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Reproductive Infections in Cattle in Tanzania – Lessons for Control Priorities

C. Mathew[1, 3*], S. Klevar[2], T. Løken[1], R.H. Mdegela[3], G. Mwamengele[3], E. Skjerve[1], J. Godfroid[4] and M. Stokstad[1]

[1]*Norwegian University of Life Science, Oslo, Norway*

[2]*Norwegian Veterinary Institute, Oslo, Norway*

[3]*Sokoine University of Agriculture, Morogoro, Tanzania*

[4]*University of Tromsø, Tromsø, Norway*

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***Corresponding author:** Coletha Mathew (BVM, MVM), Department of Production Animal Clinical Sciences, Norwegian University of Life Science, P.O. Box 8146, Dep 0033 Oslo, Norway, Tel: +47 67230000, Fax: 67 23 06 91; E-mail: coletha.mathew.mtenga@nmbu.no or colethamat@yahoo.com*

Abstract

Reproductive disorders have negative impact on performance in cattle worldwide. Studies on infections causing reproductive disorders in Tanzania are few and fragmented, which complicates targeted disease prevention. To investigate the prevalence of selected infections and their associations with reproductive disorders and risk factors in cattle under different management systems, a cross-sectional study was conducted in two bordering regions in the southern highlands in Tanzania. Herd and individual animal level data were collected by direct observation and a semi-structured questionnaire interview of the farmer. Sera from 658 cattle from 202 herds were analyzed using a commercial ELISA kits for antibodies to Bovine Viral Diarrhea Virus (BVDV), *Brucella* spp. and *Neospora caninum*. The logistic regression model identified herd size (odds ratio (OR): 14.5), location (OR: 23.1) and management system (grazing strategy) (OR: 22.7) as risk factors for *Brucella* spp. The same risk factors were also identified for BVDV herd size (OR: 2.8), location (OR: 12.7) and management system (OR: 2.9). History of abortion was associated with seropositivity for *Brucella* spp. (OR: 4.6). No risk factors, including location and presence of dogs, nor any association with reproductive disorders were identified for *N. caninum.* In one region the herd level sero-prevalence was 66.7% for BVDV and 36.1% for *Brucella* spp., while in the other it was 6.5% for BVDV and 0.6% for *Brucella* spp. In total, BVDV specific antibodies were found in 15.2% of the animals in 17.9% of the herds, and *Brucella* spp. specific antibodies were detected in 5.4% of the animals in 7.4% of the herds. Anti- *N. caninum* antibodies were found in 4.5% of animals in 8.4% of the herds. In conclusion, prevalence and impact of BVDV and *Brucella* spp. differed significantly between geographically closely related areas, most probably due to differences in management system that affects the potential for survival of the agents in the population. This shows that all control measures must be based on accurate epidemiological knowledge of the occurrence of the infection. Low-prevalence areas are highly susceptible for introduction of infection, while in the high-prevalence areas control measures must be implemented to reduce the impact and the risk of transferring *Brucella* spp. from livestock to humans.

Keywords: Abortion; Antibody-ELISA; Bovine; *Brucella* spp; BVDV; *N caninum;* Pestivirus; Reproductive-Disorders; Serology

Introduction

Livestock keeping is a major agricultural activity in Tanzania. Although the cattle population is large, the production output is disproportionately low and management systems are diverse. Smallholder dairy production dominates the urban and peri-urban areas, while pastoralism dominates the rural areas. All types of management systems, from large industrialized dairy herds to traditional pastoralism, where big herds are pastured more freely, may be present in the same area. The herd size, management system and degree of contact between cattle herds, as well as contact with other livestock and wild animals, are highly variable.

Reproductive disorders contribute significantly to suboptimal performance and production in cattle. Studies on reproductive performance including estimation of the frequency of abortion and stillbirth have been reported in different parts of Tanzania but little is known of different risk factors associated with reproductive disorders [1-3]. Causes of reproductive disorders are broadly categorized as infectious and non-infectious. *Brucella* spp., Bovine Viral Diarrhea Virus (BVDV) and *Neospora caninum* are known to be among the most common infections associated with reproductive disorders in many parts of the world, but the information about which ones are implicated in reproductive disorders in cattle in Tanzania is scarce [4]. These infections may cause different reproductive disorders including early

embryonic death, abortion, stillbirth and fetal malformations [5-7]. In addition, *Brucella* is an important zoonotic agent, and its seroprevalence in cattle varies between regions in Tanzania [8-11]. In Tanzania, the prevalence of antibodies against BVDV has been found to be 12% and 17% in cattle and wildlife populations respectively [12,13]. Neosporosis caused by the protozoan parasite *N. caninum*, has emerged as one of the most frequently diagnosed causes of abortion in cattle in many parts of the world [14]. In Tanzania, only a few reports exist on *N. caninum* in cattle and canid populations [15,16].

All three infections are generally considered endemic in the cattle populations in Tanzania, as in the rest of Africa. Climatic factors and the diverse management systems of the cattle industry are likely to influence the epidemiology of these infections, but the impact of these infections on reproductive disorders has received little attention.

The aim of the present study was to investigate the occurrence of selected infections and their impact on reproductive disorders in cattle under different management systems in the southern highlands of Tanzania. Specifically, the study was carried out to establish the i) animal and herd level prevalence of serum antibodies to *Brucella* spp., BVDV and *N. caninum*, ii) the association between serostatus and reproductive disorders, and iii) management and other risk factors associated with serostatus and reproductive disorders.

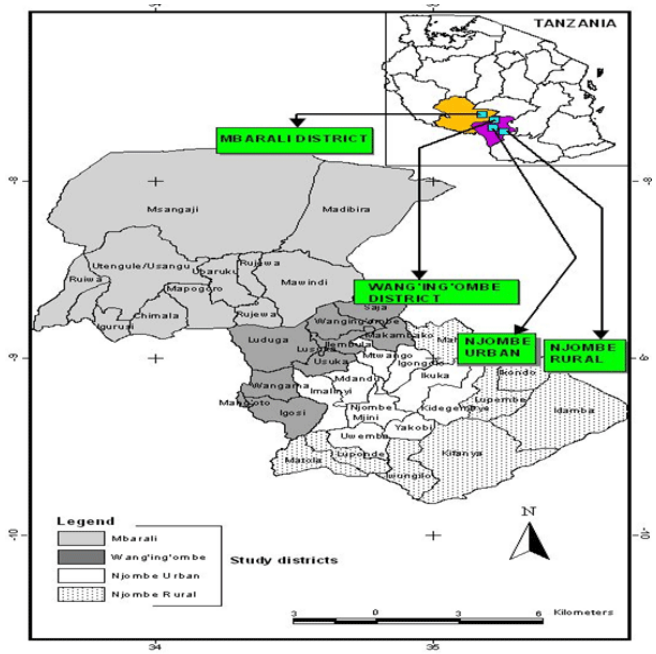
Materials and Methods

Study design

The study was a cross-sectional including selected dairy and pastoral herd in four districts in two regions. Epidemiological information regarding the selected animals and herds were collected by interviews and direct observation.

Study area

The study was part of a larger research and education program (EPINAV) taking place in the same area the study was conducted in Mbeya and Njombe regions in the southern highlands of Tanzania (Figure 1). Njombe is located in the altitude between 1600-1800m above sea level with annual rainfall of about 1000-1600mm and temperature ranges from 12-23oC. Mbarali is in altitude of about 1252m above sea level with average temperature between 25-30oC and mean annual rainfall of about 450-650 mm. In the Mbeya region, the Mbarali district was included, and in the Njombe region, the Wanging`ombe district, Njombe urban and Njombe rural districts were included. For practical reasons, the herds identified were in a limited number of villages; fifteen villages in the Njombe region and nine in the Mbeya region all of which participated in EPINAV program. Contact between villages, farmers and researchers were already established and well-functioning due to the EPINAV program. The herds selected were thus a mix of randomly selected herds in villages selected more by convenience.



	Region (Districts)	
Herd size	**Mbeya** (Mbarali)	**Njombe** (Rural, Urban,Wanging`ombe)
> 100 cattle	1	0
7–100 cattle	13	5
1–6 cattle	28	155

Figure 1: A map of Tanzania showing the study areas and the associated table indicating size of herds from the study regions.

Sampling strategy and sample size

The sample size was determined based on an 50% individual prevalence, 95% level of confidence and 5% absolute precision [17]. This provided a minimum sample size of 385 cattle. Due to the diversity of the production and management systems in the area, the total sample size was increased to 658. Inclusion criteria for herds were: presence of at least one female aged six months or above and that the farmer was willing to participate. A total of 201 herds were selected. Simple random sampling technique was used to select cattle in medium and large herds. In addition, serum from 200 cattle in a large herd with about 350 cattle and 28 breeding bulls in the primary selected herds were sampled as subgroups.

Blood sampling

About 5 ml of whole blood was aseptically collected from each animal. The blood samples were left at room temperature for a maximum of 12 hours for serum separation. Serum samples were then pipette into sterile tubes, transported on ice to a local laboratory and immediately frozen at approximately -20ºC. The material was shipped on ice, then kept frozen at -20ºC until analysis.

Collection of epidemiological information

The farmers were interviewed by enumerators with good knowledge of the local language using a structured questionnaire including questions on relevant biodata, past or present occurrence of reproductive disorders, management and possible risk factors for the past three years. The animal level biodata included age, sex, breed, source, parity and Body Condition Score (BCS) while the herd level data included location, herd size and management strategy. Reproductive disorders included abortions, stillbirth, and delivery of weak/malformed calves, dystocia and retention of fetal membranes.

Serological examination

All sera were analyzed at the Norwegian Veterinary Institute in Norway. Positive and negative control sera provided by the kits were included in all tests. The presences of antibodies to *Brucella* spp. were analyzed using indirect ELISA commercial kits following the manufacturer's instructions (SVANOVA® Brucella-Ab I-ELISA Svanova Biotech AB-Uppsala). The sensitivity and specificity provided by the manufacturer were 95.1% and 97.6%. Serum samples with ≥ 15 % positivity (PP) values were considered positive and PP value < 15 were considered negative. Anti-BVDV antibodies were analyzed using indirect ELISA commercial kit following the manufacturer's instructions (SVANOVA® BVDV -Ab I-ELISA Svanova Biotech AB-Uppsala), with a sensitivity of 99% and a specificity of 96% according to the manufacturer. Serum samples with PP values ≥ 10 were considered positive and PP value < 10 as negative. About 200 BVDV antibody negative samples were subjected to BVDV antigen test using commercial ELISA kit following the manufacturer's instructions (IDEXX BVDV Antigen Test kit/ serum plus Idexx Switzerland AG/ Switzerland). *N. caninum* specific antibodies were analyzed using an indirect ELISA commercial kit following manufacturer's instructions (SVANOVA® Neospora -Ab I-ELISA Svanova Biotech AB-Uppsala). The sensitivity and specificity provided by the manufacturer were 99% and 96%. Serum samples with PP values ≥ 20 were considered positive and PP value < 20 as negative.

Data analysis

Analysis of data was done using STATA version 12 for Windows (Stata Corp., Collage station, TX, USA) with herd as primary sampling unit. Most of the independent variables were categorical. Continuous variables were converted to categorical variables. Associations between dependent variables (infection status and reproductive history) and independent variables were estimated using univariable logistics regression adjusted for herd clustering effect at individual animal level. With consideration to biological plausibility of the factors in addition to their statistical relevance a final multivariable logistic regression model was formed using backward elimination procedure (inclusion criteria $P \leq 0.05$ of the likelihood ratio test). Tabular analysis using Goodman and Kruskal's gamma was used to determine association between the infections. Prevalence estimation for the males and the big herd subpopulations was done separate from other animals in the general study population. Some animals were not included in the analysis due to lack of reliable information.

Results

Herds, animals and management

Out of the 201 primary sampled herds, 183 had one to six cattle (small-scale herds) and 18 had seven to 100 (medium-scale herds). In addition one large-scale herd with about 350 cattle was included as a subpopulation group. From Njombe region, 155 herds were small-scale and five medium-scale. In Mbeya region (Mbarali district referred to Mbarali in Tables and Figures), 28 herds were small-scale 13 herds were medium-scale and one was large scale. In Njombe, all herds kept cross-bred dairy cattle, while in Mbeya both dairy and zebu cattle herds were present. In total, there were 392 female cross-bred dairy cattle (Holstein Friesian and Ayrshire crossed with Zebu) from 186 herds and 66 female zebu cattle from 15 herds sampled. Female cattle included in the final analysis were 65 heifers without calves (nulliparous), 94 with one calving (primiparous) and 229 with two or more calvings (multiparous) while 70 of them we did not get their information on parity. From one large-scale herd of cross-bred dairy cattle, 200 sera were collected and the 28 breeding bulls were from 12 herds; nine bulls from Njombe and 19 from Mbeya.

None of the sampled cattle were vaccinated against the studied infections. Most of the cattle in Mbeya region were kept on pasture during the day and indoors at night, while the majority of cattle in Njombe were confined in open barns with concrete walls or branches of trees with earthened, wooden or concrete floor. For zero grazed animals, roughage was obtained from communal grazing land with little supplementation from agricultural leftovers and industrial by-products. Grazing was on communal land except for a few herds that grazed on the farm.

Seroprevalence of BVDV, *Brucella* spp. and *N. caninum* and association between the infections

The overall, animal prevalence for BVDV, *Brucella* spp. and *N. caninum* antibodies were 15.2%, 5.4%, and 4.5% respectively. No serum was positive for BVDV antigens. Herd level prevalence (at least one positive animal) for BVDV, *Brucella* spp. and *N. caninum*, were at 17.9%, 7.4%, and 8.4%, respectively. In Mbeya region the herd level sero-prevalence was 66.7% for BVDV and 36.1% for *Brucella* spp. (animal level was 38.3% and 17.8% respectively) while in Njombe region it was 6.5% for BVDV and 0.6% for *Brucella* spp. (animal level was 5.7% and 0.3% respectively). The sero-prevalence for all the three infections in Njombe and Mbarali is shown in Figure 2. *Brucella* spp. and BVDV sero-positivity were associated with each other both at animal ($\gamma = 0.64$) and herd level ($\gamma = 0.9$). BVDV and *N. caninum* was not associated with each other at animal level ($\gamma = 0.01$) but a weak association was observed on herd level ($\gamma = 0.38$). *Brucella* spp. and *N. caninum* was weakly associated at animal level ($\gamma = 0.04$) but much more at herd level ($\gamma = 0.58$). The large-scale herd, from which 200 sera were collected, had a sero-prevalence of 73.1%, 47.8%, and 5.6%, for BVDV, *Brucella* spp. and *N. caninum* respectively. Out of the 28 breeding males, 32.1% were seropositive to BVDV, 14.3% to *Brucella* spp. and 10.7% to *N. caninum.*

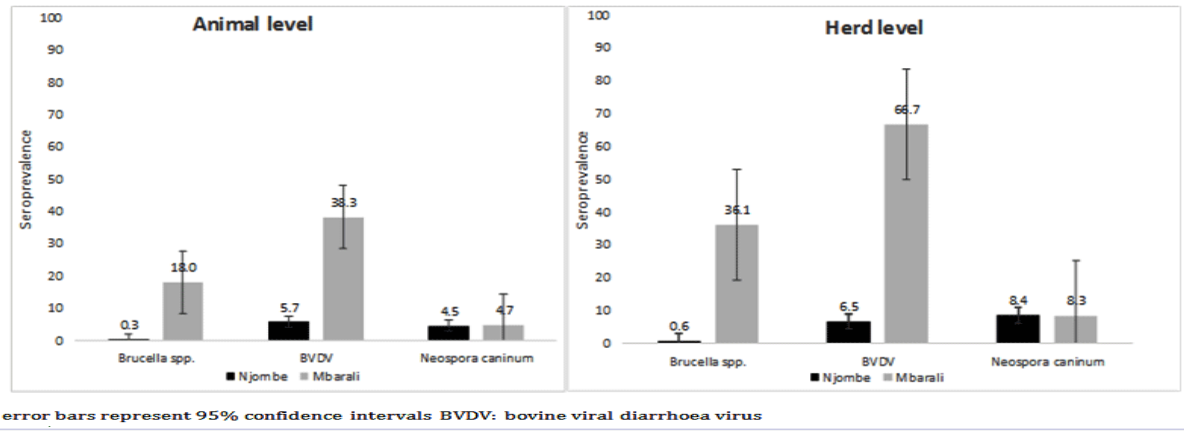


Figure 2: Prevalence of serum antibodies to *Brucella* spp., bovine viral diarrhea virus and *Neospora caninum* in cattle in the southern highlands in Tanzania

Prevalence of reproductive disorders

Reproductive disorders were observed in 98 animals with an overall prevalence of 33% (95% CI: 28-39). Table 1 indicates proportions for each disorder. Retained placenta and abortion were the most frequent encountered reproductive disorders. Dystocia was encountered in 29 animals, but due to missing information in many herds this parameter was not included in statistical analysis. Mbeya had higher proportions of abortion on animal and herd level than Njombe (Table 1).

Table 1: Prevalence of reproductive disorders in cattle in the southern highlands of Tanzania

Disorders (n)	P=Animal level prevalence (%)					P=Herd level prevalence (%)			
	P	95% CI	Location	p	95% CI	P	95% CI	p	95% CI
Abortion (38)	11.3	8-16	Njombe	7.0	4-11	11.6	7.7-17	7.8	4-13
			Mbarali	23.4	14-35			27.8	16-45
Retained placenta (51)	17.2	13-20	Njombe	18.2	12-25	22.6	17-29	23.4	17-31
			Mbarali	14.3	7-27			19.4	9-36
Stillbirth (5)	1.7	0.7-4	Njombe	1.4	0.4-4	2.6	1-6	1.9	0-6
			Mbarali	2.6	0.6-9			5.6	1-19
Malformations (4)	1.4	0.5-4	Njombe	1.4	0.4-4	1.6	0.5-5	1.9	0-5
			Mbarali	1.3	0.1-8			0	-

CI: Confidence Interval, n: number of cases

Association between sero-status and risk factors

At animal level, hypothesized risk factors for the three infectious agents were location, breed and parity. *Brucella* spp. sero-positivity was significantly associated with both location and breed while BVDV was associated with only breed. Altogether, zebu cattle were more likely to be seropositive for *Brucella* spp. and BVDV than crossbred dairy cattle while the prevalence of *N. caninum* was not affected by breed (Table 2). There was no association between *N. caninum* sero-positivity and presence of dogs on the farm (Table 2).

Table 2: Association between animal (n=292) level sero-status for *Brucella* spp., BVDV and *Neospora caninum* and hypothesized risk factors in a multivariable logistic regression model in cattle in the southern highlands of Tanzania

Risk factors	Level	Prevalence (95% CI)	OR	95% CI	*p*
		***Brucella* spp.**			
Breed	Dairy Cross	1.6 (0.5-5.0)	1.00	-	-
	Local	35 (22-51)	5.34	1.22-23.5	0.03
Location	Njombe	0.46 (0.07-3.1)	1.00	-	-
	Mbarali	22.1 (13.4-34.1)	21.5	1.9–248	0.01

Parity	Primiparous	2.3 (0.6-8.3)	1.00	-	-
	Multiparous	7.8 (4.0-15.0)	3.72	0.65–21.3	0.14
BVDV					
Breed	Dairy Cross	7.9 (4.6-13.6)	1.00	-	-
	Local	50 (37.6-62.3)	4.9	1.76–13.6	0.002
Location	Njombe	6.5 (3.1-13.4)	1.00	-	-
	Mbarali	33.8 (23.2-46.3)	2.89	0.92–9.1	0.09
Parity	Primiparous	10.5 (5.6-18.7)	1.00	-	-
	Multiparous	15.1 (10.4-21.5)	1.44	0.69-3.1	0.33
Neospora caninum					
Breed	Dairy Cross	5.2 (2.7-9.6)	1.00	-	-
	Local	5.0 (0.7-28)	0.37	0.04-3.53	0.38
Location	Njombe	4.2 (2.0-8.7)	1.00	-	-
	Mbarali	7.8 (2.9-19.4)	3.12	0.74–13.2	0.12
Parity	Primiparous	2.3 (0.6-9.0)	1.00	-	-
	Multiparous	6.3 (3-3-11.7)	3.18	0.77–13.2	0.11

CI: Confidence Interval, BVDV: Bovine Viral Diarrhoea Virus, OR: Odds Ratio, p: associated p values from multivariable logistic regression

At herd level, location of the herd, size of the herd and management system were hypothesized as potential risk factors for sero-positivity to the infections. *Brucella* spp. sero-positivity was significantly associated with all the risk factors. BVDV sero-positivity was significantly associated with location while *N. caninum* sero-positivity was not associated with any of the risk factors (Table 3).

Table 3: Association between herd (n=201) level sero-status for *Brucella* spp., BVDV and *Neospora caninum* and hypothesized risk factors in a multivariable logistic regression model in cattle in the southern highlands of Tanzania.

Risk factors	Level	Prevalence (%) (95%CI)	OR	95% CI	*p*
***Brucella* spp.**					
Location	Njombe	0.63 (0.09-4.3)	1.00	-	-
	Mbarali	36.5 (23.3-52.2)	23.1	1.96-292	0.013
Herd size	Small-scale (≤ 6)	2.7 (1.1-6.4)	1.00	-	-
	Medium-scale (6-100)	61.1 (37.7-80.3)	14.5	2.2–94.4	0.005
Management system	Indoor	1.1 (0.3-4.4)	1.00	-	-
	Outdoor	63.6 (42.1-80.8)	22.7	3.45-150	0.15
BVDV					
Location	Njombe	6.9 (3.8-12.0)	1.00	-	-
	Mbarali	63.4 (47.7-76.7)	12.7	4.7-34.8	< 0.001
Herd size	Small-scale (≤ 6)	13.7 (9.4-19.4)	1.00	-	-
	Medium-scale (6-100)	66.7 (42.7-84.3)	2.8	0.65–11.8	0.17
Management system	Indoor	11.7 (7.7-17.4)	1.00	-	-
	Outdoor	72.7 (50.9-87.3)	2.9	0.75-11.3	0.12
Neospora caninum					
Location	Njombe	9.4 (5.7-15.0)	1.00	-	-
	Mbarali	9.8 (3.7-23.4)	0.69	0.15-3.1	0.62
Herd size	Small-scale (≤ 6)	8.7 (5.4-13.8)	1.00	-	-
	Medium-scale (6-100)	16.7 (5.4-41.1)	2.1	0.36-12.2	0.40
Management system	Indoor	8.9 (5.5-14.1)	1.00	-	-
	Outdoor	13.6 (4.4-35.0)	1.4	0.19–9.9	0.74
Presence of dogs	Yes	9.7(5.2-17.1)	1.05	0.4-2.7	0.8
	No	9.3(4.9-16.9)	1.0	-	-

CI: Confidence Interval, BVDV: Bovine Viral Diarrhoea Virus, OR: Odds Ratio, p: associated p values from multivariable logistic regression

Association between reproductive disorders and risk factors

Factors associated with reproductive disorders were breed, parity of the animal, location, herd size and management system. Both at animal and herd level only abortion gave a model with explanatory power. At animal level, abortion was associated with herd size (OR: 4.4 CI 1.7-11.2). At herd level, abortion was mainly associated with size of the herd (OR: 5.7, CI 1.6-20.6). Other reproductive disorders did not show any significant association with any of the risk factors.

Association between reproductive disorders and sero-status

At the animal level, *Brucella* spp. sero-positivity were significantly associated with history of abortion (OR: 4.6, 95% CI 1.5-14.2), while other disorders were not associated with any of the infections. At herd level, abortion was also strongly associated with *Brucella* spp. (OR: 15.5, 95% CI 4.6-51.3) and BVDV (OR: 5, 95% CI 1.9-12.9) while *N. caninum* was not associated with any of the reproductive disorders. A combined *Brucella* spp. and BVDV sero-positivity was associated with abortion both on animal (OR: 11.7, 95% CI 2.7-50.3) and herd level (OR: 10.1, 95% CI 2.9-35.5).

Discussion

These results indicated that BVDV, *Brucella* spp. and *N. caninum* antibodies are present in the study area. BVDV sero-prevalence was the highest, followed by *Brucella* spp. and *N. caninum.* Furthermore, important differences in the seroprevalence of *Brucella* spp. and BVDV and the frequency of abortions were revealed between the two bordering regions in the study area. This indicates high diversity in the epidemiological pattern of these agents within a geographically closely related areas. These differences are most likely influenced by many factors. Since the heterogeneous livestock production system in the study area is typical for African conditions, a complex epidemiology is probably a general pattern.

The observed sero-prevalence for *Brucella* spp. calls for attention, as human brucellosis originates from animals [18]. Veterinary public health measures need to be in place as this is a zoonotic infection and consumption of unpasteurized dairy products is still a practice in some communities in Tanzania. *Brucella abortus* ,biovar 3 has earlier been isolated from an aborted cattle fetus from the large-scale herd included, illustrating the risk for transmission. *Brucella abortus* biovar 1 has been detected in the Katavi-Rukwa ecosystem in Tanzania [8,19]. Previous studies have reported the prevalence of brucellosis in cattle to range from 2.2-12.3 % in different regions and management systems in Tanzania [10,11,20,21]. Similarly the present study indicates a difference in sero-prevalence in two geographically very closely areas. Interestingly, Njombe, with a total of 160 herds investigated, had only one seropositive animal, which could be a false positive, and therefore, it is possible to regard the area as *Brucella* free. This is further supported by information from a local milk factory. They require that farmers test their animals for brucellosis before milk is accepted, and no positive animal has been detected for the past five years (personal communication). The prevalence of brucellosis in Mbarali could be explained by management strategies which allow for more direct or indirect contacts between infected and susceptible animals, as has been observed elsewhere [22]. High prevalence of abortion, strong association with *Brucella* spp. on both animal and herd level, and isolation of the agent in the same area suggest that *Brucella abortus* causes abortion in this area.

The prevalence of BVDV was found to be higher than that detected in 18 regions about 25 years ago, but more similar to that observed in the northern parts of the country [13,23]. With this relatively high sero-prevalence, the cattle population most likely also includes Persistently Infected (PI) animals, but such animals are frequently weak-born, unthrifty and underperform and are often eliminated from the herd early in life under these management conditions [24]. Since only animals over six months of age were included in the present study, this might explain why no PI animals were detected. It is not unlikely that BVDV could also has been introduced directly or indirectly from outside as most herds were open, but the general trend of very small herds and little contact probably limits the survival of BVDV in Njombe.

The higher prevalence of both BVDV and *Brucella* spp. in the two subgroups investigated is interesting, since both subgroups represent particular risk of inter-herd transmission. Breeding males represent a risk because they are commonly moved from herd to herd for natural breeding, and the large-scale herd as it represents typical procedures of replacement heifers for smaller herds.

The low sero-prevalence for *N. caninum* indicates a different epidemiological pattern from BVDV and *Brucella* spp. Contrary to our findings, in Ethiopia, a higher sero-prevalence for *N. caninum* than BVDV and *Brucella* spp. has been reported, and is regarded as more important for reproductive performance [25,26]. This highlights the difference in epidemiological patterns for these infections in African countries. Presence of infected dogs, which shed infective oocysts in the environment is crucial to dissemination into the cattle population. Investigation of the dog population in the area would have been valuable to explain if a low prevalence in the dogs may be the main reason for the observed low prevalence in cattle. The lack of association between presence of dogs and *N. caninum* sero-prevalence could be because exposure is more evenly distributed as stray dogs move easily between farms. This lack of association between presence of dogs at farm and *N. caninum* seropositivity has also been reported in Ireland [27].

The observed association between larger herds and *Brucella* spp. sero-positivity is in accordance with other observations [28,29]. Evidence suggests that when *Brucella* spp. is introduced into herds, a large proportion of animals will be infected and the infection will persist for a longer time [30-32]. Sharing of pasture and drinking water facilitate transmission of most infectious diseases, which is in line with the present findings of grazing as risk factor for *Brucella* and BVDV infections [33]. This might be caused by a higher degree of contact with

animals from other herds [34]. In addition the pasture may have been contaminated with infectious agents from animal secretion particularly with *Brucella* spp. since it is common for cattle to give birth outdoor which contaminate the surrounding environment.

The trend in this study that *Brucella* spp. and BVDV prevalence is linked to breed, has also been observed earlier for *Brucella* spp. [35]. However, all the zebu cattle herds in the present study were located in the Mbeya region with the higher seroprevalence of *Brucella* spp. and BVDV so the finding that Zebu cattle was more likely to be seropositiv for these infections should be carefully interpreted. Since breed, location, grazing strategy and management are often interlinked, confounding effects are possible.

The quality of the information gained from the interviews is a concern, as written recordings by farmers are uncommon. The data on reproductive disorders were the only reproductive performance information that was regarded suitable for analysis. Since *Brucella* spp. typically gives abortion where it is easily observed, the consequences on reproductive performance is most likely better estimated than for BVDV, which often leads to early embryonic death and repeated breeding/prolonged calving interval [36]. The impact of BVDV on reproduction is therefore probably underestimated in the present study.

The present findings indicate that co-infections with BVDV and *Brucella* spp. may have a greater influence on occurrence of reproductive disorders than mono-infection. Immunosuppressive properties of BVDV is known, and although the mechanisms of abortion caused by BVDV is unclear, it has been speculated that pathological changes induced in the placenta may allow other pathogens to cross the fetal membrane barriers [37,38]. The most likely explanation for the co-infections in this study is, however, that they share the same risk factors.

Serological investigations and cross sectional design has both advantages and disadvantages as methods to establish the prevalence of infection. In the absence of vaccination, sero-positivity can be regarded as an earlier infection. For all three infections, animals are generally sero-positive for several years after the infection [39-41]. The risk period for reproductive disorders caused by the agent is only when during pregnancy, and when the agent is actually present. Later, the animal will be fully or partly protected, which leads to underestimation of the association between infection and reproductive performance. Collection of reproductive history for the past three years, as in this study, reduces this challenge.

Conclusion

Antibodies to all the three studied infections were detected in cattle in the area, but the impact of the infections seems to be highly variable. For BVDV and *Brucella* spp., the prevalence was high but variable, with some areas almost free from *Brucella* spp. and very low BVDB. Location, herd size and grazing strategy influence the sero-prevalence. The high-prevalence area represent a risk to the low-prevalence area especially because purchase of replacement stock is common. In the high prevalent areas, the infections have a significantly impact both on cattle reproduction and possibly human health consequences.

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Ethical approval

The protocol for field studies and collection of animal material was approved by the University Ethics committee using guidelines from the Code of Conduct for Research Ethics of Sokoine University Agriculture SUA/VET/012/04. Farmer's verbal consent was sought before embarking on data and biological material collection.

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