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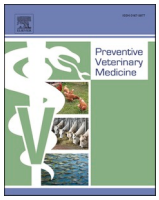

Seroepidemiology of selected transboundary animal diseases in goats in Zambia

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ABSTRACT

Transboundary pathogens of goats present significant constraints to the livelihoods of millions of farmers in countries such as Zambia. Consequently, this study aimed to investigate the seroprevalence of *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), foot and mouth disease virus (FMDV), *Brucella* spp., Crimean Congo haemorrhagic fever virus (CCHFV), and Rift Valley fever virus (RVFV) in Zambian goats. Another aim was to identify associations between seroprevalence and different predictor variables, such as trade and border proximity. From September to October 2019, 962 serum samples were collected from goats in seven Zambian districts, four of which have an international border while the remaining three do not. A questionnaire survey was conducted with each household, focusing on trade routines, management strategies and herd disease history. Animal-level seroprevalence adjusted for herd-level clustering was 8.2 % (95 % confidence interval [CI] 7.5–9.0) for Mccp, 12.9% (95% CI 12.0–13.7) for FMDV, 13.0 % (95% CI 12.1–13.9) for *Brucella* spp., 3.3 % (95% CI 2.8–3.7) for CCHFV, and 0.4 % (95 % CI 0.3–0.7) for RVFV. The association between herd-level seroprevalence and border proximity and trade appeared negligible, with the exception of selling goats at least twice a year which was identified as a potential risk factor for *Brucella* spp. (OR 4.1, 95 % CI 1.1–16.0, $p = 0.040$). In addition, a positive association between herd-level seroprevalence of FMDV and a herd size of 21 goats or more (OR 3.3, 95 % CI 1.0–11.1, $p = 0.049$) was detected. Also, positive associations between animal-level seroprevalence of *Brucella* spp. and increasing age (OR 7.7, 95 % CI 1.5–40.7, $p = 0.016$), and CCHFV and keeping pigs in the household (OR 2.7, 95 % CI 1.0–7.1, $p = 0.044$), were found. For FMDV (OR 3.8, 95 % CI 1.4–10.9, $p = 0.011$) and *Brucella* spp. (OR 4.5, 95 % CI 1.2–17.3, $p = 0.031$) on the other hand, animal-level seroprevalence was significantly higher in households without pigs. To the best of the authors' knowledge, this is the first study to describe the presence of antibodies for CCPP and CCHF in the Zambian goat population. While the association between seroprevalence and trade and border proximity generally appeared negligible, it is recommended that their influence is further evaluated in future studies, preferably through in-depth longitudinal studies incorporating impacts of different biosecurity measures and trade variations, linked to for example seasonality and trade peaks.

Abbreviations: CCHF, Crimean-Congo haemorrhagic fever; CCPP, Contagious caprine pleuropneumonia; FMD, Foot and mouth disease; Mccp, *Mycoplasma capricolum* subsp. *capripneumoniae*; RVF, Rift Valley fever.

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1. Introduction

Goats play an important role in securing the livelihoods of resource-limited smallholder farmers in countries such as Zambia, but the sector's productivity is severely constrained by infectious diseases (FAO, 2013). In Zambia, goats are important for income generation, food security and various traditional events. Nevertheless, very little is known about pathogen prevalence among Zambian small ruminants since only a small number of studies have been conducted (Hussein et al., 1985; Davies et al., 1992; Ahmadu et al., 2004; Muma et al., 2006; Goma et al., 2007; Simukoko et al., 2007; Qiu et al., 2013; Laohasinnarong et al., 2015; Nyimba et al., 2015; Musinguzi et al., 2017; Nyirenda et al., 2017a, 2017b; Simulundu et al., 2017; Chambaro et al., 2020; Lysholm et al., 2022a, 2022b). Furthermore, there are currently no ongoing national control programs or vaccination campaigns for goat diseases in Zambia.

Transboundary diseases are generally highly infectious and capable of rapid cross-regional spread, irrespective of international borders, and have severe socioeconomic effects aside from their impact on food security, trade and general animal welfare. There are numerous transboundary diseases that affect small ruminants such as contagious caprine pleuropneumonia (CCPP), foot and mouth disease (FMD), brucellosis, Crimean Congo haemorrhagic fever (CCHF) and Rift Valley fever (RVF) (OIE and FAO, 2004; Thomson and Penrith, 2017). Contagious caprine pleuropneumonia is caused by the bacterium *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) and causes morbidity and mortality rates of up to 100% and 80–100% respectively in naïve populations (Iqbal Yatoo et al., 2019). Contagious caprine pleuropneumonia is considered as one of the most severe goat diseases known and the annual global burden has been estimated to 507 million USD (Iqbal Yatoo et al., 2019). *Mycoplasma capricolum* subsp. *capripneumoniae* has never been detected in Zambia although it is present in neighbouring Tanzania (Kusiluka et al., 2000a, 2000b; Kgotlele et al., 2016; Chota et al., 2020). In previous studies in Tanzania, animal-level seroprevalence in goats have ranged from 3.3% to 52.1% (Swai et al., 2013; Mbyuzi et al., 2014; Torsson et al., 2017; Chota et al., 2019) depending on e.g. study area and design and type of laboratory test used. Herd-level seroprevalence in Tanzania has in one study been estimated to 9.6% (Swai et al., 2013). Due to the frequent cross-border trade of livestock and lack of physical barriers between the two countries, allowing animals to graze on either side of the border, there is a clear risk of cross-border spread and introduction of CCPP into Zambia (Karimuribo et al., 2014).

Foot and mouth disease (FMD), caused by foot and mouth disease virus (FMDV), is endemic in Zambia (Sinkala et al., 2014b). While mortality due to FMDV generally is low, morbidity is high and the virus can cause severe negative effects on production, trade and animal welfare (Grubman and Baxt, 2004; OIE and FAO, 2004). Therefore, FMD is one of the most regulated livestock diseases in the world and the annual economic impact in endemic regions, resulting from visible production losses and costs of vaccinations, have been estimated to 6.5–21 billion USD (Knight-Jones and Rushton, 2013). Small ruminants will often only develop mild clinical signs that are easily missed, and can therefore act as viral reservoirs for more sensitive species such as cattle (Grubman and Baxt, 2004). In Zambia, most previous research has focused on cattle (Perry and Hedger, 1984; Hamoonga et al., 2014; Sinkala et al., 2014a, 2014b), but in a recent study conducted close to the Tanzanian border, animal- and herd-level seroprevalence in sheep and goats was estimated to 1.03% and 3.14%, respectively (Lysholm et al., 2022b). In neighbouring Botswana and Zimbabwe, animal-level seroprevalences of the SAT1–3 serotypes have been estimated to 9.32% (Hyera et al., 2006) and 1.50% (Bhebhe et al., 2016), respectively, while in Tanzania, a 44.3% seroprevalence of antibodies to all seven FMDV serotypes has been found (Torsson et al., 2017).

There is a significant number of transboundary goat diseases that are zoonotic and hence can have severe negative effects on both human and animal health, e.g. brucellosis, CCHF and RVF. Brucellosis is an endemic bacterial disease in Zambia (Bell et al., 1977; Muma et al., 2006). The disease is caused by members of the *Brucella* genus, and is a common health hazard for people in contact with susceptible animals and animal products (Corbel, 2006; Seleem et al., 2010). Goats are typically affected by the subspecies that is most virulent to humans, namely *Brucella melitensis*, and sporadically also by *Brucella abortus* and *Brucella suis*. The economic burden in sheep and goat populations in India has been estimated to 120 million USD (Singh et al., 2015), but this figure does not incorporate e.g. costs related to human disease or veterinary treatment. Animal-level seroprevalence in small ruminants in previous studies in Zambia has ranged from 0% to 1.65% (Muma et al., 2006; Lysholm et al., 2022b), while 10.1% of sheep and goats at two small livestock markets in Zambia were seropositive (Lysholm et al., 2022a). In cattle in Zambia, animal- and herd-level seroprevalence have ranged from 6.0% to 24% and 21–58%, respectively (Muma et al., 2006, 2007a, 2007b, 2012, 2013; Chimana et al., 2010; Mfune et al., 2021). Humans are often infected when they assist at parturition, through the consumption of unpasteurised milk or undercooked meat, or during slaughter and carcass-dressing procedures (Corbel, 2006; Seleem et al., 2010). Human cases of brucellosis are occurring regularly in Zambia, and previous studies have detected seroprevalence ranging from 5% to 20% (Muma et al., 2008; Mubanga et al., 2021), with regular contact with livestock identified as a risk factor (Muma et al., 2008).

CCHF is a viral disease caused by Crimean Congo haemorrhagic fever virus (CCHFV) that is mainly transmitted by ticks from the *Hyalomma* genus, although humans can also become infected during the slaughter of a viraemic animal (Whitehouse, 2004) or when consuming undercooked meat (Fazlalipour et al., 2016). While no human case of CCHFV in Zambia has been documented to date, seroprevalence in cattle has been estimated to 8.4% (Kajihara et al., 2021). As clinical signs in viraemic animals are often mild or unapparent, animals can act as reservoirs for humans who can then develop a serious and sometimes deadly disease (Whitehouse, 2004).

RVF is an endemic viral disease in Zambia (Dautu et al., 2012), caused by Rift Valley fever virus (RVFV) that is predominately transmitted by mosquitoes, e.g. *Aedes* spp. and *Culex* spp., and close direct contact, for example during the slaughter of viraemic individuals (Bird et al., 2009). The virus typically appears in epizootic outbreaks every five to 25 years, which are characterised by a high number of abortions and neonatal mortalities in sheep, cattle and goats in particular, together with influenza-like disease in humans. RVF is considered endemic in Zambia although no outbreaks have been reported in the last three decades (Dautu et al., 2012). In the epizootic outbreak in 2006–2007 in Tanzania, the cost due to livestock deaths was estimated to 6 million USD and a considerable negative impact on trade was observed (Ahmed et al., 2018). Seroprevalence in ruminants in Zambia has in previous studies ranged from 2.3% to 80%, with the lower prevalences detected during interepidemic periods and the higher during outbreaks (Hussein et al., 1985; Morita, 1988; Davies et al., 1992; Samui et al., 1997; Saasa et al., 2018; Lysholm et al., 2022b). Furthermore, the seropositivity rate in small ruminants at two small livestock markets in Zambia has been estimated to 0.84% (Lysholm et al., 2022a). In previous studies on humans in Zambia, detected seroprevalence has been 2.6% (Hasebe et al., 1989) and 11% (Morita, 1988). Among the workers at a cattle abattoir in Zambia, five out of 53 (9.4%) had antibodies for RVF (Morita, 1988).

Stimulating farmer participation in trade is often highlighted as a way of alleviating poverty (Delgado et al., 1999; ILRI, 2002), but trade can also contribute to increased dissemination of infectious diseases (Fèvre et al., 2001; Kilpatrick et al., 2006). Livestock trade, animal movements and gatherings at markets often occur at a higher frequency in areas close to international borders, and FMD outbreaks have been shown to be clustered in areas close to international borders in Zambia and Tanzania (Picado et al., 2011; Hamoonga et al., 2014; Sinkala et al., 2014a; Allepuz et al., 2015).

This study aimed to investigate the seroprevalence of CCPP, FMD,

brucellosis, CCHF and RVF in goats in Zambia, and to identify associations between seroprevalence and different predictor variables, such as trade and border proximity.

2. Materials and methods

2.1. Study area and design

The study was designed to provide a cross-sectional snapshot of the prevalence of Mccp, FMDV, *Brucella* spp., CCHFV and RVFV in seven Zambian districts. The main pathogen selection criteria were being capable of causing disease in goats, availability of a well-functioning commercial ELISA test, and being listed as a priority pathogen by the OIE and FAO in the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs) (OIE and FAO, 2004). While Mccp is not listed in the GF-TADs, it was included after discussions within the author group and with local partners, due to its transboundary nature and potential severe impact on small ruminant populations (Iqbal Yatoo et al., 2019). Furthermore, Mccp was selected because it is present in e.g. neighbouring Tanzania, but it is currently unclear whether the pathogen is circulating in Zambia (Karimuribo et al., 2014). FMDV was included because it has been identified as the principal animal pathogen of global concern (OIE and FAO, 2004), and since, while it is endemic in Zambia, previous research has been focused on cattle (Perry and Hedger, 1984; Banda et al., 2014; Hamoonga et al., 2014; Sinkala et al., 2014a, 2014b). *Brucella* spp., CCHFV and RVFV were selected as they are zoonotic transboundary animal pathogens with potential impacts on both human and animal health (OIE and FAO, 2004). Furthermore, *Brucella* spp. and RVFV are endemic in Zambia, but few studies have been conducted that focused on the presence of antibodies in goats (Bell et al., 1977; Muma et al., 2006; Dautu et al., 2012). CCHFV nucleic acid has been isolated from ticks in Zambia (Kajihara et al., 2021), but no studies have been conducted that investigates CCHFV antibodies in small ruminants. Sheeppox and goatpox virus were not included as no seropositive small ruminants were found in Zambia in a previous study conducted by the authors (Lysholm et al., 2022b). While the collected samples were analysed for peste-des-petits-ruminants virus (PPRV), the results were excluded from the present study.

The study districts were purposively selected based on their location, the density of goat-keeping households, and their national and international trade activity. Four districts with an international border were selected: Chavuma district bordering Angola, Chililalombwe district bordering the Democratic Republic of the Congo (DRC), Siavonga district bordering Zimbabwe, and Vubwi district bordering both Malawi and Mozambique. Three other districts without an international border were also selected: Chibombo, Mazabuka and Monze (Fig. 1).

The study was conducted in two different strata: districts that have an international border and districts that do not. The individual animal was the primary sampling unit. The sample size per stratum was calculated using the Epitools online calculator 'Sample size to estimate a true prevalence with an imperfect test' at www.epitools.ausvet.com.au (Ausvet, Australia), a calculator based on the instructions and formulae in Humphry et al. (2004). The sample size calculation was assuming an infinite population, applying a confidence interval of 95%, a margin of error of 5%, assumed true prevalence of 50%, and the sensitivity and

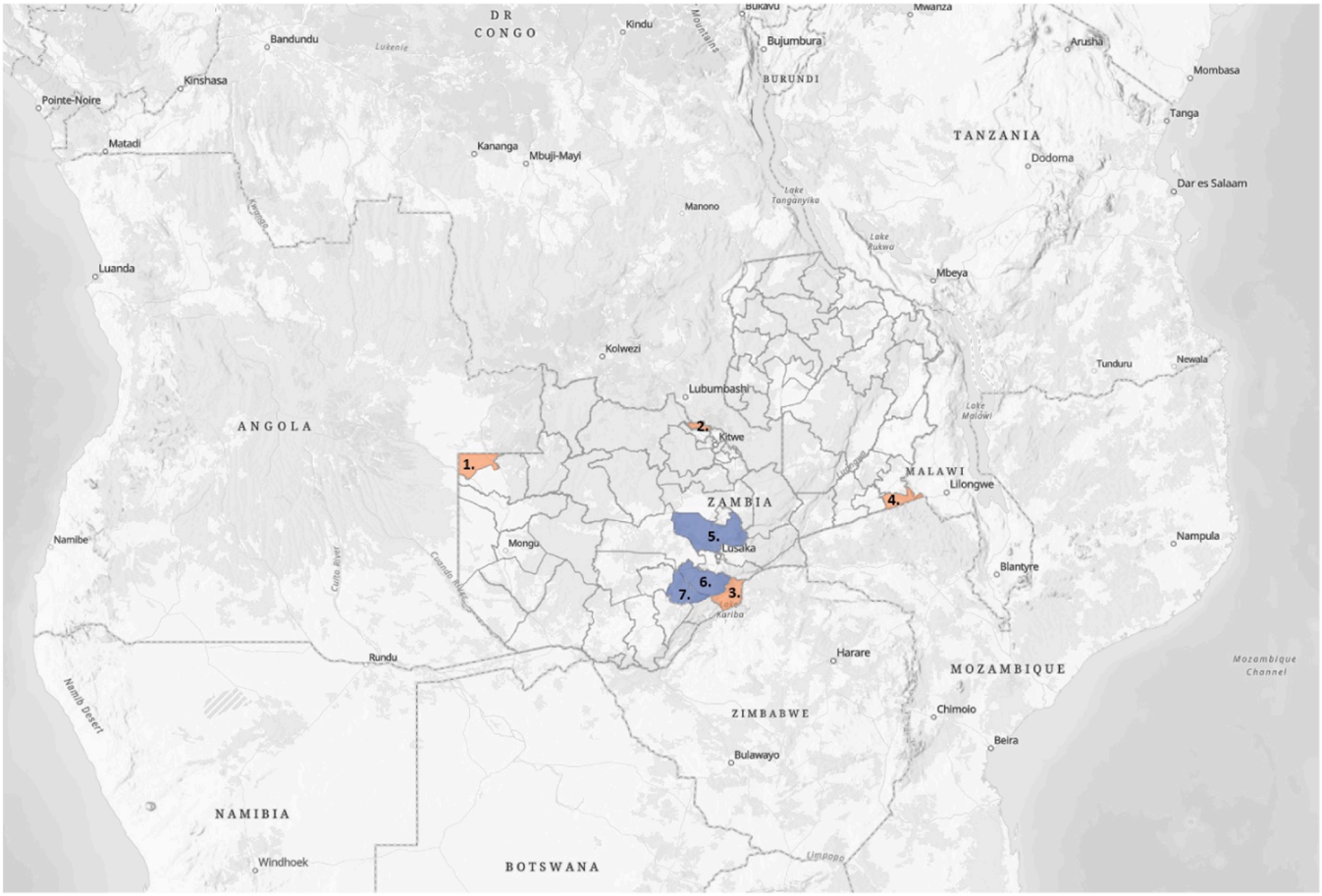

Fig. 1. Map of Zambia showing the locations of the visited districts. 1 =Chavuma, 2 =Chililalombwe, 3 =Siavonga, 4 =Vubwi, 5 =Chibombo, 6 =Mazabuka, 7 =Monze
Source: Esri, USGS | Esri, © OpenStreetMap contributors, HERE, Garmin, FAO, NOAA, USGS.

specificity values of the FMD non-structural protein (NSP) competitive enzyme-linked immunosorbent assay (cELISA) in order to generate the largest required sample size. This gave a sample size of 392, which was subsequently rounded up to 480 to adjust for errors during sample procurement and analysis. As the characteristics of the goat population in Zambia largely is unknown, the sample size calculation was based on a simple random sample and did hence not take clustering in e.g. herds and villages into account. The design effect was not estimated due to the lack of information on pathogen prevalence in Zambia and on the composition of the goat population.

In four of the seven districts, random village selection was performed using village lists provided by the districts' veterinary officials. In Chibombo, Monze and Siavonga districts, complete village lists were too time-consuming to generate and therefore an initial random selection was made of veterinary camps within the districts. Village lists were subsequently compiled from the selected camps and a random selection performed from these lists. In Chibombo, five out of seven veterinary camps were included in the random village selection, in Monze, five out of twenty veterinary camps were included, and in Siavonga, three out of five were included. In each district, ten villages were randomly selected, except in Chililalombwe, which was subdivided into six peri-urban areas, from which five were chosen for sample collection. The lists were subsequently modified in collaboration with local veterinary personnel if selected villages or peri-urban areas were inaccessible or lacked goat-owning households. Three households per village were visited in Chavuma, Siavonga and Vubwi districts, four households per village in Chibombo, Mazabuka and Monze districts, and eight households per peri-urban area were chosen in Chililalombwe district. Household inclusion criteria included being a goat farmer in the selected village and consenting to participate in the study. All the households were selected using snowball sampling methodology (Kendall et al., 2008), and in many instances the selected households were situated in close proximity and shared grazing lands. In each household, four goats were sampled. If the selected household did not keep enough goats to reach the desired sample size, more goats were sampled in the next households. Kids younger than four months of age were excluded to avoid interference by maternal antibodies acquired from colostrum.

2.2. Sample and data collection

The households were visited in September and October 2019. Blood samples were collected from the jugular vein using sterile needles and vacutainer tubes without additives (BD vacutainer, Plymouth, UK). The blood samples were then left standing in a cool box to coagulate and separate. At the end of each day, the serum was separated, transferred to cryotubes and placed in a freezer at − 20 °C. The samples were later transferred to a freezer for long-term storage at − 80 °C. For each sampled animal, information on age, sex, origin and disease signs, both on the day of sample collection and within the last year, was recorded. A questionnaire in English was used to collect information on trade routines, management practices, contact with domestic and wild ruminants and herd disease history. The questionnaire was translated into the local language by an enumerator who asked the questions orally, clarified misunderstandings if necessary and manually recorded the answers in English on the questionnaire sheet. The questionnaire took approximately 20–30 min to complete, contained a mix of open and closed questions and was pre-tested prior to being used in the study.

2.3. Laboratory analysis

Laboratory analysis was performed using commercially available enzyme-linked immunosorbent assays (ELISA) to detect antibodies for the selected transboundary and zoonotic pathogens. The following kits were used: IDEXX CCPP Ab test (no information on sensitivity, specificity 99.6%; Hoofddorp, The Netherlands), *ID Screen FMD NSP competition* (sensitivity 100%, specificity 100%; ID-vet, Grabels, France), ID Screen Rift Valley Fever Competition Multi-species (sensitivity 100%, specificity 100%; ID-vet, Grabels, France), ID Screen CCHF Double Antigen Multi-species (sensitivity 100%, specificity 100%; ID-vet, Grabels, France) and *Svanovir Brucella-Ab C-ELISA* (sensitivity 100%, specificity 100%; Boehringer-Ingelheim Svanova diagnostics, Uppsala, Sweden). The sensitivity and specificity values presented above are figures quoted by the manufacturers. For the *Brucella* spp. and RVFV ELISAs, sensitivity and specificity have been evaluated in independent studies, which estimated the sensitivity and specificity of the *Brucella* spp. ELISA to be 99.4% and 98.9% respectively (Biancifiori et al., 2000), and 91–100% and 100% respectively for RVFV (Kortekaas et al., 2013). In addition, the performance of the CCHFV ELISA on cattle serum samples has been evaluated in an independent study and found to perform well under field conditions in Uganda, with high concordance with results yielded from the immunofluorescence assay (Balinandi et al., 2021). All the kits were utilised, validated and interpreted according to the manufacturers' instructions. For the RVFV c-ELISA, results could be positive, negative or doubtful. Doubtful results were considered negative in the statistical analysis.

2.4. Statistical analysis

True prevalence was calculated using the apparent prevalence as well as the sensitivity and specificity of the statistical test, in accordance with Rogan and Gladen (1978) and using the 'Estimated true prevalence and predictive values from survey testing' at https://epitools.ausvet.com.au (Ausvet, Australia). Adjustment of seroprevalence estimates for clustering within herds and adjustment with sample weights was done on animal-level data. The data was analysed for associations between seroprevalence and potential predictor variables using Stata IC 16/1 (StataCorp LLC, USA). Univariable and multivariable analyses were conducted on both animal-level and herd-level data for the respective pathogens. A herd was considered seropositive for a pathogen if at least one of the sampled animals tested positive for that pathogen. In addition, analyses were performed to find common features in herds where none of the tested animals was seropositive for any of the included pathogens. Predictor variables included in the analyses were age, sex, district, selling frequency, buying frequency, buying from other countries, selling to other countries, presence of community members buying from other countries, contact with domestic ruminants from other herds, contact with cattle from other herds, contact with wild ruminants, herd size, presence of cattle, sheep and pigs in the household, and administration of acaricides and anthelmintic drugs. Univariable analysis was conducted using the Chi2 test or Fisher's exact test where applicable. Also, multilevel mixed-effects logistic regression analysis was performed. In the animal-level analysis, village and herd were included as random variables, and in herd-level analysis, village was included as a random variable. All variables with a p-value of 0.25 or less in the univariable analyses were included in the multivariable analysis, unless multicollinearity was detected. Multicollinearity was tested in all the models using variance inflation factor (VIF), and a cut-off value of ten was used.

The analyses were guided by directed acyclic graphs (Fig. 2), which identified 'district' as a confounding variable which therefore was retained in all models. Also, the frequencies of buying and selling new goats were always included in the initial models, as these were of special interest for the scope of the study. Initially, the full model was run, and the variable with the highest conjoined p-value using the Wald Test was removed in a stepwise backward elimination procedure, which was continued until only significant variables remained. Confounding was controlled for in each step, and a variable was judged to be a confounder when it affected the coefficient of other variables with $> 20\%$. However, no confounder was identified. Selection of the best fitting model was subsequently performed using Akaike information criteria (AIC). Residual plots were also examined visually according to Dohoo et al. (2003). A p-value of < 0.05 was considered statistically significant, but

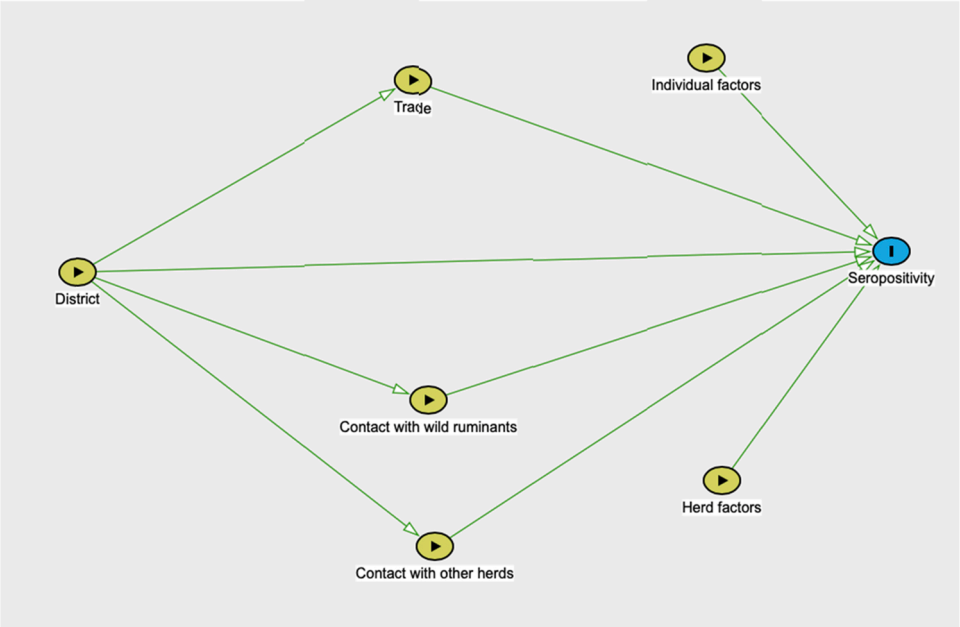

Fig. 2. Directed acyclic graphs illustrating potential variables associated with pathogen seropositivity. Trade includes buying and selling frequency, and buying from and selling to other countries. Contact with other herds refers to contact with small ruminants and cattle from other households. Individual factors include sex and age, and herd factors herd size and presence of cattle and pigs in the household. http://www.dagitty.net.

p-values of < 0.10 are also presented in the results to show potential associations between a variable and seroprevalence. Clustering within herds and villages was estimated by computing the intra-class correlation coefficient (ICC), using the estat icc command.

For all pathogens, tests were performed to establish whether the seroprevalence was higher in districts that have or do not have an international border in univariable logistic regression. However, as the results differed considerably between districts within the same category, we concluded that the seroprevalence was more dependent on the district itself rather than on its proximity to an international border. Therefore, the variable 'district' was initially included in the multivariable analysis, while 'border proximity' was omitted. Later, the analysis was rerun on herd-level data with border proximity as a fixed variable and district and village as random variables, to further explore the association between border proximity and seroprevalence.

3. Results

3.1. Characteristics of study population

In total, serum samples were obtained from 962 goats originating from 280 different households. Of these, 484 goats from 160 households originated from a district that has at least one international border, and 478 animals from 120 households originated from a district that does not have an international border. The sex distribution was 84 % female and 16 % male. All goats were local mixed breeds, occasionally cross-bred with Boer goats. Approximately 15 % of the sampled animals were less than a year old, 68 % were between one and three years old, and 16% were over three years old, while age was not recorded for 1 %. Almost 94 % of the participating farmers used communal grazing grounds for their goats, some for the whole year, but more commonly combined with another grazing strategy such as herding or tethering during periods when crops are in the fields. The remaining herds were herded, allowed to graze on an enclosed field or tethered in one location with food brought to them by the farmer (so-called 'cut and carry') (Table 1). In general, the visited herds either had frequent contact with ruminants from other herds or no contact at all, with 75 % and 57 % in contact with small ruminants or cattle respectively from other herds on at least a monthly basis. Herd size ranged from two to 185 goats, with a median of 15 goats. None of the visited farmers vaccinated their goats for any disease, and only 45 % and 47 % respectively regularly dewormed or treated their goats with acaricides, ranging in frequency from

Table 1
Description of the goats and goat herds sampled as part of the study in Zambia in 2019.

		n	Proportion of total (%)
Individual			
Total		962	100
Sex	Female	806	83.8
	Male	151	15.7
	Unknown	5	0.5
Age	< 1 year	142	14.8
	1–3 years	651	67.7
	> 3 years	158	16.4
	Unknown	11	1.1
Location	Border districts	484	50.3
	Non-border districts	478	49.7
Herd			
Total		280	100
Grazing strategy	Communal grazing whole year	79	28.2
	Communal grazing combined with e. g. herding and tethering	184	65.7
	Grazing on fenced grazing land	3	1.1
	Herding whole year	12	4.3
	Tethering whole year	2	0.7
Selling frequency	At least twice a year	105	37.5
	At least once every two years	107	38.2
	More rarely or never	58	20.7
	Unknown	10	3.6
Buying frequency	At least twice a year	43	15.4
	At least once every two years	51	18.2
	More rarely or never	182	65.0
	Unknown	4	1.4
Location	Border districts	160	57.1
	Non-border districts	120	42.9

weekly to yearly.

Approximately 76% of the visited households sold goats regularly, ranging in frequency from monthly to once every two years (Table 1). The farmers most often sold to traders, followed by farmers and villagers buying for home consumption. Some farmers also sold their animals at markets, to restaurants or to slaughterhouses. About 34% bought goats when need arose, ranging in frequency from monthly to once every two years. The vast majority bought from other farmers in their village, followed by farmers in nearby villages. Only four farmers occasionally bought goats from traders. Selling goats to other countries was relatively common, especially in the border districts where 22% of the farmers had sold to buyers from neighbouring countries at least once. However, only 2.5% had bought goats from other countries or had neighbours who had, and all of these respondents lived in a border district and bought from the neighbouring country, except for one household in Chibombo district that bought from the DRC.

The majority of the sampled animals (83%) showed no overt clinical signs at the time of the visit, and 73% had also not been clinically ill in the last year, according to their owners. However, 62% of the farmers had experienced coughing in their herd in the past year, 60% diarrhoea, 45% ocular and/or nasal discharge, 45% abortions and 44% had problems with kid mortalities.

3.2. Seroprevalence

Apparent animal-level seroprevalence adjusted for herd-level clustering was 8.2% (95% CI 7.5–9.0) for Mccp, 12.9% (95% CI 12.0–13.7) for FMDV, 13.0% (95% CI 12.1–13.9) for *Brucella* spp., 3.3% (95% CI 2.8–3.7) for CCHFV, and 0.4% (95% CI 0.3–0.7) for RVFV. Both unadjusted and adjusted animal-level seroprevalence estimates are presented in Tables 2 and 3. Apparent herd-level seroprevalence was 17.1% (95% CI 12.9–22.1) for Mccp, 22.1% (95% CI 17.4–27.5) for FMDV, 18.2% (95% CI 13.9–23.2) for *Brucella* spp., 8.9% (95% CI 5.9–12.9) for CCHFV and 0.7% (95% CI 0.1–2.6) for RVFV (Tables 4 and 5). True animal-level seroprevalence for *Brucella* spp. and RVFV was 8.0% (95% CI 6.33–10.0) and 0.2% (0.06–0.83%) respectively, if the sensitivity and specificity values from independent studies were used (Biancifiori et al., 2000; Kortekaas et al., 2013). For Mccp, true seroprevalence could not be calculated as no information was available on the sensitivity of the diagnostic test. For FMDV and CCHFV, the calculated true prevalence and apparent prevalence were the same as both manufacturers report 100% sensitivity and 100% specificity of the respective ELISAs.

Antibodies to Mccp, FMDV and *Brucella* spp. were found in all the districts visited, while antibodies to CCHFV were detected in all districts except Chililalombwe. Antibodies to RVFV were only present in one three-year old female goat in Vubwi district, and one four-year old male goat in Chililalombwe district whose origin was unknown as it had been purchased at a nearby small livestock market. Furthermore, approximately 70% of the goats and 50% of the herds were not seropositive for any of the included pathogens, and of the animals that were seropositive, only 3.5% were seropositive for two pathogens, and 0.3% for three pathogens simultaneously. Goats that were seropositive for Mccp were more likely to also be seropositive for CCHFV ($p < 0.01$, OR 4.2). Otherwise, no associations of seropositivity of different pathogens were found.

3.3. Predictor variable analysis

Logistic regression was performed to detect associations between pathogen seroprevalence and border proximity (Tables 6 and 7). Multilevel mixed-effects logistic regression analysis was performed for all pathogens except RVFV since only two animals were seropositive for this pathogen. Predictor variables associated with herd-level seropositivity identified in the multivariable analyses are shown in Table 8 for Mccp, Table 9 for FMDV, Table 10 for *Brucella* spp. and Table 11 for CCHFV. Predictor variables associated with herd-level seronegativity for

Table 2
Apparent animal-level seroprevalence for Mccp, FMDV and *Brucella* spp. detected in the study, unadjusted as well as adjusted for herd-level clustering.

		Mccp			**FMDV**			***Brucella* spp.**		
		Positive (analysed)	Unadjusted seroprevalence % (95% CI)	Adjusted seroprevalence % (95% CI)	Positive (analysed)	Unadjusted seroprevalence % (95% CI)	Adjusted seroprevalence % (95% CI)	Positive (analysed)	Unadjusted seroprevalence % (95% CI)	Adjusted seroprevalence % (95% CI)
Total		97 (962)	10.1 (8.25–12.2)	8.21 (7.50–8.97)	105 (962)	10.9 (9.01–13.1)	12.9 (12.0–13.7)	86 (959)	8.97 (7.24–11.0)	13.0 (12.1–13.9)
Sex	Female	88 (806) 9	10.9 (8.85–13.3)	9.27 (8.43–10.2)	88 (806)	10.9 (8.85–13.3)	13.6 (12.6–14.6)	77 (804)	9.58 (7.63–11.8)	13.8 (12.8–14.9)
	Male	(151)	5.96 (2.76–11.0)	3.88 (2.73–5.23)	16 (151)	10.6 (6.18–16.6)	8.86 (7.19–10.8)	8 (150)	5.33 (2.33–10.2)	8.16 (6.53–10.1)
Age	< 1 year	6 (142)	4.23 (1.57–8.97)	1.48 (0.84–2.56)	8 (142)	5.63 (2.46–10.8)	4.41 (3.15–14.6)	5 (142)	3.52 (1.15–8.03)	6.36 (4.86–8.14)
	1–3 years	72 (651)	11.1 (8.75–13.7)	9.33 (8.42–10.3)	82 (651)	12.6 (10.1–15.4)	13.4 (12.4–14.6)	69 (648)	10.7 (8.38–13.3)	14.5 (13.4–15.6)
	> 3 years	17 (158)	10.8 (6.39–16.7)	10.4 (8.25–12.7)	15 (158)	9.49 (5.41–15.2)	21.4 (18.6–24.6)	9 (158)	5.70 (2.64–10.5)	9.96 (7.89–12.3)
Border proximity	Yes	15 (484)	3.10 (1.74–5.06)	2.96 (2.38–3.68)	20 (484)	4.13 (2.54–6.31)	4.35 (3.63–5.19)	59 (483)	12.2 (9.43–15.5)	20.5 (19.0–22.0)
	No	82 (478)	17.2 (13.9–20.8)	13.4 (12.2–14.7)	85 (478)	17.8 (14.5–21.5)	21.3 (19.8–22.9)	27 (476)	5.67 (3.77–8.15)	5.61 (4.79–6.54)
District	Chavuma	3 (122)	2.46 (0.51–7.02	2.20 (1.31–3.45)	5 (122)	4.10 (1.34–9.31)	3.28 (2.18–4.76)	5 (121)	4.13 (1.36–9.38)	18.2 (15.7–21.2)
	Chibombo	21 (160)	13.1 (8.31–19.4)	8.30 (6.32–10.6)	5 (160)	3.12 (1.02–7.14)	3.63 (2.41–5.41)	3 (160)	1.88 (0.39–5.38)	1.45 (0.71–2.70)
	Chililalombwe	1 (122)	0.82 (0.02–4.48)	0.40 (0.08–1.10)	4 (122)	3.28 (0.90–8.18)	1.22 (0.61–2.30)	2 (122)	1.64 (0.20–5.80)	5.25 (3.83–7.08)
	Mazabuka	57 (160)	35.6 (28.2–43.6)	30.9 (28.0–34.1)	29 (160)	18.1 (12.5–25.0)	22.0 (19.4–24.9)	22 (160)	13.8 (8.82–20.1)	14.9 (12.6–17.3)
	Monze	4 (158)	2.53 (0.69–6.35)	3.22 (2.32–4.41)	51 (158)	32.3 (25.1–40.2)	30.7 (28.2–33.5)	2 (156)	1.28 (0.16–4.55)	0.99 (0.52–1.76)
	Siavonga	2 (120)	1.67 (0.20–5.89)	0.60 (0.21–1.49)	6 (120)	5.00 (1.86–10.6)	7.46 (5.71–9.53)	50 (120)	41.7 (32.7–51.0)	47.2 (43.6–50.7)
	Vubwi	9 (120)	7.50 (3.49–13.8)	15.3 (11.9–19.6)	5 (120)	4.17 (1.37–9.46)	6.98 (4.51–10.0)	2 (120)	1.67 (0.20–5.89)	1.19 (0.30–2.80)

Table 3
Apparent animal-level seroprevalence for CCHFV and RVFV detected in the study, unadjusted as well as adjusted for herd-level clustering.

		CCHFV			RVFV		
		Positive (analysed)	Unadjusted seroprevalence % (95% CI)	Adjusted seroprevalence % (95% CI)	Positive (analysed)	Unadjusted seroprevalence % (95% CI)	Adjusted seroprevalence % (95% CI)
Total		33 (962)	3.43 (2.37–4.78)	3.26 (2.79–3.72)	2 (957)	0.21 (0.02–0.75)	0.43 (0.26–0.65)
Sex	Female	28 (806)	3.47 (2.32–4.98)	3.24 (2.74–3.80)	1 (803)	0.12 (0.00–0.69)	0.10 (0.04–0.26)
	Male	5 (151)	3.31 (1.08–7.56)	3.54 (2.47–4.88)	1 (149)	0.67 (0.02–3.68)	1.93 (0.16–3.00)
Age	< 1 year	2 (142)	1.41 (0.17–5.00)	1.17 (0.60–2.15)	0 (140)	0 (0–2.60)†	0 (0–0.41)†
	1–3 years	24 (651)	3.69 (2.38–5.44)	3.47 (2.92–4.11)	1 (648)	0.15 (0.00–0.86)	0.13 (0.04–0.31)
	> 3 years	7 (158)	4.43 (1.80–8.92)	5.01 (3.59–6.84)	1 (158)	0.63 (0.01–3.48)	2.52 (1.52–3.90)
Border proximity	Yes	8 (484)	1.65 (0.72–3.23)	1.72 (1.26–2.26)	2 (479)	0.42 (0.05–1.50)	0.86 (0.56–1.30)
	No	25 (478)	5.23 (3.41–7.62)	4.79 (4.03–5.65)	0 (478)	0 (0–0.77)†	0 (0–0.13)†
District	Chavuma	1 (122)	0.82 (0.02–4.48)	0.16 (0.00–0.68)	0 (122)	0 (0–2.98)†	0 (0–0.45)†
	Chibombo	1 (160)	0.62 (0.02–3.43)	0.74 (0.24–1.72)	0 (160)	0 (0–2.28)†	0 (0–0.54)†
	Chililalombwe	0 (122)	0 (0–2.98)†	0 (0–0.46)†	1 (117)	0.85 (0.02–4.67)	2.43 (1.47–3.77)
	Mazabuka	19 (160)	11.9 (7.30–17.9)	11.3 (9.37–13.6)	0 (160)	0 (0–0.28)†	0 (0–0.41)†
	Monze	5 (158)	3.16 (1.04–7.23)	2.20 (1.48–3.25)	0 (158)	0 (0–2.31)†	0 (0–0.31)†
	Siavonga	5 (120)	4.17 (1.37–9.46)	4.93 (3.48–6.64)	0 (120)	0 (0–3.03)†	0 (0–0.47)†
	Vubwi	2 (120)	1.67 (0.20–5.89)	2.11 (0.96–4.30)	1 (120)	0.83 (0.02–4.56)	1.29 (0.44–3.18)

† One-sided confidence interval (97.5%)

Table 4
Apparent herd-level seroprevalence for Mccp, FMDV and *Brucella* spp. detected in the study.

		Mccp		FMD		*Brucella* spp.	
		Positive (analysed)	% seroprevalence (95% CI)	Positive (analysed)	% seroprevalence (95% CI)	Positive (analysed)	% seroprevalence (95% CI)
Total		48 (280)	17. 1 (12.9–22.1)	62 (280)	22.1 (17.4–27.5)	51 (280)	18.2 (13.9 – 23.2)
District	Chavuma	3 (40)	7.50 (1.57–20.4)	5 (40)	12.5 (4.19–26.8)	3 (40)	7.50 (1.57 – 20.4)
	Chibombo	11 (40)	27.5 (14.6–43.9)	5 (40)	12.5 (4.19–26.8)	3 (40)	7.50 (1.57 – 20.4)
	Chililalombwe	1 (40)	2.50 (0.06–13.2)	3 (40)	7.50 (1.57–20.4)	2 (40)	5.00 (0.61 – 16.9)
	Mazabuka	21 (40)	52.5 (36.1–68.5)	14 (40)	35.0 (20.6–51.7)	10 (40)	25.0 (12.7 – 41.1)
	Monze	3 (40)	7.50 (1.57–20.4)	24 (40)	60.0 (43.3–75.1)	2 (40)	5.00 (0.61 – 16.9)
	Siavonga	2 (40)	5.00 (0.61–16.9)	6 (40)	15.0 (5.71–29.8)	29 (40)	72.5 (56.1 – 85.4)
	Vubwi	7 (40)	17.5 (7.34–32.8)	5 (40)	12.5 (4.19–26.8)	2 (40)	5.00 (0.61 – 16.9)

† One-sided confidence interval (97.5%)

Table 5
Apparent herd-level seroprevalence for CCHFV and RVFV detected in the study.

		CCHF		RVFV	
		Positive (analysed)	% seroprevalence (95% CI)	Positive (analysed)	% seroprevalence (95% CI)
Total		25 (280)	8.93 (5.86–12.9)	2 (280)	0.71 (0.09–2.56)
District	Chavuma	1 (40)	2.50 (0.06–13.2)	0 (40)	0 (0–8.81)†
	Chibombo	1 (40)	2.50 (0.06–13.2)	0 (40)	0 (0–8.81)†
	Chililalombwe	0 (40)	0 (0–8.81)†	1 (40)	2.50 (0.06–13.2)
	Mazabuka	14 (40)	35.0 (20.6–51.7)	0 (40)	0 (0 −8.81)†
	Monze	4 (40)	10.0 (2.79–23.7)	0 (40)	0 (0–8.81)†
	Siavonga	4 (40)	10.0 (2.79–23.7)	0 (40)	0 (0–8.81)†
	Vubwi	2 (40)	5.00 (0.61–16.9)	1 (40)	2.50 (0.06–13.2)

† One-sided confidence interval (97.5%)

Table 6
Association between district location and herd-level seropositivity for Mccp, FMDV and CCHFV in Zambia, using logistic regression analysis.

	Mccp		FMDV		CCHFV	
	OR % (95% confidence interval)	p-value	OR % (95% confidence interval)	p-value	OR % (95% confidence interval)	p-value
Border districts	*Baseline*	*Baseline*	*Baseline*	*Baseline*	*Baseline*	*Baseline*
Inland districts	4.66 (2.33–9.29)	< 0.001	4.14 (2.26–7.61)	< 0.001	4.83 (1.86–12.5)	0.001

all surveyed pathogens are shown in Table 12. Animal-level predictor variables identified in multivariable analyses are displayed in Supplementary Tables 1–4. Unless otherwise specified, the results presented here are analyses made of herd-level data.

In univariable analyses, herd-level seroprevalence of Mccp (OR 4.7, 95% CI 2.3–9.3, $p < 0.001$), FMDV (OR 4.1, 95% CI 2.3–7.6, $p < 0.001$) and CCHFV (OR 4.8, 95% CI 1.9–12.5, $p = 0.001$) was significantly higher in districts that did not have an international border. For RVFV, the two seropositive goats originated from Chililalombwe and Vubwi, i. e. two districts that have an international border, but the herd-level seroprevalence was not significantly different in districts with or without an international border ($p = 0.508$). For *Brucella* spp. on the

Table 7
Association between district location and herd-level seropositivity for *Brucella spp.*, and herd-level seronegativity for all included pathogens, using logistic regression analysis.

	***Brucella* spp.**		**Seronegative**	
	OR % (95% confidence interval)	p-value	OR % (95% confidence interval)	p-value
Border districts	2.03 (1.05–3.92)	0.034	3.13 (1.91–5.13)	< 0.001
Inland districts	*Baseline*	*Baseline*	*Baseline*	*Baseline*

Table 8
Predictor variables associated with herd-level seropositivity for Mccp in multilevel mixed effects logistic regression. p-values < 0.05 are in bold. n = 266.

Fixed herd-level variables		**Mccp**		
		Odds ratio (OR)	OR 95% confidence interval	p-value
District	Chavuma	2.87	0.03–277	0.652
	Chibombo	75.2	0.75–7576	0.066
	Chililalombwe	*Baseline*	*Baseline*	*Baseline*
	Mazabuka	987	7.45–130794	**0.006**
	Monze	12.4	0.12–1229	0.283
	Siavonga	2.60	0.03–260	0.684
	Vubwi	36.8	0.40–3379	0.118
Selling frequency	At least twice a year	*Baseline*	*Baseline*	*Baseline*
	At least once every two years	0.45	0.11–1.89	0.276
	More rarely or never	1.20	0.20–7.05	0.841
Buying frequency	At least twice a year	*Baseline*	*Baseline*	*Baseline*
	At least once every two years	1.03	0.15–7.10	0.979
	More rarely or never	3.03	0.53–17.5	0.215
Constant		< 0.01	0.00–0.06	0.002
Random effects parameters		Estimate	Std.Err.	95% confidence interval
	Village	7.89	4.24	2.75–22.6

other hand, herd-level seropositivity was significantly higher in districts that have an international border (OR 2.0, 95% CI 1.1–3.9, p = 0.034). Herds that were seronegative for the surveyed pathogens were significantly more common in districts with international borders (OR 3.1, 95% CI 1.9–5.1, p < 0.001).

In the multivariable analyses, herd-level seroprevalence varied considerably between districts. For OR, 95% CI and p-values, please see Tables 8–12. In the analysis, herd-level seroprevalence was significantly higher in Mazabuka district for Mccp, *Brucella* spp. and CCHFV, and in Monze district for FMDV, both of which are inland districts. Furthermore, herd-level seroprevalences of Mccp and FMDV were higher in the inland districts Chibombo and Mazabuka, respectively, but these findings were not statistically significant. In addition to the findings above, a significant association was found between seropositivity for Mccp and the inland district Chibombo in animal-level data. Only one significant association with a border district was found, namely Siavonga district and seropositivity for *Brucella* spp. Also, potential associations were observed between animal-level seropositivity for Mccp and FMDV and the border district Vubwi, but these findings were not statistically significant. Furthermore, herds that were seronegative for all surveyed pathogens were significantly more common in Chavuma, Chibombo, Chililalombwe and Vubwi district, all of which except Chibombo have at least one international border. In the multivariable analysis, where district was included as a random variable (data not presented here),

Table 9
Predictor variables associated with herd-level seropositivity for FMDV in multilevel mixed effects logistic regression. p-values < 0.05 are in bold. n = 255.

Fixed herd-level variables		**FMDV**		
		Odds ratio (OR)	OR 95% confidence interval	p-value
District	Chavuma	2.68	0.41–17.7	0.306
	Chibombo	1.53	0.21–11.1	0.672
	Chililalombwe	*Baseline*	*Baseline*	*Baseline*
	Mazabuka	4.93	0.81–30.0	0.083
	Monze	22.7	3.62–143	**0.001**
	Siavonga	1.67	0.27–10.3	0.582
	Vubwi	4.78	0.65–35.0	0.124
Selling frequency	At least twice a year	*Baseline*	*Baseline*	*Baseline*
	At least once every two years	0.52	0.20–1.32	0.168
	More rarely or never	1.49	0.53–4.24	0.452
Herd size	1–10	*Baseline*	*Baseline*	*Baseline*
	11–20	2.75	0.86–8.81	0.088
	21 or more	3.34	1.01–11.1	**0.049**
Keeping pigs in household	No	2.46	0.88–6.94	0.088
	Yes	*Baseline*	*Baseline*	*Baseline*
Constant		0.01	0.00–0.07	< 0.001
Random effects parameters		Estimate	Std.Err.	95% confidence interval
	Village	0.58	0.51	0.10–3.26

Table 10
Predictor variables associated with herd-level seropositivity for *Brucella* spp. in multilevel mixed effects logistic regression. p-values < 0.05 are in bold. n = 255.

Fixed herd-level variables		***Brucella* spp.**		
		Odds ratio (OR)	OR 95% confidence interval	p-value
District	Chavuma	3.34	0.36–30.8	0.287
	Chibombo	1.78	0.24–13.3	0.579
	Chililalombwe	2.92	0.28–30.0	0.368
	Mazabuka	9.55	1.39–65.4	**0.022**
	Monze	*Baseline*	*Baseline*	*Baseline*
	Siavonga	110	13.9–865	**< 0.001**
	Vubwi	1.32	0.08–22.0	0.847
Selling frequency	At least twice a year	4.13	1.07–16.0	**0.040**
	At least once every two years	1.67	0.42–6.66	0.466
	More rarely or never	*Baseline*	*Baseline*	*Baseline*
Herd size	1–10	*Baseline*	*Baseline*	*Baseline*
	11–20	0.69	0.19–2.57	0.579
	21 or more	2.13	0.64–7.12	0.217
Keeping pigs in household	No	2.62	0.75–9.16	0.133
	Yes	*Baseline*	*Baseline*	*Baseline*
Constant		< 0.01	0.00–0.05	< 0.001
Random effects parameters		Estimate	Std.Err.	95% confidence interval
	Village	0.31	0.68	0.00–23.1

border proximity was in general not significantly associated with seroprevalence. The only exception was Mccp, where seroprevalence was significantly higher in inland districts compared to districts with one or more international borders (OR 23.6, 95% CI 1.5–379.2, p = 0.026).

Trade was generally not associated with seroprevalence in this study. Selling goats twice a year or more was positively associated with herd-

Table 11
Predictor variables associated with herd-level seropositivity for CCHFV in multilevel mixed effects logistic regression. p-values < 0.05 are in bold. n = 237.

Fixed herd-level variables		CCHFV		
		Odds ratio (OR)	OR 95% confidence interval	p-value
District	Chavuma	2.72	0.07–95.8	0.583
	Chibombo	*Baseline*	*Baseline*	*Baseline*
	Mazabuka	46.1	2.58–823	**0.009**
	Monze	3.58	0.18–72.4	0.405
	Siavonga	4.89	0.25–97.1	0.298
	Vubwi	2.50	0.11–56.3	0.565
Keeping cattle in household	No	*Baseline*	*Baseline*	*Baseline*
	Yes	8.96	0.76–106	0.082
Constant		< 0.01	0.00–0.05	< 0.001
Random effects parameters		Estimate	Std.Err.	95% confidence interval
	Village	2.34	1.69	0.57–9.66

level seropositivity for *Brucella* spp. (OR 4.1, 95% CI 1.1–16.0, p = 0.040) compared to households that never sold goats. Also, herds that were seronegative for all surveyed pathogens were more common in the group that sold goats regularly, but rarely, in this case at least once every two years (OR 2.6, 0.8–8.3, p = 0.098), compared to households that never sold goats. However, this finding was not statistically significant. No significant associations were found between seroprevalence and buying frequency.

Furthermore, herds with 21 goats or more were more likely to be seropositive for FMDV (OR 3.3, 95% CI 1.0–11.1, p = 0.049) on herd-level data, compared to herds consisting of ten goats or less. A similar tendency was observed for *Brucella* spp. (OR 3.5, 95% CI 1.0–12.3, p = 0.054) on animal-level data, although the association was non-significant. Furthermore, associations were found between seropositivity for CCHFV and keeping pigs on animal-level data (OR 2.7, 95% CI 1.0–7.1, p = 0.044) and cattle on herd-level data (OR 9.0, 95% CI 0.8–106, p = 0.082), although for cattle, the association was non-significant. For FMDV (OR 3.8, 95% CI 1.4–10.9, p = 0.011) and *Brucella* spp. (OR 4.5, 95% CI 1.2–17.3, p = 0.031) on the other hand, households without pigs were more likely to be seropositive on the animal-level data. In addition, goats aged three years or above were more likely to be seropositive for *Brucella* spp. (OR 7.7, 95% CI 1.5–40.7, p = 0.016) compared to younger animals. A similar trend was observed for FMDV (OR 2.95, 95% CI 0.8–10.5, p = 0.095), although this association was not significant. Lastly, households that administered anthelmintic drugs on a yearly basis were significantly more likely to be seronegative for the surveyed pathogens (OR 5.0, 95% CI 1.1–23.6, p = 0.044), compared to households that dewormed more frequently.

The study found some associations between seropositivity and certain clinical signs. Goats seropositive for Mccp were significantly more likely to have had nasal and ocular discharge in the past year (OR 21.0, 95% CI 6.2–71.3, p < 0.01), while animals seropositive for FMDV were significantly more likely to originate from herds that had reported a problem with kid mortalities during the past year (OR 1.7, 95% CI 1.1–2.5, p = 0.015). Herds seropositive for *Brucella* spp. were significantly more likely to have experienced abortions during the past year (OR 5.6, 95% CI 2.7–11.6, p < 0.01). Lastly, goats that were seropositive for CCHFV were significantly more likely to be suffering from mange at the time of sampling (OR 3.7, 95% CI 1.2–11.3, p = 0.019).

Clustering within villages and herds was estimated by computing ICC, and the results revealed considerable clustering within villages for most, and within herds for all, pathogens. Highest level of clustering was observed for Mccp, where village-level and herd-level ICC was 0.55 and 0.66, respectively. For CCHFV, computed village and herd-level ICC was both 0.30. For FMDV and *Brucella* spp., the ICC's revealed considerable clustering within herds (0.35 and 0.39 respectively), while between-village heterogeneity was low (0.13 and <0.01).

Table 12
Predictor variables associated with herd seronegativity for Mccp, FMDV, Brucella spp., CCHFV and RVFV in multilevel mixed effects logistic regression. p-values < 0.05 are in bold. n = 245.

Fixed herd-level variables		Odds ratio (OR)	OR 95% confidence interval	p-value
District	Chavuma	39.2	3.69–416	**0.002**
	Chibombo	17.3	2.03–148	**0.009**
	Chililalombwe	127	9.24–1745	**< 0.001**
	Mazabuka	*Baseline*	*Baseline*	*Baseline*
	Monze	1.97	0.24–16.0	0.527
	Siavonga	2.56	0.28–23.3	0.404
	Vubwi	20.5	1.99–211	**0.011**
Selling frequency	At least twice a year	1.56	0.48–5.10	0.463
	At least once every two years	2.64	0.84–8.33	0.098
	More rarely or never	*Baseline*	*Baseline*	*Baseline*
Buying frequency	At least twice a year	1.53	0.45–5.20	0.493
	At least once every two years	0.67	0.24–1.82	0.429
	More rarely or never	*Baseline*	*Baseline*	*Baseline*
Presence of community members buying small ruminants from other countries	No	*Baseline*	*Baseline*	*Baseline*
	Yes	5.01	0.31–81.5	0.258
Herd size	1–10	1.32	0.45–3.91	0.615
	11–20	1.54	0.54–4.37	0.416
	21 or more	*Baseline*	*Baseline*	*Baseline*
Frequency of administration of anthelmintic treatments	At least once every three months	*Baseline*	*Baseline*	*Baseline*
	At least once a year	4.98	1.05–23.6	**0.044**
	More rarely or never	3.13	0.77–12.8	0.112
Contact frequency with cattle from other herds	At least once a month	*Baseline*	*Baseline*	*Baseline*
	More rarely or never	1.39	0.45–4.28	0.567
Keeping sheep in household	No	*Baseline*	*Baseline*	*Baseline*
	Yes	1.49	0.43–5.18	0.534
Constant		0.01	0.00–0.14	0.001
Random effects parameters		Estimate	Std.Err.	95% confidence interval
	Village	2.06	1.12	0.71–5.98

4. Discussion

This study investigated the animal-level and herd-level seroprevalence of Mccp, FMDV, *Brucella* spp., CCHFV and RVFV in goats in seven districts in Zambia. To the best of our knowledge, this is the first study to describe the presence of antibodies to Mccp and CCHFV in goats in Zambia. Furthermore, the serological results were used to explore the associations between seroprevalence and different predictor variables, such as trade and border proximity.

Mccp is a highly contagious pathogen that is known to be present in Tanzania (Kusiluka et al., 2000a, 2000b; Kgotlele et al., 2019; Chota et al., 2020), but has yet to be detected in Zambia (Karimuribo et al., 2014). In this study, the detected animal-level seroprevalence adjusted for herd-level clustering was 8.2%, while herd-level seroprevalence was estimated to 17.1%. These values are comparable with, or lower than, results from neighbouring Tanzania, where animal- and herd-level

seroprevalences range from 3.3% to 52.1% and 9.6–45.7% respectively (Swai et al., 2013; Mbyuzi et al., 2014; Torsson et al., 2017; Chota et al., 2019). None of the farmers who participated in the study reported vaccinating their goats, and according to the district veterinary personnel who assisted during the sampling procurement, no vaccination campaigns for goats have ever been conducted in the areas visited. It is therefore unlikely that the seroprevalence detected is a result of previous vaccinations. Seropositive goats for Mccp were found in all the districts surveyed and in all age groups, which may indicate active circulation of Mccp in goats in Zambia. However, this should be verified in future studies.

FMDV is endemic in Zambia and numerous outbreaks in cattle have been described (Perry and Hedger, 1984; Banda et al., 2014; Hamoonga et al., 2014; Sinkala et al., 2014a, 2014b). In this study, the detected adjusted animal-level seroprevalence was 12.9 %, and herd-level seroprevalence was 22.1 %. These results are higher than findings in a study conducted in 2018 in Zambia, where animal- and herd-level seroprevalence in sheep and goats close to the border to Tanzania was estimated to 1.03 % and 3.14 %, respectively (Lysholm et al., 2022b). The seroprevalence in this study can also be compared with findings in neighbouring Botswana, Tanzania and Zimbabwe, where animal-level seroprevalence of 9.3 % (Hyera et al., 2006), 39.4% (Torsson et al., 2017) and 1.5 % (Bhebhe et al., 2016), respectively, were found. In this study, seropositive goats were found in all study districts and age groups, which indicates widespread exposure to FMDV in goats in Zambia.

Like FMDV, *Brucella* spp. are considered endemic in Zambia (Bell et al., 1977; Muma et al., 2006) and seropositive cattle have been detected in various districts across the country, including Chibombo, Mazabuka and Monze which were targeted in the present study (Muma et al., 2006, 2007a, 2007b, 2012, 2013; Chimana et al., 2010; Mfune et al., 2021). In this study, seropositive goats were found in all the districts visited, and the adjusted animal-level seroprevalence of *Brucella* spp. was 13.0 %, while herd-level seroprevalence was estimated to 18.2 %. This result is higher compared to previous studies, where no seropositive goats were found around two national parks on the Kafue flats (Muma et al., 2006), close to Monze and Mazabuka district that were included also in this study. Also, the detected seroprevalence is higher than what was found in small ruminants in Zambia close to the Tanzanian border (Lysholm et al., 2022b), while it is similar to the proportion of seropositivity in small ruminants at two small livestock markets in Zambia (Lysholm et al., 2022a). The result is also similar to, or higher than, results from studies in Tanzania, where animal-level seroprevalence ranges from 0 % to 20 % (Mellau et al., 2009; Assenga et al., 2015; Shirima and Kunda, 2016; Ntirandekura et al., 2021).

For CCHFV, the adjusted animal-level seroprevalence was 3.3 %, and herd-level seroprevalence 8.9 %. This result is in line with a recent study on cattle in Zambia, where detected seroprevalence was 8.4 % (Kajihara et al., 2021). Furthermore, our results are similar to two studies that analysed the seroprevalence of CCHFV in goats in Senegal and the Democratic Republic of Congo (DRC), where animal-level seroprevalence was 6.9% (Mangombi et al., 2020) and 5.9 % respectively (Sas et al., 2017). In this study, seropositive goats were detected in all districts except Chililalombwe, which borders the DRC. However, on the Congolese side of the border in Lubumbashi, goats seropositive for CCHFV have been found, indicating a possible circulation of the virus in the area (Sas et al., 2017). Despite their relative proximity, the absence of seropositive goats in Chililalombwe district may be due to the trade of small ruminants primarily being uni-directional from Zambia to the DRC (Lysholm et al., 2020).

The adjusted animal-level seroprevalence of RVFV in this study was 0.4 %, while herd-level seroprevalence was 0.7%. RVFV is considered endemic in Zambia, and the results of this study were lower than those found in previous studies, where animal-level seroprevalence in cattle, sheep and goats has been 2.26 % or higher (Hussein et al., 1985; Davies et al., 1992; Samui et al., 1997; Saasa et al., 2018). In a recent study in small ruminants in Zambia close to the Tanzanian border, animal- and herd-level seroprevalence was 2.26 % and 5.62 %, respectively (Lysholm et al., 2022b). The latter study was conducted in a region in Zambia that receives more rainfall (Makondo and Thomas, 2020), which could lead to more favourable conditions for the mosquito vectors. The result is however similar to the 0.84% proportion of seropositivity detected in sheep and goats at two small livestock markets in Zambia, where the sampled animals primarily originated from southern and central parts of the country (Lysholm et al., 2022a). While we did not find any seropositive goats in Mazabuka district, a recent study detected a 13.5% seroprevalence in cattle serum samples collected in 2014 in the same district (Saasa et al., 2018). Although the age of the tested cattle was not included in that paper, one possible explanation for the difference in seroprevalence is that cattle often are kept longer and allowed to reach a higher age compared to small ruminants. Our results were also considerably lower than those of studies on small ruminants in Tanzania, where animal-level seroprevalence ranges from 5.4 % to 12 % (Sumaye et al., 2013; Kifaro et al., 2014; Wensman et al., 2015), and Mozambique, where animal-level seroprevalence ranges from 10 % to 35 % (Fafetine et al., 2013; Blomström et al., 2016; Moiane et al., 2017). The low seroprevalence in this study is in line with the absence of epizootic RVFV outbreaks in the last three decades (Dautu et al., 2012). The detected results can thus either be due to false positive laboratory results or low interepizootic viral circulation. More research is needed to elucidate whether RVFV is currently circulating in the country in the absence of epizootic outbreaks, something that has previously been found for example in Tanzania (Sumaye et al., 2013; Wensman et al., 2015), Kenya (Mbotha et al., 2018) and Zambia itself (Davies et al., 1992; Saasa et al., 2018).

The association between border proximity and pathogen seroprevalence was investigated in both univariable and multivariable analyses. For one pathogen, namely *Brucella* spp., seroprevalence was significantly higher in districts with an international border. However, this was largely due to the high seroprevalence in Siavonga district, where animal- and herd-level seroprevalence was 41.7% and 72.5% respectively. If Siavonga district was excluded, animal- and herd-level seroprevalence in districts with an international border was 2.48% and 5.83% respectively, i.e. significantly lower than in the inland districts. The reason for the high *Brucella* spp. seroprevalence in Siavonga should be further investigated in future studies, and our results demonstrates a clear need for control measures in this area that aims to reduce disease burden in both the animal and human population. For Mccp, FMDV and CCHFV on the other hand, seroprevalence was significantly higher in districts situated in inland Zambia. Furthermore, the study found that herds that were seronegative for all the included pathogens were significantly more common in districts situated by one or more international borders. However, while the pathogen seroprevalence generally was significantly higher in the surveyed inland districts, it is unlikely that border proximity is a true protective factor, but rather that the higher seroprevalence in areas further away from international borders is due to other factors. For example, long-distance trade and movement of small ruminants tend to be more common in the districts of Central and Southern Provinces where farmers are generally considered to be business oriented and likely to engage in trade (Lubungu et al., 2012; Namonje-Kapembwa et al., 2016; Chapoto and Subakanya, 2019). These provinces also have the largest goat populations in Zambia (Ministry of Fisheries and Livestock et al., 2019). In the present study, the surveyed farmers who lived in non-border districts were significantly more likely to both sell and buy goats ($p = 0.002$ and <0.001) and had significantly larger herd sizes with a median value of 22, compared with the border districts where the median herd size was 10. Nevertheless, these aspects cannot explain all of the differences in seroprevalence between districts with an international border and those inland. For example, in Chililalombwe district, seroprevalence was low for all the included pathogens, even though the proportion of farmers regularly buying animals and the herd sizes was similar to Chibombo, Mazabuka

and Monze districts. Hence, based on the present findings, there appears to be limited association between seroprevalence of the surveyed pathogens in the included districts, and border proximity. This conclusion is in part supported by the fact that in the multivariable analysis where district was included as a random variable, border proximity only had a significant impact on the seroprevalence of Mccp. However, more studies are needed to fully elucidate the potential impact of border proximity on the seroprevalence of transboundary pathogens in Zambia, preferably also surveying other pathogens and areas of the country.

These results are in contrast with previous findings in Tanzania, where outbreaks of FMDV in cattle often occur close to international borders, as well as along major roads and railways (Picado et al., 2011; Allepuz et al., 2015). The same has been observed in earlier Zambian studies, where hotspots of FMDV outbreaks in cattle have been identified in regions bordering Tanzania, Botswana and Namibia, as well as on the Kafue Flats in central Zambia (Hamoonga et al., 2014; Sinkala et al., 2014a). In this study, none of the hotspot border areas were included, while two districts on the Kafue Flats were surveyed simultaneously with an ongoing outbreak, which may have influenced the FMDV seroprevalence detected.

Trade was generally not associated with seroprevalence in this study. More than 75% of the respondent households in this study sold small ruminants on a regular basis. Selling animals twice a year or more was significantly associated with increased herd-level seroprevalence of *Brucella* spp. compared to households that never sold goats. One possible explanation for this is that the farmers often sold to traders, who are moving between households and villages taking already purchased animals with them, allowing them to intermingle with local goats before the traders move on with what they have bought (Namonje-Kapembwa et al., 2016; Lysholm et al., 2020). This practice of allowing an assemblage of goats from source villages along internal trade routes may contribute to pathogen spread. Furthermore, selling animals regularly but rarely, i.e. once every two years, was associated with an increased chance of a herd being seronegative for all surveyed pathogens compared to households that never sold goats. The reason for this finding should be investigated further in future studies.

Buying animals constitute a risk for introducing pathogens into the herd and has been identified as risk factors associated with seroprevalence of FMDV (Osmani et al., 2021) and *Brucella* spp. (Asmare et al., 2013; Nthiwa et al., 2019) in previous studies. In this study, only about a third of the farmers regularly bought small ruminants and the majority of these bought from farmers within the same village. As most of the small ruminants grazed on communal pastures the whole or parts of the year, regular contact is likely between herds and the pool of source animals from which the farmer would be buying. This could explain the fact that this study did not detect any association between buying frequency and seroprevalence.

Interestingly, an association was found between keeping pigs in addition to goats and reduced seroprevalence for FMDV and *Brucella* spp. on animal-level data. This finding seems counterintuitive, as while pigs and goats generally are infected by different *Brucella* species, cross-infections do occur (Díaz Aparicio, 2013), and pigs are generally considered highly efficient transmitters of FMDV (Alexandersen and Donaldson, 2002; Kitching and Hughes, 2002). A potential explanation is the regular outbreaks of deadly diseases in the Zambian pig population, such as African swine fever (Simulundu et al., 2018). As a result, pig farmers may be more knowledgeable of measures to protect their animals from disease, which may also contribute to a reduced prevalence of disease among goats. For CCHFV on the other hand, associations were found between seropositivity and keeping cattle in herd-level data, and pigs in animal-level data, although the association with cattle was not statistically significant. Cattle are susceptible to CCHFV and can serve as amplifying hosts, while the role of pigs in the viral cycle is currently unclear (Spengler et al., 2016). Both species can also contribute to an increased risk of CCHFV infection by augmenting the tick burden in an area. Furthermore, herd size of 21 goats or more was associated with increased seroprevalence of FMDV on herd-level data and *Brucella* spp. on animal-level data, compared to smaller herds, although the association was non-significant for *Brucella* spp. Also, increasing age was identified as a potential risk factor for *Brucella* spp. and FMDV, although the association was non-significant for FMDV. These findings were as expected since the goats in large herds are at closer contact with more animals compared to in small herds which may facilitate pathogen dissemination, and since older animals have been exposed to pathogens for a longer time (Megersa et al., 2009). Lastly, among the herds that were seronegative for all the included pathogens, a significant association was found with administration of anthelmintic drugs on at least a yearly basis compared to on a trimonthly basis. While this study did not investigate prevalence of endo- or ectoparasites, it seems counterintuitive that more herds were seropositive for the included pathogens in the group that dewormed more often. One potential explanation is that these herds could be experiencing more clinical signs of disease, such as diarrhea, and were therefore deworming their animals more frequently.

In addition, the study detected associations between various clinical signs reported by the farmers and seropositivity for certain pathogens. These included associations between seropositivity for Mccp and ocular and nasal discharge, FMDV and kid mortalities, and *Brucella* spp. and abortions. Interestingly, animals seropositive for CCHFV were found to be more likely to suffer from mange at the time of sampling. This can indicate poor usage of ascarides, predisposing the animal to tick exposure and hence increasing the risk of CCHFV infection. However, frequency of administration of acaricides was included in the statistical analysis, and no association was found with CCHFV seroprevalence.

Despite the interesting results generated by this study, it did have some limitations. Designing and executing a completely randomized study, and calculating sample weights for adjustment of estimates, is very challenging in Zambia, for example because of the lack of a registry of sheep and goat farmers and of the small ruminant population. The sample size calculation in this study was based on a simple random sample, and hence did not take aspects such as clustering of positive cases within e.g. herds and villages into account. To reduce the potential effects of clustering, we opted to collect samples in a comparatively large number of households and villages. Furthermore, the sample size calculation assumed a 50% true prevalence, and the sensitivity and specificity values from the ELISA with the lowest values were used, to yield a large necessary sample size. Ideally, however, a multi-staged sample design should have been used (Thrusfield, 2005) to account for clustering in different levels in a systematic way.

Another study limitation is the fact that the predictor variable analyses was limited by the study design, which was primarily focused on generating representative seroprevalence estimates. While the list of included predictor variables is quite extensive, important aspects such as e.g. environmental conditions influencing the burdens of ticks and mosquitoes, and thereby possibly also the prevalence of CCHFV and RVFV, were not considered. Also, the districts without an international border were purposively chosen based on the density of goat-keeping households and their accessibility from the capital Lusaka, and the districts visited in this study were situated in close proximity to one other. This may have biased the study results to some extent as it would have reduced the independence of the study subjects, since neighbouring districts are more likely to have similar environmental conditions such as temperature and rainfall patterns, as well as to share management traditions, access to common exposure routes such as livestock markets, as well as pathogen spectrum and outbreak patterns, for example. Choosing districts further away from one other would probably have added valuable information to the study results. Furthermore, the analysis of the associations between seroprevalence and trade was in this study limited to the frequency of buying and selling, but did not account for aspects such as usage of different trade alternatives such as livestock markets, or biosecurity routines. This would be highly relevant for investigation in future studies.

5. Conclusions

This study offers the first description of the presence of antibodies to Mccp and CCHFV in the Zambian goat population, with the findings indicating widespread exposure to Mccp, FMDV, *Brucella* spp. and CCHFV in goats in Zambia. The association between seroprevalence and proximity to an international border was interpreted to be negligible in this study. For most of the surveyed pathogens, no significant associations between trade and seroprevalence were found, except for *Brucella* spp. and selling goats at least twice a year. As relatively few households buy new animals regularly, this aspect as well as the impact of different trade alternatives and biosecurity routines should be investigated in larger studies in future.

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Ethical considerations

In the household study, informed written consent was acquired prior to data collection. For farmers who were unable to sign the consent sheet, a thumbprint was obtained. The data collection was performed in collaboration with Zambian district veterinary officers, and district veterinary assistants or livestock assistants were present at all data collection sites. The study received ethical approval from the International Livestock Research Institution (ILRI) Institutional Research Ethics Committee (ILRI-IREC2018–04).

Conflict of interest statement

The authors declare no conflict of interest.

Data Availability

The data that supports the findings in this study are available on reasonable request from the corresponding author..

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2022.105708.

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