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Sero-Epidemiological Study of Selected Zoonotic and Abortifacient Pathogens in Cattle at a Wildlife-Livestock Interface in South Africa

Abiodun A. Adesiyun,[1,2,*] Darryn L. Knobel,[3,4,*] Peter N. Thompson,[1,5,*] Jeanette Wentzel,[5,6] Francis B. Kolo,[3] Agatha O. Kolo,[3] Anne Conan,[4] and Gregory J.G. Simpson[1,5,*]

Abstract

A cross sectional sero-epidemiological study was conducted on cattle in a communal farming area adjacent to Kruger National Park at a wildlife-livestock interface in South Africa. A total of 184 cattle were screened for exposure to 5 abortifacient or zoonotic pathogens, namely *Coxiella burnetii*, *Toxoplasma gondii*, *Chlamydophila abortus*, *Neospora caninum*, and Rift Valley fever virus (RVFV) using enzyme-linked immunosorbent assays. In addition, the virus neutralization test was used to confirm the presence of antibodies to RVFV. The seroprevalence of *C. burnetii*, *T. gondii*, *C. abortus*, *N. caninum*, and RVFV antibodies was 38.0%, 32.6%, 20.7%, 1.6%, and 0.5%, respectively, and varied between locations ($p < 0.001$). Seroprevalence of *C. burnetii* and *T. gondii* was highly clustered by location (intraclass correlation coefficient [ICC] = 0.57), and that of *C. abortus* moderately so (ICC = 0.11). Seroprevalence was not associated with sex or age for any pathogen, except for *C. abortus*, for which seroprevalence was positively associated with age ($p = 0.01$). The predominant mixed infections were *C. burnetii* and *T. gondii* (15.2%) and *C. burnetii*, *T. gondii*, and *C. abortus* (13.0%). The serological detection of the five abortifacient pathogens in cattle indicates the potential for economic losses to livestock farmers, health impacts to domestic animals, transmission across the livestock-wildlife interface, and the risk of zoonotic transmission. This is the first documentation of *T. gondii* infection in cattle in South Africa, while exposure to *C. burnetii*, *C. abortus*, and *N. caninum* infections is being reported for the first time in cattle in a wildlife-livestock interface in the country.

Keywords: cattle, wildlife-livestock interface, zoonoses, seroprevalence, intraclass correlation coefficient

Introduction

In cattle, pathogens belonging to several broad groups—bacteria, parasites, and viruses—are important zoonotic and abortifacient agents (Sager et al. 2001, Baudin et al. 2016, Kaveh et al. 2017). While some typically cause clinical disease, others are underreported because of their often asymptomatic infection in animals (Ayinmode et al. 2017, Salifu et al. 2019). Exposure of apparently healthy cattle to pathogens of zoonotic and reproductive significance is often detected by serological assays (Sun et al. 2015, Ayinmode et al. 2017).

Coxiella burnetii, the causative agent of Q-fever, and certain members of the Chlamydiales order are zoonotic, intracellular, gram-negative bacteria, with abortigenic potential in humans (Eldin et al. 2017, Ghaoui et al. 2018) in ruminants and worldwide geographical distribution (Frangoulidis and Fischer 2015). *C. burnetii* is excreted in milk,

[1]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.
[2]Department of Basic Veterinary Sciences, Faculty of Medical Sciences, University of the West Indies, St Augustine, Trinidad and Tobago.
[3]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.
[4]Center for Conservation Medicine and Ecosystem Health, Ross University School of Veterinary Medicine, Basseterre, St. Kitts and Nevis.
[5]Centre for Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.
[6]Hans Hoheisen Wildlife Research Station, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.
*All these authors share first authorship.

feces, and birth products of infected animals. Infection in animals mainly occurs through inhalation of aerosolized particles (Angelakis and Raoult 2010). Human infections are mostly associated with infections in ruminants (Szymańska-Czerwińska et al. 2015). Q-fever is a disease of public health significance, which can also cause financial losses to animal owners (Gwida et al. 2014). Serological evidence of *C. burnetii* infections in ruminants has been reported in several countries (Knobel et al. 2013, Haider et al. 2015, Pexara et al. 2018). In South Africa, the last documented report of *C. burnetii* in animals was in 1987, where 7.8% of cattle in the former Transvaal province, South Africa were seropositive for the pathogen (Gummow et al. 1987).

Toxoplasma gondii is the causative agent for toxoplasmosis in both animals and humans. Animals become infected through ingestion of infectious oocytes and bradyzoites in tissues and the inhalation of infectious aerosolized oocyte particles (Saeij et al. 2005). *T. gondii* infections in both livestock and humans may cause reproductive problems such as late abortions and fetal death (Cañón-Franco et al. 2014); however, infections in livestock and wild animals often remain unnoticed due to the asymptomatic nature of infections. Clinical manifestations are reported in ~20% of toxoplasmosis cases (Amouei et al. 2019). In South Africa, *T. gondii* infections have been demonstrated in sheep (Abu Samra et al. 2007), free-roaming chacma baboons in the Kruger National Park (McConnell et al. 1973), and recently in cats tested in the Western Cape (Hammond-Aryee et al. 2015), but not to our knowledge in cattle.

Chlamydophila abortus is a recognized cause of abortion in small ruminants (Loureiro et al. 2017, Heidari et al. 2018), cattle (Osman et al. 2012, Wheelhouse et al. 2015), and humans (Pospischil et al. 2002). It is an important zoonotic agent and has been associated with abortions and other reproductive problems in livestock (Borel et al. 2006, Hireche et al. 2016). Seroprevalence studies on exposure to *C. abortus* have been conducted in apparently healthy dairy cattle in Jordan (Talafha et al. 2012), riverine buffalo in Egypt (Osman et al. 2012), free-ranging European bison in Poland (Salwa et al. 2007), and in wild mammals in Serengeti National Park in Tanzania (Pospichil et al. 2012). The only available reports of chlamydophilosis in animals in South Africa date back to 1973, where it was diagnosed in domestic and laboratory animals (Pienaar and Schutte 1975) and associated with neonatal deaths in beef herds (Ehret et al. 1975).

Neosporosis is an infectious disease caused by *Neospora caninum*, an obligate intracellular cyst-forming protozoan, and is considered a major cause of miscarriage in cattle, water buffalo, and other livestock in many parts of the world (Sager et al. 2001, Bruhn et al. 2013, Talafha and Al-Majali 2013, Reichel et al. 2015). In South Africa, exposure to *N. caninum* has been serologically documented in dogs (Jardine and Dubey 1992, Jacobson and Jardine 1993), in apparently healthy cattle and aborted fetuses (Jardine and Last 1995, Njiro et al. 2011), and from a dead white rhinoceros calf on a wildlife breeding farm (Williams et al. 2002). Although antibodies to *N. caninum* have been reported in humans (Tranas et al. 1999, Lobato et al. 2006), the parasite has not been demonstrated in human tissues, and therefore, the zoonotic potential is uncertain (Dubey et al. 2007). The unknown status of the pathogen as a zoonosis may have potential impact on the epidemiology and control of neosporosis and *N. caninum*.

Rift Valley fever (RVF) is so named because the first outbreak was reported by Kenyan veterinary officers in the Rift Valley in 1915; the virus was isolated in 1931 (Daubney et al. 1931). Subsequently, numerous outbreaks have been reported periodically in African countries and the Middle East (Yemen and Saudi Arabia) (Hoogstraal et al. 1979, Meegan and Bailey 1988). RVF, caused by Rift Valley fever virus (RVFV) of the genus *Phlebovirus* in the family *Bunyaviridae*, is a well-known viral zoonosis that affects domestic animals and humans by causing acute fever and disease (Baudin et al. 2016, Fafetine et al. 2016, Sweileh 2017). The virus is transmitted to vertebrate hosts by the bite of infected mosquitoes, usually *Aedes* and *Culex* species. RVF mainly affects domestic animals (cattle, goats, sheep, and camels) and generally causes abortions in pregnant females and high mortality in young animals (Anyangu et al. 2010). Transmission to humans most commonly occurs via contact with infected tissues, blood, or other body fluids during handling or slaughter of infected animals, although exposure may also occur via mosquito bites and possibly by consumption of raw milk (Swanepoel and Coetzer 2004).

South Africa experiences episodic large outbreaks of RVF at 25- to 30-year intervals (Pienaar and Thompson 2013). The latest series of outbreaks occurred from 2008 to 2011, involving all nine provinces, but the source of virus was not established. Evidence of interepidemic transmission of RVFV has been found in several African countries, including in livestock in South Africa (Van den Bergh et al. 2019) and in African buffalo (*Syncerus caffer*) in the Kruger National Park (LaBeaud et al. 2011). This suggests that the virus may be maintained by circulation between vectors and hosts in some areas. The potential role of wildlife in the maintenance of RVFV has been reviewed (Olive et al. 2012) with the conclusion that there is no definitive evidence for existence of a wild mammal reservoir.

The detection of antibodies to zoonotic agents, including *C. burnetii*, *Rickettsia* spp., and *Leptospira* spp. at a prevalence of 60%, 84%, and 22%, respectively, in apparently healthy farmers, herders, and veterinary personnel in contact with the cattle in our study area (Simpson et al. 2018), indicates the potential for cattle to be sources of these zoonoses. In that study, the authors reported that 98% of the people were positive for at least one of the nine tested zoonoses.

Considering the dearth of information on the occurrence of the pathogens in cattle on communal farms, the primary objective of the study was to determine the prevalence of exposure to *C. burnetii* and the causative agents for toxoplasmosis, chlamydophilosis, neosporosis, and RVF in cattle on communal farms at the wildlife-livestock interface and to identify factors associated with exposure.

Methodology

Source of sera

Serum samples had already been collected from 1,990 head of cattle in the study area, which falls within the site of the Mnisi Community Programme, an initiative by the University of Pretoria and the Mnisi Traditional Authority to promote sustainable livelihoods through research into human health, animal production and resource utilization. The area, where 15,000 cattle are owned by 1,300 communal farmers,

lies in the Lowveld region of Mpumalanga Province, South Africa and shares 75% of its boundary with wildlife reserves. The Mnisi community falls within the control zone for foot and mouth disease (FMD), which is maintained in the African buffalo population in the adjacent wildlife reserves. In the control zone, all cattle are registered at a dip tank (one to two dip tanks per village) and subjected to weekly inspections at that dip tank by the state veterinary services. Fifteen dip tanks were operational in the study area; however, serum samples were tested from 10, primarily because of the quality of the samples. From April through September 2013, cattle registered in the 10 dip tanks were ear tagged and tattooed with a unique number. Of the 10,624 cattle registered in the 10 dip tanks at the end of June 2013, 9,952 (94%) were ear-tagged and 1,990 (19%) were sampled. All serum samples were stored in a bank at the Hans Hoheisen Wildlife Research Station (HHWRS) located in Mpumalanga province. Figure 1 shows the locations of the dip tanks relative to the Kruger National Park and other wildlife reserves.

Sample size determination and sample selection

Overall, a total of 10,624 cattle were enrolled in the 10 dip tanks for HHWRS. A serum sample was collected from every fifth animal, using a systematic random sampling procedure for enrolment resulting in 1,990 samples, which constituted the serum bank. To estimate the sample size for the current study, for a 95% confidence interval (CI), the following formula (Thrusfield 2007) was used: $n = [1.96^2\ P_{exp}\ (1 - P_{exp})]/d^2$, where n = required sample size, P_{exp} = estimated prevalence of selected zoonoses, and d = desired absolute precision. For the study, P_{exp} was estimated at 50% and d was 7.5%. The estimated minimum sample size for the study was therefore 171. Samples were randomly selected from the sera in the serum bank, stratified by dip tank and proportional to the total population of animals at each dip tank, giving a total sample size of 184 sera to be tested.

Information on the location of cattle herds in farming areas (dip tanks), sex, and age was obtained from the data captured at the time of specimen collection to contribute to the serum bank.

Serological testing for pathogens

All sera were tested for antibodies to *C. burnetii*, *T. gondii*, *C. abortus*, *N. caninum*, and RVFV. IDEXX IgG indirect enzyme-linked immunosorbent assay (ELISA) kits (IDEXX, Liebefeld-Bern, Switzerland) were used to detect IgG immunoglobulins against *C. burnetii*, *T. gondii*, *C. abortus*, and *N. caninum*. Interpretations of the results were based on the criteria protocols stipulated by the kit manufacturers. The respective diagnostic sensitivity (DSe) and specificity (DSp) provided by the manufacturer for each of the IDEXX test kits to detect antibodies are as follows: *C. burnetii* (100%, 100%), *T. gondii* (100%, 87.2%), *C. abortus* (89–95%, 100%), and *N. caninum* (90.6–98.8%, 98.9–99.95%). For the detection of IgG immunoglobulins to RVFV, the IgG NP-based ELISA kit (DSe 91–100%, DSp 100%; IDVet, Grabels, France) (Kortekaasa et al. 2013) was used. The test results were interpreted using the criteria stipulated by the kit manufacturer. Considering that RVF is a notifiable disease in South Africa, it was mandatory to perform confirmatory tests for its diagnosis. For this study, the virus neutralization test (VNT) as described by the OIE guidelines (Organization for Animal Health [OIE] 2012) was used to confirm the ELISA-positive samples.

Statistical analyses

For each of the five pathogens, seroprevalence was calculated by dip tank and overall with exact 95% CIs. To estimate the degree of clustering, the intraclass correlation coefficient (ICC or ρ) was calculated for each pathogen as follows (Fleiss et al. 2003):

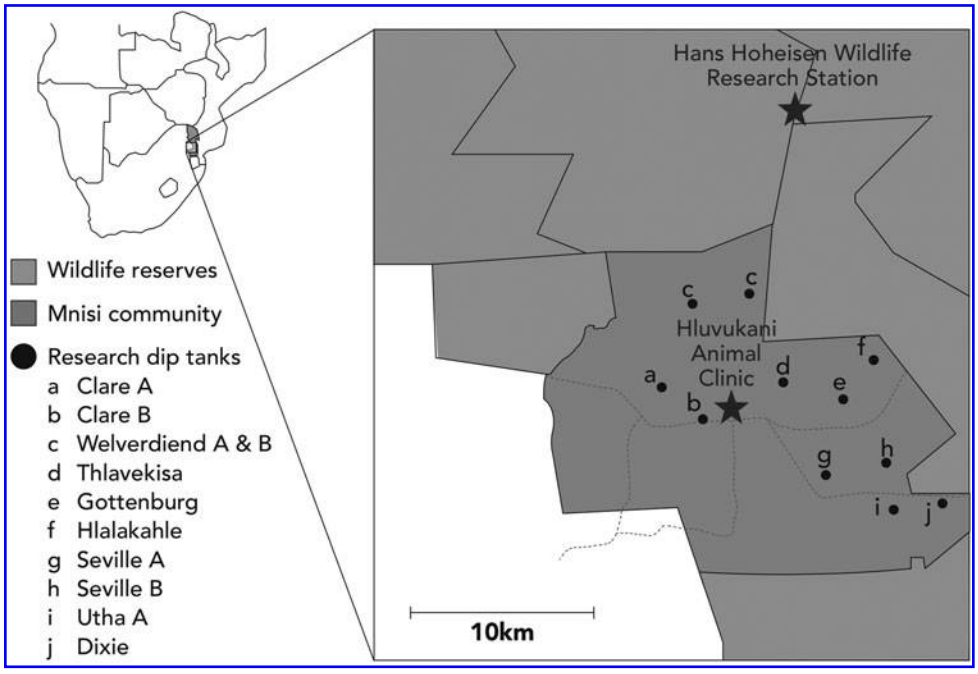

FIG. 1. Locations of dip tanks in the Mnisi Community, South Africa, relative to the wildlife reserves (Simpson et al. 2018).

Table 1. Demographic Data on Animals Tested in the Study

Farming area	No. of samples tested	No. (%) of animals tested					
		Sex		Age group (years)			
		Female	Male	<1	1–2	>2–5	>5
Clare A	21	15 (71.4)	6 (28.6)	6 (28.6)	3 (14.3)	6 (28.6)	6 (28.6)
Clare B	19	14 (73.7)	5 (26.3)	2 (10.5)	4 (21.1)	6 (31.6)	7 (36.8)
Dixie	16	10 (62.5)	6 (37.5)	2 (12.5)	6 (37.5)	2 (12.5)	6 (37.5)
Gottenburg	19	15 (78.9)	4 (21.1)	2 (10.5)	5 (26.3)	5 (26.3)	7 (36.8)
Hlalakahle	18	14 (77.8)	4 (22.2)	3 (16.7)	4 (22.2)	5 (27.8)	6 (33.3)
Seville A	18	15 (83.3)	3 (16.7)	4 (22.2)	3 (16.7)	5 (27.8)	6 (33.3)
Seville B	19	13 (68.4)	6 (31.6)	2 (10.5)	5 (26.3)	6 (31.6)	6 (31.6)
Thlavekisa	17	12 (70.6)	5 (29.4)	5 (29.4)	2 (11.8)	5 (29.4)	5 (29.4)
Utha A	18	11 (61.1)	7 (38.9)	5 (27.8)	4 (22.2)	5 (27.8)	4 (22.2)
Welverdiend A&B	19	13 (68.4)	6 (31.6)	6 (31.6)	3 (15.8)	6 (31.6)	4 (21.1)
Total	184	132 (71.7)	52 (28.3)	37 (20.1)	39 (21.2)	51 (27.7)	57 (31.0)

$$\rho = \frac{\sum_{i=1}^{K}\left\{Y_{i+}(Y_{i+}-1) - 2p(n_i-1)Y_{i+} + n_i(n_i-1)p^2\right\}}{\sum_{i=1}^{K} n_i(n_i-1)p(1-p)}$$

where K is the number of dip tanks, Y_{i+} is the number of seropositive animals at dip tank i, n_i is the number of animals tested at dip tank i, and p is the overall seroprevalence. Associations of dip tank, age, and sex with seropositivity for each of the pathogens were assessed first by cross-tabulation and the Fisher exact test, and then using mixed-effects logistic regression models, with dip tank as a random effect, to adjust for confounding and clustering. Since linearity could not be assumed, age was categorized into quartiles. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test. Differences in seroprevalence between the various pathogens were assessed using a mixed-effects logistic regression model with random effects for individual nested within dip tank. Data were analyzed using Stata 14 (StataCorp, College Station, TX). The level of significance was set at alpha = 0.05.

A risk surface was created from the seroprevalence of each pathogen at each dip tank using inverse distance weighting and displayed on a map using ArcGIS 10.2 (Esri Corporation, Redlands, CA).

Ethics Committee approval

The study protocol was approved by the Animal Ethics Committee (AEC) of the University of Pretoria (V018-15) and by the Department of Agriculture, Forestry and Fisheries (DAFF). Specimens were collected from cattle under approved protocol V032-11.

Results

Distribution of samples tested by age and sex

Table 1 displays the distribution of cattle sampled across 10 farming areas studied by age and sex. A total of 184 head of cattle were sampled, of which females (71.7%) outnumbered the males (28.3%). The lowest and highest number of animals sampled belonged to the <1-year-old age group (20.1%) and the >6-year-old age group (31.0%), respectively.

Seroprevalence of pathogens

Overall, seroprevalence varied significantly between pathogens ($p < 0.001$) (Table 2). The seroprevalence (and 95% CIs) of *C. burnetii*, *T. gondii*, *C. abortus*, *N. caninum*, and

Table 2. Prevalence of Selected Zoonoses in Mnisi Area by Location of Farms Tested

Farming area	No. of animals tested	No. (%) of animals seropositive				
		Coxiella burnetii	Toxoplasma gondii	Chlamydophila abortus	Neospora caninum	*Rift Valley fever*
Clare A	21	17 (81.0)	16 (76.2)	8 (38.1)	0 (0.0)	0 (0.0)
Clare B	19	18 (94.7)	16 (84.2)	5 (26.3)	1 (5.3)	0 (0.0)
Dixie	16	14 (87.5)	15 (93.8)	6 (37.5)	0 (0.0)	0 (0.0)
Gottenburg	19	12 (63.2)	9 (47.4)	10 (52.6)	0 (0.0)	0 (0.0)
Hlalakahle	18	4 (22.2)	3 (16.7)	1 (5.6)	0 (0.0)	0 (0.0)
Seville A	18	2 (11.1)	1 (5.6)	1 (5.6)	0 (0.0)	0 (0.0)
Seville B	19	0 (0.0)	0 (0.0)	2 (10.5)	0 (0.0)	0 (0.0)
Thlavekisa	17	0 (0.0)	0 (0.0)	1 (5.9)	1 (5.9)	1 (5.9)
Utha A	18	0 (0.0)	0 (0.0)	2 (11.1)	0 (0.0)	0 (0.0)
Welverdiend A&B	19	3 (15.8)	1 (5.3)	2 (10.5)	1 (5.3)	0 (0.0)
Total (%)	184	70 (38.0)	61 (33.2)	38 (20.7)	3 (1.6)	1 (0.5)
95% CI (%)		31.0–45.5	26.4–40.5	15.0–27.2	0.3–4.7	0.01–3.0

CI, confidence interval.

RVFV was 38.0% (31.0–45.5%), 33.2% (26.4–40.5%), 20.7% (15.0–27.2%), 1.6% (0.3–4.7%), and 0.5% (0.01–3.0%), respectively.

Seven of the 10 farming areas had cattle seropositive to *C. burnetii*, with seroprevalence ranging from 11.1% (Seville A) to 94.7% (Clare B; $p<0.001$). Cattle seropositive for *T. gondii* were also detected in 7/10 farming areas with seroprevalence ranging from 5.3% (Welverdiend A&B) to 93.8% (Dixie; $p<0.001$). Antibodies to *C. abortus* were detected in cattle from all 10 farming areas with the seroprevalence ranging from 5.6% (Seville A and Hlalakahle) to 52.6% (Gottenburg; $p=0.001$). Only three farming areas (Clare B, Thlavekisa and Welverdiend A&B) had cattle seropositive for *N. caninum*—one animal each (5–6%). Antibodies to RVFV were detected in only 1/17 cattle in Thlavekisa farming area by ELISA and confirmed by VNT. The mixed-effects logistic regression models for *C. burnetii*, *T. gondii*, and *C. abortus* showed statistically significant variation in seroprevalence between dip tanks ($p<0.001$, $p<0.001$ and $p=0.002$, respectively). The ICC estimates for *C. burnetii* and *T. gondii* showed a very high degree of clustering within dip tanks ($\rho=0.57$ for both), whereas for *C. abortus* the clustering was moderate ($\rho=0.11$). For *N. caninum* and RVFV, the variation between dip tanks was not significant ($p=1.000$ for both) and there was no evidence of clustering ($\rho=0$). The Hosmer-Lemeshow goodness-of-fit test indicated adequate model fit ($p>0.400$) for all models, except for RVFV, where it was not possible to assess due to the single positive outcome.

The spatial distribution of seroprevalence for each of the five pathogens is shown as risk surfaces produced by interpolation using inverse distance weighting in Fig. 2. It is evident that *T. gondii* and *C. burnetii* show a very similar spatial pattern of seroprevalence, being highest at Clare A, Clare B, and Dixie, intermediate at Gottenburg, and lowest in the other six areas. A somewhat similar pattern was seen for *C. abortus*, although it was highest at Gottenburg.

Seroprevalence of pathogens by age and sex

The seroprevalence of the five pathogens in the studied animals based on age and sex is shown in Table 3, with statistical significance determined using the mixed-effects logistic regression models. For *C. burnetii*, *T. gondii*, and *C. abortus*, although there was a general pattern of increasing seroprevalence with age, this was statistically significant only for *C. abortus*, with an increase in seroprevalence from 5.4% (<1-year-old) to 29.8% (≥5-year-old).

There were no significant differences in seroprevalence between sexes for any of the pathogens.

Frequency of single and multiple infections by zoonoses

The frequency of single and mixed infection in individual cattle tested is shown in Table 4. Mixed infections of *C. burnetii* and *T. gondii* (15.4%) and *C. burnetii*, *T. gondii*, and *C. abortus* (13.0%) were the most frequently encountered. For single infections, *C. burnetii* (7.6%) and *C. abortus* (4.9%) were most frequently detected. The only animal seropositive for RVFV did not have antibodies to the other four pathogens, while the three *N. caninum*-positive cattle had mixed infections. A total of 96 cattle (52.2%) were negative for antibodies to all 5 pathogens.

Discussion

The seroprevalence of 38.9% found for *C. burnetii* antibodies by ELISA is considerably higher than the prevalence of 7.8% recorded in South Africa in 1987 (Gummow et al. 1987). Although both studies were conducted in South Africa, the difference in the seroprevalence detected may partly be attributed to factors such as the period when the samples were collected for testing (March 1985 to July 1986 vs. April to September 2013), the location and management of cattle farms (Transvaal province vs. Mpumalanga province), and most importantly, serological tests used (Complement Fixation Test vs. ELISA). These factors have the potential to affect exposure and detection of *C. burnetii* (Muleme et al. 2016, Plummer et al. 2018, Larson et al. 2019).

Regarding the risk factors for exposure of cattle to *C. burnetii*, significant associations have been primarily linked with management practices (Carbonero et al. 2015, Nokhodian et al. 2016, Boroduske et al. 2017). In South Africa, the practice of communal kraaling at night may have contributed to the exposure of cattle to *C. burnetii* through milk, aerosol, and vectors. In our study, there was a general increase in the seropositivity to *C. burnetii* with age, but the difference was not statistically significant as reported by others (Knobel et al. 2013, Carbonero et al. 2015).

This study, to our knowledge, is the first documentation of the pathogen in livestock in the wildlife-livestock interface, which borders the largest wildlife park in the country. Of potential zoonotic relevance is the report that 61% of the herders and veterinary personnel associated with the cattle under study were positive for *C. burnetii* IgG antibodies (Simpson et al. 2018). The likely reason is that these personnel had close contact with the cattle on farms and at dip tanks where these animals congregate weekly for vector control and active surveillance for FMD. Human exposure to *C. burnetii* has been reported to occur through direct contact with milk, urine, feces, or semen from infected animals as well as inhalation of aerosolized particles from animal placentas, parturient fluids, aborted fetuses, and environmental dust (Tissot-Dupont and Raoult 2008, Vanderburg et al. 2014). It is known that *C. burnetii*, a zoonosis, can cause abortion in livestock resulting in economic losses through abortion and death of affected animals. It is currently unknown whether wildlife in the Kruger National Park are infected by *C. burnetii* or what risk of transmission of the pathogen at the wildlife-livestock-human interface exists in the area. It is pertinent to mention that Simpson et al. (2018) reported that febrile patients and workers (farmers, herders, and veterinary staff) associated with dip tanks had seroprevalence of Q-fever, 37.8% and 60.9%, respectively. Also, acute febrile patients at local clinics who had reported attending dip tanks were 9.39 times more likely to be exposed to Q-fever (Berrian et al. 2019). Considering that the seroprevalence to *C. burnetii* antibodies was detected to be highest (38%) of the five pathogens studied, there is therefore a need to conduct additional studies on cattle and humans at the interface to directly assess the risk posed by *C. burnetii*-positive cattle to human contacts.

In our study, the seroprevalence of antibodies to *T. gondii* was relatively high (33.2%) compared to the other pathogens tested for in the current study. It is possible that communal farm management system practiced in the study area, as well

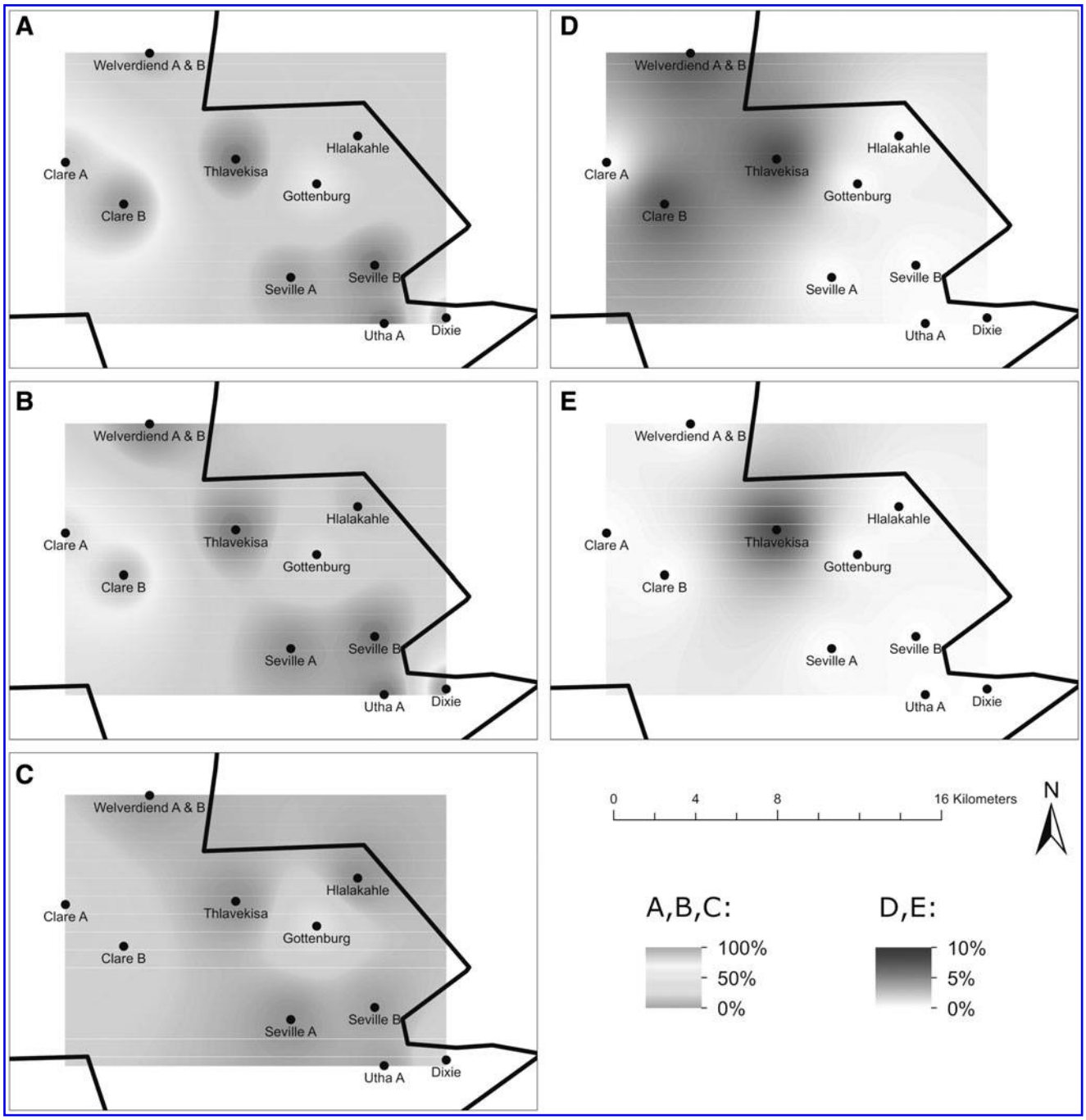

FIG. 2. Spatial distribution of seroprevalence to selected zoonotic and abortifacient agents in cattle at dip tanks in the Mnisi communal area: risk surfaces produced by interpolation using inverse distance weighting. **(A)** *Coxiella burnetii*; **(B)** *Toxoplasma gondii*; **(C)** *Chlamydophila abortus*; **(D)** *Neospora caninum*; **(E)** Rift Valley fever virus.

as the kraaling of cattle at nights have the potential to increase exposure to *T. gondii*, particularly to cats, known definitive host of the pathogen (Dubey 2008, Cenci-Goga et al. 2011). Although the study was not designed to determine cat demography, it is known that in these rural communities where the study was conducted, free-roaming (owned but not confined to houses) cats exist. The absence of cat control and the potential of cat access to animal feed may, in part, explain the high prevalence of *T. gondii* antibody detection. In South Africa, although *T. gondii* infections were earlier documented in sheep (Abu Samra et al. 2007), in free-roaming chacma baboons in Kruger National Park (McConnell et al. 1973), and recently in cats sampled in the Western Cape region (Hammond-Aryee et al. 2015), no published report exists on toxoplasmosis in cattle. Our study is therefore the first documentation of *T. gondii* infection in cattle in South Africa.

In our study, 20.7% of the cattle tested were seropositive for *C. abortus* and cattle with antibodies to the pathogen were detected in all 10 farming areas. This is an indication of the ubiquitous nature of the pathogen. The study showed age to

Table 3. Seroprevalence of Zoonotic and Abortigenic Pathogens in Cattle by Sex and Age

Variable	No. of cattle tested	No. (%) of seropositive				
		Coxiella burnetii	Toxoplasma gondii	Chlamydophila abortus	Neospora caninum	Rift Valley fever
Sex						
Female	132	49 (37.1)	46 (34.8)	31 (23.5)	2 (1.5)	1 (0.8)
Male	52	21 (40.4)	15 (28.8)	7 (13.5)	1 (1.9)	0 (0.0)
Age group (years)						
<1	37	11 (29.7)[a]	9 (24.3)[a]	2 (5.4)[a]	1 (2.7)[a]	0 (0.0)[a]
1–2	39	19 (48.7)[a]	14 (35.9)[a]	11 (28.2)[b]	1 (2.6)[a]	0 (0.0)[a]
>2–5	51	19 (37.3[a]	15 (29.4)[a]	8 (15.7)[ab]	0 (0.0)[a]	0 (0.0)[a]
>5	57	21 (36.8)[a]	23 (40.4)[a]	17 (29.8)[b]	1 (1.8)[a]	1 (1.8)[a]

Sex: no significant differences between males and females. Age: within columns, values with no superscripts in common differ significantly ($p < 0.05$; mixed-effects logistic regression model, adjusting for sex and location).

be positively associated with seropositivity for *C. abortus*, a finding which was expected since it has been established, and that exposure or infections by most pathogens in cattle increase with age corresponding with increasing exposure to the pathogen over time in the farm environment (Beechler et al. 2013, Cañón-Franco et al. 2014, Mugizi et al. 2015).

The last reports of chlamydophilosis in South Africa were in 1975 in domestic and laboratory animals (Pienaar and Schutte 1975). It was associated with neonatal deaths in beef herds (Ehret et al. 1975). Our study therefore provides the most recent data of the occurrence of exposure to the pathogen in South Africa and the first report in a wildlife-livestock interface.

It is of disease transmission relevance to have detected similar spatial distribution patterns and frequency of coinfections among the three high-seroprevalence pathogens (*C. burnetii*, *T. gondii*, and *C. abortus*) in our study, suggesting common risk factors for exposure of cattle to these pathogens. The high degree of clustering, particularly of *C. burnetii* and *T. gondii*, evidenced by the large variation in seroprevalence between locations and their high ICCs, is likely due to risk factors that vary geographically within the study area; these require further investigation. These three zoonotic pathogens, two bacteria (*C. burnetii* and *C. abortus*) and a protozoan parasite (*T. gondii*), also share common modes of transmission, mainly through ingestion, transplacental, and/or inhalation (Saeij et al. 2005, Frangoulidis and Fischer 2015, Szymańska-Czerwińska et al. 2015, Hireche et al. 2016, Heidari et al. 2018), which could have contributed to the similar patterns of occurrence in our study.

Table 4. Prevalence of Single and Multiple Infections

Pathogen	No. (%) of cattle positive[a]
Coxiella burnetii only	14 (7.6)
Chlamydophila abortus only	9 (4.9)
Toxoplasma gondii only	4 (2.2)
Rift Valley fever only	1 (0.5)
Neospora caninum only	0 (0.0)
C. burnetii and *T. gondii*	28 (15.2)
C. burnetii, *C. abortus* and *T. gondii*	24 (13.0)
C. abortus and *T. gondii*	3 (1.6)
C. burnetii and *C. abortus*	2 (1.1)
C. burnetii, *T. gondii* and *N. caninum*	1 (0.5)
C. burnetii and *N. caninum*	1 (0.5)
Negative[b]	96 (52.2)

[a]Of a total of 184 head of cattle from the 10 farming areas.
[b]Negative for all five pathogens tested.

It is evident that the seroprevalence of IgG antibodies to *N. caninum* in the cattle tested is very low (1.6%). Since the specificity of the test used in our study was reported by the manufacturer to be 99–100% and in other studies 94% (Wapenaar et al. 2007) and 93% (Alvarez-García et al. 2013), the possibility exists that the positive test results in our study may have been false positives. Since we were unable to confirm the results using a more specific test, they should be interpreted with caution; however, our investigation indicates that, if it is present, there is at most a low level of infection of cattle in the wildlife-livestock interface studied. The low seroprevalence of *N. caninum* in our investigation may reflect the low level of infection of cattle in the wildlife-livestock interface studied. Serologically, the pathogen had earlier been found in apparently healthy cattle and in aborted fetuses in South Africa (Jardine and Last 1995, Njiro et al. 2011). Since *N. caninum* is an important abortifacient, it is imperative that efforts be made to keep the infection rate in cattle low to reduce the possible exposure of the wildlife population in the neighboring Kruger National Park, where the agent has, to date, not been documented in the wildlife population.

Although the seroprevalence of antibodies to RVFV in the current study was very low (0.5%), the result is still significant as RVF is a reportable disease in South Africa. There is evidence of long-term, low-level circulation of RVFV in free-roaming African buffalo in the Kruger National Park (Beechler et al. 2013). In addition, a high seroprevalence and high rate of seroconversion to RVFV has recently been found in livestock adjacent to wildlife reserves in KwaZulu-Natal (Van den Bergh et al. 2019). The detection of antibodies to RVFV, although at a low seroprevalence, cannot be ignored, considering the potential economic impact of abortions and deaths of cattle and infection in human contacts as documented by others (Lernout et al. 2013, Oyas et al. 2018, Msimang et al. 2019). It may therefore be prudent to institute an active surveillance system for RVFV in the wildlife-livestock interface studied. The potential transmission of some of the five agents tested between the wildlife in

neighboring Kruger National Park and cattle in the wildlife-livestock interface exists. Three pathogens (*C. burnetii*, *C. abortus*, and *N. caninum*) have not been previously documented in the wildlife in the Kruger National Park, but they have been detected serologically in wildlife in other countries (Martino et al. 2004, Bandyopadhyay et al. 2009, Almería 2013, San-Miguel Ayanz et al. 2017, González-Barrio and Ruiz-Fons 2019). The possibility therefore exists that the three pathogens detected in the cattle tested may be transmitted to the wildlife population across the fence. Serological evidence of *T. gondii* (McConnell et al. 1973) and RVFV (Beechler et al. 2013, Fagbo et al. 2014) in wildlife in the Kruger National Park exists; further investigation into the possible transmission of these pathogens between livestock and wildlife is required.

Conclusions

The study confirmed the existence of antibodies to five pathogens in cattle reared in an area bordering the largest wildlife reserve in South Africa, the Kruger National Park. The pathogens, except for RVFV, are either documented for the first time in cattle in South Africa or at a wildlife-livestock interface for the first time in over 30 years. The fact that the three pathogens with the highest seroprevalence (*C. burnetii*, *T. gondii*, and *C. abortus*) are known abortifacients and zoonotic agents has important economic and zoonotic implications. Although *N. caninum* is not a confirmed zoonosis and had a low seroprevalence, it is a known abortifacient, and we have demonstrated its occurrence for the first time in cattle at the wildlife-livestock interface. The presence of these important pathogens in cattle at the wildlife-livestock interface indicates the need to institute a surveillance system in the area to monitor the potential for transmission of these pathogens to humans and wildlife.

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Address correspondence to:
Abiodun A. Adesiyun
Department of Production Animal Studies
Faculty of Veterinary Science
University of Pretoria
Pretoria
Onderstepoort 0110
South Africa

E-mail: abiodun.adesiyun@up.ac.za