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Widespread exposure to Crimean-Congo haemorrhagic fever in Uganda might be driven by transmission from *Rhipicephalus* ticks: Evidence from cross-sectional and modelling studies

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SUMMARY

Background: Crimean-Congo haemorrhagic fever (CCHF) is a widespread tick-borne viral infection, present across Africa and Eurasia, which might pose a cryptic public health problem in Uganda. We aimed to understand the magnitude and distribution of CCHF risk in humans, livestock and ticks across Uganda by synthesising epidemiological (cross-sectional) and ecological (modelling) studies.

Methods: We conducted a cross-sectional study at three urban abattoirs receiving cattle from across Uganda. We sampled humans ($n = 478$), livestock ($n = 419$) and ticks ($n = 1065$) and used commercially-available kits to detect human and livestock CCHF virus (CCHFV) antibodies and antigen in tick pools. We developed boosted regression tree models to evaluate the correlates and geographical distribution of expected tick and wildlife hosts, and of human CCHF exposures, drawing on continent-wide data.

Findings: The cross-sectional study found CCHFV IgG/IgM seroprevalence in humans of 10·3% (7·8–13·3), with antibody detection positively associated with reported history of tick bite (age-adjusted odds ratio = 2·09 (1·09–3·98)). Cattle had a seroprevalence of 69·7% (65·1–73·4). Only one *Hyalomma* tick (CCHFV-negative) was found. However, CCHFV antigen was detected in *Rhipicephalus* (5·9% of 304 pools) and *Amblyomma* (2·9% of 34 pools) species. Modelling predicted high human CCHF risk across much of Uganda, low environmental suitability for *Hyalomma*, and high suitability for *Rhipicephalus* and *Amblyomma*.

Interpretation: Our epidemiological and ecological studies provide complementary evidence that CCHF exposure risk is widespread across Uganda. We challenge the idea that *Hyalomma* ticks are consistently the principal reservoir and vector for CCHFV, and postulate that *Rhipicephalus* might be important for CCHFV transmission in Uganda, due to high frequency of infected ticks and predicted environmental suitability.

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Research in Context

Crimean-Congo haemorrhagic fever (CCHF) is a tick-borne zoonotic viral infection that is of major public health concern throughout Africa and Eurasia but remains poorly understood. Human infections typically arise due to isolated spill-over transmission events, usually through tick-bites or contact with infectious livestock in abattoirs and farms, and more rarely human-to-human transmission. CCHF outbreaks are a threat to public health services, can lead to outbreaks with high case fatality rates (5–30%) and are difficult to prevent and treat. Detection of acute infections is rare but limited public health surveillance means that the incidence and distribution of CCHF are likely underestimated, and risk drivers poorly understood, particularly across Africa. This hinders rapid diagnosis and supportive treatment, as well as disease control and prevention efforts.

Evidence before this study

Since surveillance improvements in 2010, there has been an increase in reported CCHF cases from Uganda (32 confirmed cases up to 2019), where the disease had not previously been detected since 1988. Recent case investigations indicated that the ecological characteristics of CCHF virus (CCHFV) transmission in Uganda, in particular the primary tick reservoir species, might differ from those documented elsewhere. Given the paucity of knowledge about CCHF in East Africa, work to characterise the distribution and drivers of infection and disease in this region is urgently needed. The complex natural history of CCHFV and sporadic nature of human infections requires a multidisciplinary, One Health approach integrating disease ecology, epidemiology and public health methods.

Added value of this study

We aimed to improve understanding about CCHF in Uganda by bringing together evidence from observational epidemiology and ecological modelling. We conducted a cross-sectional study in Uganda which found high CCHFV seropositivity in humans and cattle, and viral antigen present in ticks, sampled at three urban abattoirs receiving cattle from across Uganda. In parallel, we developed models to evaluate the correlates and geographical distribution of expected tick and wildlife hosts, and of human exposures, drawing on continent-wide data from across Africa. Taken together, these approaches provide complementary evidence that CCHF exposure risk is widespread across Uganda, and that the primary tick species involved in maintenance and transmission might be different from those implicated elsewhere.

Implication of all the available evidence

Transmission of CCHFV appears to be much more widespread and common amongst ticks, livestock and people in Uganda than the rare incidence of confirmed acute infection would suggest, and the strongest risk factor for human exposure was tick bite. It is likely that the true burden of CCHF is underestimated, with health security implications. Although ticks of the genus *Hyalomma* are generally considered to be the primary maintenance host and vector for CCHFV in nature, they appear to be uncommon in most of Uganda. Instead, our study strongly suggests that *Rhipicephalus appendiculatus* ticks might play a substantial role in maintenance and transmission in this area. There is a need for integrated, landscape-level eco-epidemiological studies to inform surveillance and control efforts for CCHF in Uganda.

Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a widely-distributed tick-borne zoonotic viral disease affecting humans, causing subclinical or mild nonspecific febrile illness, with severe haemorrhagic disease in some individuals.[1-3] Non-specific symptoms, including fever, headache, vomiting, diarrhoea and muscular pain,[2] make differentiation of CCHF from other common febrile illnesses challenging, so many milder cases likely go undiagnosed. In the subset of cases developing severe bleeding,[4] case fatality estimates vary from <5% to 30%.[2]

Ticks are both the reservoir host and vector for the causative agent, *Crimean-Congo haemorrhagic fever orthonairovirus* (CCHFV). Species of the genus *Hyalomma* are considered the principal virus reservoir and vector,[5] although some species from other genera (notably *Rhipicephalus*) show experimental evidence of competence.[3] Ticks can become infected vertically (i.e., from parent to offspring), or horizontally when co-feeding alongside infected ticks or on a viraemic animal.[3,6] Wild mammals (mainly ungulates, lagomorphs and rodents) and livestock (such as cattle, sheep and goats) are hosts for immature and adult ticks, and therefore important in CCHFV ecology.[5,7-9] Although infected wildlife and livestock are usually asymptomatic[10], viraemia can last from 2 to 15 days, and species that experience prolonged viraemia (e.g., some lagomorphs) might act as amplifying hosts.[10] Human infections occur via tick bite or exposure to infected animal fluids or tissues, and livestock are considered the main source of transmission to humans, although much remains unknown about mechanisms and drivers of spillover.[11,12] Risk factors for CCHFV in humans include living in a rural area, tick-bite, and professions handling livestock (such as farmers, veterinarians, abattoir workers and butchers).[2,4,13-15] Livestock movement may play an important role in dispersing infected ticks and CCHFV.[2]

The geographic distribution of CCHF spans from western China, across southern Asia to the Middle East, Spain, the Balkans, and most of Africa.[1-3] Acute human cases are only sporadically reported, and so regions with low case numbers may represent absence of viral circulation or a lack of detection. Large numbers of cases detected through surveillance in hotspots such as Turkey[16] suggest that the true incidence is often higher than detected. Understanding of CCHF epidemiology is particularly limited in sub-Saharan Africa where it may represent a cryptic health burden. Previous mapping efforts have been hindered by patchy data in Africa,[17,18] and evidence of CCHFV exposure has been found in supposedly low-risk areas, such as Ghana and Sierra Leone.[19,20] Indeed, infrequent acute case detections often contrast with seroprevalence data indicating substantial exposure in humans and livestock.[21] CCHF appears to be prevalent and/or increasing in many places[2,13,22,23], highlighting the need to better understand the epidemiology, distribution and ecological drivers.

In Uganda, following the introduction of viral haemorrhagic fever surveillance in 2010, an increase in CCHF case detection occurred (>30 cases), after a period of no reported cases since 1988.[4,23-25] It is likely that CCHFV is endemic in Uganda,[13] however, the distribution and burden of human CCHF, and the distribution of tick, livestock and wildlife species involved in viral transmission and persistence, are poorly understood.[17] Case investigations imply that CCHF ecology in Uganda might differ from elsewhere, with no evidence of infected *Hyalomma* ticks near to a recent acute case.[13]

We reasoned that the rarity of observed cases and the virus' complex natural history required a multidisciplinary, One Health approach in Uganda, integrating ecology, epidemiology and public health methods. We therefore conducted a cross-sectional study at three Ugandan abattoirs to investigate the prevalence of CCHFV exposure among humans and cattle, and the tick species associated with CCHFV. In parallel, we conducted a modelling study to map the distribution of ecological drivers (tick and mammal host distribution) and human CCHF risk across Uganda, and to infer key socio-ecological drivers of risk.

Methods

Cross-sectional study of humans, livestock and ticks in abattoirs

Study population, design and setting. Between March-July 2020, a cross-sectional study was conducted at three urban abattoirs in Uganda (Kampala City Main Abattoir, Entebbe Main Abattoir and Wakiso City Livestock Slaughter House), collecting survey data and blood samples from humans and cattle and picking ticks from the cattle. Our rationale was that cattle from across Uganda congregate in these central urban abattoirs, and we included workers tending live cattle, involved in slaughter and processing meat products, and without direct contact with cattle or products. This ensured sampling of animals originating from across a broad geographical area and humans with varying levels of livestock exposure. Supp. Fig. 1 summarises the study flow.

Humans. We recruited abattoir workers aged ≥18 years, who provided written informed consent. Participants answered a short questionnaire collecting demographic and socio-clinical characteristics, job type and duration of exposure, and provided a blood sample. A small token of appreciation was provided for participating.

Livestock. Cattle from owners who consented were restrained in crush pens for blood to be drawn and ticks to be picked. District of origin was recorded.

Ticks. Blunt forceps were used to pick ticks (including from the head, ears, neck, feet, perineum, udder, and tail) into ventilated corning centrifuge tubes. Ticks were kept in cold boxes prior to transfer to the Arboviruses laboratory (National Reference Laboratory) in Entebbe for identification, processing and −80 °C storage.

Laboratory procedures. Human and livestock blood, collected in serum separating and EDTA tubes, was kept on ice before laboratory transfer for processing and −80 °C storage. Commercially available enzyme-linked immunosorbent assay (ELISA) kits (VectoCrimean-CHF-IgG and IgM ELISA test kits; Vector-Best, Novosobirsk, Russia), with internal negative and positive controls, were used to test for human CCHFV-specific IgM and IgG antibodies to determine recent and previous CCHF infection, respectively. A commercially available "CCHF Double Antigen Multi-species" (Innovative Diagnostics (ID Vet), Grabels, France) ELISA was used to detect CCHFV antibodies in cattle.

Adult ticks were placed on Petri dishes, cleaned with 70% ethanol and taxonomically identified using a stereo-microscope and methods described by Walkers and colleagues.[26] Ticks from the same animal were pooled by species and feeding status before being crushed. Pools were then washed with saline containing antibiotics (1000 units of penicillin and 500 mg of streptomycin per 1 ml of saline) and ground (SPEX, 2000 GENO/GRINDER) for 30–60 s and the suspension centrifuged at 2000 rpm for 5 min. The supernatant was tested using a CCHFV-antigen ELISA kit (Vector-Best, Russia) to detect virus presence.

Statistical analysis. The target sample size for human participants was 484 abattoir workers to give ±2·3% precision on prevalence estimates with a 5% confidence level, assuming 8% of slaughterhouse workers would be positive for IgG, a standard error of 0·013, and accounting for 10% sample failure. Data were analysed using Stata 15·1 (College Station, TX, USA). CCHFV antibody prevalence was estimated with 95% confidence interval (CI) and described according to participant characteristics. Logistic regression was used to calculate age-adjusted odds ratios (aOR) to investigate how antibody seroprevalence (IgM and/or IgG) varied by participant characteristics. For cattle, we present CCHFV antibody prevalence by region and district of cattle origin and, for tick pools, antigen positivity by region and district of cattle origin and tick genera and species. A map[27] showing districts of livestock origin and tick species was constructed using ArcGIS 10·6.1 Fig. 1.

Ethical approval. Ethical approval was obtained from the Uganda Virus Research Institute (UVRI) Research and Ethics Committee (REC); University College London Research Ethics Committee; and the Uganda National Council for Science and Technology.

Modelling and mapping host distributions and CCHF risk

Modelling aimed to understand how potential tick hosts of CCHFV (including those identified in our cross-sectional study) and human CCHF exposure risk are distributed across Uganda and evaluate the importance of host suitability and socio-ecological covariates in predicting CCHF risk. We developed boosted regression trees (BRT) models to map environmental suitability for tick or mammal host species, and human exposure risk (probability of human CCHF infection). Because the distributions and drivers of host and virus ranges occur at macroecological (i.e., continental) scales, we fitted models to geolocated data from across Africa to ensure salient information was inferred from across CCHF's full range (also reducing issues of data sparsity in Uganda). We initially hypothesised that *Hyalomma* suitability would be high in areas of Uganda with CCHF cases, and that *Hyalomma* and mammal host suitability would be influential predictors of human infection risk.

Models of tick and mammal hosts. We identified tick and mammal species with evidence of substantially contributing to CCHFV transmission in Africa in the literature,[3,5,6,10] or with detected CCHFV antigen in our observational study (Supp. Table 1). These included four species of *Hyalomma* (*Hyalomma truncatum, H. rufipes, H. impeltatum and H. dromedarii*), and three other tick species: *Rhipicephalus (Boophilus) decoloratus* (because recent studies have identified CCHFV in this species in Uganda),[13] *R. appendiculatus* and *Amblyomma variegatum* (because these species contained CCHFV antigen in our observational study; see Results). For mammals, we identified one genus (hares, which comprise two main African species, *Lepus microtis* and L. *capensis*) with strong evidence of acting as competent amplifying hosts (i.e., facilitating CCHFV transmission among ticks; Supp. Table 1). We collected geolocated data on occurrences of each of these species from the largest compiled database of African tick records[28-30] and the Global Biodiversity Information Facility (Supp. Text 1).

For each species, we projected environmental suitability in 8 km grid cells across Africa using an ensemble of 200 BRT models[31] fitted to the geolocated occurrences and an equal number of randomly sampled pseudoabsences, considering climate and landscape covariates (bioclimatic variables and long-term vegetation indices) as predictor variables[32] (Supp. Table 2–3). Each submodel was fitted to a training subset (50% of data for the five species with a very large dataset i.e., >1500 points, and 75% for all other species) and the remaining holdout data were used as a test set to evaluate out-of-sample (OOS) predictive ability. To reduce the confounding effects of geographical sampling bias, train-test splits were probabilistically selected using a spatially-structured approach[33] and points were thinned in highly sampled areas (Supp. Text 1). Overall ensemble predictive ability was calculated as the mean OOS area under the receiver operator curve (AUC) statistic across all submodels.

Models of human CCHF exposure risk. We define exposures as locations where human CCHF acute cases were detected (1960–2020) and applied the same modelling approach as above. We compiled an up-to-date dataset of geolocated CCHF records in Africa including Messina et al.'s database[34] (to 2012) and later surveillance reports and scientific literature (Supp. Text 2, Supp. Fig. 2). Here, covariates included hypothesised socio-ecological drivers (agricultural land use, livestock densities, vegetation dynamics and climate; Supp. Table 2) and tick and mammal host distributions (modelled suitability for *Hyalomma* spp., *Rhipicephalus* spp., *A. variegatum*, and *Lepus* spp.). We fitted an ensemble of 200 BRT mod-

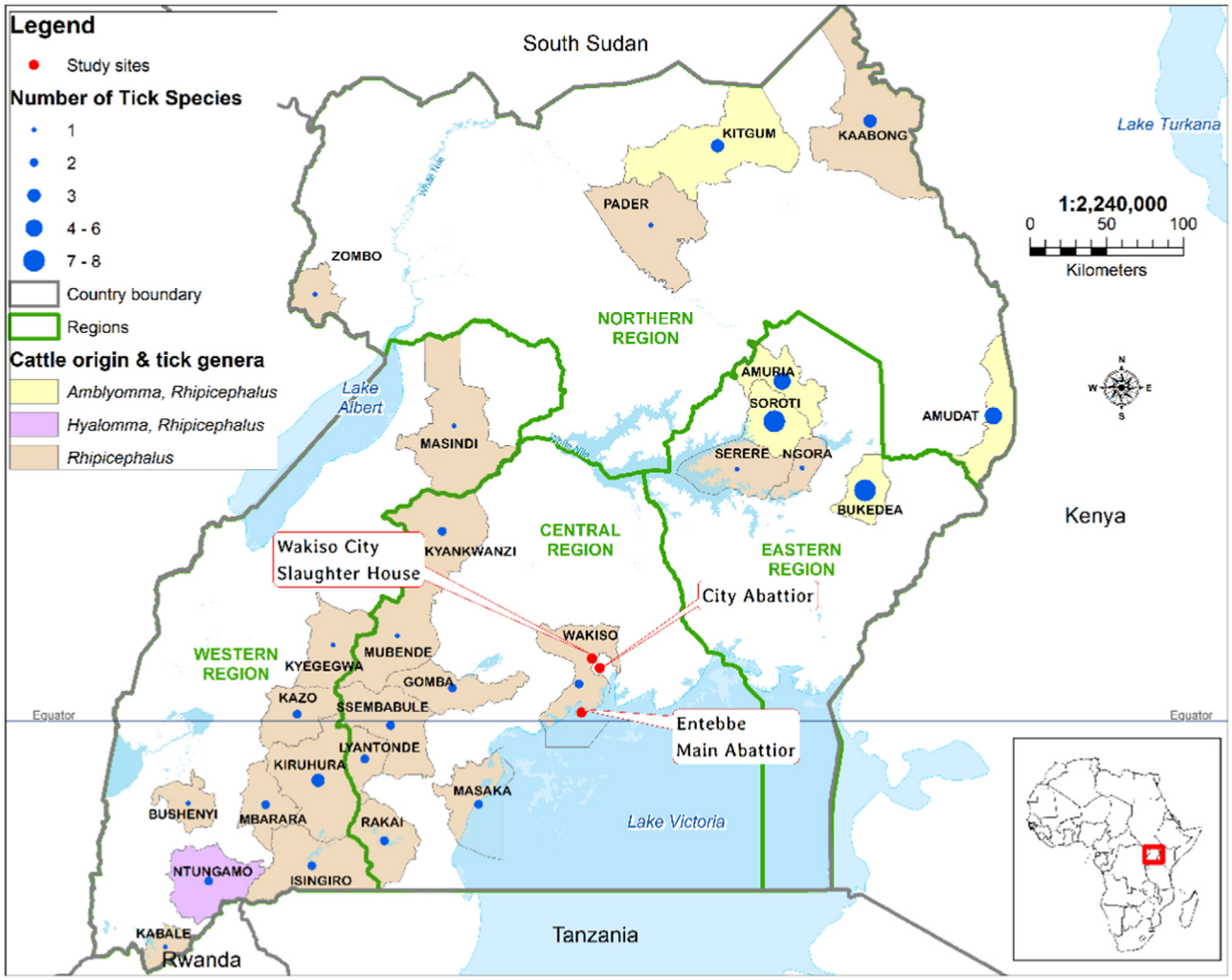

Fig. 1. Distribution of cattle origin and associated tick species from cross-sectional study. Study abattoirs are shown as red points. Shaded polygons indicate the districts of origin for all the cattle (n = 292; Table 2) enroled in the study. Colour shading indicates the tick genera that were picked from the cattle originating in each district, and point size indicates the number of tick species that were picked from cattle from each district. CCHFV seroprevalence was similar in cattle originating in Western, Central and Eastern regions, and notably lower in cattle originating from the Northern region (Table 2).

els, holding out 25% of data each time for spatial cross-validation (Supp. Text 2). We extracted partial dependency plots and variable importance from each submodel and used the ensemble to map exposure risk in Uganda and Africa-wide.

Role of funding source

The study sponsors had no role in study design, data collection, analysis and interpretation, or in report writing. All authors had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

Results

In total, 478 human participants took part in our observational study, 380 from Kampala City, 51 from Entebbe Main, and 47 from Wakiso City abattoirs. The median age at interview was 32 years (range: 18–71 years), 401 (83·9%) were males and 215 (45·0%) resided in Kampala district (Table 1). Most reported their main occupation involved direct contact with slaughtered animal body parts (307, 64%) and had been in this role for two years or more (384, 80%). Just over half reported a history of tick bite (259, 55%). All participants provided blood samples; 14 (2·9% (95% CI: 1·7–4·9) were positive for IgM, providing evidence of recent CCHF infection, and 37 (7·7% (5·7–10·5) were positive for IgG, consistent with previous CCHF infection (two were positive for both IgM and IgG). In total, 49 participants had antibodies (IgM and/or IgG) to CCHFV, giving a combined seroprevalence of 10·3% (7·8–13·3). After adjusting for age, only reported tick bite was associated with CCHFV seropositivity (aOR 2·09 (1·09–3·98), Supp. Table 4).

We sampled 419 cattle, which originated from across Uganda, with a small number from Tanzania (Table 2, Fig. 1). Around half of cattle (220 (52·5%)) came from central Uganda (Table 2). Blood was collected from all animals and 292 (69·7% (65·1–73·4)) were seropositive for CCHFV antibodies, with the highest seroprevalence in those from Eastern Uganda (73·2% (57·7–84·5)) and Central Uganda (73·2% (66·9– 78·6)) and lowest in those from Northern Uganda (19/38 (50·0% (34·6–65·4).

1065 ticks were picked from 242 cattle, and we created 523 tick pools of the same species picked from the same animal (Table 2). Of these, 32 pools (6·1%) tested positive for CCHFV antigen. Only one *Hyalomma* tick was identified (non-engorged, *Hyalomma truncatum*), which tested negative for CCHFV antigen. The CCHFV antigen positive ticks were all *Rhipicephalus* or *Amblyomma* species (Table 3). among non-engorged ticks, 17 (6·3%) of 272 tick pools

Table 1
Characteristics of participants testing positive for Crimean-Congo haemorrhagic fever virus (CCHFV) IgG and/or IgM antibodies.

	Positive antibodies		Denominator
	Number	% (95% CI)	
Antibodies	49	10.3 (7.8, 13.3)	478
Age (years)			
<25	10	9.4 (5.1, 16.7)	106
25–34	13	8.0 (4.7, 13.3)	162
35–44	13	10.7 (6.3, 17.6)	121
≥ 45	13	14.6 (8.7, 23.6)	89
Sex			
Male	41	10.2 (7.6, 13.6)	401
Female	8	10.4 (5.3, 19.5)	77
Tribe			
Baganda	30	9.3 (6.6, 13.1)	321
Banyankole	3	8.3 (2.7, 22.9)	36
Others	16	13.2 (8.3, 20.5)	121
Religion			
Muslims	21	10.4 (6.9, 15.4)	202
Catholics	11	7.4 (4.1, 12.9)	149
Other Christians	17	13.4 (8.5, 20.5)	127
Abattoir			
Kampala City	34	8.9 (6.5, 12.3)	380
Entebbe Main	9	17.6 (9.4, 30.6)	51
Wakiso City Slaughterhouse	6	12.8 (5.8, 25.7)	47
Place of residence			
Kampala	18	8.4 (5.3, 12.9)	215
Wakiso	25	11.0 (7.5, 15.8)	227
Others	6	16.7 (7.7, 32.5)	36
Education status			
None/primary	22	10.6 (7.1, 15.6)	207
Secondary/Tertiary	27	10.0 (6.9, 14.2)	271
Main occupation			
No direct contact with cattle or products	8	9.4 (4.7, 17.7)	85
Tending live cattle	4	4.7 (1.8, 11.8)	86
Slaughter/process/sell meat products	37	12.1 (8.9, 16.2)	307
Duration on main occupation			
< 2 years	9	9.6 (5.0, 17.4)	94
2–5 years	14	13.1 (7.9, 20.9)	107
> 5 years	26	9.4 (6.5, 13.4)	277
[†]Ever been injured at work in the abattoir			
No	21	9.7 (6.3, 14.4)	217
Yes	28	10.7 (7.5, 15.1)	261
[†]Ever use PPE at work in the abattoir			
No	8	14.6 (7.4, 26.5)	55
Yes	41	9.7 (7.2, 12.9)	423
Ever been bitten by a tick			
No	15	6.8 (4.2, 11.1)	219
Yes	34	13.1 (9.5, 17.8)	259
Time since last tick bite			
< 6 months	12	10.6 (6.1, 17.8)	113
≥ 6 months	21	15.8 (10.5, 23.0)	133
Past diagnosis with tick-born infection			
No	47	10.5 (7.9, 13.7)	449
Yes	2	6.9 (1.7, 23.8)	29
Past hospitalisation with febrile haemorrhage disease			
No	46	10.4 (7.8, 13.6)	444
Yes	3	8.8 (2.9, 24.1)	34
Ever helped livestock give birth			
No	39	10.1 (7.4, 13.5)	388
Yes	10	11.2 (6.1, 19.7)	89
Perceived risk for zoonotic infection			
None	28	10.7 (7.5, 15.1)	261
Yes	20	9.3 (6.1, 14.1)	214
Heard about CCHF in the past			
No	32	9.1 (6.5, 12.5)	353
Yes	17	13.6 (8.6, 20.8)	125

N; Number,%; percentage, IgG; Immunoglobulin G, IgM; Immunoglobulin M, PPE; personal protective equipment, CI; confidence interval. Missing values for: ever helped cattle give birth = 1, time since last tick bite = 233, perceived risk for zoonotic infection = 3. Religion; other include Adventists, Anglicans and Pentecostals, tribe; other included 23 tribes with less than 25 individuals. Main occupation: no direct contact with cattle or products (administration, restaurant, security, etc.), tending live cattle (cattle traders, veterinarian, herdsmen, transporters) and slaughtering, processing or selling meat products (slaughtering, skinning, cleaning or contact with meat, offal or blood, etc.).

[†] Restricted to those in occupations involving contact with cattle or processing and selling animal products (tending live cattle and slaughtering and processing meat).

Table 2
Region and district of origin for cattle and tick pools testing positive for Crimean-Congo haemorrhagic fever virus antibodies or antigen.

Regions/Districts	Cattle			Tick pools				
	Total	Positive antibodies		Total	Antigen positive		Cattle* (Number)	Ticks Number
		Number	% 95% (CI)		Number	% (95% CI)		
All	419	292	69·7 (65·1, 73·4)	523	32	6·1 (4·4, 8·5)	242	1065
Eastern Uganda	41	30	73·2 (57·7, 84·5)	114	0	114	33	286
Amuria	1	1		8	0		1	18
Bukedea	19	15		51	0		15	135
Mbale	4	0		–	–		–	–
Ngora	1	1		1	0		1	2
Serere	2	1		3	0		2	6
Soroti	14	12		8	0		14	125
Central Uganda	220	161	73·2 (66·9, 78·6)	226	19	8·4 (5·4, 12·8)	121	411
Gomba	14	12		17	0		11	39
Kassanda	6	1		–	–		–	–
Kiboga	7	6		–	–		–	–
Kyankwanzi	16	9		17	3		9	33
Lyantonde	51	43		64	3		34	114
Masaka	2	1		4	0		2	5
Mubende	1	1		–	–		–	–
Nakaseke	41	29		21	2		12	28
Rakai	68	46		81	10		45	146
Ssembabule	4	4		3	1		2	5
Wakiso	10	9		19	0		6	41
Northern Uganda	38	19	50·0 (34·6, 65·4)	77	2	2·6 (0·6, 9·8)	28	185
Amudat	20	7		43	2		15	101
Kaabong	7	3		9	0		3	20
Kitgum	8	8		16	0		7	34
Pader	2	0		5	0		2	19
Zombo	1	1		4	0		1	11
Western Uganda	113	76	67·3 (58·1, 75·3)	98	11	11·2 (6·3, 19·2)	57	164
Bushenyi	1	1		2	0		1	3
Isingiro	3	2		8	2		2	13
Kabale	2	2		1	0		1	3
Kazo	3	3		5	1		2	12
Kiruhura	35	28		30	5		20	49
Kyegegwa	13	2		1	0		1	1
Masindi	2	2		5	0		2	9
Mbarara	46	32		40	2		23	67
Ntungamo	8	4		2	1		5	7
Tanzania	7	6	85·7 (41·8, 98·0)	8	0	–	3	19

N; Number,%; percentage, CI; confidence interval.
* Number of cattle from which ticks came from.

Table 3
Genera and species of ticks tested for Crimean-Congo haemorrhagic fever virus antigen.

Tick classification	Non-engorged tick pools				Engorged tick pools				Total tick pools	Total tick numbers
	Positive	% (95% CI)	Total	Tick (N)	Positive	% (95% CI)	Total	Tick (N)		
All	19	5·6 (3·4, 8·6)	339	660	13	7·1 (4·1, 11·8)	184	405	523	1065
Genera										
Amblyomma	1	2·9	34	89	0	0	10	21	44	110
Hyalomma	0	0	1	1	–	–	–	–	–	1
Rhipicephalus	18	5·9	304	570	13	7·5	174	384	478	954
Species										
Adults										
Amblyomma lepidum	0	0	2	3	–	–	–	–	2	3
Amblyomma variegatum	1	3·6	28	82	0	0	6	14	34	96
Hyalomma truncatum	0	0	1	1	–	–	–	–	1	1
Rhipicephalus appendiculatus	17	6·3	272	520	12	9·9	121	256	393	776
Rhipicephalus decoloratus	0	0	12	19	0	0	8	17	20	36
Rhipicephalus evertsi	0	0	10	19	0	0	2	3	12	22
Rhipicephalus microplus	0	0	1	1	–	–	–	–	1	1
Rhipicephalus pravus	0	0	3	3	–	–	–	–	3	3
Rhipicephalus pulchellus	0	0	3	4	–	–	–	–	3	4
Larvae										
Rhipicephalus	–	–	–		0	0	1	4	1	4
Nymphae										
Amblyomma	0	0	4	4	0	0	4	7	4	11
Rhipicephalus	1	33·3	3	4	1	2·4	42	104	45	108

N; Number,%; percentage, CI; confidence interval.

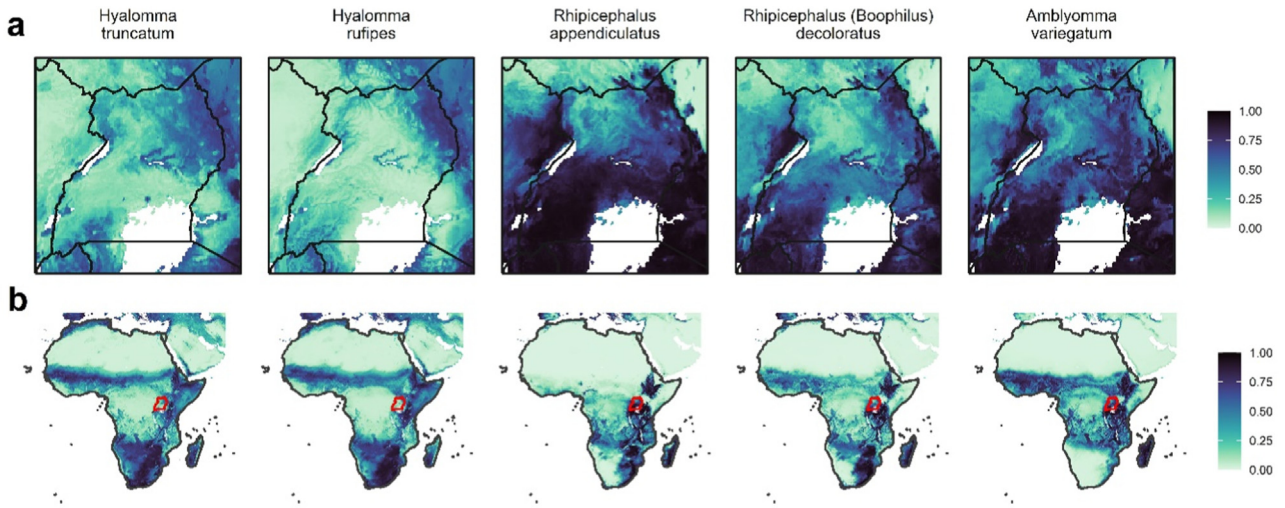

Fig. 2. Environmental suitability for potential tick hosts of CCHFV in Uganda and Africa-wide. Map colour scale shows environmental suitability for each of the 5 tick species with a substantial number of occurrences in Uganda, defined as mean predicted probability of occurrence from an ensemble of 200 boosted regression trees models fitted for Africa-wide tick occurrence data (Methods, Supp. Table 3). Columns are per-species, with the top row showing suitability in Uganda and its immediate surrounds (A), and the bottom row showing Africa-wide predicted suitability with Uganda outlined in red (B).

containing *R. appendiculatus* and 1 (3·6%) of 28 tick pools containing *A. variegatum* tested positive. Among engorged ticks, 12 (9·9%) of 121 tick pools containing *R. appendiculatus* tested positive, while none of the 6 tick pools containing *A. variegatum* tested positive (Table 2). *Rhipicephalus* ticks were picked from cattle originating from all regions of Uganda, whereas *Amblyomma* spp. were only picked from cattle originating in Northern and Eastern regions. The *Hyalomma truncatum* came from an animal from the Western region (Fig. 1).

Model ensembles for all tick and *Lepus* species showed good predictive ability under spatial cross-validation (mean AUC between 0·8 and 0·93; Supp. Fig. 3). Contrary to our hypothesis but consistent with the cross-sectional study results, most of Uganda – including Central and Western regions where most CCHF cases are documented – had very low predicted environmental suitability for *Hyalomma* species (Fig. 2, Supp. Figs. 3,4). Patches of higher suitability are found in the northeast and southwest of the country, including the origin district of the animal from which the single *Hyalomma* tick was recovered (Ntungamo; Figs. 1, 3a). In contrast, much of Uganda is suitable for *R. (Boophilus) decoloratus, A. variegatum* and *R. appendiculatus,* with high predicted suitability for the latter throughout the Central and Western regions (Fig. 2a-b). The most influential predictors of environmental suitability for all ticks were vegetation-related (NDVI mean and seasonality), followed by precipitation and temperature factors (Supp. Fig. 3). *Lepus* hare species are widespread across grassland and savannah biomes throughout Africa, and there is high predicted suitability for L. *microtis* across Uganda (Supp. Fig. 5).

The model of CCHF exposure showed good predictive ability (mean AUC = 0·88, range 0·76–0·95 across all submodels). Spatial predictions show high socio-ecological suitability for human CCHF infection throughout the Western, Central and Eastern regions of Uganda, and low suitability in much of the Northern region (Fig. 3a-b). These predictions are consistent with cattle seroprevalence in the cross-sectional study (Table 2, Fig. 1). The three most influential predictors of CCHF exposure across the continent were, in order, agricultural land use, vegetation seasonality and *Lepus* spp. suitability (median variable importance > 10%), with lower influence of *Hyalomma* spp. suitability, *Rhipicephalus* spp. suitability, minimum temperature of the coldest month and cattle density (Fig. 3c-b). Consequently, the model predicts a heterogeneous distribution of CCHF exposure across Africa (Fig. 4b) with risk highest in agricultural and pastoral areas with lower vegetation seasonality and high suitability for hare species (Fig. 3c). It should be noted that our approach does not explicitly adjust for differences in surveillance effort between countries and regions. Neither *Hyalomma* nor *Rhipicephalus* were among the consistently highest-ranked predictors of CCHF exposure (Fig. 3d) and there are several areas – especially Uganda, but also parts of the Congo basin and West Africa – where predicted CCHF risk is high despite low *Hyalomma* suitability (Fig. 3a, Supp. Fig. 6).

Discussion

Our cross-sectional and modelling studies provided complementary and independent evidence to support two main conclusions. First, CCHFV transmission appears much more widespread among ticks, livestock and people in Uganda than the low incidence of confirmed cases might suggest. Differing infection levels in ticks and cattle from different regions (Tables 2 and 3), and models of human CCHF exposure (Fig. 3) suggest that risk may be particularly high in Central and Western Uganda. Second, the principal tick vectors involved in CCHFV maintenance and transmission in Uganda might differ from elsewhere in the disease's range, with our results together suggesting that *Hyalomma* species are relatively uncommon in Uganda, and instead implicating *Rhipicephalus* ticks (here, particularly *R. appendiculatus*).

Around one in ten abattoir workers had serological evidence of recent and/or previous CCHF infection. This was lower than the 17%–30% in at-risk professionals reported in a recent global systematic review.[35] Few studies have reported on the prevalence of CCHF serology in abattoir workers, but the IgM seroprevalence of 3% in our study, suggesting recent infection, is lower than reported among abattoir workers from Senegal (7%),[36] Ghana (13%),[20] Turkey (13%)[37] and Iran (16%),[38] while the IgG seroprevalence of 8%, suggesting previous infection, is similar to 6% among healthy Ugandan blood donors[39] but lower than 13% reported in nomadic people from rural Senegal.[36]

Conducting this study in three central abattoirs enabled the sampling of cattle from across most of Uganda. The seroprevalence of 70% in cattle is high, but comparable to 75% previously reported in cattle from five districts in Uganda[40] and consistent with a recent systematic review, reporting seropositivity from 1 to 79%.[35] The highest seroprevalence in our study was in cattle from the

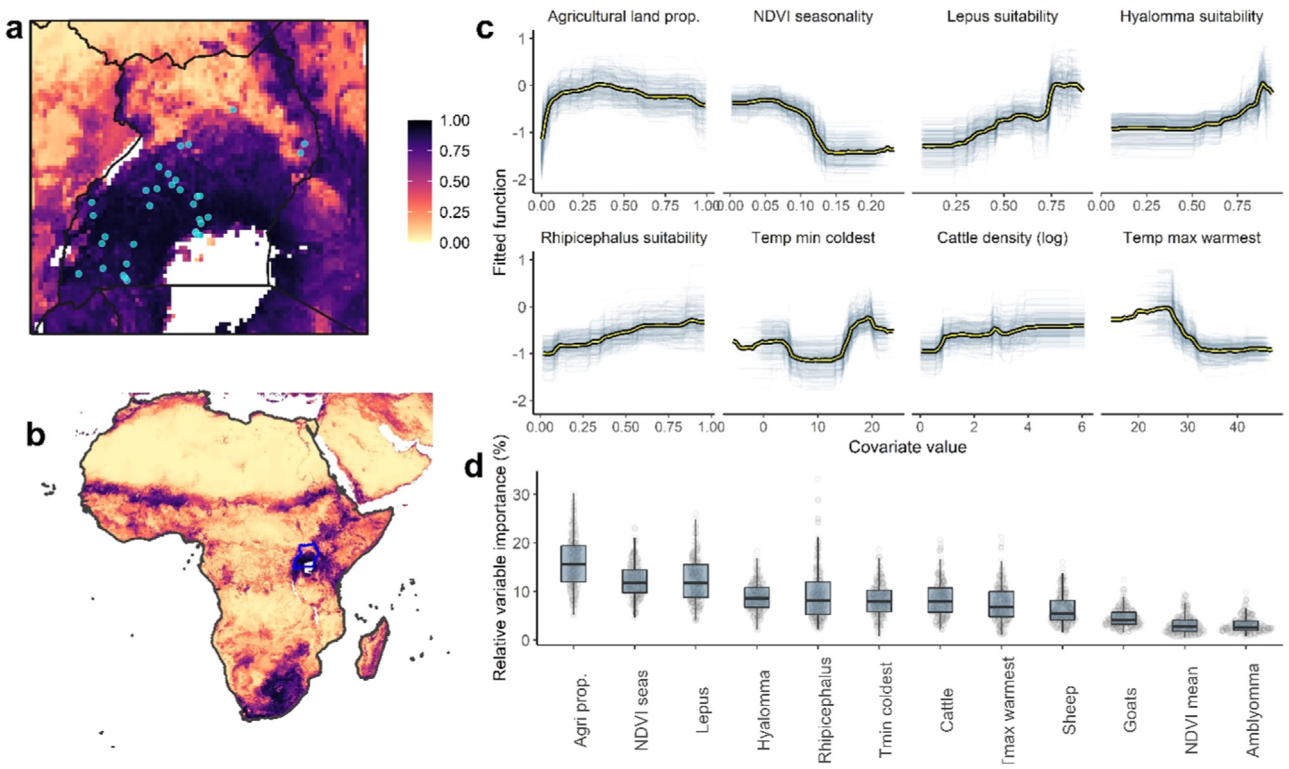

Figure 3. Geographical distribution and socio-ecological predictors of human CCHF exposure. Top map (A) shows mean predicted suitability for CCHF exposure for Uganda and its surroundings, with overlaid blue points showing localities of human acute cases recorded in Uganda to date. Bottom map (B) shows per grid-cell mean predicted suitability for CCHF exposure across Africa (with Uganda boundaries shown in blue). Figures show partial dependency plots (C) and relative covariate importance (D), for the ensemble of 200 boosted regression trees modelled fitted to the distribution of human CCHF records. The yellow line in (C) shows the mean partial dependency function, with the shape of the function describing the relationship with the covariate. Individual blue lines represent individual submodels, with a wider spread indicating higher uncertainty in the effect of each covariate. Points and boxplots in (D) show and summarise the importance of each variable to predicting exposure for each submodel, with covariates ordered from left to right by median importance.

Central and Eastern regions (70%), while the lowest was the Northern region (50%). Although cattle might have been infected outside of their region of origin, these differences are consistent with predicted risk from our human exposure model, which is also lower in Northern Uganda. Although abattoirs bring together cattle from disparate regions making it possible to study ticks from across the country, caution is needed when interpreting results, as ticks may drop off and attach during cattle transportation.

Serological evidence of CCHFV exposure in humans and cattle, and the association of seropositivity with previous tick bite highlights an important potential occupational hazard to abattoir workers, however, our study might be underpowered to detect other risk factors. Other limitations for the cross-sectional study include the convenience sampling method, self-reported data from participants, and potential for cross-reactivity in serological assays.

Despite *Hyalomma* ticks being generally considered the key hosts and vectors for CCHFV, only one, non-infected *Hyalomma* was recovered in our cross-sectional study, and our models predict generally low environmental suitability for all modelled *Hyalomma* species across Uganda. In contrast, we found that *Rhipicephalus appendiculatus* and *Rhipicephalus (Boophilus) decoloratus*[13,41] have a widespread distribution across Uganda (Fig. 2). One potential confounder in our ecological models is heterogeneity in tick survey effort, however, Uganda was not notably under sampled for ticks overall (Supp Fig. 4a).

The main predictors of human CCHF exposure included several factors that might jointly affect ecological suitability for tick and CCHFV maintenance, and human-tick and human-livestock contact (agricultural land use, vegetation dynamics, hare and tick suitability, cattle density; Fig. 3c). Our models included an extra decade of data and additional covariates than previous risk maps,[34] and thus predict a more contiguous distribution of CCHF exposure across Africa. However, data remain sparse, which is reflected in wide uncertainty in covariate response curves, and our models probably underpredict risk in low-surveillance areas[42]. One notable result is the importance of *Lepus* suitability as a predictor of CCHF exposure (Fig. 3). Hares are among the few mammals with robust evidence of CCHFV host competence, experience prolonged viraemia and are important hosts for immature ticks (including *Rhipicephalus* and *Hyalomma).*[5] This genus might play a substantial role in setting the geographical limits to CCHFV endemic maintenance in nature, which requires further investigation. Alternatively, since *Lepus* species are associated with grassland and pastoral biomes, they might be acting as proxy for broader ecological community conditions required to maintain CCHFV across Africa.

Overall, we found evidence of CCHFV as a widespread health risk in Uganda. Our epidemiological and ecological approaches together suggest that *Rhipicephalus* ticks, rather than *Hyalomma*, might be important for transmission in this region. We found many *Rhipicephalus* ticks, consistent with a similar survey in western Kenya, which showed *Ambylomma* and *Rhipicephalus* as the most prevalent ticks in the region[43]. Although *Rhipicephalus* have demonstrated vector competence for CCHFV in experimental settings, and a recent study in Iran also detected CCHFV antigen in an *R. appendiculatus* specimen[44], evidence for a substantial role in viral maintenance and transmission in nature has been inconclusive.[3]

Numerous additional factors could make Uganda an unusual transmission setting for CCHFV, including the high prevalence of pastoral livelihoods and large volumes of cattle (and wild ungulate)

movement across the country. Livestock and human travel from areas of higher *Hyalomma* density such as northern Uganda or Tanzania could theoretically be responsible for CCHFV dispersal. For example, in 2013 and 2018 respectively, human cases were reported as imported from South Sudan and Rwanda into Uganda[2], and mass movement of livestock for sacrifice during religious festivals is suggested to have contributed to CCHF outbreaks elsewhere[45]. In our study, 86% (6/7) of cattle from Tanzania were seropositive for CCHFV, but the small number requires cautious interpretation, and the role of livestock trade in the spread of CCHF in the region remains unclear.

Our results suggest further research into the eco-epidemiology of CCHF is needed in Uganda, for example, through sampling livestock, wild mammals and ticks in pastoral and agricultural ecosystems including cattle markets, farms and national parks. More broadly, our results call into question the common assumption that *Hyalomma* tick populations are necessary to maintain CCHFV transmission in nature and suggest that this disease's ecologies may vary across its broad geographical range.

Author contributions

SAL, RG, DK, KEJ, IA, JJL, and NF conceptualised and designed the study. SAL and RG wrote the first draft of the manuscript, with further contributions from SAL, RG, DK, GN, JM, SB, LO, KEJ, IA, JJL, and NF. SAL and DK led on questionnaire and sampling design for the cross-sectional study, and the fieldwork was undertaken by SAL, DK, GN, JM, and SB. Cross-sectional statistical analysis was done by SAL, with supervision by IA and NF. Laboratory work was led by DK, with tick identification by GN. Modelling was done by RG, with contributions by LO, and supervision by KEJ. SAL, KEJ, IA, JJL and NF obtained funding. All authors had full access to all data in the study and contributed to data interpretation, reviewed successive drafts, and approved the final version of the manuscript. The corresponding author had final responsibility for the decision to submit for publication.

Data sharing

All code and data used for the modelling analyses are accessible and permanently archived at https://doi.org/10.5281/zenodo.6778254. Applications for relevant anonymised data from the observational study should be submitted to nigel.field@ucl.ac.uk.

Declaration of Competing Interest

The authors have no associations that might pose a conflict of interest.

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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.2022.09.016.

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