Crimean-Congo Hemorrhagic Fever Virus Seroprevalence in Human and Livestock Populations, Northern Tanzania

Ellen C. Hughes, William de Glanville, Tito Kibona, Blandina Theophil Mmbaga, Melinda K. Rostal, Emanuel S. Swai, Sarah Cleaveland, Felix Lankester, Brian J. Willett, Kathryn J. Allan

Author affiliations: University of Liverpool Institute of Infection Veterinary and Ecological Sciences, Liverpool, UK (E.C. Hughes); University of Glasgow College of Medical Veterinary and Life Sciences, Glasgow, Scotland, UK (E.C. Hughes, W. de Glanville, S. Cleaveland, K.J. Allan); Washington State University, Pullman, Washington, USA (E.C. Hughes, F. Lankester); Nelson Mandela African Institute of Science and Technology, Arusha, Tanzania (T. Kibona); Global Animal Health Tanzania, Arusha, Tanzania (T. Kibona, F. Lankester); Kilimanjaro Christian Medical University College, Kilimanjaro, Tanzania (B.T. Mmbaga); EcoHealth Alliance, New York, New York, USA (M.K. Rostal); Ministry of Livestock and Fisheries, Dodoma, Tanzania (E.S. Swai); MRC-University of Glasgow Centre for Virus Research, Glasgow, UK (B.J. Willet)

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We conducted a cross-sectional study of Crimean-Congo hemorrhagic fever virus (CCHFV) in northern Tanzania. CCHFV seroprevalence in humans and ruminant livestock was high, as were spatial heterogeneity levels. CCHFV could represent an unrecognized human health risk in this region and should be included as a differential diagnosis for febrile illness.

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tickborne orthonairovirus with potential to cause severe Crimean-Congo hemorrhagic fever (CCHF) disease in humans, which can lead to human-to-human transmission (*1*). CCHFV is a World Health Organization priority pathogen for research and development (*2*). Although a wide range of wild and domestic animals can be infected (*3*), CCHFV does not typically cause clinical disease in nonhuman species (*1*). In eastern Africa, intermittent outbreaks of CCHF disease in humans have occurred in Uganda since 2013 (*4*), but the epidemiology of CCHFV remains poorly understood. Northern Tanzania, neighboring Uganda, has been identified as an area likely to be at high risk for human disease caused by CCHFV, because competent tick vectors and suitable environmental conditions exist in the region (*5*), but no clinical CCHF cases have yet been reported in the country.

To investigate CCHFV exposure in northern Tanzania, we performed serologic testing on human and ruminant livestock serum samples collected in 2016 during an investigation of several zoonotic pathogens (*6*) (Appendix, https://wwwnc.cdc.gov/EID/article/30/4/23-1204-App1.pdf). The study used a multilevel sampling frame of 351 humans and 7,456 randomly selected livestock in linked households in Arusha and Manyara Regions (Figure). We tested serum samples by using the ID Screen CCHF Double Antigen Multi-species ELISA (IDvet, https://www.innovative-diagnostics.com) (Appendix). We estimated seroprevalence by using the Survey package in R (The R Foundation for Statistical Computing, https://www.r-project.org) (*7*). We assessed species-level differences in seroprevalence by using a mixed-effects model with household and village as random effects. We investigated patterns of spatial autocorrelation in village-level seroprevalence by using the Moran *I* statistic and assessed correlation of village-level seroprevalence between species pairs by using the Pearson correlation coefficient (ρ) (Appendix).

Overall, seroprevalence was high in all livestock species: cattle 49.6% (95% CI 40.0%–59.2%), goats 33.8% (95% CI 21.7%–47.5%), sheep 27.8% (95% CI 17.0%–40.6%) (Table; Figure). Sheep and goats had significantly lower odds of exposure than cattle: sheep OR was 0.32 (95% CI 0.27–0.37, $p \leq 0.001$) and goats OR 0.45 (95% CI 0.39–0.51; $p \leq 0.001$). Village-level seroprevalence ranged widely in all species but values were consistent with those reported elsewhere in East Africa (*3*) (Table). The finding of higher seroprevalence in cattle than in sheep and goats is also consistent with other settings in Africa (*3*) and might reflect differences in host feeding preferences of *Hyalomma* spp. ticks, considered chief vectors of CCHFV (*1*). However, further work is required to understand the relative contribution of different host species to viral maintenance, and their relationship to human infection risk.

Overall, human seroprevalence was 15.1% (95% CI 11.7%–19.2%), but village-level seroprevalence varied widely between study sites (Table). Seroprevalence was similar to that reported in health-care-seeking patients in Kenya in 2012 (*8*), but higher than the 1.2% seroprevalence reported in community participants elsewhere in Tanzania (*9*). However, interpretation of those regional comparisons is challenging in light of the substantial observed between-village variation in our study (Table).

Assessment of spatial autocorrelation via Moran *I* statistic showed no evidence of village-level spatial

Table. Seroprevalence of Crimean-Congo hemorrhagic fever virus in human and livestock populations, northern Tanzania*

Species	No. tested	Overall seroprevalence (95% CI)	Seroprevalence range per village (95% CI)		Moran *I* statistic (p value)
			Low	High	
Cattle	3,015	49.6 (40.0–59.2)	5.3 (1.2–9.4)	76.6 (70.3–82.8)	−0.09 (0.60)
Sheep	2,059	27.8 (17.0–40.6)	0.0 (0–3.9)	70.3 (55.5–85.0)	−0.09 (0.57)
Goats	2,382	33.8 (21.7–47.5)	0.0 (0–5.8)	79.6 (68.3–90.8)	−0.10 (0.61)
Human	351	15.1 (8.5–23.8)	0.0 (0.0–16.1)	50 (30.7–69.2)	0.43 (0.001)

*Serum samples were collected in northern in 2016 and tested for antibodies to Crimean-Congo hemorrhagic fever virus. Moran I statistic and associated p value are shown for the village level (Appendix).

autocorrelation in livestock (Table), suggesting that although context-specific drivers, such as husbandry practices and local agroecology are likely involved, drivers of exposure were not observable at this scale. In contrast, we observed significant positive spatial autocorrelation in the village-level human seroprevalence (Moran *I* statistic 0.43; p≤0.001) and clustering of higher seroprevalence villages in the western part of Manyara (Figure). In addition, species-pair correlations showed that village-level human and livestock seroprevalence were not well correlated (cattle, ρ = 0.34, p = 0.142; sheep, ρ = 0.35, p = 0.13; goats, ρ = 0.42, p = 0.062), and we saw high human seroprevalence in some low livestock seroprevalence locations and vice versa (Appendix). That heterogeneity, combined with differences in spatial distribution, could suggest different drivers of exposure in livestock and human populations. However, discrepancies in sample size could exaggerate those differences, so further linked investigation into human and livestock exposure and patterns of tick infection are required. Further exploration of specific risk factors is ongoing and could provide clarity on drivers of exposure.

The high human exposure levels to CCHFV implies that clinical CCHF is a potentially serious, underdiagnosed health risk in this population and suggests that CCHF should be included as a differential diagnosis for undifferentiated febrile illness in northern Tanzania. However, evidence of human seropositivity in the absence of clinical cases is common, even where health professionals are familiar with CCHF diagnosis (*8*,*10*). The causes of disease emergence in such populations are poorly understood, and further research into regions like northern Tanzania, where the virus is endemic but human disease has not been reported, is critical to understanding human disease risk.

In conclusion, we found that CCHFV is circulating widely in livestock across northern Tanzania. CCHFV seroprevalence in the region shows high

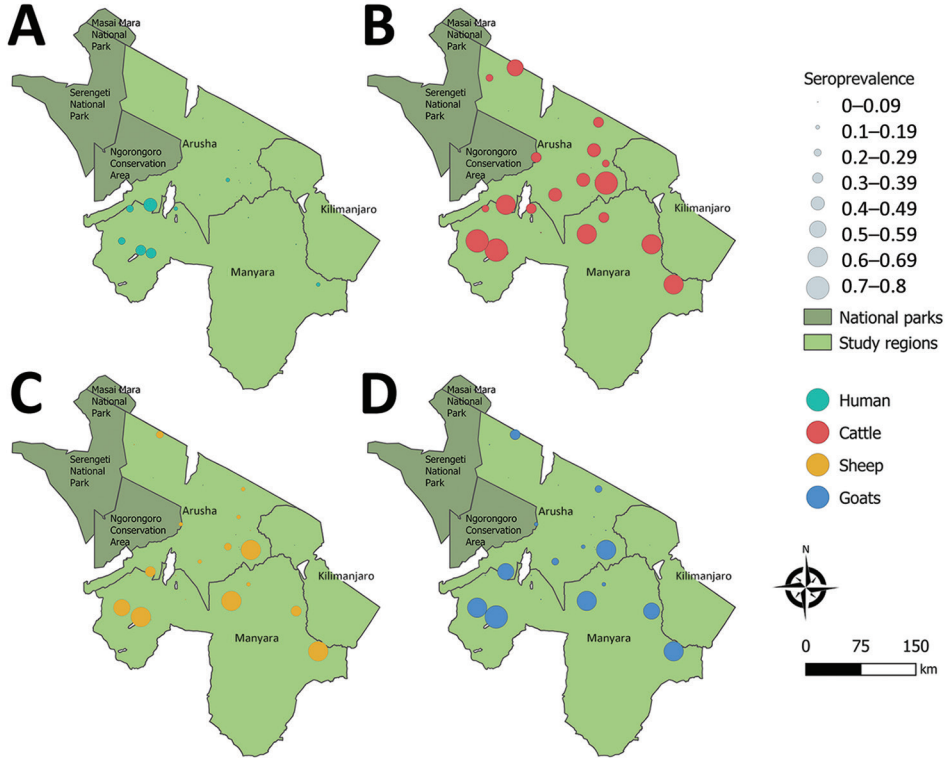

Figure. Sampling area for study of seroprevalence of Crimean-Congo hemorrhagic fever virus in human and livestock populations, northern Tanzania. Circles indicates seroprevalence rates for humans (A), cattle (B), sheep (C), and goats (D). The pictured region is near Uganda, where human Crimean-Congo hemorrhagic fever cases have been documented (*4*).

spatial heterogeneity and further investigations are needed to understand drivers of exposure. In addition, high human seroprevalence demonstrates widespread exposure of the population to CCHFV and suggests that CCHF should be included as a differential diagnosis for febrile illness in this region.

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About the Author

Dr. Hughes is a veterinarian and post-doctoral researcher who works for the Global Burden of Animal Diseases Programme at the University of Liverpool, Liverpool, UK. Her research interests include emerging and endemic zoonoses, disease burden estimation, and One Health approaches to animal and human health.

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Address for correspondence: Ellen C. Hughes, c/o Prof. Brian Willett, MRC-University of Glasgow Centre for Virus Research, Garscube Campus, 464 Bearsden Rd, Glasgow, Scotland G61 1QH, UK; email: ellen.hughes@liverpool.ac.uk

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Appendix

Study Design

Samples were collected as part of a cross-sectional sero-survey undertaken in 2016 to investigate several zoonotic pathogens, including *Brucella* spp., *Coxiella burnetii* and Rift Valley Fever Virus (RVFV), in livestock and humans across a range of agricultural systems (*1*,*2*). This study was designed to achieve a range of aims with a target of 400 households to estimate a household-level prevalence of 50% for multiple outcomes, with around 5% error at the 95% confidence interval. Livestock sample sizes at the household-level were based on a desire to detect at least one seropositive animal of each species (cattle, goat, sheep) in the household with 95% herd sensitivity if the herd was "exposed," given an expected within-herd prevalence of target diseases of 40%, and assumed diagnostic test sensitivity of 90% and specificity of 100%. This meant a target of 10 randomly selected animals of each species per household, or sampling all animals present where herd/flock sizes of a particular species were 10 or less (*3*). Animals less than 6 months were excluded. Livestock samples were collected using a multilevel sampling approach and resulted in a total of 3,015 cattle, 2,382 goats, and 2,059 sheep sampled from 417 households across 20 villages. Villages were randomly selected using generalized random tessellation stratified sampling to ensure spatial balance (*2*,*4*). Human serum samples (n = 351) were collected from a random selection of 113 of these households across 17 villages, as well as four additional village sites without linked livestock samples. In these households, all willing people aged five or above were sampled. GPS coordinates were not recorded for sites where livestock sampling did not also occur, so these do not appear on the maps (Figure).

Laboratory Testing

Samples from all species were heat-treated at 56°C for two hours in Tanzania before shipping to the UK (license no. TARP(S)2016/49) for analysis at the MRC-University of Glasgow Centre for Virus Research using a commercially produced, species-independent, double antigen sandwich ELISA (IDvet, Grabels, France). Manufacturer-reported sensitivity and specificity values for the ID Screen® CCHF Double Antigen Multi-species (IDvet, Grabels, France) are shown in Appendix Table 1. Further details of the ELISA can be found in Sas et al. 2018 (*5*) and discussion of potential cross-reactivity in Hughes 2022 (*6*).

Cross-reactivity with related orthonairoviruses is a potential issue with serologic testing for CCHFV, although recent large-scale studies have suggested limited evidence of cross-reactivity (*7*,*8*). In livestock, the performance of the ID Screen® CCHF Double Antigen Multi-species (IDvet, Grabels, France) has been shown to correlate highly with results obtained using other methods for CCHF antibody detection, including immunofluorescent assays, in-house indirect species-specific ELISAs against both glycoprotein Gc and nucleoprotein (NP) (*9*,*10*). The assay has also been used in the testing of human samples (*11*), including in conjunction with other commonly used human-specific assays to retrospectively confirm the earliest case of CCHF in Spain, published in EID in 2021 (*12*).

Statistical Analysis

Seroprevalence was calculated for each species both at a population and village-level. Overall seroprevalence and 95% confidence intervals were calculated using the *Survey* package in R, using village and household as cluster identifiers (village = primary sampling unit, household = secondary sampling unit) (*13*). Village-level seroprevalence was calculated as the number of positives/total number sampled in each village with binomial confidence intervals.

Village-level seroprevalence for all species was plotted on maps of the study area. Maps were created in QGIS version 3.16.0 (*14*). All statistical analyses were performed in R statistical environment, version 3.6.1 (*15*). Mixed-effects logistic regression models were implemented using the *lme4* package (*16*). Random effects were included at the village and household level. Moran's I was calculated using the *spdep* package in R (*17*). A Moran's I statistic of 1 is equivalent to perfect spatial clustering, while a value of −1 represents perfect dispersal.

Statistical significance was set at $p \leq 0.05$. Moran's I statistic was calculated using the village level seroprevalence and did not include analysis of co-variables. As such, it is a test for spatial autocorrelation of seroprevalence for the village context only, and does not account for specific variables that may drive exposure within village units.

Ethics

The study protocols, questionnaires, and consent documents were approved by the Kilimanjaro Christian Medical Centre (KCMC) (approval no. 832) and National Institute of Medical Research (NIMR) (approval no. 2028) ethics committees, and University of Glasgow Medical, Veterinary and Life Sciences (MVLS) Ethics Committee (approval no. 200140152). Permission to carry out the study in Tanzania was provided by the Tanzania Commission for Science and Technology (permit no. 2014–244-ER-2005–141). Written informed consent or assent for sample collection and questionnaire administration was collected from all participants. Samples were imported into the UK under license TARP(S)2016/49. Permission to publish was granted by the Director of Veterinary Services, Tanzania (Act No. 17 of 2003).

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Appendix Table 1. Manufacturer-reported sensitivity and specificity values for the antigen test used in this study*

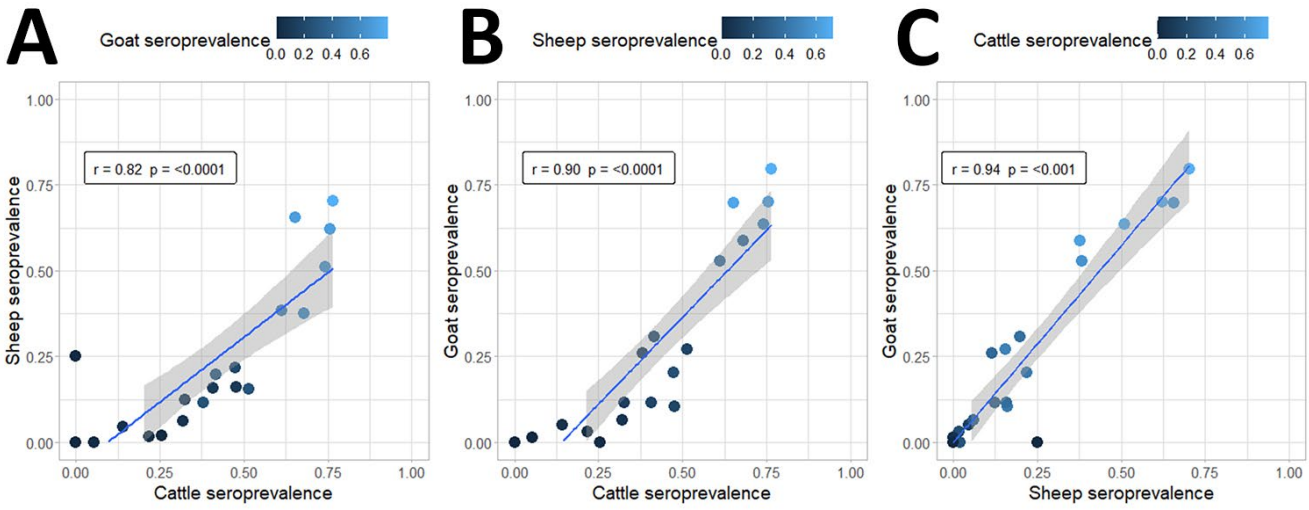
Species	% Specificity (95% CI)	% Sensitivity (95% CI)
Cattle	100 (99.1–100), n = 402	97.9 (92.6–99.4), n = 95
Sheep	100 (99.1–100), n = 402	99.0 (94.7–99.8), n = 102
Goats	100 (99.1–100), n = 402	100 (95.1–100), n = 74
Humans	100 (98.5–100), n = 257	NA

*ID Screen CCHF Double Antigen Multi-species (IDvet, Grabels, France).
NA, not available.

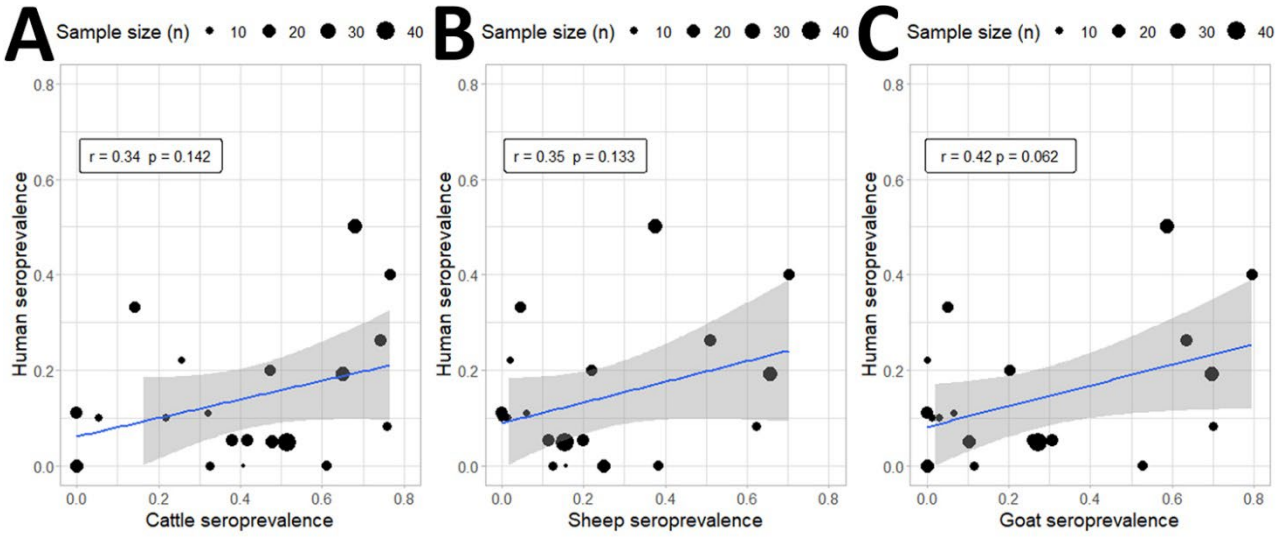
Appendix Table 2. Odds of exposure to Crimean-Congo hemorrhagic fever in sheep and goats compared to cattle*

Variable	Odds ratio (95% CI)	p value
Intercept	0.75 (0.41–1.36)	0.341
Species		
Cattle	Referent	Referent
Goat	0.45 (0.39–0.51)	<0.001
Sheep	0.32 (0.27–0.37)	<0.001

*Odds ratio, 95% CIs, and p values from an all-species mixed effect logistic regression model with species as a fixed effect, and village and household as random effects.



Appendix Figure 1. Correlation between village-level seroprevalence by species-pairs. A) Cattle and sheep seroprevalence compared with goat seroprevalence; B) cattle and goat seroprevalence compared with sheep seroprevalence; C) sheep and goat seroprevalence compared with cattle seroprevalence. Points are colored according to the village-level log odds of the third species.



Appendix Figure 2. Correlation between village-level human and livestock seroprevalence. Graphs show human seroprevalence compared with seroprevalence of cattle (A), sheep (B), and goat (C) seroprevalence. Points are sized according to the village-level sample size.