 doi:10.20944/preprints202011.0318.v1

Article

Seroprevalence of bovine brucellosis in selected districts of Zambia

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Abstract: Brucellosis is an infectious zoonosis that has huge economic and public health implications globally. The disease is prevalent in humans, livestock and wildlife in Sub Saharan Africa. A cross-sectional study was conducted between 6th May 2017 and 31st July 2020 during which 1712 sera from 177 cattle herds in Southern, Western and Eastern provinces of Zambia was collected and screened against brucellosis. Rose Bengal Test and c-ELISA were used in serial testing for detection of antibodies against *Brucella* species. **Results**: A total of 127 animals and 37 herds tested positive, giving an overall individual and herd seroprevalence of 7.42% (CI: 0.61-0.86) and 20.9% respectively. Namwala district recorded the highest seroprevalence (12.2%) while Lundazi had the lowest (0%). A higher seropositivity was observed among female animals (8.5%), those aged between 11 and 17 years (14.1%) and pregnant cows (13.8%). **Conclusions**: *Brucella* seroprevalence among traditional cattle in Zambia remains high. It is vital that control programmes against bovine brucellosis are introduced in order to reduce its zoonotic impact on human health and increase animal production.

Keywords: Cattle; brucellosis; seroprevalence, Zambia

1. Introduction

Brucellosis is an infectious zoonotic disease of economic and public health importance that affects livestock, wildlife and humans worldwide [1]. The disease is caused by a gram-negative cocco bacilli bacterium of the *Brucella* genus, which currently contains 12 host specific species [2]. The main cause of brucellosis in cattle is *brucella abortus*, however, other species have been isolated [3-5]. The associated clinical signs include abortions, infertility, reduced milk production, calf mortality, hygroma, epididymitis and orchitis [6]. Transmission between animals is through contact with aborted foetus, placenta, vaginal discharges and milk from infected animals. Reduction in animal reproduction and production contributes to serious economic losses in the livestock industry, while humans are at risk of *Brucella* infections through occupational exposure to animals and consumption of unpasteurized milk [7]. This underscores the public health importance of the disease.

Diagnosis of brucellosis is done using bacteriological, molecular and serological methods [1]. Bacteriological tests are highly specific and confirmatory compared to other methods and are therefore referred to as the "Gold standard" [1,3]. In order to increase the likelihood of isolating the *Brucella* organisms, bacteriological samples collected for culture often include milk [8], hygroma fluids [9], foetal materials and vaginal discharges up to six weeks post abortion or parturition [1,10]. Serology tests detect antibodies directed against epitopes associated with the smooth lipopolysaccharide (S-LPS). The common serology tests used are Rose Bengal Test (RBT), Serum Agglutination Test (SAT), Compliment Fixation Test (CFT), Milk Ring Test (MRT) and Enzyme-linked Immuno Assay (ELISA) [11]. These tests are recommended by the World Organisation for Animal Health [1]. In brucellosis sero-diagnosis, serial tesing is recommended in order to increase the testing strategy (Godfroid et al., 2010). The common serial test combination uses RBT and cELISA for screening and confirmation, respectively.

Most developed countries have managed to eradicate bovine brucellosis [12]. However, it is still endemic in most Sub Saharan African countries at varying seroprevalence [13-18]. In Southern Africa, the accurate brucellosis picture is not clearly known because most reports are based on non-representative laboratory results. In South Africa for instance, a 5.5 % seroprevalence reported recently by [19] showed that the disease control scheme introduced in 1968 had an impact in reducing the disease prevalence. In Namibia, brucellosis is endemic in communal herds and commercial farms in low proportions **[**20**].** In Zimbabwe, despite implementing vaccination programme, brucellosis prevalence varies across provinces with the latest report indicating a 30.1% herd seroprevalence ([18], while Angola reported a 40.1% in cattle herds ([21]. In Malawi, [22] reported a seroprevalence of 7.7% in dairy cattle in the northern region that borders with the Eastern Province of Zambia.

In Zambia, a few studies that were done in wildlife and cattle in Southern and Western provinces almost eight years ago estimated the disease seroprevalence in smallholder and traditional cattle [23-26] while the status is unknown in Eastern Province and other parts of the country. Therefore, this study aimed to estimate the seroprevalence of bovine brucellosis in Southern, Western and Eastern provinces in order to fill the knowledge gap on the current epidemiological status of the disease in the high cattle farming areas of Zambia. Such information is vital in mapping and understanding the epidemiological situation of the disease that can be used in the control of brucellosis in Zambia and other counties in the region with similar settings.

2. Materials and Methods

This cross-sectional study was carried out in five purposively selected districts of Namwala, Choma and Monze (Southern), Senanga (Western) and Lundazi (Eastern) of Zambia. Southern province is a highly productive cattle rearing area that lies between latitudes 16^0S and 30^0S and longitudes 27^0E and 00^0E, it has a total land surface area of 85,283 Km^2, an estimated human population of 1907,784 ([27] and a cattle population of 2,105,891 [28]. Pastoral or nomadic cattle - grazing system is practiced, where animals are grazed in the Kafue flats/flood plains in dry seasons and moved to upper areas during the wet season [24]. Western province lies between latitudes 14^0S and 17^0S and longitudes 22^0E and 25^0E and has a total land area of about 126, 386 km^2. The province has a human population of 1007,855 ([27] and an estimated cattle population of 890,288 [28]. Western province has dominant sandy soils and the Barotse floodplain of the Zambezi River which naturally waters the grasslands. Over three quarters of the cattle in Western Province are pastured in the floodplain. They are managed under a system of transhumance and move between the floodplains (January to July) and adjacent uplands (rest of the year) [29]. Pastoral livestock farming is the mainstay of the province's economy followed by fish and crop farming. The five districts were purposively selected proportional to the size of cattle population from each province. The three

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provinces were purposively selected because they have higher cattle populations in Zambia. Livestock lists obtained from the veterinary offices were used to proportionately estimate the number of cattle to be sampled in veterinary camps of each study district in respective provinces.

The required sample size was calculated using the formula $n = z^2 * p * (1 - p) / d^2$; Where: z=1.96, p= expected herd seroprevalence of 32% [14] d= desired absolute precision of 10% and confidence level of 95%. The resulting sample size of 84 was multiplied by the design effect (D) of 1.9 calculated using the formula D = 1+ (b-1) roh [30]. The average number of samples per cluster (b) was 10 and intra cluster correlation coefficient or rate of homogeneity (roh) was 0. 1 [31]. The final sample size was 160 herds. At least 10 animals were expected to be sampled per herd, bringing the total sample size of cattle to 1600, whereby 100, 30 and 30 herds were sto be ampled in Southern, Western and Eastern provinces respectively giving a total herd size of 160.

A total of 1,712 cattle was sampled, whereby 152 herds were sampled in Southern (96), Western (30) and Eastern (26) provinces respectively. 5 ml of blood was aseptically collected from the jugular vein into labelled sterile plain vacutainer tubes. In the field, serum was separated using a portable field centrifuge and stored in pre-labelled cryovial tubes at -20°C until transported to UNZAVET for laboratory for analysis. All serum samples were screened for *Brucella* spp. antibodies using The Rose Bengal test kit (Central Veterinary Laboratory, New Haw, Addelestone Surrey KT153NB, UK) according to the test procedure recommended by the OIE Terrestrial Manual ([1]. A sample was considered positive if any visible sign of agglutination was observed. All positive sera were retested using competitive Enzyme-Linked Immunosorbent Assay (cELISA) using the (INGEZIM BRUCELLA COMPAC 2.0, Spain) test kit as per manufacturer's instuctions and as described by [14]. Samples were considered positive for brucellosis if they tested positive on both RBT and cELISA.

Data analysis

Data were entered into a spread-sheet (Microsoft Excel 2010 version, Redmond, USA), proportions were estimated for the individual and herd brucella prevalence. The individual animal seroprevalence was calculated by dividing the number of RBT and c-ELISA positive animals by the total number of animals that were tested. Herd level prevalence was calculated by dividing the number of herds with at least one reactor on RBT and c-ELISA by the number of all herds tested. Associations between hypothesised risk factors and the outcome variables were assessed and statistical analysis was computed using Chi-square test and logistic regression using statistical software STATA version 16. The $p < 0.05$ was considered as a level of significance.

3. Results

A total of 129 animals from 37 herds were seropositive giving the overall individual animal (Table 1) and herd (Table 2) levels district brucellosis seroprevalence of 7.54% (CI: 0.62-0.87) and 21.1% (0.15-0.27) respectively. Namwala district had the highest herd seroprevalence (33.9%, CI: 0.21-0.46) followed by Monze (32.4%,CI: 0.17-0.47) while we did not detect any seropositivity in Lundazi (Table 2). Southern province had the highest individual animal (8.97%, CI:0.07-0.11) (Table 3) and herd (28.5%, CI;0.20-0.36) (Table 4). Animal seropositivity was significantly higher in female animals (8.5%, p=0.00), those aged 11 years and above (14.1%,p=0.01) as well as pregnant cows (13.8%,p=0.00). Some animals with hygromas (33.3%), history of abortion (5.6%) and infertility (11.1%) were also seropositive (Table 5).

The odds ratio suggested that animals in Namwala were more likely (OR=8.54) to test positive than those from other districts; and animals aged 11 years and above were also more likely (OR=2.67)

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to test positive than other age groups. On the other hand, pregnant animals and those with abortion history were more likely to test positive (OR=4.28, OR=6.25) (Table 5).

Table 1. Individual animal seroprevalence by district

District	n	Positive	Seroprev.%.	CI	p-value
Lundazi	251	0	0	-	
Choma	185	4	2.15	0.01-0.04	
Monze	326	21	6.44	0.04-0.09	
Namwala	602	75	12.45	0.10-0.15	
Senanga	347	29	8.35	0.05-0.11	0.000
Total	**1712**	**129**	**7.53**	**0.62-0.87**	

Pos.—Positive; Seroprev.—Seroprevalence; CI—Confidence interval; n—number

Table 2. Herd level seroprevalence by district

District	Herd no	Positive	Seroprev.%	CI	p-value
Lundazi	26	0	0.00	-	
Choma	23	2	8.60	0.03-0.20	
Monze	37	12	32.4	0.17-0.47	
Namwala	59	20	33.9	0.21-0.46	
Senanga	30	3	10.0	0.00-0.21	0.001
Total	**175**	**37**	**21.14**	**0.15-0.27**	

Pos.—Positive; Seroprev.—Seroprevalence; CI—Confidence interval; n—number

Table 3. Individual animal seroprevalence by province

Province	n	Pos	Animal seroprev.%	CI	P -value
Southern	1114	100	8.97	0.07-0.11	
Western	347	29	8.35	0.05-0.11	
Eastern	251	0	0.00	-	0.000
Total	**1712**	**129**	**7.53**	**0.62-0.87**	

Pos.—Positive; Seroprev.—Seroprevalence; CI—Confidence interval; n—number

Table 4. Herd level seroprevalence by province

Province	n	Pos	Animal seroprev.%	CI	P -value
Southern	1114	100	8.97	0.07-0.11	
Western	347	29	8.35	0.05-0.11	
Eastern	251	0	0	-	0.000
Total	**1712**	**129**	**7.53**	**0.62-0.87**	

Pos.—Positive; Seroprev.—Seroprevalence; CI—Confidence interval; n—number

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Table 5. Association between animal characteristics and individual animal level seropositivity assessed by logistic regression

Variable	Category	Pos/tested	Seropositivity (%)	Odds ratio	95% CI	p-value
Province	S/P	100/1114	8.97	Ref		
	W/P	29/347	8.35	0.92	0.60-1.42	0.72
	E/P	0/251	0	1	-	-
District	Choma	4/185	2.15	Ref		
	Monze	21/326	6.44	3.13	1.05-9.27	0.04
	Namwala	75/602	12.46	6.47	2.33-17.95	0.00
	Senanga	29/347	8.36	4.14	1.43-11.99	0.01
	Lundazi	0/251	0	1	-	-
Age	0-5 yrs	58/985	5.89	Ref		
	6-10 yrs	60/649	9.24	1.62	1.11-2.36	0.01
	>11 yrs	11/78	14.10	2.62	1.31-5.23	0.01
Sex	Female	116/1349	8.60	0.39	0.21-0.71	0.00
	Male	13/363	3.58			
Reproductive status	Bull	13/361	3.60	Ref		
	Cow	63/829	7.60	2.20	1.19-4.05	0.01
	Lactating	33/370	8.92	2.62	1.35-5.06	0.00
	pregnant	20/145	13.79	4.28	2.06-8.86	0.00
History	Had calves < 3 months old	126/1674	7.53	Ref		
	Abortion	2/25	8.0	1.06	0.24-4.58	0.929
	Infertility	0/6	0	1	-	-
	hygroma	1/3	33.3	6.14	0.55-68.21	0.139

S/P-Southern province; W/P-Western province; E/P-Eastern province; RBPT—Rose Bengal Plate Agglutination Test; cELISA—competent Enzyme-Linked Immunosorbent Assay; Pos.—Positive; Seroprev.—Seroprevalence; CI—Confidence interval

4. Discussion

This study aimed at estimating the brucella seroprevalence and identifying the risk factors. The overall brucellosis seroprevalence at animal and herd levels was 7.53% and 21.1% respectively in our study. Although we didn't detect seropositivity in Eastern province, Southern province had the highest herd and individual animal seroprevalences respectively (28.5%, 8.97%), followed by Western (8.35%).The individual animal seroprevalence in this study was slightly higher than the 6% reported in smallholder dairy cattle in Lusaka and Southern provinces by [25]. The herd seroprevalence was also comparable to the previously reported 20.7% in Southern province [26] but lower than the 46.2-74% in the livestock-wildlife interface area of the Kafue flats [24] among traditional cattle in Zambia. The herd seroprevalence was slightly higher in Southern province and Namwala district despite being statistically insignificant. The odds ratio also suggested that animals in Namwala were more likely (OR=8.54) to test positive than those from other districts. The observed high seropositivity at

 doi:10.20944/preprints202011.0318.v1

individual animal and herd level can be attributed to the fact that the province has the highest traditional cattle population in Zambia, which predominantly relies on communal grazing in the Kafue flood plains [24] . Movement of cattle herds to the plains, in search of greener pasture is high which results in inter-herd interactions and consequent spread of infection [32]. The slight increase observed in the herd and animal prevalence over the decade possibly shows that the disease has become stable over the years, thereby reaching an endemic state. The continuing lack of control measures towards brucellosis in Zambia is evidenced by governments disease control programs which prioritize livestock diseases that are considered more important (e.g. FMD,ECF and CBPP) compared to others [33]. This is leading to unmitigated transmission and has possibly contributed to this plateau state. The traditional cattle sector constitutes a significant proportion of the cattle production system in Zambia [34], hence this sustained disease pressure is worrying due to the economic and public health risk it poses to pastoral communities. The absence of *Brucella* seropositivity observed in Lundazi district, Eastern province is inconclusive as it may be attributed to the small sample size, however it is interesting to note that a 7.7% was observed in Northern Malawi [22], which borders with Lundazi district in Zambia, an area where we conducted our sampling. We recommend a more conclusive study in Eastern province to further investigate the disease epidemiology.

Our herd seroprevalence is comparatively lower than the 25.6%, 29.2%, 32%, 40.1% and 45.9% reported in Ethiopia, Tanzania, Cameroon, Nigeria, and Angola [13,14; 16,17,21]. In comparison to findings from studies in other countries in Southern Africa, our herd seroprevalence is comparatively lower than the 9.9% and 30.1% reported in Zimbabwe [35]. At animal level, our findings are higher than the 5.5 % reported in South Africa [19], but comparable to the 7.4 % in Northern Malawi [22]. The result variations observed may be attributed to several factors including sampling techniques and sample sizes, different diagnostic tests and interpretations used as well as seasonal cattle movements in search of pasture amidst droughts.

Seropositivity was significantly higher in female animals (8.6%, p=0.00), those aged 11 and above years (14.1%,p=0.01) and pregnant cows (13.8%,p=0.00).This is in agreement with findings from other similar studies [13,16, 24, 35] and consistent with the known relationship between *brucella* status and age and sex. Animals aged 11 years and above were more likely (OR=2.67) to test positive than other age groups. On the other hand, pregnant animals and those with abortion history were more likely to test positive (OR=4.28, OR=6.25). It has been documented that female and older animals tend to have increased chances of testing positive for brucellosis [36]. As the animal reaches sexual maturity, the levels of growth stimulating factors for *Brucella* organisms become high [37], while constant exposure to the *brucella* organisms increases with age. The high seroprevalence among pregnant cattle can be explained by the elevated erythritol levels in the placental and fetal fluids during the third trimester [38]. These high sugar levels stimulate growth and multiplication of the bacteria in the reproductive organs.

The combined use of RBT and cELISA tests serially in our study maximized the specificity of the reported results.

5. Conclusions

The overall brucellosis seroprevalence at animal and herd levels was 7.53% and 21.1% respectively**.** seropositivity was significantly higher in female animals, those aged between 11 years and above and pregnant cows. It is interesting to note that the *brucella* seroprevalence seems to have

maintained enzootic stability over a decade in Southern and Western provinces. There is need to develop and implement multisectoral One Health surveillance and control strategies to minimize the disease burden in animals and consequently in humans (Godfroid et al., 2013).

Supplementary Materials: Are available on request

Author Contributions: "Conceptualization, R.L.M. ; methodology, R.L.M, JBM and J.G; software, R.L.M, J.B.M.; validation, H.B.M. and J.G.; formal analysis, R.L.M, M.M, I.S; investigation, R.L.M, M.M, I.S.; resources, R.L.M.; data curation, R.L.M.; writing—original draft preparation, R.L.M writing—review and editing, F.S, J.B.M, H.B.M and J.G.; visualization, R.L.M; supervision, J.B.M., H.B.M. and J.G.; project administration, R.L.M; funding acquisition, R.L.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the African Centre for Infectious Diseases in Humans and Animals UNZA (ACEIDHA-UNZA) and the Ministry of Higher Education scholarship.

Acknowledgments: We acknowledge the veterinary officers and assistants in the study provinces, ACEIDHA -UNZA and the Ministry of higher Education.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Ethical Statement: Blood and serum samples from cattle were collected with consent from animal owners and as per biosafety and bioethical standards of "Ethical Research Board" of ERES CONVERGE , Zambia (Ref No. 2018-Dec-004).

References

1. OIE (2018) Bovine brucellosis. In: OIE (ed) Terrestrial manual. Version adopted by the World Assembly of Delegates of the OIE in May 2016
2. Whatmore, A. M., Davison, N., Cloeckaert, A., al Dahouk, S., Zygmunt, M. S., Brew, S. D., Perrett, L. L., Koylass, M. S., Vergnaud, G., Quance, C., Scholz, H. C., Dick, E. J., Hubbard, G., & Schlabritz-Loutsevitch, N. E. (2014). Brucella papionis sp. nov., isolated from baboons (Papio spp.). *International Journal of Systematic and Evolutionary Microbiology*, *64*(2014), 4120–4128.
3. A.A., Fasina, F.O., Katsande, C.T., Dogonyaro, B.B., Potts, A., Matle, I., Gelaw, A.K. and Van Heerden, H., 2019. Seroprevalence and characterization of Brucella species in cattle slaughtered at Gauteng abattoirs, South Africa. *Veterinary Medicine and Science*, *5*(4), pp.545-555.
4. Menshawy, A., Perez-Sancho, M., Garcia-Seco, T., Hosein, H. I., García, N., Martinez, I., ... & Alvarez, J. (2014). Assessment of genetic diversity of zoonotic Brucella spp. recovered from livestock in Egypt using multiple locus VNTR analysis. *BioMed Research International*, *2014*.
5. Muendo, E. N., Mbatha, P. M., Macharia, J., Abdoel, T. H., Janszen, P. V., Pastoor, R., & Smits, H. L. (2012). Infection of cattle in Kenya with Brucella abortus biovar 3 and Brucella melitensis biovar 1 genotypes. *Tropical animal health and production*, *44*(1), 17-20.
6. Ducrotoy, M., Bertu, W. J., Matope, G., Cadmus, S., Conde-Álvarez, R., Gusi, A. M., ... & Moriyón, I. (2017). Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta tropica*, *165*, 179-193.

7. Godfroid, J., Scholz, H. C., Barbier, T., Nicolas, C., Wattiau, P., Fretin, D., ... & Saegerman, C. (2011). Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive veterinary medicine*, *102*(2), 118-131.
8. Hoffman, T., Rock, K., Mugizi, D.R., Muradrasoli, S., Lindahl-Rajala, E., Erume, J., Magnusson, U., Lundkvist, Å., Boqvist, S., 2016. Molecular detection and characterization of Brucella species in raw informally marketed milk from Uganda. Infect. Ecol. Epidemiol. 6, 32442–32442. https://doi.org/10.3402/iee.v6.32442
9. Sanogo, M., Abatih, E., Thys, E., Fretin, D., Berkvens, D. and Saegerman, C., 2012. Risk factors associated with brucellosis seropositivity among cattle in the central savannah-forest area of Ivory Coast. *Preventive veterinary medicine,* 107(1-2), pp.51–56.
10. Poester, F.P., Samartino, L.E. and Santos, R.L., 2013. Pathogenesis and pathobiology of brucellosis in livestock. *Rev sci tech*, 32(1), pp.105–115.
11. Godfroid, J., Nielsen, K., & Saegerman, C. (2010). Diagnosis of brucellosis in livestock and wildlife. *Croatian medical journal*, *51*(4), 296–305. https://doi.org/10.3325/cmj.2010.51.296
12. Franc, K. A., Krecek, R. C., Häsler, B. N., & Arenas-Gamboa, A. M. (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC public health*, *18*(1), 125. https://doi.org/10.1186/s12889-017-5016-y
13. Asgedom, H., Damena, D. & Duguma, R. Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district, Ethiopia. *SpringerPlus* **5,** 851 (2016). https://doi.org/10.1186/s40064-016-2547-0
14. Sagamiko FD, Muma JB, Karimuribo ED, Mwanza AM, Sindato C, Hang'ombe BM. Sero-prevalence of Bovine Brucellosis and associated risk factors in mbeya region, Southern highlands of Tanzania. *Acta Trop*. 2018;178:169-175. doi:10.1016/j.actatropica.2017.11.022
15. Mfune, R.L., 2015. *Epidemiological study of bovine brucellosis in smallholder dairy cattle in Lushoto and Rungwe districts, Tanzania* (Doctoral dissertation, Sokoine University of Agriculture).
16. Awah-Ndukum, J., Mouiche, M., Bayang, H. N., Ngwa, V. N., Assana, E., Feussom, K., Manchang, T. K., & Zoli, P. A. (2018). Seroprevalence and Associated Risk Factors of Brucellosis among Indigenous Cattle in the Adamawa and North Regions of Cameroon. *Veterinary medicine international*, *2018*, 3468596. https://doi.org/10.1155/2018/3468596
17. Ogugua, A.J., Akinseye, V.O., Cadmus, E.O. *et al.* Prevalence and risk factors associated with bovine brucellosis in herds under extensive production system in southwestern Nigeria. *Trop Anim Health Prod* **50,** 1573–1582 (2018). https://doi.org/10.1007/s11250-018-1597-4
18. Vhoko K, Iannetti S, Burumu J, Ippoliti C, Bhebhe B, De Massis F. Estimating the prevalence of Brucellosis in cattle in Zimbabwe from samples submitted to the Central Veterinary Laboratory between 2010 and 2014. *Vet Ital*. 2018;54(1):21-27. doi:10.12834/VetIt.1111.61912
19. Kolo, F.B., Adesiyun, A.A., Fasina, F.O., Katsande, C.T., Dogonyaro, B.B., Potts, A., Matle, I., Gelaw, A.K. and Van Heerden, H., 2019. Seroprevalence and characterization of Brucella species in cattle slaughtered at Gauteng abattoirs, South Africa. *Veterinary Medicine and Science*, *5*(4), pp.545-555.
20. Madzingira, O., & Sezuni, P. M. (2017). Serological prevalence and public health significance of brucellosis on a dairy farm in Namibia from 2011 to 2014. *BMC research notes*, *10*(1), 620. https://doi.org/10.1186/s13104-017-2933-x
21. Mufinda, F., Boinas, F., & Nunes, C. (2015). Prevalence and Factors Associated with Cattle Brucellosis in Animal Herds of the Namibe Province in Angola. *Alexandria Journal of Veterinary Sciences*, *47*(1), 7. https://doi.org/10.5455/ajvs.188809
22. Tebug, Stanly Fon, Gilson R. Njunga, Mizeck G.G. G Chagunda, Jacob P. Mapemba, Julius Awah-Ndukum, and Steffi Wiedemann. 2014. "Risk, Knowledge and Preventive Measures of Smallholder Dairy Farmers in Northern Malawi with Regard to Zoonotic Brucellosis and Bovine Tuberculosis." *Onderstepoort J Vet Res* 81 (1): 1–6. https://doi.org/10.4102/ojvr.v81i1.594.
23. Chimana, H.M., Muma, J.B., Samui, K.L., Hangombe, B.M., Munyeme, M., Matope, G., Phiri, A.M., Godfroid, J., Skjerve, E. and Tryland, M., 2010. A comparative study of the seroprevalence of brucellosis in commercial and small-scale mixed dairy–beef cattle enterprises of Lusaka

province and Chibombo district, Zambia. *Tropical Animal Health and Production*, *42*(7), pp.1541-1545.

24. Muma, J.B., Samui, K.L., Siamudaala, V.M. *et al.* Prevalence of antibodies to *Brucella* spp. and individual risk Factors of Infection in Traditional Cattle, Goats and Sheep Reared in Livestock–Wildlife Interface Areas of Zambia. *Trop Anim Health Prod* **38,** 195–206 (2006). https://doi.org/10.1007/s11250-006-4320-
25. Muma JB, Pandey GS, Munyeme M, Mumba C, Mkandawire E, Chimana HM. Brucellosis among smallholder cattle farmers in Zambia: public health significance. *Trop Anim Health Prod*. 2012;44(4):915-920. doi:10.1007/s11250-011-9987-x
26. Muma, J.B., Syakalima, M., Munyeme, M., Zulu, V.C., Simuunza, M. and Kurata, M., 2013. Bovine tuberculosis and brucellosis in traditionally managed livestock in selected districts of Southern Province of Zambia. *Veterinary medicine international*, *2013*.
27. CSO.(2018). Zambia in figures 2018. Lusaka: https://www.zamstats.gov.zm/phocadownload/Dissemination/Zambia%20in%20Figure%202018.pdf
28. Ministry of Fisheries and Livestock (2017) The 2017/18 Livestock and Aquaculture Census Report: Ministry of Fisheries and Livestock, Zambia
29. Simwinji, N., 1997. Summary of Existing Relevant Socio-economic and Ecological Information on Zambia's Western Province and Barotseland. *IUCN-The World Conservation Union Regional Office for Southern Africa, Harare*, pp.1-56.
30. Groves, R.M., Fowler Jr, F.J., Couper, M.P., Lepkowski, J.M., Singer, E. and Tourangeau, R., 2011. *Survey methodology* (Vol. 561). John Wiley & Sons.
31. Zolzaya, B., Selenge, T., Narangarav, T. *et al.* Representative Seroprevalences of Human and Livestock Brucellosis in Two Mongolian Provinces. *EcoHealth* **11,** 356–371 (2014). https://doi.org/10.1007/s10393-014-0962-7
32. Sinkala, Y., Simuunza, M., Pfeiffer, D.U., Munang'andu, H.M., Mulumba, M., Kasanga, C.J., Muma, J.B. and Mweene, A.S., 2014. Challenges and economic implications in the control of foot and mouth disease in sub-Saharan Africa: Lessons from the Zambian experience. *Veterinary medicine international*, *2014*.
33. Animal Health Act, 2010. The Animal Health (Control and Prevention 01 Animal Disease) Order, 2014. Available at http://www.parliament.gov.zm/node/3376 (Accessed: 20 October 2020)
34. Mumba, C., Häsler, B., Muma, J.B., Munyeme, M., Sitali, D.C., Skjerve, E. and Rich, K.M., 2018. Practices of traditional beef farmers in their production and marketing of cattle in Zambia. *Tropical animal health and production*, *50*(1), pp.49-62. Accessed 31 Nov 2019
35. Gomo, C., De Garine-Wichatitsky, M., Caron, A. & Pfukenyi, D.M., 2012, 'Survey of brucellosis at the wildlife-livestock interface on the Zimbabwean side of the great Limpopo transfrontier conservation area', *Tropical Animal Health and Production* 44, 77–85. **http://dx.doi.org/10.1007/s11250-011-9890-5**
36. Madut NA, Muwonge A, Nasinyama GW, Muma JB, Godfroid J, Jubara AS, et al. (2018) The sero-prevalence of brucellosis in cattle and their herders in Bahr el Ghazal region, South Sudan. PLoS Negl Trop Dis 12(6): e0006456. https://doi.org/10.1371/journal.pntd.0006456
37. Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D., Jacobs, D.E., Ikede, B.O., McKenzie, R.A., Colwell, D., Osweiler, G. and Bildfell, R.J., 2007. Veteriary medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses.
38. Petersen, E., Rajashekara, G., Sanakkayala, N., Eskra, L., Harms, J., & Splitter, G. (2013). Erythritol triggers expression of virulence traits in Brucella melitensis. *Microbes and infection*, *15*(6-7), 440–449. https://doi.org/10.1016/j.micinf.2013.02.002
39. Godfroid, J., Al Dahouk, S., Pappas, G., Roth, F., Matope, G., Muma, J., Marcotty, T., Pfeiffer, D., Skjerve, E., 2013. A "One Health" surveillance and control of brucellosis in developing countries: Moving away from improvisation. Comp. Immunol. Microbiol. Infect. Dis. 36, 241–248. https://doi.org/10.1016/j.cimid.2012.09.001