Seroprevalence of human Brucellosis and associated risk factors among high risk occupations in Mbeya Region of Tanzania

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Abstract

Background

Brucellosis is an infectious zoonotic disease that affects humans, livestock and wildlife.

Methods

A cross-sectional study was conducted in Mbeya region between November 2015 and January 2016 to investigate the seroprevalence of human brucellosis and identify associated risk factors among individuals in risky occupations in Mbeya Region. A total of 425 humans from six occupational categories were serially tested for *Brucella* antibodies using the Rose Bengal Plate

Test (RBPT) and competitive Enzyme Linked Immunosorbent Assay (c-ELISA), for screening and confirmation, respectively. A questionnaire survey was administered to participants collect epidemiological data.

Results

The overall seroprevalence among the high risk occupational individuals was 1.41% (95% CI: 0.01-0.03). Seroprevalence among the different occupations were as follows: shepherds 1.33% (95% CI: 0.14-0.22); butcher men 5.26% (95% CI: 0.10-0.17) and abattoir workers 1.08% (95% CI: 0.39-0.49). Seroprevalence was noted to vary according to occupation type, milk consumption behaviour, age and sex. Butcher men recorded the highest seroprevalence (5.0%) while individuals who consumed unboiled milk had a higher seroprevalence (1.56%) compared to those who drunk boiled milk. High seropositivity (2.25%) was observed among the age group of 1-10 years while male individuals had a higher seroprevalence (1.41%) than females (0%). Butcher men were at higher risk of exposure compared to other professions.

Conclusion

Our findings show the presence of brucellosis in occupationally exposed individuals in Mbeya region. There is need to sensitize the exposed individuals in order to reduce the risk of acquiring *Brucella* infections from animals and animal products This also calls for public health awareness about the disease, and implementation of control measures that will prevent further spread of brucellosis within and outside the study area..

Keywords: Brucellosis, human, occupation, prevalence, risk factors.

Author summary

Brucellosis is a bacterial zoonosis that has evolved to establish itself as an occupational and food-borne disease Worldwide. It is responsible for huge economic losses incurred by livestock keepers and poses a public health risk to humans in most developing countries. In Tanzania, which has the 3rd highest cattle population in Africa, many studies that have been done show that brucellosis exists in livestock, especially in cattle and wildlife. However, very few studies have reported on human brucellosis. The disease has been reported to occur in humans who have direct exposure to cattle or cattle products like livestock farmers, abattoir workers, veterinarians, shepherds and farm workers in many developing countries. A few studies in Tanzania have reported seroprevalences among these high-risk occupations; however, the disease has not been fully described in Mbeya region. This study was therefore aimed at filling these information gaps and contributing to the existing body of knowledge.

Introduction

Brucellosis is a major zoonotic disease of public health and economic importance affecting domestic animals, wildlife and humans [1]. It is the second most important zoonotic disease in the world after Rabies [2]. Brucellosis is distributed worldwide but is common in countries that do not have good standardized and effective public health and domestic animal health programmes [3]. Although the genus *Brucella* consists of twelve species, it is noteworthy that this list may change as other species continue to be discovered [4]. Among the *Brucella* species, zoonotic infections are mainly attributed to *B. melitensis*, *B. abortus,* and *B. suis* [5], while *B. canis* has been mainly reported as an occupational hazard to veterinarians and laboratory workers [6]. Human brucellosis is a highly debilitating infection that presents as an acute febrile flu-like illness [7]. It is characterized by symptoms such as fever, anorexia, fatigue, headaches,

66 depression and weight loss that may easily be confused with malaria or typhoid [7]. The source
67 of human infection always resides in domestic or wild reservoirs.

68 Human cases continue to occur because of the traditional use of raw milk products and
69 following close contact with infected animals [8, 9]. It has been observed that most cases of
70 human brucellosis occur in rural areas where half of the people live in close proximity to their
71 livestock, consume raw milk and make cheese using unhygienic methods [7]. Although few
72 reports on human brucellosis exist, documentation of human cases of brucellosis in Sub-
73 Saharan Africa is scarce, particularly reports relating to isolation of the causative agents. In
74 sub-Saharan Africa, the prevalence of human brucellosis has been reported with varying
75 seroprevalence ranging from 0.02% to 31.8% [10, 11, 12, 13, 14]. In Tanzania, several studies
76 have been done in different regions including Katavi, Manyara, Morogoro, Northern Tanzania
77 and Mwanza which have reported human brucellosis at seroprevalence ranging from 0.6 to
78 48.4% [15,16, 17, 18,19, 20,21]. However, there is no previous report on the disease among
79 the high-risk human population in Mbeya region. Therefore, this study was aimed at
80 establishing the seroprevalence and associated risk factors of human brucellosis among high-
81 risk occupations in Mbeya region, Tanzania.

82

83 Materials and methods

84 Study area

85 The study was carried out in Mbeya Region in the Southern highlands of Tanzania between
86 November 2015 and January 2016 in three selected districts namely; Mbarari, Mbeya and
87 Momba. Geographically, Mbeya region lies about 5500 feet above sea level and experiences

subtropical highland climate with humid summers and dry winters. The temperature ranges between -6°C in the highlands and 29°C on the lowlands, while the average rainfall is 900mm per year. Details of the study area have been described in our earlier publication [22]. According to the 2012 national census, the region has an estimated human population of about 2,707,[23] among which 1, 297,738 are males and 1, 409, 672 are females. A majority of the population (1, 809,298) dwell in the rural areas whereas 898, 112 are found in urban areas.

Study population

The study population consisted of all individuals above 18 years that were involved in the cattle value chain. They were grouped into six categories; livestock professionals, shepherds, butcher men, abattoir workers, milk vendors and consumers of animal products. Sampling priority was given to individuals with direct contact/exposure to animals.

Study design and sample size calculation

This was a cross-sectional study that was strategically designed in order to determine the seroprevalence of human brucellosis in high-risk individuals. A total of 425 humans comprising 75 shepherds from 37 *Brucella* positive herds, 11 livestock professionals, 57 butcher men, 186 abattoir workers from 4 abattoirs, 72 persons engaged in cattle milking and 24 animal product consumers were recruited in the study. The numbers of shepherds to be sampled were pre-determined from known *Brucella* positive herds based on our earlier study [22]. Among the households with infected cattle herds, only 37 were enrolled out of 53 herds. The selected study region encompassed a strategic population of individuals whose culture encourages the use of animal products for proteins, thus predisposing them to zoonotic diseases.

Data collection

A phlebotomist aseptically collected 5ml of blood from the participant's brachial vein using a sterile disposable syringe into pre labelled plain vacutainer tubes. The samples were then incubated overnight at room temperature and centrifuged at 3000 *xg* to get clear serum. All collected samples were assigned identification numbers and stored in a mobile refrigerator until shipment to the University of Zambia laboratory where they were stored in at -20 degree until they were examined for *Brucella* antibodies.

A pre-tested structured questionnaire was administered to the participants from whom blood was drawn in order to collect information on demographic data, socioeconomic data, exposure to animals and animal products, consumption of dairy and animal source products and the presence of specific symptoms like fever, headaches, sweats, sleeping difficulties, fatigue, weight loss, joint pain, muscle pain and back pain.

Serological testing

Rose Bengal plate test

All collected sera samples were screened using Rose Bengal Plate Test (RBPT), antigen manufactured by Ubio Biotechnology Systems Pvt Ltd for detection of *Brucella* antibodies according to the test procedure recommended by OIE [1]. Briefly, 20µl of RBPT antigen and 20µl of the test serum were placed alongside on one well of the glass plate and mixed thoroughly. The slide was gently rocked for 4 minutes and thereafter, any visible agglutination was considered as a presumptive positive result.

Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA)

RBPT positive sera were then subjected to competitive Enzyme-Linked Immunosorbent Assay (c-ELISA) as a confirmatory test, adopting a test procedure and interpretation of results as recommended by the manufacturer (Svanova Biotech AB SE-751 Uppsala, Sweden) and described by [24].

According to the ELISA kit manufacturer, serum was regarded as positive if the PI value was ≥ 30%. Only individuals that tested positive to both RBPT and c-ELISA were regarded as *Brucella* seropositive.

Data management and analysis

Data obtained from the serological tests and a questionnaire survey was stored in an Excel® spreadsheet database before being imported into STATA 13® statistical software for analysis. Categorical variables were summarized as frequency and percentages; continuous variables were summarized as mean or standard deviation (SD). P-values of 0.05 or less were considered statistically significant. A person was considered to be seropositive when tested positive to both RBPT and c-ELISA. The degree of association between each risk factor was assessed using the chi-square test and for all analysis, a p-value of ≤ 0.05 was taken as significant.

Ethical consideration

Ethical approval (reference number NIMR/HQ/R.8a/Vol.1X/2050) was obtained from the Medical Research Committee of the United Republic of Tanzania prior to the study. Individual written consent was obtained from guardians for individuals that were less than 18 years prior to enrolment. Informed consent was obtained from all participants using written and verbal explanation of the study purpose and procedure using the Swahili language.

Results

A total of 425 individuals working in the cattle value chain in Tanzania were included in the study (Table 1). The overall human brucellosis prevalence was 1.41% (95% CI: 1.7-2.6). No female participant (n=334) tested positive for brucellosis even though these were in the majority compared to males (n=91). *Brucella* seroprevalence was recorded in three occupational categories out of the six that were considered in our study (Table 2). It was also observed that 75.3% of respondents (n=320) consumed raw milk and only 24.7% (n=10) consumed boiled milk and none had a history of consuming pasteurized milk (Table 2). The predictor variables were assessed for collinearity using Pearson's Chi-square test and revealed a strong association between the occupation of an individual and his/her sex (P-value=<.0001) and age category (P-value=<.0001).

Table 1: Human blood sample distribution by study district and occupation in Mbeya Region

District	Shepherd	Livestock Professional	Milking personnel	Abattoir worker	Butcher men	Animal product consumer	**Total**
Mbarari	45	7	64	50	30	24	**220**
Momba	22	1	0	9	13	0	**45**
Mbeya	8	3	8	127	14	0	**160**
Total	**75**	**11**	**72**	**186**	**57**	**24**	**425**

Seroprevalence of human brucellosis

There was no statistical association between *Brucella* seropositivity and all the hypothesized risk factors evaluated in univariate analysis (Table 2). However, results indicated an apparent variation of seroprevalence by occupation, milk consumption behavior, age and sex. Butcher men recorded the highest seroprevalence (5.0%) while a higher seroprevalence (1.56%) was recorded among individuals who consumed unboiled milk compared to drinkers of boiled milk (Table 2). High seropositivity (2.25%) was observed among the age group of 1-10 years while male individuals had a higher seroprevalence (1.41%) than females (0%) as shown in Table 02.

Table 2: **Results of univariate analysis of seroprevalence of human brucellosis by different variables**

Variable	Level	No	Distribution	Prop. positive (%)	95% CI	P-value
Sex	Male	91	21.4	1.41	0.18-0.26	0.19
	Female	334	78.6	0.00	0.74-0.82	
Area	Mbarari	220	51.8	1.36	0.47-0.57	0.64
	Momba	45	10.6	0.00	0.08-0.14	
	Mbeya	160	37.6	1.88	0.33-0.42	
Age	1-10 yrs	159	37.4	2.25	0.33-0.42	0.27
	11-20 yrs	196	46.1	1.02	0.41-0.51	
	21-30 yrs	65	15.3	0.00	0.12-0.20	
	31-40 yrs	5	0.1	0.00	0.00-0.03	
Occupation	Shepherd	75	17.6	1.33	0.14-0.22	0.17

	Livestock officer	11	2.5	0.00	0.01-0.05	
	Butcher men	57	13.4	5.26	0.10-0.17	
	Abattoir worker	186	43.7	1.08	0.39-0.49	
	Milking	72	17.0	0.00	0.14-0.21	
	Animal product	24	5.6	0.00	0.04-0.08	
Source of milk	Cattle	425	1	1.41	-	-
	Goat	0	-	0.00		
Milk consumption	Unboiled	320	75.3	1.56	0.71-0.79	0.65
	Boiled	105	24.7	0.95	0.21-0.29	
Consuming raw blood	No	425	1	1.41	-	-
	Yes	0				
Assisting parturition	No	312	73.4	1.60	0.69-0.77	0.58
	Yes	113	26.6	0.88	0.23-0.31	

Discussion

Brucellosis is among the diseases categorized as a neglected zoonosis by the WHO. This is so because, despite its wide distribution and potentially harmful effect on human health, it has not been given due attention compared to other diseases. Generally, the public health importance of brucellosis is acknowledged throughout the world; however people in certain occupations or settings still face increased risk of exposure to the *Brucella* pathogen**.** These may include many players in the livestock value chain starting from livestock keepers, veterinarians/livestock officers, animal handlers, laboratory workers, slaughterhouse workers, butcher-men and consumers of animal products (meat, milk, geese).

The aim of this study was to estimate the seroprevalence of human brucellosis and identify associated risk factors among individuals engaged in risky professions in Mbeya Region. The study found an overall seroprevalence of 1.41% among shepherds, butcher men and abattoir workers in Mbeya region. This seropositivity is lower than the 2.2% reported [1] in the Kilimanjaro region and the 48.4% reported by [20] in Mwanza region. The difference can be attributed to differences in geographical locations and the use of a single but highly sensitive test (Rapid agglutination test) in the previous study. In other parts of Africa, brucellosis studies among high risk occupations in Ghana and Nigeria in slaughterhouses found seroprevalences of 9.6% [25] and 24.1% [11] while a study in Sudan found varying prevalences of 9.5%, 15.3%, 24.4% and 26.5% among veterinarians, meat inspectors, abattoir workers and animal handlers, respectively [26]. The findings in Ghana, Nigeria and Sudan were higher than those found in this study in all occupational groups. These result does not justify the lower levels of human brucellosis in our study area as most of the people had a tendency of taking antibiotics regularly [27]. In so doing, they could treat brucellosis unknowingly and thereby negatively cause the existing problem of antimicrobial drug resistance. Brucellosis has also been reported in slaughterhouse workers in Iran with the prevalence of 7.8% [28] and 9.8% [9]. In Lahore

district of Pakistan, the prevalence of brucellosis in abattoir workers was found to be 21.7%, which was higher compared to that observed in our study area [30]. This can be explained by the fact that both Pakistan and Iran depend on sheep and goat meat and milk for protein sources, which are likely to be contaminated with *B. melitensis*. In humans, *B. melitensis* is highly infectious compared to *B. abortus* [31], which is likely to be the problem in the study area. This practice followed in marketing and distribution of sheep and goat meat as well as milk products, in particular, makes the enforcement of hygienic measures very difficult [7].

Risk factors associated with *Brucella* seropositivity

The prevalence of human brucellosis in occupationally exposed individuals in the Mbeya region of Tanzania has been noted to vary with the occupational category, milk consumption behaviour, age and sex, although this was not statistically significant. The butcher men had a higher risk of exposure to brucellosis than shepherds and abattoir workers. This could be attributed to that fact that there is increased risk of injury (knife-cuts) among the butcher men compared to the other categories, thus increasing the exposure risk to the Brucella pathogen. High prevalence of brucellosis in males can be explained by the fact that most of activities in cattle value chain are carried out by males than females. Since majority of butchermen are males who has high risk of acquiring brucellosis, it can be the reasons for the high prevalence of the disease. This is similar to findings by other studies [32, 33]. However, none of these have establish the risk that shepherds have towards brucellosis in Tanzania despite the fact that they are at the starting point of the livestock value chain. Therefore, this is the first report in Tanzania to establish the risk that shepherds have towards brucellosis. These findings are in contrast to those by [20] who reported a high risk of exposure to brucellosis abattoir workers. The high disease prevalence among butcher men could be because they spend longer periods handling animal carcasses usually without protective wear and are more likely to be injured

when cutting meat and are in close contact with blood and tissues of infected animals. Hence they are at higher risk of infection than other groups. *Brucella* seropositivity was higher in males (1.41%) than females (0%), similar to findings by [11] in Nigeria but contrary to findings by [17] in Morogoro. Seroprevalence was higher in individuals below 20 years of age (2.25%). These findings agree with those by [34] in Uganda. This can be attributed to the traditional role that young men play in livestock management among the pastoral communities. The young males start rearing livestock at a young age and are in direct contact with animal and animal products during their daily livestock activities, which increases their risk of exposure to brucellosis. In our study, 75.3% of people consumed raw milk which is higher than that reported by [35]. This can be explained by the fact that over 70% of milk sales in Tanzania is produced by pastoral farmers who do not believe or know that milk could be a potential source of infection to humans; and are not ready to subject their milk to any form of [36]. Milk is a major vehicle for transmission of *Brucella* infection and individuals with a history of consumption of raw milk were more likely to be infected [34].

Conclusion and Recommendations

Our findings demonstrate the presence of human brucellosis in occupationally exposed individuals, specifically abattoir workers, butcher men and shepherds in the Mbeya region. Given that we applied random sampling strategy to obtain the sample size (from our earlier study), the study findings can be generalized to the region. The results from this study indicate that more work needs to be done to educate the occupationally exposed individuals on brucellosis and its associated risks. Therefore, there is need to create public awareness, design and implement control measures that will prevent further spread of the disease within and

253 outside the study area. We recommend regional and multi-sectoral collaboration, especially
254 among veterinarians and medical professionals using the one health approach in order to
255 combat the disease.

256 Limitations encountered in this study

257 Some of the limitations were that some shepherds from certain cattle herds that had been
258 screened earlier could not be screened due to the migratory nature of agro-pastoralism in search
259 of water and pasture.

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264 Author contributions

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Conflict of Interest: The authors declare no conflict of interest on the study.

References

1. OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: (Mammals, Birds and Bees). Rev Sci Tech. 2012;819–1404.
2. Abubakar M, Mansoor M, Arshed MJ. Bovine brucellosis old and new concepts with Pakistan perspective. Pak Vet J. 2011;32(2):147-155.
3. Kunda J, Cleavant S, Fitspatri S, Nigel F, Kambarage D, Shirima G, et al. Brucellosis in Arusha and Manyara regions, Tanzania: A challenge to public health. Tanz Med J. 2005;20(1):1-4.
4. Scholz HC, Revilla-Fernández S, Al Dahouk S, Hammerl JA, Zygmunt MS, Cloeckaert A, et al. *Brucella vulpis sp*. nov., isolated from mandibular lymph nodes of red foxes (*Vulpes vulpes*). Int J Syst Evol Microbiol. 2016;66(5):2090-8.
5. Seleem M, Boyle SM, Sriranganthan N. Brucellosis: a re-emerging zoonosis. Vet Microbiol. 2010;140:392-398.
6. Lucero NC, Corazza R, Almuzara MN, Reynes E, Escobar GI, Boeri E, et al. Human *Brucella canis* outbreak linlked to infection in dogs. Epidem Infect. 2010;138(2): 280-285.

294 7. Corbel MJ. Brucellosis in humans and animals. World Health Organization; 2006.
295 8. Godfroid J. Brucellosis in livestock and wildlife: zoonotic diseases without pandemic
296 potential in need of innovative one health approaches. Arch Public Health.
297 2017;75(1):34.
298 9. Yumuk Z, O'callaghan D. Brucellosis in Turkey-an overview. Int J Infect Dis.
299 2012;16:e 228-e235.
300 10. Magwedere K, Bishi A, Tjipura-Zaire G, Eberle G, Hemberger Y, Hoffman LC, et al.
301 *Brucellae* through the food chain: the role of sheep, goats and springbok (*Antidorcus*
302 *marsupialis*) as sources of human infections in Namibia. J S Afr Vet Assoc.
303 2011;82(4):205-12.
304 11. Aworh MK, Okolocha E, Kwaga J, Fasina F, Lazarus D, Suleman I, et al. Human
305 brucellosis: seroprevalence and associated exposure factors among abattoir workers in
306 Abuja, Nigeria-2011. Pan Afr Med J. 2013(*16*)
307 12. Nizenyimana G, Mwiine FN. Comparative *Brucella abortus* antibody prevalence in
308 cattle under contrasting husbandry practices in Uganda. J S Afr vet.Assoc. 2013;.84(1).
309 13. Ragasa G, Mekonnen D, Yamuah L, Tilahun H, Guta T, Gebreyohannes A, et al.
310 Human brucellosis in Traditional pastoral communities in Ethiopia. Int J Trop.Med.
311 2009;59-64.
312 14. Muma, J.B., Samui, K., Lund, A., Nielsen, K., Chimana, H., Chisenga C, et al.
313 Brucellosis in rural communities in Zambia and factors associated with increased anti-
314 *Brucella* spp. antibody presence. Z J Sci Tech. 2008;12 (2): 9-18
315 15. Assenga JA, Matemba LE, Muller SK, Malakalinga JJ, Kazwala RR. Epidemiology of
316 *Brucella* infection in the human, livestock and wildlife interface in the Katavi-Rukwa
317 ecosystem, Tanzania. BMC vet res. 2015;11(1), p.189.

16. John K, Fitzpatrick J, French N, Kazwala R, Kambarage D, Mfinanga GS, et al. Quantifying risk factors for human brucellosis in rural northern Tanzania. PloS one. 2010;5(4):e9968.

17. James LW. Studies on human brucellosis in the Mikumi Selous Ecosystem, Morogoro, Tanzania. Doctoral dissertation, Sokoine University of Agriculture.2013.Accessed fromhttp://www.suaire.suanet.ac.tz:8080/xmlui/bitstream/handle/123456789/938/LAWRENCIA%20WANKYO%20JAMES.pdf?sequence=1&isAllowed=y

18. Bouley AJ, Biggs HM, Stoddard RA, Morrissey AB, Bartlett JA, Afwamba IA, et al. Brucellosis among hospitalized febrile patients in northern Tanzania. Am J Trop Med Hyg. 2012;87(6):1105-1111.

19. Mngumi EB, Mirambo MM, Wilson S, Mshana SE. Predictors of specific anti-*Brucella* antibodies among humans in agro-pastoral communities in Sengerema district, Mwanza, Tanzania: the need for public awareness. Trop Med Health. 2016;44: 34.

20. Mirambo MM, Mgode GF, Malima ZO, John M, Mngumi EB., Mhamphi GG. Et al.. Seroposotivity of *Brucella spp*. and *Leptospira spp*. antibodies among abattoir workers and meat vendors in the city of Mwanza, Tanzania: A call for one health approach control strategies. PLoS Negl Trop Dis. 2018;12(6):e0006600.

21. Carugati M, Biggs HM, Maze MJ, Stoddard RA, Cash-Goldwasser S, Hertz JT, et al. Incidence of human brucellosis in the Kilimanjaro Region of Tanzania in the periods 2007–2008 and 2012–2014. Trans R Soc Trop Med and Hyg. 2018;112(3)136-143.

22. 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. 2017;11:22 https://doi.org/10.1016/j.

341 23. NBS. Tanzania National Bureau of statistics. 2012. Available from: http://nbs.go.tz
342 cited 10 February 2019.
343 24. Muma JB, Lund A, Nielsen K, Matope G, Munyeme M, Mwacalimba K, et al.
344 Effectiveness of Rose Bengal test and fluorescence polarization assay in the diagnosis
345 of *Brucella spp*. infections in free range cattle reared in endemic areas in Zambia. Trop
346 Anim Health Prod. 2009 Jun 1;41(5):723-9.
347 25. Amegashie EA, Owusu-Dabo E, Salifu SP, Awuah AA, Baffour-Awuah S, Addofoh
348 N, et al. Sero-prevalence and occupational risk factors for *Brucella* infection among
349 slaughter house workers and butchers in Kumasi, Ghana. J Epidemiol Res. 2017;(3)1.
350 26. Zein AM. Seroprevalence of Brucellosis among high- risk occupational groups in
351 Northern State, Sudan. Khartoum Med J. 2014;07(2):994-999.
352 27. Pradel G, Schilitzer M. Antibiotics in malaria therapy and their effect on the parasite
353 epicoplast. Curr Mol Med. 2010;10(3):335-49.
354 28. Baheshti S, Rezaian GR, Azad F, Faghiri Z, Taheri F. Seroprevalence of Brucellosis
355 and Risk Factors Related to High Risk Occupational Groups in Kazeroon, South of
356 Iran. Int J Occup Environ Med. 2010;1(2):62-68.
357 29. Nikokar I, Hosseinpour M, Asmar M, Pirmohbatei S, Hakeimei F, Razavei MT.
358 Seroprevalence of Brucellosis among high risk individuals in Guilan, Iran. J Res Med
359 Sci. 2011;16 (10):1366-1371.
360 30. Mukhtar F. Brucellosis in high risk occupational group: Seroprevalence and analysis of
361 risk factors. J Pak Med Assoc. 2010;60:1031.
362 31. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map
363 of human brucellosis. Lancet Infect Dis. 2006 Feb 1;6(2):91-9.

32. Swai ES. Human Brucellosis: Seroprevalence and Risk Factors Related to High Risk Occupational Groups in Tanga Municipality, Tanzania. Zoonoses Public Health. 2009;56(4):183-187.

33. Zein AM. Seroprevalence of Brucellosis among high-risk occupational groups in Northern State, Sudan. Khartoum Med J. 2014 Feb 28;7(2):994-9.

34. Tumwine G, Matovu E, Kabasa JD, Owiny DO, Majalija S. Human brucellosis: sero-prevalence and associated risk factors in agro-pastoral communities of Kiboga District, Central Uganda. BMC Public Health. 2015;15(1):900.

35. Staal SJ, Kaguongo WN. The Ugandan dairy subsector targeting development opportunities. Nairobi, Kenya: International Livestock Research Institute (ILRI). 2003:30 pp.

36. Njombe AP, Msanga Y, Mbwambo N, Makembe N. The Tanzania dairy industry: Status, opportunities and prospects. In Ministry of Livestock and Fisheries Development. African Dairy Conference and Exhibition, Dar es Salaam, Tanzania. 2011:22pp.

Supporting Information

S1 Checklist. STROBE Checklist (DOC)

S2 Ethical Approval. Approval letter from Ministry of Health (MOH), Tanzania for collection of human blood samples (PDF)

S3 Dataset. Human dataset used for analysis (XLS)