Estimating the prevalence of Brucellosis in cattle in Zimbabwe from samples submitted to the Central Veterinary Laboratory between 2010 and 2014

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Keywords
Brucellosis, Sero-prevalence, Surveillance, Zimbabwe, Zoonosis.

Summary
Brucellosis is a disease caused by bacteria belonging to the genus *Brucella*, which is endemic in sub-Saharan African countries, including Zimbabwe. Zimbabwe has a widespread dairy industry with peri-urban dairy establishments built in order to improve milk availability to rural communities. This study has been the first attempt to estimate the prevalence of brucellosis in Zimbabwe as a whole, using samples submitted to the Central veterinary laboratory between 2010 and 2014. A total of 156 farms were tested with Rose Bengal Test (RBT), Complement Fixation Test (CFT) and Milk Ring Test (MRT). Parallel testing was used to determine whether or not a farm was to be considered positive: 30.1% (95% C.I.: 23.5% - 37.8%) of the farms tested were found positive (47/156). Harare district had the highest number of sample submissions with 6 out of 17 farms testing positive (P = 37.5%; 95% C.I.: 18.4% - 61.7%). Awareness of milk-borne zoonoses is reportedly generally low in farmers (41.5%). This is even more the case in small-holder farmers who have higher likelihoods of selling or ingesting raw milk compared with dairy farmers. The results show the need to carry out surveillance of brucellosis in cattle in Zimbabwe to understand the spatial distribution of the disease in the country. This is particularly relevant given the zoonotic and economic implications of this disease.

Valutazione della prevalenza della Brucellosi bovina in Zimbabwe da campioni conferiti al Laboratorio Veterinario Centrale (CVL) dal 2010 al 2014

Parole chiave
Brucellosi, Sieroprevalenza, Sorveglianza, Zimbabwe, Zoonosi.

Riassunto
La brucellosi è una malattia sostenuta da batteri appartenenti al genere *Brucella*, endemica nei Paesi dell'Africa sub-Sahariana, tra cui lo Zimbabwe. Lo Zimbabwe ha un settore lattiero-caseario ampiamente diffuso con stabilimenti caseari periurbani costruiti per migliorare la disponibilità del latte alle comunità rurali. Questo studio rappresenta il primo tentativo di stimare la prevalenza della brucellosi in Zimbabwe, utilizzando campioni conferiti al Laboratorio Veterinario Centrale (CVL) dal 2010 al 2014. In totale sono state testate 156 aziende con test del Rosa bengala (RBT), test di fissazione del complemento (CFT) e Ring test su latte (MRT). Una interpretazione dei test in parallelo è stata usata per determinare se un'azienda doveva essere considerata positiva: il 30,1% (95% C.I.: 23,5% - 37,8%) delle aziende è risultato positivo (47/156). Il numero più alto di campioni conferiti proveniva dal distretto di Harare con sei aziende su diciassette risultate positive (P = 37,5%; 95% C.I.: 18,4% - 61,7%). La consapevolezza delle zoonosi trasmesse mediante latte e prodotti derivati è piuttosto bassa negli allevatori e soprattutto in quelli di aziende di piccole dimensioni che hanno maggiori probabilità di vendere o consumare latte crudo. I risultati di questo lavoro dimostrano la necessità di programmare la sorveglianza della brucellosi bovina in Zimbabwe per comprendere la distribuzione spaziale della malattia nel Paese. Ciò è particolarmente rilevante in considerazione delle implicazioni zoonotiche ed economiche di questa malattia.

Introduction

Brucellosis is a contagious disease caused by gram negative, facultative, intracellular bacteria belonging to the genus *Brucella*. The agent is classified by the World Organisation for Animal Health (OIE) as a Risk group 3 pathogen, requiring a minimum of biocontainment level 3 (OIE 2009).

The disease affects domestic ruminants, horses, dogs, some wildlife species, marine mammal species, and human beings (Gomo *et al.* 2012). There is an epidemiological link among wildlife, domestic animal, and human brucellosis, (Godfroid *et al.* 2013), although mechanisms and factors affecting the transmission among species are still subject to research; particularly the effect of captive wildlife on the disease epidemiology (Godfroid 2002).

The main clinical signs in domestic livestock include late abortions, reproductive failure, orchitis, epididymitis, and hygromas (Nicoletti 2013). The disease also has important economic consequences, due both to the trade bans of live animals or commodities in areas where it is endemic and not controlled and to the production losses that occur in livestock following outbreaks.

Brucellosis causes several symptoms in human beings including undulating fever, headaches, weakness and weight loss (Nicoletti 2013). Among *Brucella* species, the most pathogenic for humans is *Brucella melitensis*, followed by *Brucella abortus* and then by *Brucella suis* (Acha and Szyfres 2003). The zoonotic nature of the marine *Brucella* (*Brucella ceti*) has been documented in several studies (Brew *et al.* 1999, Sohn *et al.* 2003).

Brucellosis is suspected when clinical signs are evident (*i.e.* abortion); however, laboratory diagnosis or bacteriological investigation are essential for confirmation of the infection. Several tests are available for serological diagnosis, but not all of them are approved for international trade. Currently, the tests prescribed for international trade of cattle are the Rose Bengal test (RBT), the Buffered Plate Agglutination test (BPAT), the ELISAs, the Complement Fixation test (CFT), and the Fluorescence Polarisation assay (OIE 2009).

Some species of *Brucella* (*B. abortus, B. melitensis* and *B. suis*) have several biovars, which can be differentiated through culture, serology and polymerase chain reaction (PCR) (Godfroid *et al.* 2013, OIE 2009). Studies carried out in Zimbabwe revealed the presence of *B. abortus* biovar 1 and biovar 2, and also the presence of *B. melitensis* biovar 1 (Matope *et al.* 2009).

Brucellosis is widespread in the world (Benkirane 2006), but its prevalence varies considerably depending on the area and farming systems concerned (Matope *et al.* 2009). Several countries have been successful in eradicating this disease. While prevalence is steadily decreasing in some countries, especially those that benefited from financial support from various sponsors, a dramatic increase of the prevalence is being reported in the Gulf countries and, to some extent, in the rest of the South Mediterranean area (Benkirane 2006). The disease is also still endemic in many African countries (Faye *et al.* 2005). Between 1996 and 2003, Brucellosis has been reported in Angola, Burkina Faso, Ethiopia, and Kenya (Benkirane 2006).

In sub Saharan African countries, including Zimbabwe, brucellosis is endemic (Karimuribo *et al.* 2007). Its importance might be higher in extensive and pastoral farming systems, because it is more difficult to control (Matope *et al.* 2009). Zimbabwe has a widespread dairy industry, although most establishments are still in urban and peri- urban areas. Dairy establishments were built in districts outside urban areas to improve milk availability in remote areas (Matope et al 2009).

Historically, brucellosis control was given more importance in commercial rather than in communal farming systems in Zimbabwe (Madsen 1989, Mohan *et al.* 1996). A contagious abortion (CA) accreditation scheme for the control of brucellosis in Zimbabwe started in 1982 on a voluntary basis with the aim of controlling and, eventually, eradicating the disease from dairy herds around the country (Borland and Moyo 1995). The accreditation scheme became compulsory for all commercial producers since 1991. Under this scheme, all female dairy cattle that were at least 18 month old were bled 3 times a year. If all 3 bleedings were negative, upon the fourth negative result, the farmer would receive an accreditation certificate. Accreditation certificates were renewed annually under the condition that bulk milk samples submitted to the laboratory for the Milk Ring test (MRT) every 3 months were negative over 1 year (Reg. 1995). Farmers with accreditation certificates were offered higher premiums for their milk by the industry (Reg. 2004).

Research has been carried out in the past in Zimbabwe to estimate the prevalence of Brucellosis at village or district level, as prevalence varies particularly in communal areas due to restocking from commercial herds (Gomo *et al.* 2012, Matope *et al.* 2009). However, no survey for estimating the prevalence of the disease in the country has been carried out following the agrarian reform of 1999 to 2000; when there was an increase in cattle movement across the country and following a period during which the Brucellosis Accreditation Scheme (BAS) was not fully enforced.

This paper describes the results of a preliminary survey for the estimation of the prevalence of

brucellosis in the country. The survey is based on samples submitted to the Central Veterinary Laboratory (CVL) over a period of 5 years (2010-2014).

Materials and methods

Study areas

Zimbabwe is divided into 8 administrative provinces, namely: Mashonaland East, Mashonaland West, Mashonaland Central, Matebeleland North, Matebeleland South, Masvingo, Midlands, and Manicaland. These administrative provinces are further divided into 60 districts.

This retrospective study was carried out using samples collected from these provinces over 5 consecutive years (2010-2014). Among the 8 provinces, no samples were received from the 19 districts listed below during the study period, thus this districts are not represented in Table I:

- Manicaland province: Buhera, Chimanimani;
- Mashonaland Central province: Rushinga, Mount Darwin;
- Mashonaland East province: Murehwa, Mutoko, Mudzi, UMP;
- Mashonaland West province: Kariba;
- Masvingo province: Chivi, Zaka, Gutu;
- Matebeleland North province: Hwange, Tsholotsho;
- Matebeleland South province: Bulilima, Beitbridge;
- Midlands province: Gokwe North, Zvishavane, Mberengwa.

Study population

Zimbabwe has an estimated cattle head census of 5,388,187 (Department of Veterinary Services annual report, 2012; unpublished data) (Figure 1). The estimated number of dairy herd is 30,000 (registered dairy operators). Cattle husbandry ranges from intensive commercial farming to extensive pastoral farming in small-holder and communal areas. The vaccination history of cattle is mostly unknown. Despite dairy farms are required by legislation to vaccinate calves between 3 and 9 months of age using the *B. abortus* Strain 19 vaccine, (Matope *et al.* 2011), the proportion of farms following this prespriction is unknown. Both S19 and RB51 vaccines are available.

The total number of samples from cattle submitted to the CVL of Harare was 12,359. Among them, 12,240 (99.0%) came from the testing of dairy herds under the Brucellosis accreditation scheme, which is being restored; while 119 (1.0%) samples were received from communal farmers on a voluntary basis mostly following abortion storms. Milk samples were collected from registered dairy farms, in line with the Brucellosis accreditation scheme, by which they are requested to submit bulk milk samples every three months (Reg. 2004). A number ranging between 50 and 75 animals contributed on average to each bulk milk tank (Figure 2).

Sample collection

The samples were submitted to the CVL for different purposes within the study period. Blood was collected by veterinary field personnel in plain tubes, stored between 0 and 4 °C and transported to the CVL in Harare for testing. All milk samples were collected in sterile bottles from bulk milk tanks, stored between

Table I. *Percentage of cattle farms positive to Brucellosis in Zimbabwe according to the provinces of origin.* Harare and Bulawayo were reported separately due to the relatively high number of farms tested.

Province	Number of positive farms	Number of tested farms	% Positive	95% Lower Confidence Level	95% Upper Confidence Level
Bulawayo	3	3	100.0%	39.8%	99.4%
Harare	6	16	37.5%	18.4%	61.7%
Manicaland	1	6	16.7%	3.7%	57.9%
Mashonaland Central	3	10	30.0%	10.9%	61.0%
Mashonaland East	1	44	2.3%	0.5%	11.8%
Mashonaland West	2	15	13.3%	4.0%	38.3%
Masvingo	5	16	31.3%	14.2%	56.0%
Matebeleland North	5	7	71.4%	34.9%	91.5%
Matebeleland South	12	16	75.0%	50.1%	89.7%
Midlands	9	23	39.1%	22.1%	59.4%
Total	**47**	**156**	**30.1%**	**23.5%**	**37.8%**

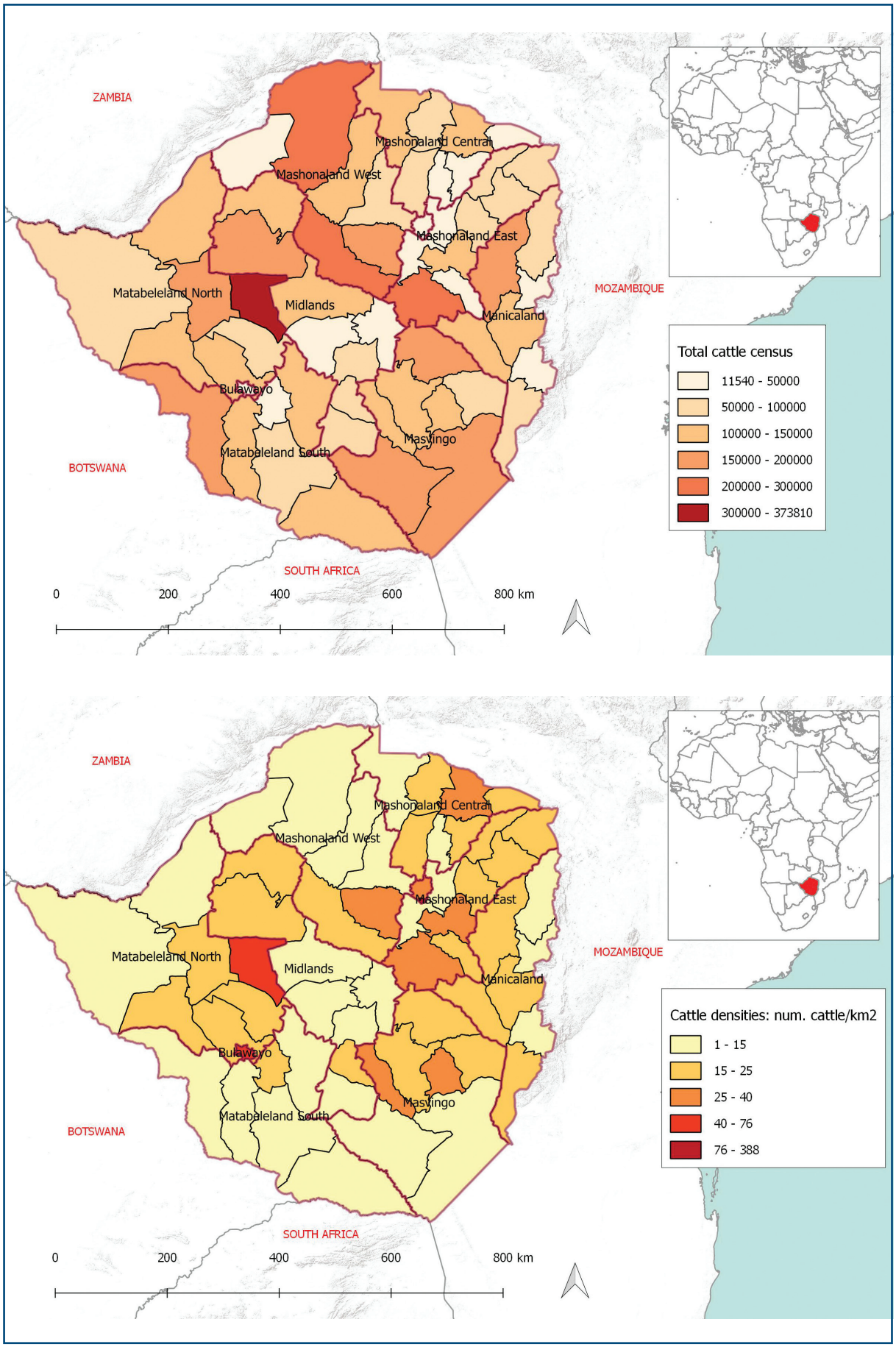

Figure 1. *Distribution and density of cattle per district in Zimbabwe.* The darker coloured districts have the highest cattle densities.

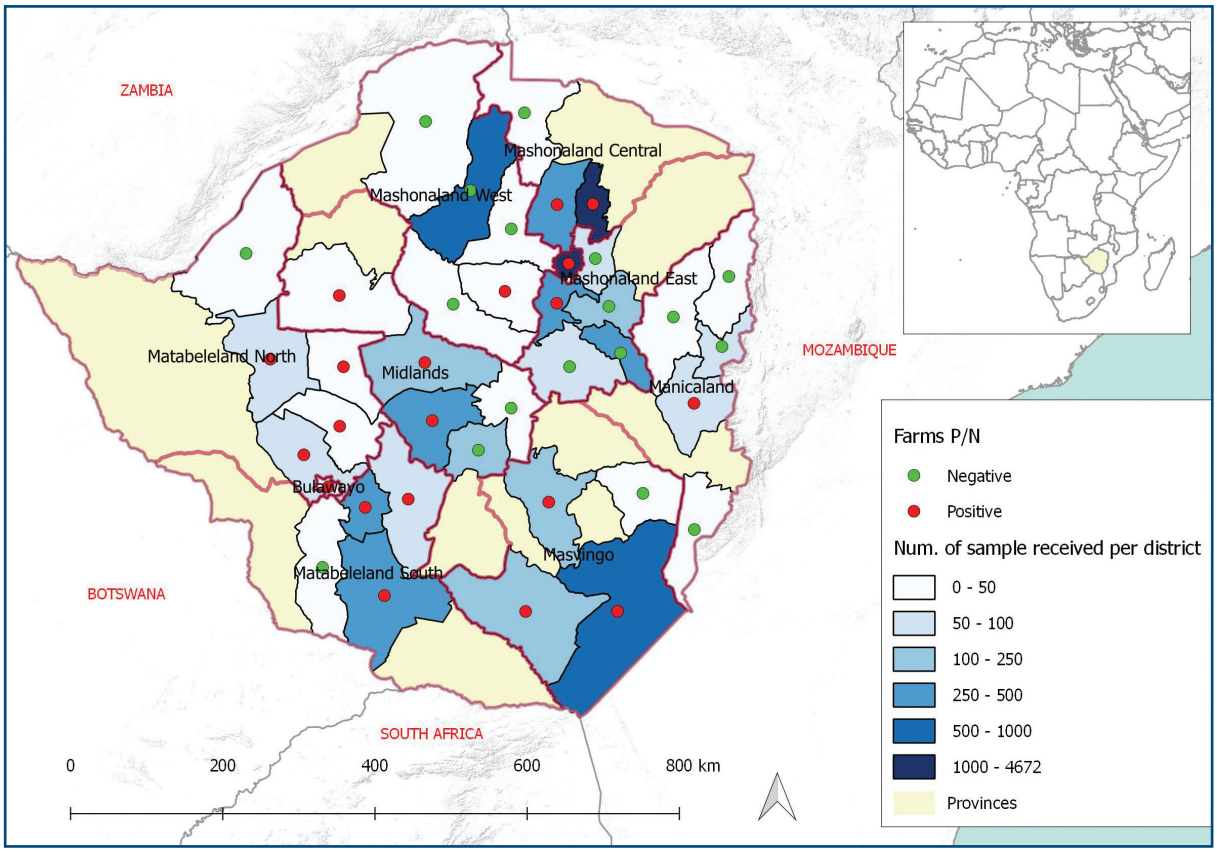

Figure 2. *Distribution of the number of samples per district and the presence of positive* Brucella *farms per district.* Light yellow areas indicate the 19 districts from where no samples were received. Harare, which had the highest number of sample submissions, had 6 out of a total of 16 farms testing positive (P = 37.5%; 95% C.I.: 18.4%-61.7%).

0 and 4 °C and transported to the laboratory for testing. Milk samples were collected routinely by diary services personnel, who collect bulk milk tank samples from commercial farms 3 times a year.

Sample testing

Parallel testing was used to determine whether or not a farm was to be considered positive. If at least one bulk milk sample during the 5 years tested positive and/or serology of an individual animal revealed a positive reaction during the previous 5 years, the farm was considered positive.

Milk samples were tested using the milk ring test (MRT). Any positive sample reaction was considered as being suggestive of herd infection.

Approximately 2 ml of serum were harvested from each plain tube containing clotted blood into cryotubes following centrifugation at 2500 rpm for 15 minutes. The serum was usually immediately tested, or stored at - 20 °C while awaiting testing. Most serum samples (68.2%) were first tested using the rose Bengal test (RBT) to screen for antibodies against *Brucella*. Due to unavailability of the RBT test at the CVL during a certain time within the 5 years of this study, 2,569 serum samples (31.8% of the total serum samples) were tested using only the complement fixation test (CFT) (South African National Accreditation System - SANAS - accredited). Rose Bengal test - Complement Fixation test serial testing was performed for most RBT positive samples (815/843; 96.7%). A positive serology reaction was considered as showing positivity in an animal.

All test methods and procedures were adapted from the manual of standards for diagnostic tests and vaccines (OIE 2008). *Brucella abortus* antigen was acquired from Onderstepoort Biological products (OBP) (Pretoria, South Africa). Guinea pig complement was acquired from CVL stabled laboratory animals. Haemolytic serum was acquired from bioMérieux, (Midrand, South Africa); while the national reference standards (positive and negative serum control) were from CVL stores.

Data analysis

Sample information and results were entered into a Microsoft® Access database (MS-Access 2013). Microsoft® Excel (MS-Excel 2013) was used to make the descriptive analysis of the data. Maps were produced using Quantum GIS software (QGIS 2.6.1.).

Table II. *Number of samples tested for bovine brucellosis per test.* CFT was used to test more samples at the CVL during the study period.

Test	Number of positive samples	Number of tested samples	% Positive	95% Lower Confidence Level	95% Upper Confidence Level
MRT	72	4,320	1.7%	1.3%	2.1%
RBT	843	4,220	20.0%	18.8%	21.2%
CFT	2,137	6,276	34.1%	32.9%	35.2%

Results

A total of 12,359 samples were tested from 41 districts (8,079 blood/serum samples and 4,280 milk samples). One-hundred and fifty-six farms submitted samples for testing. Of these, 144 were small to large-scale dairy establishments, while 12 were communal farms. Samples submitted per district ranged from 1 (Guruve) to 4,672 (Harare), with an average of 328 samples per district. Parallel testing was used to determine whether or not a farm was to be considered positive. Results are shown in Table II.

Discussion

The data analysed were collected over 5 years, and this poses a possible bias due to the differing number of samples sent per year. Sample flow to the CVL generally increased from 2010 to 2014. Hence, to capture the most submissions per district, all the years were included in the analysis. The herd prevalence of 30.1% might be high because of the bias in sample collection: some of the analysed data was from samples collected for diagnostic purposes and may be difficult to interpret them has surveillance data. Also, according to the clinical history filled in for the samples in the specimen submission forms, some of the serum samples were collected from aborted cows or cows that had contributed to a positive bulk milk tank analysis using MRT.

Cows with a history of abortion were considered more likely of being sero-positive (Matope *et al.* 2011). The use of parallel testing instead of serial testing to determine whether or not a farm was to be considered positive was due to the fact that some samples/farms were tested using just one test. There is likely an overestimation of prevalence in CFT test results, as most samples tested using CFT were for confirmatory purposes after a positive RBT test or following abortion storms. Underestimation of prevalence is also possible because of the relatively low specificity of MRT.

However, previous studies carried out in the country (before 1989 and between 1992 and 1996) suggested that the prevalence of the disease in commercial herds generally ranged from 10.0-53.0%; whereas in communal or smallholder farms it ranged between 0.0 and 16.0% (Madsen 1989, Mohan *et al.* 1996). More recent studies in Chiredzi district suggest a sero-prevalence of 8.3% in communal cattle (Gomo *et al.* 2012). Other studies show a range in herd prevalence between 8% (95% CI: 0.0 %, 18.9 %) to 40% (95 % CI: 22.1%, 58.0 %) in smallholder dairy farms in 6 selected areas of the country (Matope *et al.* 2011). Matope and colleagues (Matope *et al.* 2011) established that cows between 2 and 4 years of age have a higher risk of being sero- positive than cows older than 7 years. Bulk MRT samples are likely to have been drawn mostly from high milk producing cows in commercial farms. However, risk factors such as age and vaccination status could not be established in this study. Vaccination using *B. abortus* S19, which is known to interfere with brucellosis diagnosis, can also over-estimate prevalence due to the persistence of antibodies following vaccination (Dorneles *et al.* 2015). Different areas have different management systems and are likely to have also different prevalence rates for brucellosis (Matope *et al.* 2011).

An average national herd prevalence of 30.1% is therefore possible, although it cannot be concluded from the results of this study. The results of this paper clearly show the need to carry out sero-surveillance for brucellosis in cattle in Zimbabwe, in order to understand the spatial distribution of the disease in the country. This is particularly important given the zoonotic and economic implications of Brucellosis. Awareness of milk-borne zoonoses was found to be generally low among farmers (41.5%), and more-so in small holder farmers who have higher likelihoods of selling or ingesting raw milk compared to dairy farmers (Mosalagae 2011). It is, therefore, critical that during the resuscitation of the BAS, small holder farmers will be encouraged to take part in the scheme.

References

Acha N.P. & Szyfres B. 2003. Zoonoses and communicable diseases common to man and animals., 3rd ed., Vol. 1. Pan American Health Organization (PAHO), Washington, DC.

Benkirane A. 2006. Ovine and caprine brucellosis: World distribution and control/eradication strategies in West Asia/North Africa region. *Small Ruminant Res*, **62**, 19-25.

Borland P. & Moyo H. 1995. FAO Workshop Report: Regional exchange network for market oriented dairy development. Section II: Country papers on dairy development and status of the dairy sector Zimbabwe dairy sector: milk production, marketing and processing. http://www.fao.org/docrep/004/w3199e/W3199E02.htm/.

Brew S.D., Perrett L.L., Stack J.A., MacMillan A.P. & Staunton N.J. 1999. Human exposure to *Brucella* recovered from a sea mammal. *Vet Rec*, **144**, 483.

Dorneles E.M.S., Sriranganathan N. & Lage A.P. 2015. Recent advances in *Brucella abortus* vaccines. *Vet Res*, **46**, 76. doi: 10.1186/s13567-015-0199-7.

Faye B., Castel V., Lesnoff M., Rutabinda D. & Dhalwa J. 2005. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). *Prev Vet Med*, **67**, 267-281.

Godfroid J. 2002. Brucellosis in wildlife. *Rev Sci Tech*, **21**, 277-286.

Godfroid J., Garin-Bastuji B., Saegerman C. & Blasco J.M. 2013. Brucellosis in wildlife. *Rev Sci Tech*, **32**, 27-42.

Gomo C., De Garine-Wichatitsky M., Caron A. & Pfukenyi D. 2012. Survey of brucellosis at the wildlife livestock interface on the Zimbabwean side of the Greater Limpopo Transfrontier Conservation Area. *Trop Anim Health Prod*, **44** (1), 77-85.

Karimuribo E.D., Ngowi H.A., Swai E.S. & Kambarage D.M. 2007. Prevalence of brucellosis in crossbred and indigenous cattle in Tanzania. *Livestock Research for Rural Development*, **19**, 148.

Madsen M. & Anderson E.C. 1995. Serologic survey of Zimbabwean wildlife for brucellosis. *J Zoo Wild Med*, **26** (2), 240-245.

Madsen M. 1989. The current status of brucellosis in Zimbabwe. *Zimbabwe Vet J*, **20**, 133-145.

Matope G., Bhebhe E., Muma J.B., Oloya J., Madekurozwa R.L., Lund A. & Skjerve E. 2011. Seroprevalence of brucellosis and its risk factors in cattle from smallholder dairy farms in Zimbabwe. *Trop Anim Health Prod*, **43**, 975-979.

Matope G., Bhebhe E., Muma J.B., Skjerve E. & Djonne, B. 2009. Characterization of some *Brucella* species from Zimbabwe by biochemical profiling and AMOS- PCR. *BMC Research Notes*, **2**, 261.

Mohan K., Makaya P.V., Muvavarirwa P., Matope G., Mahembe E. & Pawandiwa A. 1996. Brucellosis surveillance and control in Zimbabwe: bacteriological and serological investigation in dairy herds. *Onderstepoort J Vet Res*, **63**, 47-51.

Mosalagae D., Pfukenyi D.M. & Matope G. 2011. Milk producers' awareness of milk- borne zoonoses in selected smallholder and commercial dairy farms of Zimbabwe. *Trop Anim Health Prod*, **43**, 733-739.

Nicoletti P. 2013. Bovine brucellosis. *In* The Merck veterinary manual online. http://www.merckvetmanual.com/reproductive-system/brucellosis-in-large-animals/brucellosis-in-cattle.

Regulations of the Animal Health Act (Reg.) 1995. Animal Health (Brucellosis) Department of Veterinary Services, Zimbabwe.

Regulations of the Animal Health Act. Amendment N.2 (Reg.) 2004. Animal health (Brucellosis). Department of Veterinary Services, Zimbabwe.

Sohn A.H., Probert W.S., Glaser C.A., Gupta N., Bollen A.W., Wong J.D., Grace E.M. & McDonald W.C. 2003. Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. *Emerg Infect Dis*, **9**, 485-488.

World Organisation for Animal Health (OIE). 2008. Manual of the diagnostic tests and vaccines for terrestrial animals. Vol. 1, 5th Edition. Office International des Epizooties, Paris, OIE, France, 409-438.

World Organisation for Animal Health (OIE). 2009. Bovine brucellosis. OIE Terrestrial Manual Chapter 2.4.3. Paris, OIE.