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Risk factors for Crimean-Congo Haemorrhagic Fever (CCHF) virus exposure in farming communities in Uganda

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SUMMARY

Background: Crimean-Congo Haemorrhagic Fever (CCHF) is an emerging human-health threat causing sporadic outbreaks in livestock farming communities. However, the full extent and the risks associated with exposure of such communities has not previously been well-described.

Methods: We collected blood samples from 800 humans, 666 cattle, 549 goats and 32 dogs in districts within and outside Ugandan cattle corridor in a cross-sectional survey, and tested for CCHFV-specific IgG antibodies using Enzyme-Linked Immunosorbent Assays. Sociodemographic and epidemiological data were recorded using structured questionnaire. Ticks were collected to identify circulating nairoviruses by metagenomic sequencing.

Results: CCHFV seropositivity was in 221/800 (27·6%) in humans, 612/666 (91·8%) in cattle, 413/549 (75·2%) in goats and 18/32 (56·2%) in dogs. Human seropositivity was associated with livestock farming (AOR=5·68, p<0·0001), age (AOR=2·99, p=0·002) and collecting/eating engorged ticks (AOR=2·13, p=0·004). In animals, seropositivity was higher in cattle versus goats (AOR=2·58, p<0·0001), female sex (AOR=2·13, p=0·002) and heavy tick infestation (>50 ticks: AOR=3·52, p=0·004). CCHFV was identified in multiple tick pools of *Rhipicephalus appendiculatus*.

Interpretation: The very high CCHF seropositivity especially among livestock farmers and multiple regional risk factors associated exposures, including collecting/eating engorged ticks previously unrecognised, highlights need for further surveillance and sensitisation and control policies against the disease.

Introduction

Crimean-Congo Haemorrhagic fever (CCHF) is a tick-borne viral infection caused by the CCHF virus (CCHFV), a member of the genus *Orthonairovirus* and family *Nairoviridae*.[1] The virus circulates in an enzootic tick-vertebrate-tick cycle, involving a variety of domestic animals and wildlife, with humans usually acting as dead-end host.[2] Whereas the disease is transient in animals, it can manifest as a devastating haemorrhagic fever in humans, with a case fatality rate (CFR) of up to 40%.[3] CCHF is listed on the World Health Organization (WHO) priority diseases requiring accelerated research and development, as the disease has potential for causing

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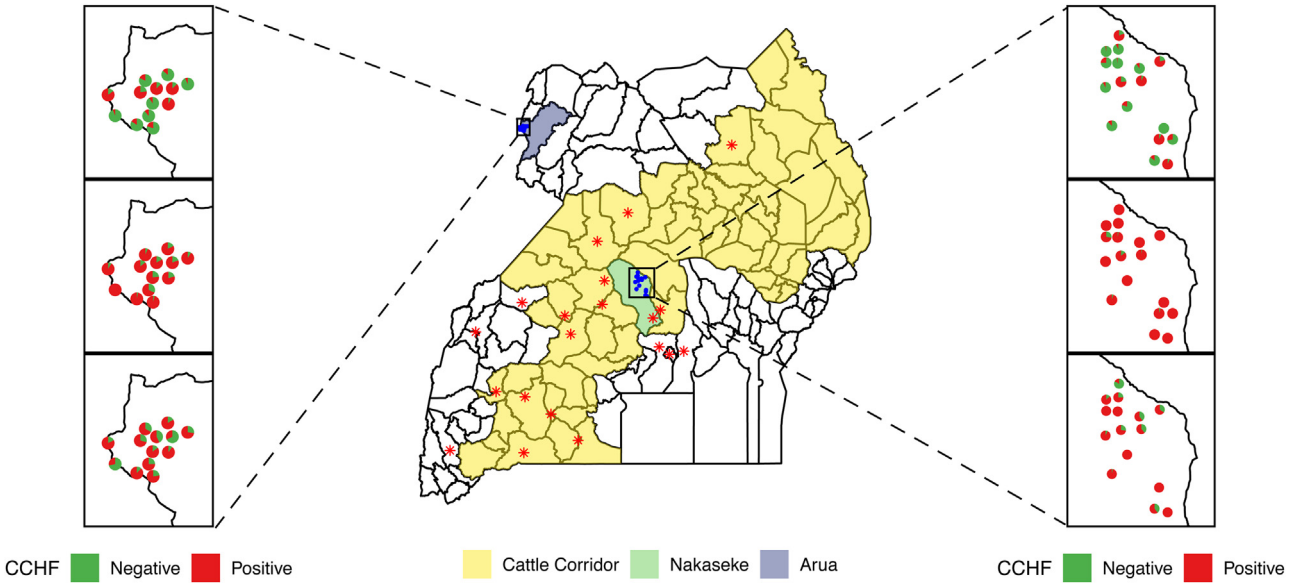

Fig. 1. Study area: Arua district, North-Western and Nakaseke, Central Uganda· The blue dots are the sampled farmers, the red asterisks show previous CCHF outbreaks while the yellow belt is Uganda's cattle corridor region. The green and red pies indicate the proportions of CCHF negative and positive farmers (A), cattle (B) and goats (c) on each farm.

public health emergencies without available medical countermeasures.[4]

In Uganda, CCHF cases have been detected since 1958.[5] However, in the last decade the frequency of reported outbreaks has increased, with new human cases emerging in historically non-hotspot areas.[6,7] Despite the rising incidence of CCHF in Uganda, surveillance activities are still limited to hospitalized human cases, and are almost non-existent in the animal population.[6,8,9] As in other countries, the main risk factors for human outbreaks in Uganda remain only partially characterized and the burden of disease is not well documented across the country.[10] As recently highlighted[7,11], zoonotic spillover of CCHFV involves close interaction of humans with animals, ticks and the environment. Therefore, it is critical to study not only human cases but surrounding hosts and vectors to gain a more useful understanding of the disease and to address knowledge gaps. In this study, we aimed to determine the seroprevalence of CCHF and elucidate the risk factors associated with transmission in human and animal populations in areas of Uganda, considered to be high risk (Nakaseke district) and low risk (Arua district) from CCHF. These areas lie within and outside the Ugandan's Cattle Corridor, respectively. Additionally, we collected ticks from animals and the environment to confirm the presence of nairoviruses by sequencing in the selected study areas.

Materials and Methods

Ethics

This study was undertaken as part of the ArboViral Infection study (AVI) approved by the Research Ethics Review Committee at the College of Veterinary Medicine, Animal Resources and Biosecurity of Makerere University, Kampala, Uganda (Reference Number: SVARREC/20/2018) and by the Uganda National Council for Science and Technology (Reference Number: HS 2485). Informed written consent was obtained from all study participants before they, and their animals, were enrolled into the study.

Study design

We selected two Ugandan districts of Arua (outside the Cattle Corridor) and Nakaseke (within the Cattle Corridor) for cross-sectional sampling, from December 2018 to March 2019 (Fig. 1). According to the available historical and recent records of CCHF occurrence in Uganda[6,7,12], Nakaseke district has recorded several CCHF cases, and was classified as a high-risk (exposed) area, while Arua district has not reported any human cases, and was classified as a low-risk (control) area.

Selection of study participants

A multistage probability sampling approach was used in which one Sub County was randomly selected from the available list of Sub Counties in the respective districts. Thereafter, a list of households that kept livestock, or not, within the selected Sub Counties was made. From each of these two categories of households, a random selection of households to enrol in the study was made depending on a predetermined sample size of 800 humans and 1,215 animal samples.[13] Once a household was selected, all members within it who were aged 8 years and above were assigned random numbers and at least three of them were selected randomly and asked to enrol in the study. Blood and tick samples were collected from a minimum of 25% of randomly selected animals from household herds.

Data collection

Socio-demographic and CCHF risk factor information was recorded using a structured questionnaire. Prior to administration of the structured questionnaire, a community engagement activity was carried out to explore potential additional risk factors for exposure to CCHF and the tool modified to include these. Briefly, data collected about humans included their gender, age, occupation, and predisposing factors such as their exposure to animal blood or tissues, tick bites and other socio-behavioral related activities such as crushing ticks with bare hands and history of collecting and eating engorged ticks. Animal data collected included breed, age, sex, and estimated tick infestation number. Herd-related risk factors such as livestock production systems, herd size, and tick control practices were also noted.

Sample collection and processing

Blood was drawn into 10-ml sterile vacutainer tubes (Becton Dickinson, Plymouth, UK) from humans and animals by medical and veterinary professionals respectively. In Arua, samples were transported under cold chain for initial processing at the Uganda Virus Research Institute (UVRI) plague laboratory or while in Nakaseke, samples were taken to Kinyogoga Health IV laboratory. Samples were centrifuged and serum aliquoted into 2ml sterile storage vials (Sarstedt Inc, Newton, North Carolina). Animal and human sera were heat inactivated at 56°C for 2 hours and 30 minutes, respectively, and stored at – 80°C, until further laboratory investigation at the Arbovirology laboratory based at UVRI, Entebbe, Uganda.

Livestock ticks were collected from half of the body of every selected animal, while environmental ticks were collected by both dragging and flagging methods. Ticks were transported in 70% ethanol for identification to species level using morphological keys[14,15] under a stereo- microscope (Stereo Discovery V12, Zeiss, Birkerød, Denmark). Tick pools containing 5-10 adult ticks, 10-20 nymphs and 25 larvae were made according to their collection sites, species, sex, and the host animal. All tick pools were then crushed in 0·5ml of Agencourt lysis buffer in a Genogrinder 2000 (OPS Diagnostics, Lebanon, NJ, USA), followed by downstream RNA extraction procedures as per manufacturer's instructions (Beckman Coulter).

Serological assays

Human sera was tested for CCHFV IgG using the Vector Best assay (Novosibirsk, Russia) following manufacturers' instructions. A starting sera dilution of 1:100 was used. Incubation steps were carried out at 37°C for 1 hr and followed by a wash, then a conjugate step and another incubation at 37°C for 30min. Reactions were developed at 25°C for 25 minutes before adding stop solution. Optical densities (ODs) were recorded at 450nm and 630nm as main and reference filters respectively using Gen5 software (Version 2·06, Winooski, VT, USA). The optical density cut off value (OD_{co}) was calculated by adding 0·2 to the optical density of the negative control and used for result interpretation. Samples were considered positive if their OD values were greater than or equal to the OD_{co} value.

At present, there are no approved and widely accepted assay that can serve as a gold standard for detection of antibodies against CCHFV in animal sera. In this study, as in others[16-18], we used ID Screen® CCHF Double-Antigen Multi-species ELISA kit (ID-Vet Innovation Diagnostics, France) for determining CCHFV seroprevalence in animals.

Animal sera was tested following the manufacture's test protocol. All steps were carried out at room temperature (RT) (23°C -25°C). The optical density (OD) was measured at 450 nm. Sample Positivity Percentage (S/P%) for each sample was calculated by dividing the OD value of the sample (OD_S) by OD of the positive control (OD_{PC}), multiplied by 100. Serum samples were considered positive if the value for their S/P% was over 30%.

To assess possible IgG cross-reactivity in animal sera to closely related nairoviruses, a subset of samples: 81 goat sera (41 IDVet seropositive and 40 IDVet seronegative) and 99 cattle (51 IDVet seropositive and 48 IDVet seronegative) were tested for anti-CCHFV NP and anti-NSDV NP using an inhouse antigen specific indirect ELISA (Jenner Institute, UK). Nunc MaxiSorp 96-well plates (Fisher Scientific) were coated overnight at 4°C with CCHFV NP and NSDV NP at a concentration of 2 µg/ml in PBS, as wells as without antigen. Plates were also coated with a specified concentration of a commercial animal IgG (Bio-Rad) which served as a positive control. After blocking with Blocker Casein in PBS, test samples and non-endemic negative controls (UK) at minimum 1:100 dilution were plated out for 2 hours at RT. Respective alkaline phosphatase-conjugated secondary antibodies were added depending on the animal species, anti-bovine IgG and anti-sheep/goat IgG followed by incubation for 1 hour at RT. Plates were developed using PNPP alkaline phosphatase substrate and read at 405 nm when the commercial animals' IgG control reached a specified OD405. Negative cutoffs were calculated using the formula: mean + 3·635 × standard deviation of the OD405 readings of the non-endemic negative-control serum samples, where 3·635 is the standard deviation multiplier with a 99% confidence level for n = 6 controls.[19]

Detection of nairoviruses in tick samples

Tick pools were investigated for the presence of CCHFV, NSDV, and DUGV genomes using target enrichment next generation sequencing as previously described using an ArboCap enrichment library targeting all arboviruses including nairoviruses.[20] Viral genomes were detected following *de novo* assembly using dipSPADES followed by BLASTn and mapping to the relevant nairovirus reference sequences.

Statistical analysis

Sociodemographic, epidemiological and laboratory data were analyzed using Stata Release Statistical Package (v15 Stata Corp LP, College Station, TX, USA). Sociodemographic and epidemiological characteristics were summarized using frequencies and percentages, first overall and then stratified by study groups. We estimated the seropositivity of CCHF as the number of samples that tested positive divided by total tested expressed as percentage. We performed both unadjusted and adjusted regression analysis to determine factors associated with CCHFV exposure. At the unadjusted analysis, the association between the outcome variables (CCHFV seropositivity in humans and animals) and potential risk factors, was first modelled using univariable logistic regression analysis. Multicollinearity was examined among different combinations of variables, and where there was a correlation greater than 0·5, we chose a factor most likely to be associated with CCHF infection *a priori*. A backward stepwise selection approach was used to remove factors that were not associated with the outcome in the adjusted analysis ($p>0·1$). Factors that attained $p>0.05$ were considered statistically significant. Similar considerations were made for the subsequent stratification of the analysis by human study groups and the animals. Receiver Operating Characteristic (ROC) analysis was performed to compare sensitivity and specificity for the anti-CCHFV NP in-house assay, and results presented as supplementary data.

Results

Baseline characteristics of the study population

Human participants

A total of 800 participants (386 livestock farmers and 414 non-livestock farmers) were enrolled into the study in Arua (n=422; 52·7%) and Nakaseke (n=378; 47·3%) districts (Table 1). The average age of the participants was 36·6 (SD=18·0) years, and 640 (80%) of them were males. One hundred and eighty-four (23·0%) of the participants had a history of tick bites, the presence of rodents and bats around homes was reported by 285 (35·6%) and 212 (26·5%) of the participants, respectively. Relatively few participants reported any contact with blood or tissues of slaughtered livestock (n=76; 9·5%) or wildlife and its products (n=1; 0·12%).

Table 1
Demographic characteristics and CCHF seroprevalence in humans.

	Arua (low risk area) N=422					Nakaseke (high risk area) N=378					Overall seropositivity (N=800)	
	Livestock farmers		Non-livestock farmers			Livestock farmers		Non-livestock farmers (n=228)				
Characteristics	Participant N (%)	Seropositivity N (%)	Participant N (%)	Seropositive N (%)	Area Seroprevalence	Participant N (%)	Seropositivity N (%)	Participant N (%)	Seropositive N (%)	Area Seroprevalence	Livestock farmersN=386	Non-livestock farmersN=414
Overall	236 (55·9)	95(40·2)	186 (44·1)	56 (30·1)	151 (35·8)	150 (39·7)	51(34·0)	228 (60·3)	19 (8·3)	70 (18·5)	146 (37·8)	75 (18·1)
Age groups												
8-7yrs	26 (11·0)	10 (38·5)	40 (21·5)	12 (30·0)	22 (33·3)	30 (20·0)	4 (13·3)	25 (11·0)	0 (0·0)	4 (7·3)	14 (25·0)	12 (18·5)
18-34yrs	77 (32·6)	34 (44·2)	57 (30·6)	13 (22·8)	47 (35·1)	63 (42·0)	19 (30·2)	76 (33·3)	7 (9·2)	26 (18·7)	53 (37·8)	20 (15·0)
35-50yrs	68 (28·8)	26 (38·2)	44 (23·6)	10 (22·7)	36 (32·1)	44 (29·3)	20 (45·5)	72 (31·6)	11 (15·3)	31 (26·7)	46 (41·1)	21 (18·1)
51+yrs	65 (27·5)	25 (38·5)	45 (24·2)	21 (46·7)	46 (41·8)	13 (8·7)	8 (61·5)	55 (24·1)	1 (1·8)	9 (13·2)	33 (42·3)	22 (22·0)
Gender												
Female	43 (18·22)	18 (41·9)	58 (31·2)	14 (24·1)	32 (31·7)	32 (21·3)	4 (12·5)	27 (11·8)	2 (7·4)	6 (10·2)	22 (29·3)	16 (18·8)
Male	193 (81·8)	77 (39·9)	128 (68·8)	42 (32·8)	119 (37·1)	118 (78·7)	47 (39·8)	201 (88·2)	17 (8·5)	64 (20·1)	124 (39·9)	59 (17·9)
History of tick bites												
No	189 (80·1)	75 (39·7)	173 (93·0)	52 (30·1)	127 (35·1)	89 (59·3)	28 (31·5)	165 (72·4)	14 (8·5)	42 (16·5)	103 (37·1)	66 (19·5)
Yes	47 (19·9)	20 (42·5)	13 (7·0)	4 (30·8)	24 (40·0)	61 (40·7)	23 (37·7)	63 (27·6)	5 (7·9)	28 (22·6)	43 (39·8)	9 (11·8)
Ever ate engorged ticks												
No	155 (65·7)	53 (34·2)			53 (34·2)	150 (100·0)	51 (34·0)	228 (100)	19 (8·3)	70 (18·5)	104 (34·1)	19 (8·3)
Yes	81 (34·3)	42 (51·8)			42 (51·8)						42 (51·8)	
Crushed ticks with bare hands												
No	186 (78·8)	70 (37·6)	186 (100·0)	56 (30·1)	126 (33·9)	114 (76·0)	38 (33·3)	182 (79·8)	16 (8·8)	54 (18·2)	108 (36·0)	72 (19·6)
Yes	50 (21·2)	25 (50·0)	0 (0·0)		25 (50·0)	36 (24·0)	13 (36·1)	46 (20·2)	3 (6·5)	16 (19·5)	38 (44·2)	3 (6·5)
Bat roosting around homestead												
No	132 (55·9)	49 (37·1)	125 (67·2)	42 (33·6)	91 (35·4)	130 (86·7)	44 (33·8)	201 (88·2)	16 (8·0)	60 (18·1)	93 (35·5)	58 (17·8)
Yes	104 (44·1)	46 (44·2)	61 (32·8)	14 (22·9)	60 (36·4)	20 (13·3)	7 (35·0)	27 (11·8)	3 (11·1)	10 (21·3)	53 (42·7)	17 (19·3)
Rodents around homestead												
No	64 (27·1)	21 (32·8)	74 (39·8)	29 (39·2)	50 (36·2)	149 (99·3)	50 (33·6)	228 (100)	19 (8·3)	69 (18·3)	71 (33·3)	48 (15·9)
Yes	172 (72·9)	74 (43·0)	112 (60·2)	27 (24·1)	101 (35·6)	1 (0·67)	1 (100·0)	0 (0·0)		1 (100·0)	75 (43·3)	27 (24·1)
Contact with wildlife or its products												
No	236 (100·0)	186 (100·)	236 (100·0)	56 (30·1)	151 (35·8)	149 (99·3)	50 (33·6)	228 (100·)	19 (8·3)	69 (18·3)	145 (37·7)	75 (18·1)
Yes	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	1 (0·7)	1 (100·0)	0 (0·0)	0 (0·0)	1 (100·0)	1 (100·0)	0 (0·0)
Kept dogs at home												
No	181 (76·7)	69 (38·1)	182 (97·8)	53 (29·1)	122 (33·6)	131 (87·3)	42 (32·1)	213 (93·4)	16 (7·5)	58 (16·9)	111 (35·6)	69 (17·5)
Yes	55 (23·3)	26 (47·3)	4 (2·2)	3 (75·0)	29 (49·1)	19 (12·7)	9 (47·4)	15 (6·6)	3 (20·0)	12 (35·3)	35 (47·3)	6 (31·6)
Slaughter of animals												
No	202 (85·6)	78 (38·6)	179 (96·2)	54 (30·2)	132 (34·6)	137 (91·3)	45 (32·8)	206 (90·4)	18 (8·7)	63 (18·4)	123 (36·3)	72 (18·7)
yes	34 (14·4)	17 (50·0)	7 (3·7)	2 (28·6)	19 (46·3)	13 (8·7)	6 (46·1)	22 (9·6)	1 (4·6)	7 (20·0)	23 (48·9)	3 (10·3)
History of taking care of a patient												
No	190 (80·5)	77 (40·5)	173 (93·0)	52 (30·1)	129 (35·5)	139 (92·7)	47 (33·8)	189 (82·9)	16 (8·5)	63 (19·2)	124 (37·7)	68 (18·8)
Yes	46 (19·5)	18 (39·1)	13 (7·0)	4 (30·8)	22 (37·3)	11 (7·3)	4 (36·4)	39 (17·1)	3 (7·7)	7 (14·0)	22 (38·6)	7 (13·5)

Table 1: Shows the demographic and CCHF seroprevalence of study participants, clustered by sub groups and study areas, then overall totals. Double dots " " denotes variables not applicable for a particular characteristic.

Table 2
Factors associated with CCHF exposure in humans.

	Livestock farmers (N=386)		Non-livestock farmers (N=414)		Overall (N=800)	
Characteristics	**UOR (95% CI)**	**AOR (95% CI)**	**UOR (95% CI)**	**AOR (95% CI)**	**UOR (95%CI)**	**AOR (95% CI)**
Study groups						
Non-livestock farmers	N/A	N/A	N/A	N/A	ref	ref
Livestock farmers	N/A	N/A	N/A	N/A	2·75 (1·99-3·80)**	5·68 (3·34-9·67)**
District						
Nakaseke	ref	ref	ref	ref	ref	ref
Arua	0·99 (0·79-1·24)	1·64 (1·00-2·71)*	4·74 (2·69-8·33)*	5·32 (2·95-9·57)**	1·29 (0·86-1·92)	
Gender						
Female	ref	ref	ref	ref	ref	ref
Male	2·13 (1·50-3·01)*	2·88 (1·82-4·56)**	0·94 (0·51-1·74)			
Age groups						
8-7yrs	ref	ref	ref	ref	ref	ref
18-34yrs	1·22 (0·82-1·80)	1·95 (1·20-3·20)**	0·78 (0·36-1·72)		1·33 (0·80-2·22)	2·21 (1·11-4·41)*
35-50yrs	1·15 (0·78-1·68)	3·84 (2·32-6·35)**	0·98 (0·45-2·14)		1·52 (0·90-2·56)	2·99 (1·48-6·05)**
51+yrs	1·84 (1·15-2·94)*	3·16 (1·70-5·86)**	1·25 (0·57-2·73)		1·63 (0·95-2·80)	2·13 (1·01-4·46)*
Tick bites past month						
No	ref	ref	ref	ref	ref	ref
Yes	1·01 (0·79-1·31)	1·48 (1·00-2·21)*	0·55 (0·26-1·17)		1·04 (0·72-1·50)	
Ever ate engorged ticks						
No	ref	ref	ref	ref	ref	ref
Yes	3·53 (2·65-4·71)*	18·90 (10·98-32·53)**			3·59 (2·22- 5·80)**	2·13 (1·27-3·57)**
Crushed ticks with bare hands						
No	ref	ref	ref	ref	ref	ref
Yes	1·65 (1·27-2·15)*	0·62 (0·39-0·99)*	0·29 (0·09-0·95)*		1·22 (0·81-1·83)	
Rodents in surrounding area						
No	ref	ref	ref	ref	ref	ref
Yes					1·85 (1·35-2·55)**	
Bats roosting in the area						
No	ref	ref	ref	ref	ref	ref
Yes	0·72 (0·57-0·93)*	0·51 (0·33-0·79)*	1·12 (0·61-2·02)		1·43 (1·01-2·01)*	
Keeping dogs at home						
No	ref	ref	ref	ref	ref	ref
Yes	0·70 (0·56-0·88)*		2·18 (0·80-5·94)	3·91 (1·30-11·73)*	2·31 (1·48-3·59)**	1·54 (0·93-2·55)
Slaughtered animal						
No	ref	ref	ref	ref	ref	ref
Yes	3·32 (2·42-4·57)*	2·21 (1·39-3·50)**	0·50 (0·15-1·70)		1·41 (0·85-2·33)	
Cared for the sick						
No	ref	ref	ref	ref	ref	ref
Yes	0·44 (0·33-0·58)*	0·42 (0·26-0·68)**	0·67 (0·29-1·56)		0·94 (0·60-1·49)	
Acaricide spraying						
No spraying	ref	ref	N/A	N/A	N/A	N/A
Once in 2 weeks	1·21 (0·80-1·82)	0·61 (0·34-1·09)	N/A	N/A	N/A	N/A
Once a week	3·81 (2·73-5·30)**	6·60 (3·74-11·68)**	N/A	N/A	N/A	N/A
Twice a week	14·82 (6·54-33·58)**	29·07 (11·41-74·03)**	N/A	N/A	N/A	N/A

Table 2: Show factors associated with CCHF seropositivity in humans, segregated by livestock farmers, non-livestock farmers and the overall. N/A were variables not applicable for the respective characteristic, while were collinear variables when examined among different combinations of variables and did not make it to the final model. ** were significant variables p<0.01 and * p>0.01 to P<0.05. UOR-unadjusted odds ratio, AOR-Adjusted odds ratio and ref-referenced category.

Study Animals

From the livestock farming households, a total of 1,215 animals were sampled (666 cattle, and 549 goats), of which 89·2% were females (Table 3). More than a half of the animals (n=720; 59·3%) were less than four years old. The median tick infestation was higher in cattle (48 ticks per cattle, IQR:24-86) than goats, and 743 (61·1%) animals were sprayed with acaricides at least once a week to control tick infestation.

Seroprevalence of CCHF in humans

CCHFV specific IgG antibodies were detected in 221 out of 800 human participants, an overall seroprevalence of 27·6%. As shown in Table 1, CCHFV seropositivity was significantly higher among livestock farmers (n=146; 37·8%) compared to non-livestock farmers (n=75; 18·1%) (p=0·001). Surprisingly, a higher CCHFV seropositivity was detected among participants from outwith the cattle corridor in Arua (n=151; 35·8%) compared to those from Nakaseke (n=70; 18·5%) (p=0·001). In Arua, high CCHFV seropositivity was strongly associated with the practice of collecting and eating engorged ticks (n=42, 51·8%) (p<0.0001).

Factors associated with CCHFV exposure in humans

In the overall multivariate regression model (Table 2), CCHFV seropositivity was independently associated with livestock farming (livestock farmers AOR=5·68, 95% CI:3·34-9·67, p<0·0001 compared to non-livestock farmers), eating engorged ticks (AOR=2·13, 95% CI:1·27-3·57, p=0·004), age (18-34 years [AOR=2·21, 95% CI:1·11-4·41, p=0·024], 35-50 years [AOR=2·99, 95% CI:1·48-6·05, p=0·002] and those over 50 years [AOR=2·13, 95% CI:1·01-4·45, p=0·046], all compared to 8-17 years. Participants from Arua, previously considered a low-risk district, were more likely to be seropositive compared to those from Nakaseke which was considered high risk (Table 2) in the original study design,

Among the livestock farmers, males were twice more likely to be seropositive than females (AOR=2·88, 95% CI:1·82-4·56, p<0·0001). Farmers older than 17 years were more likely to be seropositive (18-34 years [AOR=1·95, 95% CI:1·20-3·20, p=0·007], 35-50 years [AOR=3·84, 95% CI:2·32-6·35, P<0·0001] and over 50 years [AOR=3·16, 95% CI:1·70-5·86, p<0·0001]). Farmers who reported a history of tick bites were more likely to be seropositive than those who had no history of tick bites (AOR=1·48, 95% CI:1·00-2·21, p=0·050). Interestingly, it was noted that farmers

who reported ever eating engorged ticks were 19 times more likely to be seropositive. Similarly, participants with any history of contact with blood and fluids of slaughtered animals were twice more likely (AOR=2·21, 95% CI:1·39-3·50, p=0·001) to be seropositive (Table 2). Frequency of tick control by acaricide spraying of animals was also associated with CCHF seropositivity among human participants. Participants from farming households spraying once a week were 6·6 times more likely to be seropositive while those spraying twice a week were 29 times more likely to be seropositive, compared to those who did not spray their animals.

Among the non-livestock farmers, CCHFV seropositivity was independently associated with the study district and keeping dogs (Table 2). Similar to livestock farmers, seropositivity was high in Arua district (AOR=5·32, 95% CI:2·95-9·57) compared to Nakaseke district. Participants from households that reported keeping dogs were three times more likely to be seropositive than those that did not have dogs at home (AOR=3·91, 95% CI=1·30-11·73).

Seroprevalence of CCHFV in domestic animals

CCHFV antibodies were detected in 612 (91·8%) out of 666 cattle, 413 (75·2%) out of 549 goats and 18 (56·2%) out of 32 dogs using IDVet ELISA (Table 3). We detected higher seropositivity in Nakaseke, [cattle; n= 303: 97·1% and goats; n= 198: 80·8%] and among female animals, [cattle; n= 539: 93·1% and goats; n= 390: 77·2%]. CCHFV seropositivity increased with the age of the animals [cattle over 4 years, n=312: 96·3%, and goats over 4 years, n=155: 90·6%].

Anti-CCHFV NP (IDVet) and anti-NSDV NP (in-house) cross reactivity was detected in 20 (48·8%) out of the 41 IDVet seropositive goat sera and 16 (31.3%) out of the 51 IDvet seropositive cattle sera tested (Figure 1S: A and B). There was a high sensitivity and specificity in the test performance of anti-CCHFV NP specific in-house ELISA (AUC = 0.896, p<0.0001) compared with the IDVet assay (Figure 2S).

Factors associated with CCHF exposure in animals

In the animal multivariable regression model (Table 4), CCHFV seropositivity was independently associated with district [Nakaseke, AOR=2·76, 95% CI: 1·75-4·34, p<0·0001 compared to Arua], animal species [cattle, AOR=2·58, 95% CI=1·64-4·07, p<0·0001 compared with goats], female animals [AOR=2·13, 95% CI:1·32-3·42, p=0·002], tick infestation, over 50 ticks [AOR=3·52, 95% CI= 1·47-8·44, p=0·005], compared with animals that had no ticks. Animal age, 2-4 years [AOR=2·25, 95% CI:1·40-3·61, p=0·001], 4+ years [AOR=4·23, 95% CI: 2·70-6·63, p=0·0001], all compared to animals that were less than 2 years old.

Nairoviruses detected in tick samples

We detected CCHFV genomes in four out of 121 independent *Rh.appendiculatus* tick pools, NSDV in one *Rh.appendiculatus* pool and DUGV in three tick pools [two pools of *Am. variegatum* and one pool of *Rh.appendiculatus*].

Discussion

In this study, we determined the prevalence and risk factors associated with CCHFV infections in humans and animals from two districts in Uganda. Overall, the seroprevalence of CCHF was 27·6% in humans, 91·9% in cattle, 75·2% in goats and 56·2% in dogs. Previous analysis of CCHF seropravelence in similar countries ranged from 0·1-14·4% in humans and 16·5-30·3% among at-risk professionals.[21] Interestingly, we detected high seropositivity in Arua district, chosen as a historically and geographically low-risk area outwith the Ugandan cattle corridor. No clinical cases of CCHF have been reported in Arua in the recent past as compared to multiple outbreaks in Nakaseke.[7] Clinical manfestations of CCHF in humans may vary from undifferentiated fever to haemorrhagic fever and may relate to host differential immune response or existing immunity and virus genetic variation.[22] Misdiagnosis of CCHF is common, especially in the absence of classical features and low levels of disease surveillance. The surveillance system of CCHF in Uganda is hospital-based or triggered by outbreaks of haemorrhagic fever.[9] The very high seroprevalence of CCHFV in Arua and Nakaseke indicates that it may be a vastly under-estimated and widespread pathogen. Furthermore, under appreciated behavioural risk factors such as the capture and ingestion of engorged ticks are likely to contribute to significantly higher CCHF exposure amongst farming communities in traditionally low-risk areas such as Arua. Such practices should be explored in other populations with high seroprevalence in Uganda and elsewhere.

Surprisingly, we detected higher CCHF seropositivity among farmers who frequently sprayed their animals than those who did not. High frequency of acaricide application may be carried out due to a high burden of tick infestation. In such circumstances, there is a high risk of tick-borne disease transmission and exposure. Careful PPE usage during spraying and management of heavily-infested animals is indicated. We also found increased seropositivity among non-livestockfarmers from homes that kept dogs than those who did not. This intriguing finding may indicate a direct risk of CCHFV exposure or an indirect risk due to an increased exposure to ticks. In the study areas covered, there is poor tick control practices for dogs and yet as companion animals, dogs are in direct contact with their owners in the communities. Acaricide use in dogs may help to reduce the risk in affected communities.

CCHF seropositvity among cattle and goats was significantly higher in the cattle corridor site in Nakaseke than in Arua district. This was expected, as animal husbandry practices in Nakaseke are largely free-range and large herds of animals interact over a large grazing area increasing exposure risk, while in Arua, smaller numbers of animals are restricted to a smaller grazing area. Further, cattle in Nakaseke were predominantly friesian crosses, and would most likely be kept longer on farms because of their high production value, therefore, exposed for longer to CCHF than the Arua animals kept for subsistence income. Crossbred animals have also been found to be less resistant to tick-borne diseases than local breeds in Uganda.[23]

The seroprevalence of CCHF in animals shows an average prevalence of 24·6% with regional and species variation. In cattle, the global seroprevalence has been reported to range between 0·6% in Egypt and higher in Afghanistan (79·1%).[21] We found a higher seroprevalence in cattle in this study than a previous study in Uganda (75·0% of 500 cattle)[18] and a considerably higher seroprevalence than in Kenya: (28·1% of 139 cattle)[17], DRC (0·4% of 514 cattle)[24] and Sudan (between 7·0% of 299 cattle and 19·1% of 282 cattle).[25,26] Seroprevalence studies in goats and dogs in Sudan, Somalia, Niger, Senegal, Mauritania and South Africa all reported lower prevalence of infection.[16,27] Our results indicate an exceptionally high CCHF seroprevalence in cattle, goats and dogs in Uganda, with the use of similar or identical diagnostic assays. In keeping with these studies, we noted that a risk increase with older age, female sex and a higher tick burden, as previously reported.[18] Based on our assessment of cross-reactivity of the CCHFV IDvet assay and anti-NSDV NP in-house assay, a proportion of our seropositivity findings in domestic animals are likely to reflect exposure to closely-related nairoviruses. However, the finding of CCHFV being the most commonly detected nairovirus in tick pools, highlights the major contribution of this virus to seropositivity in Uganda.

Finally, we found evidence that the most likely vector of CCHFV in Uganda is *Rh. appendiculatus* rather than *Hyalomma* species which was detected infrequently. Vector competence studies are recommended, although such experiments would require high biosecurity clearance. CCHFV has however previously been iso-

Table 3
Demographic characteristics and CCHF seroprevalence in animals.

	Arua (Low risk)		Nakaseke (high risk)		Overall	
Characteristics	N	Seropositivity n (%)	N	Seropositivity n (%)	N	Seroprevalence n (%)
Cattle	354	309 (87·3)	312	303 (97·1)	666	612 (91·8)
Grazing system						
Communal/ tethering	337	296 (87·8)			337	296 (87·8)
Fence/paddocks	17	13 (76·5)	290	281 (96·9)	307	294 (95·8)
Pastoralism			22	22 (100·0)	22	22 (100·0)
Herd size						
<50	354	309 (87·3)	34	34 (100·0)	388	343 (88·4)
50-100			176	169 (96·0)	176	169 (96·0)
>100+			102	100 (98·0)	102	100 (98·0)
Animal breed						
Zebu cattle	352	307 (87·2)			352	307 (87·2)
Ankole cattle	2	2 (100·0)	23	22 (95·6)	25	24 (96·0)
Boran crosses			12	12 (100·0)	12	12 (100·0)
Friesian crosses			277	269 (97·1)	277	277 (100·0)
Animal age						
<2 yrs	160	130 (81·2)	98	93 (94·9)	258	223 (86·4)
2-4 yrs	47	42 (89·4)	37	35 (94·6)	84	77 (91·7)
>4yrs	147	137 (93·2)	177	175 (98·9)	324	312 (96·3)
Animal sex						
Male	75	62 (82·7)	12	11 (91·7)	87	73 (83·9)
Female	279	247 (88·5)	300	292 (97·3)	579	539 (93·1)
Tick infestation						
no tick			12	12 (100·0)	12	12 (100·0)
<50 ticks	103	82 (79·6)	242	233 (96·3)	345	315 (91·3)
>50+ ticks	251	227 (90·4)	58	58 (100·0)	309	285 (92·2)
Acaricide spraying						
none	143	121 (84·6)			143	121 (84·6)
Twice a week	19	15 (78·9)	7	7 (100·0)	26	22 (84·2)
Once a week	86	76 (88·4)	285	276 (96·8)	371	352 (94·8)
Once in 2 weeks	106	97 (91·5)	20	20 (100·0)	126	117 (92·9)
Goats	304	215 (70·7)	245	198 (80·8)	549	413 (75·2)
Grazing system						
Communal/ tethering	291	204 (70·1)			291	204 (70·1)
Fence/paddocks	13	11 (84·6)	219	181 (82·7)	232	192 (82·7)
Pastoralism			26	17 (65·4)	26	17 (65·4)
Herd size						
<50	268	185 (69·0)	58	41 (70·7)	326	226 (69·3)
50-100	36	30 (83·3)	68	43 (63·2)	104	73 (70·2)
>100+			119	114 (95·8)	119	114 (95·8)
Animal breed						
Boer crosses			23	17 (73·9)	23	17 (73·9)
Mubende goats			63	54 (85·7)	63	54 (85·7)
Small East African	304	215 (70·7)	159	127 (79·9)	463	342 (73·9)
Animal age						
<2 yrs	169	106 (62·7)	94	57 (60·6)	263	163 (61·9)
2-4 yrs	64	49 (76·6)	51	46 (90·2)	115	95 (82·6)
>4yrs	71	60 (84·5)	100	95 (95·0)	171	155 (90·6)
Animal sex						
Male	27	12 (44·4)	17	11 (64·7)	44	23 (52·3)
Female	277	203 (73·3)	228	187 (82·0)	505	390 (77·2)
Tick infestation						
no tick	16	8 (50·0)	33	22 (66·7)	49	30 (61·2)
<50 ticks	287	206 (71·8)	212	176 (83·0)	499	382 (76·6)
>50+ ticks	1	1 (100·0)				1 (100·0)
Acaricide spraying						
none	96	75 (78·1)			96	75 (78·1)
Twice a week	20	18 (90·0)			20	18 (90·0)
Once a week	103	64 (62·1)	223	179 (80·3)	326	243 (74·5)
Once in 2 weeks	85	58 (68·2)	22	19 (86·4)	107	77 (72·0)

Table 3: Demographic characteristics and CCHF seroprevalence in animals segregated by cattle and goats. were values not available for a particular variable, yrs-years.

Table 4
Factors associated with CCHF exposure in animals.

Characteristics	Attributes	UOR (95% CI)	p-value	AOR (95% CI)	p-value
Overall CCHF SP	84·4% (1025/1,215)				
District					
	Arua	ref		ref	
	Nakaseke	2·28(1·64-3·20)	<0·0001	2·76 (1·75–4·34)	<0·0001
Animal species					
	Goats	ref			
	Cattle	3·73 (2·66-5·24)	<0·0001	2·58 (1·64-4·07)	<0·0001
Animal breed					
	indigenous	ref		ref	
	crossbreeds	0·67 (0·34-1·33)	0·255		
Animal sex					
	Male	ref		ref	
	female	2·19 (1·43-3·33)	<0·0001	2·13 (1·32-3·42)	0·002
Animal age					
	<2yrs	ref		ref	
	2-4yrs	2·23 (1·42-3·50)	<0·0001	2·25 (1·40-3·61)	0·001
	>4yrs	5·83 (3·80-8·96)	<0·0001	4·23 (2·70-6·63)	<0·0001
Tick infestation					
	No ticks	ref		ref	
	<50 ticks	2·14 (1·21-3·79)	0·009	1·82 (0·97-3·44)	0·061
	>50+ ticks	5·39 (2·72-10·68)	<0·0001	3·52 (1·47-8·44)	0·005
Acaricide spraying					
	none	ref		ref	
	Twice a week	1·46 (0·58-3·67)	0·418	1·75 (0·65-4·69)	0·268
	Once a week	1·28 (0·86-1·89)	0·217	0·70 (0·43-1·15)	0·159
	Once in 2 weeks	1·09 (0·68-1·76)	0·719	1·06 (0·63-1·79)	0·811

Table 4; SP is overall CCHF seroprevalence in animals, while were variables that did not make to the multivariable model because of multicollinearity. UOR: univariable odds ratio. AOR: adjusted odds ratio estimated, and ref-reference category.

lated and viral sequences detected in *Rh. Appendiculatus* ticks collected while feeding on cattle in Ankole region in the late 1960s in Uganda and elsewhere.[28,29] CCHFV has also been identified in *Am. Variegatum*, and *Rh. (Boo) decoloratus* ticks[8,28,30] in other studies carried out in Uganda.

Conclusions

Our results demonstrate that CCHFV is circulating at very high prevalence in Uganda, both within and outside the cattle corridor, and that the exposure is not only limited to the livestock farmers. The virus likely represents an under-estimated cause of human disease and indicates the need for enhanced surveillance across the country in patients with febrile illness and further characterisation of the virus burden in ticks and domestic animals. Classical risk factors were found in farming communities but behavioural practices in animal husbandry and in the collection and ingestion of ticks require further validation and potential intervention strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

SAA, RT, CM, and ECT conceived the idea and designed the study, collected data, drafted the manuscript

ARA, PV, SO, GN, SB, TN, ME, SA, JS, and JJL supported SAA with collection and testing of the samples

TL, and SB supported SAA with testing of animal samples

PJ, MN, SB, PK, and AA guided SAA with data analysis and reporting.

All authors had full access to all the data of the study and had final responsibility for the decision to submit the final manuscript for publication

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.09.007.

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