Serological survey of *Brucella canis* in dogs in urban Harare and selected rural communities in Zimbabwe

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A cross-sectional study was conducted in order to detect antibodies for *Brucella canis* (*B. canis*) in dogs from urban Harare and five selected rural communities in Zimbabwe. Sera from randomly selected dogs were tested for antibodies to *B. canis* using an enzyme-linked immunosorbent assay. Overall, 17.6% of sera samples tested (57/324, 95% CI: 13.5–21.7) were positive for *B. canis* antibodies. For rural dogs, seroprevalence varied from 11.7% – 37.9%. Rural dogs recorded a higher seroprevalence (20.7%, 95% CI: 15.0–26.4) compared with Harare urban dogs (12.7%, 95% CI: 6.9–18.5) but the difference was not significant ($p = 0.07$). Female dogs from both sectors had a higher seroprevalence compared with males, but the differences were not significant ($p > 0.05$). Five and two of the positive rural dogs had titres of 1:800 and 1:1600, respectively, whilst none of the positive urban dogs had a titre above 1:400. This study showed that brucellosis was present and could be considered a risk to dogs from the studied areas. Further studies are recommended in order to give insight into the epidemiology of brucellosis in dogs and its possible zoonotic consequences in Zimbabwe. Screening for other *Brucella* spp. (*Brucella abortus*, *Brucella melitensis* and *Brucella suis*) other than *B. canis* is also recommended.

Introduction

Canine brucellosis, caused by *Brucella canis* (*B. canis*) was first discovered from episodes of abortion and reproductive failure in beagles in the USA in 1966 (Carmichael 1966). *Brucella canis* infection is a significant cause of reproductive failure in dogs worldwide (Wanke 2004). In pregnant bitches, the infection localises in the reproductive tract where it causes placentitis with subsequent abortions and stillbirths (Lopes, Nicolino & Haddad 2010). However, early embryonic deaths and resorption can occur a few weeks after mating and may be mistaken for failure to conceive (Lopes *et al.* 2010). Epididymitis, orchitis, testicular atrophy, poor sperm quality and infertility and loss of *libido* have been reported in male dogs (Carmichael & Kenney 1968; Hollett 2006). Despite being infected, many dogs in most cases remain asymptomatic and appear to be healthy (Behzadi & Mogheiseh 2011), but severe lymphadenitis involving the retropharyngeal and inguinal lymph nodes may be found (Wanke 2004). In humans, although infrequent, *B. canis* causes undulant fever (Ramacciotti 1980) or non-specific signs of recurrent fever, headache and weakness (Wallach *et al.* 2004). However, transmission to humans is reported to be rare, with only 30 cases documented worldwide since the isolation of *B. canis* in the late 1960s (Hollett 2006).

Infection due to *B. canis* is endemic in the southern states of the USA and South America but sporadic in Europe and Asia (Corrente *et al.* 2010). Except in Nigeria (Adesiyun, Abdullahi & Adeyanju 1986; Cadmus *et al.* 2011) and South Africa (Gous *et al.* 2005), there is dearth of information on canine brucellosis in Africa. The presumptive diagnosis of canine brucellosis is based on clinical signs and requires further confirmation through culture and isolation of the causative bacteria (Bae & Lee 2009). While culture and isolation is regarded as the gold-standard test for laboratory diagnosis of brucellosis, its sensitivity is low because the brucellae are fastidious micro-organisms that can easily be overgrown by contaminating bacteria. Thus, serological examinations are often used to detect evidence of exposure to *B. canis* since they are relatively easy to perform and may provide a practical advantage of estimating prevalence in populations (Bae & Lee 2009). Except for two *B. canis* isolations in two Harare dogs (Gomo 2013), the status of canine brucellosis in Zimbabwe is unknown. Hence, the objective of this study was to detect antibodies to *B. canis* in urban and rural dogs and to compare the prevalence in the two sectors. The baseline information obtained would provide a basis for future studies, management strategies and policies in controlling and preventing canine brucellosis in animals and humans.

Materials and methods

Study location and collection of serum samples

The study location and collection of serum samples has been described earlier by Dhliwayo *et al.* (2012) (Figure 1). Briefly, serum samples collected by the Animal and Wildlife Area Research and

Rehabilitation Trust (AWARE) from five rural communities (Kariba, Machuchuta, Malipati, Marumani, and Ndhlovu, Victoria Falls) during dog spay or castration campaigns were used for this study. The serum samples were collected just prior to ovariohysterectomy and orchidectomy from apparently healthy rural-owned and stray dogs with unknown medical histories. Serum samples from the Harare urban area were taken from dogs presented to private veterinary practices for routine elective surgery. All rural dogs were considered to be free-roaming, which allows contact with other dogs in the same village. In contrast, based on owners' information, urban dogs were reared in confinement in individual homes.

Testing for *Brucella canis* antibodies

The detection of IgG antibodies to *B. canis* in the collected dog sera (n = 324) was carried out using the *Brucella canis* ImmunoComb® Antibody Test Kit (also called a 'dot assay' or a modified enzyme-linked immunosorbent assay) (Biogal-Galed Laboratories, Israel) as previously described by Muhairwa *et al.* (2012). Except that for this test, a purified *B. canis* antigen is attached to the Comb, the procedure is similar to the test for canine *Leptospira* (Biogal-Galed Laboratories, Israel) described earlier by Dhliwayo *et al.* (2012). The results were read with a calibrated colour Comb Scale (graded S0 to S6), which was provided with the test kit. A scale of S3, which is equivalent to a positive immune response at a titre of 1:200 by an indirect fluorescent antibody (IFA) test, was considered as the 'cut-off' level of IgG antibodies (http://www.biogal.co.il). Hence, in this study, serum samples giving a Comb Scale score of ≥ S3 (≥ 1:200 titre) were considered to be positive for *B. canis* antibodies.

Data analysis

The overall *B. canis* seroprevalence was calculated from the total number of samples tested and expressed as a percentage. Seropositivity was examined in relation to location (urban vs rural) and sex (female vs male). The X^2–test was used to measure differences in proportions between categories and p-values of < 0.05 were considered significant. Association between seropositivity and location or sex was evaluated by calculating X^2, the relative risk (RR) and the 95% confidence interval (CI) using Win Episcope software (version 2.0).

Results

The distribution of sampled dogs and their *B. canis* seroprevalence according to different categories are shown in Table 1. A total of 324 dog serum samples were collected and the overall seroprevalence was 17.6% (95% CI: 13.5% – 21.7%). Overall, rural dogs recorded a higher seroprevalence (20.7%) compared with urban dogs from Harare (12.7%) but the difference was not significant (p = 0.07). No significant

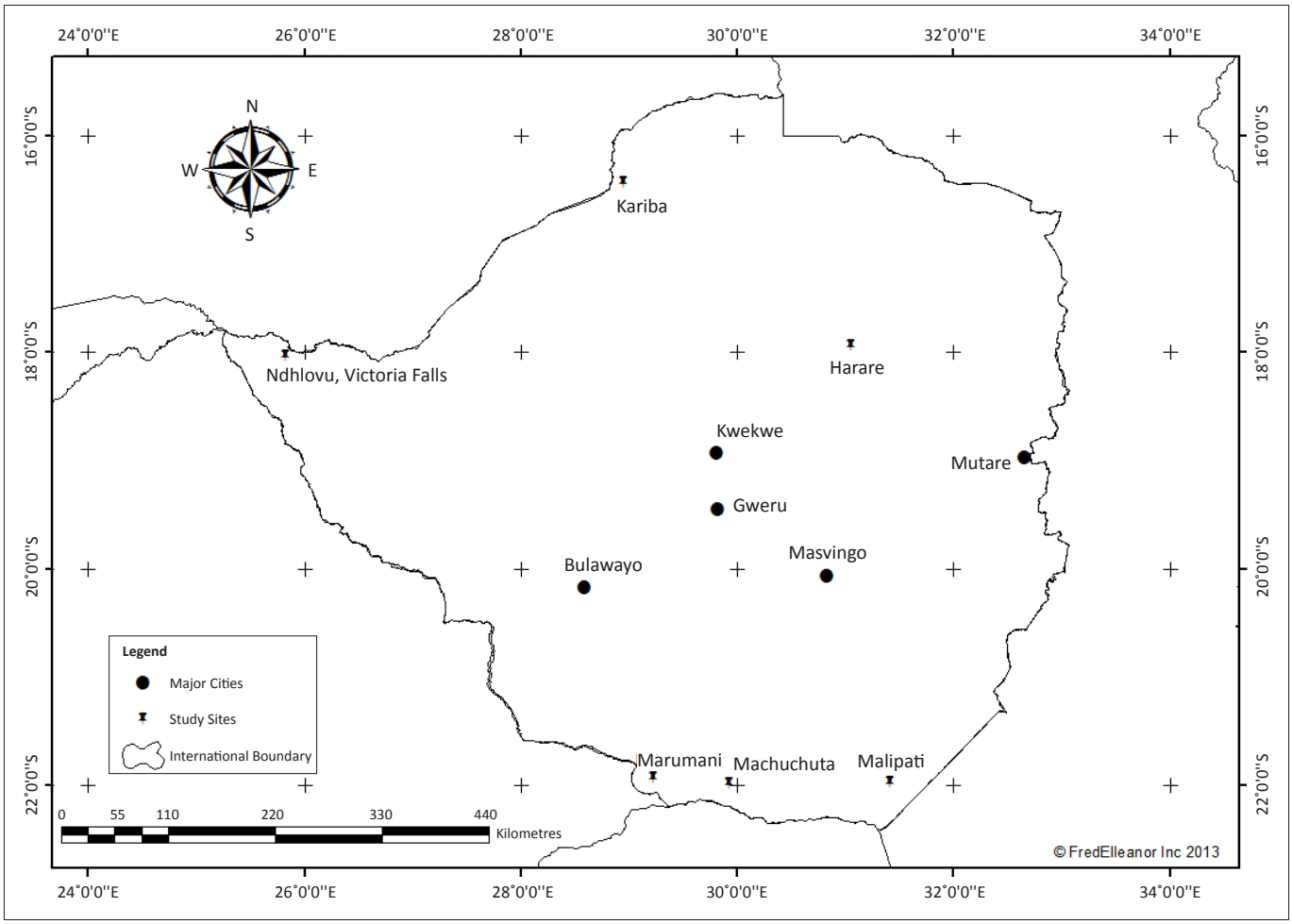

FIGURE 1: Map of Zimbabwe showing the sites where samples were collected.

(RR = 1.2, 0.9 < RR < 1.4, X^2 = 2.9, p = 0.09) association was recorded between *B. canis* seropositivity and location. For rural dogs, seroprevalence varied from 11.7% – 37.9%, with dogs from Machuchuta recording a significantly (p < 0.01) higher seroprevalence compared to those from Victoria Falls. Female dogs from both sectors had a higher seroprevalence compared with male dogs, but the differences were not significant (p > 0.05). Overall, the association between *B. canis* seropositivity and sex was not significant (RR = 1.1, 0.9 < RR < 1.5, X^2 = 0.7, p = 0.2).

The majority (64.9%) of the seropositive dogs had a titre of 1:200, whilst 22.8% of the positive dogs recorded a titre of 1:400 (Table 2). Five and two of the seropositive rural dogs had titres of 1:800 and 1:1600 respectively, whilst none of the seropositive urban dogs had a titre above 1:400 (Table 2).

Discussion

Bacteriological isolation and identification offers a definitive diagnosis of *B. canis* infection in dogs. However, bacteriological isolation is time consuming, difficult to perform and poses a health risk to personnel (Keid *et al.* 2007; Kim *et al.* 2006). For epidemiological studies to establish baseline data for canine brucellosis, serological tests of *B. canis* infection in dogs can be applied successfully without bacteriological methods (Flores-Castro *et al.* 1977). Rapid slide agglutination tests, 2-mercaptoethanol tube agglutination tests, indirect fluorescent antibody tests, agar gel immunodiffusion and enzyme-linked immunosorbent assays are some of the serological tests for *B. canis* that are routinely used (Wanke 2004). Serological cross-reactions between *B. canis* and other Gram-negatives are commonly detected using some tests, particularly the agglutination tests (Kim *et al.* 2006). In addition, titre variations between individual animals can occur when different tests are applied (Kim et al. 2006). Hence, to circumvent these problems, ELISA techniques including the 'dot-assay' that use purified species-specific antigens and/or monoclonal antibodies have been developed (Radojicic et al. 2001). Radojicic *et al.* (2001) found the dot-assay technique to be reliable and highly specific for the rapid detection of *B. canis* antibodies in dogs. Thus, given the high specificity of the 'dot-assay', the results observed in this study are likely to reflect a true exposure of the dogs to *B. canis* infection.

The present study provides the first serological evidence of *B. canis* infection in dogs in Harare urban areas and five selected rural communities of Zimbabwe. The survey shows that approximately 18% of the dogs studied had antibodies to *B. canis* by the ImmunoComb® Dot-ELISA test (Biogal, Israel). The ImmunoComb® Canine *Brucella* Antibody Test kit has a high sensitivity (98%) and a high specificity (93%) (http://www.biogal.co.il), thus reducing the possibility of false positive and false negative reactions. Despite the lack of test validation, antibodies to *B. canis* have been detected using a similar testing technique (Muhairwa *et al.* 2012; Radojicic *et al.* 2001). Therefore, since there is no vaccine for *B. canis* (Hollett 2006), the positive results obtained in the present study indicate exposure to *B. canis*. The very high titres (1:1600) observed in two of the rural dogs most likely point towards acute brucellosis at or around the time of sampling.

In Zimbabwe, there is limited information on canine brucellosis. However, two confirmed *Brucella* isolates were obtained by the Central Veterinary Laboratory from two dogs in Harare (Gomo 2013). One of the isolates was found to be the same genotype as the *B. canis* reference strain (REF RM6/66, Le Fleche *et al.* 2006), whilst the other could not be accurately assigned to a species, so was grouped with *B. canis* and *Brucellus suis* (*B. suis*) bv 3, 4 subcluster (Gomo 2013). The serological results of the present study indicate the presence of brucellosis in both urban and rural dogs. Given the isolation of *B. canis* in two Harare dogs, brucellosis could be considered to present a risk to dogs from the studied areas and further investigations are warranted. Furthermore, the

TABLE 1: Distribution of *Brucella canis* seroprevalence according to sex and location.

Category	Level	Number tested	Positive	Seroprevalence (%)	95% Confidence interval
All animals	Overall	324	57	17.6	13.5–21.7
Urban	Female	61	9	14.8	6.0–23.6
	Male	65	7	10.8	3.4–18.2
	Overall	126	16	12.7	6.9–18.5
Rural	Female	107	24	22.4	14.6–30.2
	Male	91	17	18.7	10.7–26.7
	Overall	198	41	20.7	15.0–26.4
Rural	Machuchuta	29	11	37.9	20.3–55.5
	Kariba	29	8	27.6	11.3–43.9
	Marimani	40	9	22.5	9.6–35.4
	Malipati	40	6	15.0	4.0–26.0
	Victoria Falls	60	7	11.7	3.7–19.7

TABLE 2: Distribution of *Brucella canis* seroprevalence according to location and titre.

Category	Number of positive samples	Titre 1:200		Titre 1:400		Titre 1:800		Titre 1:1600	
		n	%	*n*	%	*n*	%	*n*	%
Rural	41	23	56.1	11	26.8	5	12.2	2	4.9
Urban	16	14	87.5	2	12.5	0	-	0	-
Total	**57**	**37**	**64.9**	**13**	**22.8**	**5**	**8.8**	**2**	**3.5**

zoonotic risk of exposure of pet owners, dog handlers and veterinarians to *B. canis* cannot be overemphasised.

Although not conclusive, the data suggest that rural dogs have a higher brucellosis seroprevalence than their urban counterparts. In addition, a higher seropositivity was recorded in females and this agrees with earlier studies (Cadmus *et al.* 2011; Xiang *et al.* 2013). This has been attributed to the fact that if a single male dog is infected and mates with several females, it can transmit the infection through infected semen (Cadmus *et al.* 2011). Although the transmission of *B. canis* has been shown to occur through ingestion of contaminated material (Wanke 2004), sexual transmission is believed to be important since the organism is secreted in significant numbers in the semen of infected male dogs (Shin & Carmichael 1999). Previous studies demonstrated a higher prevalence of infection in stray compared with non-stray dogs (Chikweto *et al.* 2013; Fredrickson & Barton 1974; Lovejoy *et al.* 1976). Boebel *et al.* (1979), Brown *et al.* (1976), Thierrmann (1980) and Wooley *et al.* (1977) also reported outbreaks of brucellosis in stray dogs. In addition to natural mating, other sources of *B. canis* include the foetus, placenta, foetal fluids, urine, and vaginal discharges after an abortion or stillbirth. All rural dogs are considered to be free-roaming, thus they have contact with other dogs in the same village and this could probably place them at a greater risk of exposure to brucellosis. However, there is need for further studies to better understand the epidemiology and risk factors of canine brucellosis in urban, kennel and rural settings in the country.

Although *B. canis* is the main cause of canine brucellosis (Wanke 2004), *Bruella abortus* (*B. abortus*), *Brucella melitensis* (*B. melitensis*) and *B. suis* infections have also been reported in dogs (Baek *et al.* 2003; Barr *et al.* 1986; Cadmus *et al.* 2011; Forbes 1990; Hinic *et al.* 2010). Infection with *B. abortus* in dogs is associated with ingestion of aborted foetal tissue from infected livestock (Baek *et al.* 2003; Forbes 1990). The suspected role of dogs in spreading of *B. abortus* and *B. melitensis* to neighbouring herds, flocks and humans was reported (Baek *et al.* 2003). In Zimbabwe, *B. abortus*, *B. melitensis* and *B. suis* have been reported in livestock (Gomo 2013; Gomo *et al.* 2012; Matope *et al.* 2009; Mohan *et al.* 1996). During the present study, only *B. canis* was screened and further studies should screen for *B. abortus*, *B. melitensis* and *B. suis* to determine if dogs in the country are also exposed to these organisms.

Conclusions

The findings of this study should be viewed in the light of its limitations. Due to lack of clinical details of the studied dogs, limited conclusions can be drawn. In addition, no bacteriological confirmation of seropositive cases was done. Despite these limitations, the study showed the presence of *B. canis* seropositivity in both urban and rural dogs. The only reported *B. canis* isolation in Zimbabwe to date is that from two dogs in Harare. Further studies are recommended to give insight into the epidemiology of brucellosis in dogs and its possible zoonotic consequences in Zimbabwe. Such studies should include samples for serology as well as bacteriology from different categories of dogs originating from different ecological regions of the country. Screening for other *Brucella* species (*B. abortus*, *B. melitensis* and *B. suis*) other than *B. canis* is also recommended.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

D.M.P. (University of Zimbabwe) was the project leader and responsible for the study design, data analysis and reviewing the manuscript. G.M. (University of Zimbabwe) was the project co-leader and was responsible for the study design and manuscript writing. S.C. (University of Zimbabwe), S.D. (University of Zimbabwe), L.M. (AWARE Trust) and K.D. (AWARE Trust) were responsible for sample collection and testing, literature review and drafting the manuscript.

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