade of livestock. The epidemiological data presented in the current report provide a useful baseline of the host and spatio-temporal distribution of OWS in Indonesia, on which to build a more robust picture of monthly transmission, in order to support a control or eradication programme using SIT as a component of Integrated Pest Management (IPM). Accurate prevalence and incidence data are required, based on standardised surveillance of randomized samples of livestock, because current case densities reflect variable surveillance activity. This makes it difficult for Indonesia to regularly submit surveillance data to the World Animal Health Information Database (WAHID).

CONCLUSION

The epidemiology of traumatic myiasis caused by the OWS fly in Indonesia is complex, with multiple infestations of wounds frequent. The plasticity in host selection demonstrated by all mitochondrial lineages of the OWS fly in Indonesia is clearly advantageous to this species, as is its lack of marked seasonality, both of which facilitate colonization of new areas and diverse hosts.

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