Bovine brucellosis seroprevalence, farmers' awareness, practices and animal health extension services inputs in Mpwapwa district, Tanzania

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SUMMARY

Brucellosis is a zoonotic disease caused by gram negative cocobacillus bacteria of the genus *Brucella*. In cattle, the disease is caused by *Brucella abortus.* One of the main symptoms of brucellosis is the induction of abortion in the late term of gestation and first trimester in humans, drop in milk production resulting in economic and public health. Livestock is a key agricultural sub sector in Tanzania, depended by over 80% of rural household and contribute 5.9% of Gross Domestic Product (GDP) and cattle contribute 75% of all livestock in the country. In Mpwapwa District (Dodoma region), livestock keeping is one of the major means of economic activities, contribute 45% of district GDP and has significant contribution to the poverty reduction and food security. This district had sporadic cases of abortions in cattle and fever of unknown origin human possible related to brucellosis. Therefore, this study was aimed to determine the current seroprevalence of brucellosis in this district where there is no history of vaccination against brucellosis. A total of 545 serum samples were collected from sexually active cows and heifers in extensive farming system to detect antibodies against *Brucella abortus* using Rose Bengal Plate test(RBPT) followed by competitive ELISA(cELISA). A questionnaire to assess knowledge, attitude and practices (KAP) related to milk borne zoonosis (brucellosis) and efficiency of animal health extension services delivery was administered to 73 livestock keepers. Bovine brucellosis seroprevalence indicated that 57/545 (10.5%) cows tested were positive reactors by RBPT as screening test of which 5/57 (0.92%) confirmed positive by cELISA. 45% of the farmers have experienced several abortions in their cows, 78% were not aware of milk born zoonosis, 43% drink raw milk, 7% eat uncooked meat and 91% are not aware of the zoonotic potential of raw milk consumption. As for animal health services delivery, only 52% of farmers had access to animal health extension services and 97% of farmers have never seen samples being taken from their animals for further laboratory analysis. The findings from this study suggest that both bovine and humans are at potential risk of contracting brucellosis because of the presence of the disease in cattle population, the habit of drinking raw milk, unawareness of the disease and its impact to humans and inadequate extension service delivery.

Keywords: Rose Bengal Plate test, Enzyme Linked Immunosorbent Assay (ELISA), Knowledge and Attitude.

INTRODUCTION

Brucellosis is one of the most important bacterial zoonosis worldwide (Schelling and Zinsstag 2003). The etiological agent is a gram-negative coccobacillae belonging to the genus *Brucella. Brucella melitensis, B. abortus* and *B. suis* affect small ruminants, cattle and pigs respectively and cause severe economic loss in their productivity(OIE 2012). In Africa, different countries reported varying degree of brucellosis prevalence in bovine (Kunda J et al 2005) in earliest time between 1970's and 1990's (Eze 1977, Waghela 1977, Wernery 1979, Tekelye B 1989, Refai 1990, Mahmoud 1991, Kunda J *et al* 2005). Tanzania is having 21.3million cattle and ranked third largest country in Africa after Sudan and Ethiopia in having large number of cattle which are kept in extensive and intensive farming system (NBS, 2008). Extensive farming system is the major and widely practiced in rural areas where traditional cattle throughout their

production cycle are grazing in communal land and drink water in communal water sources. Extensive farming system is practiced by both pastoral and agropastoral communities. Intensive farming is mostly done in urban and peri-urban areas where dairy crossbreds and purebreds are kept; cattle are provided with water and feed in the house (United Republic of Tanzania 2011). In Tanzania cattle productivity is affected by number of factors including diseases, common zoonotic diseases are rabies, brucellosis, tuberculosis, anthrax and cysticercosis (MLFD 2011).

In Tanzania the first reported cases of brucellosis were dated to 1928 following abortions in exotic dairy cattles in Arusha(Kitalyi 1984). Since then the disease has been spreading and reported throughout the country (Corbel M J *et al* 1989) and caused significant economic loss and public health concern(Kunda J *et al* 2010). Studies especially in cattle have been done in different farming systems in selected regions of Tanzania. Research carried out in eastern, northern and southern zones of Tanzania in agro pastoral, pastoral and dairy farming practices have shown a range of seroprevalences between 5.7% to 15.2% (Weinhäupl I 2000, Shirima 2005, Swai 2005, Karimuribo E D 2007, G.M. Shirima 2010). Apart from the bovine prevalence, Kunda and others did a retrospective study in pastoral communities and found that, the age group of 16-35 years was mostly attended to the health care facility due to a number of reasons including brucellosis (Kunda 2005, Kunda J et al 2005)), this is the age group which is mostly engaged in livestock keeping and other economic activities in the rural areas and therefore affecting their productivity and livelihood.

In Dodoma region of Tanzania, the first case was diagnosed in 1937 at the Veterinary Research Centre Mpwapwa and a seroprevalence of 5.13% was detected in cattle using SAT (Kitalyi 1984).In 2001 at Veterinary Investigation Centre (VIC) Mpwapwa found a seroprevalence of 8.6% at Kongwa ranch and 3.7% in dairy cattle using SAT (Mghwira J 2001), but these results were not published. Since then, there have been diagnosed sporadic reported cases of brucellosis at VIC Mpwapwa laboratory on cattle abortions and in human fever of unknown origin in Mpwapwa district and seroprevalences of 13% and 44% respectively were diagnosed using Rose Bengal Plate Test (*unpublished data*).
Generally in Tanzania there is no extensive study which has been done to quantify the socioeconomic impact of brucellosis apart from existence of risk factors of the disease both in humans and livestock.

It has to be known that 90% of people in Mpwapwa district depends on agriculture and livestock keeping is their main economic activity, livestock alone contributes up to 45% of District GDP and Mpwapwa district is fairly arid and only gets good rains 2 years out of every 7 and therefore makes livestock be the sustainable and dependable source of income (MDC 2009). Apart from this fact, Mpwapwa district was thought to be at risk of brucellosis due to sporadic cases diagnosed at the VIC laboratory in Mpwapwa. The studies carried out in other selected areas of the country cannot give enough information of the prevalence and magnitude of the disease in Mpwapwa district. This implies that there is a paucity of information regarding disease status and public awareness in Mpwapwa district. All these information suggested the call for the study of brucellosis which has economic and public health concerns to the people of Mpwapwa and to the district authority.

Therefore this study wanted to establish the seroprevalence of brucellosis, identify the farmer's knowledge, attitude and practices and animal health extension services efficiency towards the disease in extensive farming system in Mpwapwa district of Dodoma region in Tanzania. This relevant information will raise awareness of the problem in Mpwapwa and hence assist policy makers and executive authorities to devise strategies for control of the disease and hence increased farm productivity, food security and assured public health.

METHODOLOGY

Description of the study area.

This study was conducted in Mpwapwa district one of the seven districts in Dodoma region. Administratively, Dodoma region has been

divided into Region, districts, division, wards, village and hamlets. According to National Livestock sample census, Dodoma region had 1,166,715 indigenous cattle covering 98.4% of the total number of cattle in the region, out of which 156,031(15%) are found in Mpwapwa district (NBS 2008) . In Mpwapwa District, livestock sector produces 45% of the district GDP and ninety percent (90%) of people in Mpwapwa depend on agriculture and livestock as their source of income(MDC 2009).

Geographically, Mpwapwa is located 120Kms from Dodoma Headquarter, the capital of Tanzania. Mpwapwa District lies between Latitude 6°00" and 7° 30"S and between Longitude 35°45" and 37° 00"East of Greenwich in the semi-arid zone of Central Tanzania (Figure 1) with a diurnal temperature range of 27°C and average rainfall ranging from 450 – 700 mm. Most rainfall found in mountainous area 7000ft above sea level and least rainfall found in plateau area 3,500ft above sea level, this discrepancy brings differences in agricultural produce every year. The major ethnic groups in this district are Gogo, Kaguru and Tiriko which are agropastoral communities keep cattles in extensive farming system. The literacy rate in Mpwapwa district is 53% (MDC 2009). The administrative divisions of the United Republic of Tanzania start from the region, district, division, ward, village and hamlets, this is controlled by Part I, Article 2.2 of the Constitution of Tanzania(Constitution 1977). Twelve out of eighteen wards (67%) in Mpwapwa District were involved in the study. The wards covered by this study were Lupeta, Mbori, Matomondo, Kimagai, Pwaga, Luhundwa, Kibakwe, Ving'hawe, Berege ,Chunyu and Chitemo

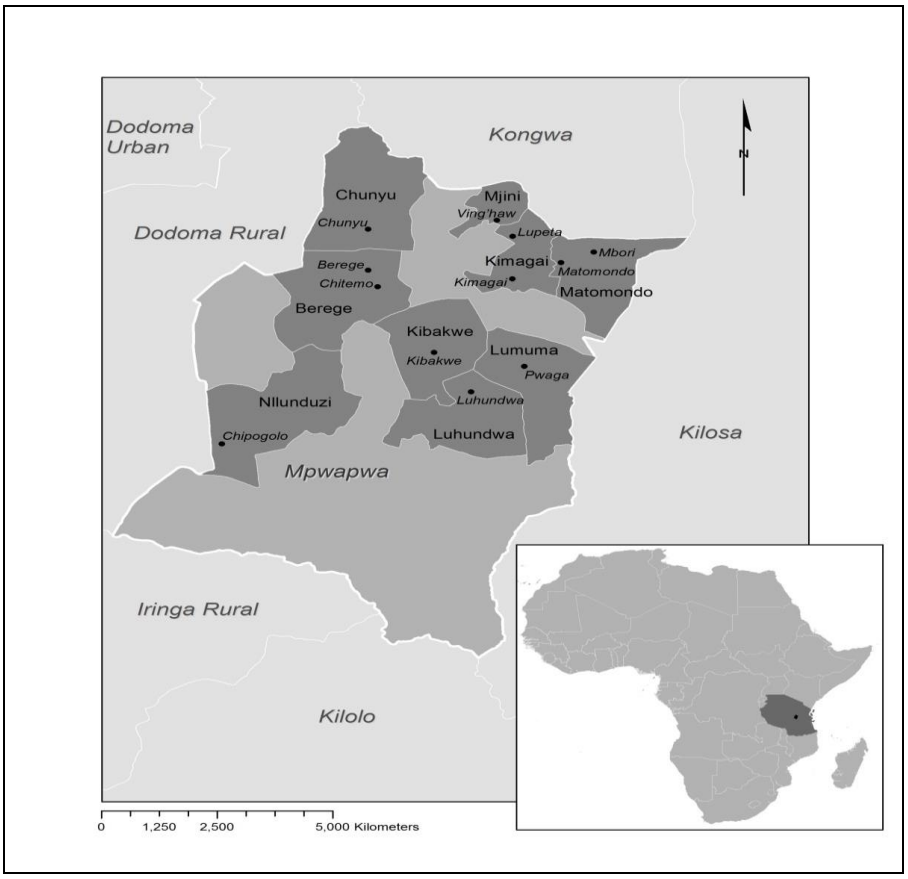

Figure 1: Location of study area in Mpwapwa District

The map shows Mpwapwa district in Dodoma region (Figure 1). On the east bordering to Kilosa district of Morogoro region, on the South bordering to Kilolo and Iringa Rural districts of Iringa region. On the north and west, bordering to Kongwa and Dodoma rural districts of Dodoma region. The named dark grey highlighted areas show the wards involved during the study area in Mpwapwa District.

Study population and design

This study was aimed at identifying the magnitude of the brucellosis disease in cattle kept by agropastoral communities in extensive farming system. Cattle population in Mpwapwa district was estimated to be 156,031 according to National bureau of the statistic report for the year 2007/8. For studying the disease in this population, the sample size was determined to assume the confidence interval of 95%, and at a level precision (e) of 0.05 (±5%) (Kasiulevičius V 2006). $n = N/1 + Ne^2$ where n= sample size, N is study population and e is precision
$n = 156031/1 + (156031)(0.05)^2 = 399$.

Therefore at least three hundred and ninety nine (399) samples were needed to be collected to get representative samples. In this study; five hundred and forty five (545) blood samples were collected. The study population involved all mature female cows including sexually active heifers, lactating cows and pregnant ones found in Mpwapwa district. A cross sectional approach was deployed during the study period of March 2013 when there is plenty pastures and animals are kept in a household kraal to identify the herds keeping cattle and hence the study units. The study units were randomly selected from the herd without replacement for sample collection. For sample collection, after getting the farmer's consent, blood sample was collected from each study unit. From each study unit, 6ml of blood was collected through jugular vein and stored in red top vacutainer tube and kept in cool box containing ice packs. In the evening the samples were kept in the refrigerator (4^oC) for twenty four hours and then were centrifuged. Serum was transferred to Eppendorf tubes and stored at -20^0C for further analysis.

Antibody detection using Rose Bengal Plate Test Procedure (RBPT)

RBPT antigen from Veterinary Laboratory Agency, New Haw, Addelestone Surrey KT153NB, UK was used for screening *Brucella* antibodies in cattle. The serological testing procedure has agreement to *FAO/WHO* procedure was conducted at Tanzania Veterinary Laboratory Agency- Mpwapwa, Dodoma by me, Isaac Mengele. Thirty (30µL) microliter of test sample serum was mixed with thirty (30µL) microliter of Rose Bengal Antigen. The mixture was then rocked for about 3 minute. Formation of any degree of agglutination was recorded as positive reaction and where no agglutination was recorded as negative.

Competitive ELISA Testing Procedure

The test samples were shipped to Ethiopia at National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia and the test was run by GetnetMekonnen. The Compelisa 160 & 400 kit® from Apha Scientific, UK was used for confirmation of all samples tested positive to RBPT. The serum samples were added to the *B. melitensis* LPS coated wells on the microtitre plates together with monoclonal antibodies specific for a common epitope on the LPS antigen. After incubation, the microplate was washed and secondary antibody conjugated with horseradish peroxidase was added and incubated. After incubation the substrate was added. On completion of the assays, optical densities at 450 nm (OD450) of samples and positive and negative controls were determined using a microplate reader (Multiskan EX, Thermo Electron Corporation, Shanghai, China).

Questionnaire Administration

A pretested structured questionnaire of predetermined questions was designed to collect information regarding the settlement, farming experience, awareness, knowledge and practices related to disease control and potential risk factors for contracting disease both in humans and animals. Direct personal investigation method was used following interviewee consent. The national language (Swahili) was used to gather relevant

information from the interviewee and fill the questionnaire. In this study, the interviewee was the livestock keepers whose animals were collected blood samples. The interview was conducted to either father or mother based on who was available.

Data Analysis

For the blood samples, the test results of each animal in a ward and hence a district was entered in a spreadsheet. Prevalence risk is the proportion of a population that has a specific disease or attribute at a specified point in time as explained by (Stevenson 2008) was used to calculate the prevalence. For the qualitative data the Epi Info version 7 was used to design a questionnaire form, data were entered and analyzed to find out the frequencies of their responses as descriptive statistics for each variable against the respective response of the interviewee. To serve the purpose, descriptive statistics can easily bring interpretation and understanding of the derived information.

RESULTS

Seroprevalence of brucellosis in Mpwapwa

A total of 545 individual bovine blood samples from 73 households in 12 wards were collected and tested for antibodies against *Brucellaabortus* using Rose Bengal Plate test and then confirmed by competitive ELISA. For Rose Bengal Plate Test, 57 cows (10.5%) reacted positive and which ewere then subjected to cELISA and five (5) were confirmed positive to give a prevalence of 0.92%.

Table 1. Diagnostic test results

Species	**No Tested**	Number of positive animals (prevalence)	
		RBPT	**cELISA**
Bovine	545	57 (10.5%)	5 (0.92%)

Based on RBPT, the test results showed that, Luhundwa and Pwaga wards had highest prevalence of brucellosis (44.4%) followed by Mbori 17.65% and Kibakwe 14.71%. Berege ward had least prevalence of 1.92% followed by Ving'hawe 3.77% (**Table 2**). For the cELISA test two samples were found in LuhundwanaPwaga giving the ward prevalence of 22.22% and 11.11% respectively and one sample (4.16%) was found in Lupeta. These results shows that three ward (25%) out of twelve were affected by the disease based on the fact that each ward had at least one positive reactors to cELISA.

Table 2. RBPT test results summary

Species	Wards	Results	
		RBPT	cELISA
		Seropositive %	Seropositive %
Bovine	Lupeta	8.33	4.16
	Mbori	17.65	0
	Matomondo	10.42	0
	Kimagai	11.84	0
	Pwaga	44.44	11.11
	Luhundwa	44.44	22.22
	Chamtumile	14.71	0
	Kibakwe	11.11	0
	Ving'hawe	3.77	0
	Berege	1.92	0
	Chitemo	5.48	0
	Chunyu	7.14	0

Overall, out of five hundred and forty five samples, fifty-seven (10.5%) samples tested positive according to RBPT for screening and for confirmatory test using most specific test cELISA 5 samples (0.9%) turned positive to the disease. Therefore, the seroprevalence of brucellosis in Mpwapwa District was 0.9%.

Knowledge, attitude and practices

The questionnaire results showed that, all households interviewed had permanent settlement and 68% of households have been keeping cattle for a period of over ten years, 24% less than ten years and 8% less than five years and 90% of these household have never attended any formal or informal livestock training.

The agro-pastoral households which were interviewed, few had understanding and knowledge of milk-borne zoonosis, only 22% of

the households were aware of the milk-borne zoonosis and how human can contract infection (**Table 4**) and only 8% tested their animals against the disease and knew how animals can contract the disease. Regarding attitude toward the utilization of the animal products, the results showed that, 92% of the household drinks milk from their animals and 43% do not boil the milk before drinking. Seventy three percent (73%) of the household sell milk to other people and 96% sell raw milk to the public (Table 3). On the other hand 7% of the households eat uncooked meat. Hundred percent of households use natural services to breed their animals and only 92% use their own bull and other 8% hires from other farmers. Forty five percent (45%) had experienced abortion in their animals by the unknown causes. And only 53% had an access and happened to seek extension services in their respective villages and wards regardless of the service quality. Twenty seven percent of famers used to vaccinate animals as a measure to control other diseases but not against brucellosis

Table 3. Knowledge, attitudes and practices

Attribute	Responses	
	Yes (%)	No (%)
Having permanent settlement	100	0
Time lapsed keeping livestock	68 (>10yrs)	32 (<10yrs)
Attended livestock training	8	92
Farmers participated in livestock research trials	10	90
Seeking livestock extension's advice	52	48
Drinking milk from their animals	92	8
Boiling milk before drinking	57	43
Selling milk to other people	73	27
Boiling milk before selling	4	96

%: Percent, >: Greater than, <: Less than

Table 4. Household responses to variable attributes

Attribute	**Responses**	
	Yes (%)	**No (%)**
Awareness of milk born disease human can get	22	78
Knowing means by which animals can get the diseases	8	92
Testing animals for the presence of diseases	8	92
Experiencing deaths of animals caused by diseases	55	45
Method for Breeding animals	100 (Natural)	0 (Artificial)
Natural, using own bull	91	9
Experiencing abortions in animals	45	55
If Yes, how many times?	38% once, 62% several	
How preventing animals from getting diseases	27 (vaccination)	73 (others)

%: Percent

DISCUSSION

Brucellosis is one of the relatively well-studied diseases in Tanzania and Sub-Saharan Africa in general. Apart from being well- studied, resources allocated to control the disease have remained to be the bottleneck and the disease still remain one *mellitensis* and *B.suis*(Godfroid J 2005, Sanogo M 2012). Being neglected, the diseases has continue to be of serious economic losses to the farmers and government through production losses and public health hazards and control and eradication program of the disease (Mekonnen 2010). Bovine brucellosis has been controlled in most of developed countries but remained the bottleneck in cattle production and public health concern in developing countries (Apan 2007, Lee 2009).

This study revealed that, there is circulating brucella among bovine in extensive farming practices in Mpwapwa district. Based on the OIE recommended tests (Biancifiori 2000), the Rose Bengal Plate screening test suggested the seroprevalence of 10.5% and competitive ELISA confirmatory test suggested the seroprevalence of 0.9%. The disagreement of the two tests is attributed by the fact that, RBPT is relatively less sensitive compared to cELISA and therefore susceptible to cross reaction with other gram negative bacteria like *Yersinia enterocolitica* O: 9, *E.coli* O: 157; *Vibrio cholera* and some *Salmonella*spp(Nielsen K et al 2004). Also is antigenically closely related Ochrobactrum(Victoria M D et al 2004) to produce false positive reactors which are interpreted as positive unlike cELISA which has been validated in cattle with high sensitivity and relatively low specificity than RBPT. The disagreement of the two tests has also been found in Northern Nigeria by Mai and others when they found a seroprevalence of 37% using RBPT and when confirmed by cELISA the results was 26.3% (Mai H M et al 2012).

Apart from the overall seroprevalence of brucellosis in Mpwapwa district being 0.9%, follow ups researches showed that, one locus of Mpwapwa had higher seroprevalence of brucellosis, before 2006 a seroprevalence of 6.72% was recorded at Kikombo in Livestock Research Centre cattle farm, following test and of the most important bacterial zoonosis worldwide. The known Brucella species circulating in the domesticated animals are *B.abortus* in Bovine, *B. mellitensi* is small ruminants and *B.suis* in pigs and B.canis in dogs (OIE 2012). Brucellosis in bovine may also be caused by other *Brucella s*pecies like *B.* slaughter policy of positive reactors, the seroprevalence become 0% by 2010 (Shirima G et al 2014), to date no test was conducted to follow up the disease status in the farm apart from existence of potential risk factors like existence of multiple livestock species and others (Muma 2007, Sanogo M 2012, Mai 2013). This and other measures to eradicate the disease have never been done elsewhere in Mpwapwa district and therefore make cattle in other areas of Mpwapwa being the potential source of infection within and outside the district. In other regions of Tanzania, seroprevalence of 6.8% was found in cattle in Katavi-Rukwa regions ecosystem (Assenga J A et al 2015) where there is interaction of domestic and wildlife, this prevalence is higher than what was found in Mpwapwa and this is purely attributed by the interaction of domestic and wild ruminants. A research study carried out in Arusha and Manyara regions, the seroprevalence of 4.9% was found in cattle which was higher than what was found in Mpwapwa district. In Arusha and Manyara higher prevalence might have been influenced by the interaction with the wildlife and other factors as well (Muma 2007, Shirima G M 2010, Mai 2013).

Cross sectional studies carried out in Nothern and Easter Uganda and Kampala economic zone revealed a seroprevalences of 7.5% and 5% respectively (Makita K et al 2011, Mugizi D R et al 2015), these are higher than what was found in Mpwapwa district. Studies carried out traditionally managed cattle in Southern Zambia and Nile Delta revealed seroprevalence of 20.7% and 12% respectively (Hegazy Y.M 2011, Muma J B et al 2013) which were higher than what was found in Mpwapwa district.

In West African country Niger, the seroprevalence of 1.3% was found in traditional cattle, sheep and goats reared in Urban, per urban and rural areas (Boukary A R et al 2013). All these findings suggest that, African countries from North, West, East and central are endemic to

the disease, this advocate for the joint effort in controlling and hence eradicating the disease.

Knowledge of a disease is an important step toward prevention and control measures(Tschopp R et al 2013). Most farmers in extensive livestock production in Mpwapwa district have been keeping cattle for more than five years and unfortunately had no knowledge (91%) about how animals can contract the disease, the situation is a bit worse in Bangladesh where over 93% of farmers had no knowledge of brucellosis (Suchandan S et al 2012) and unlike the Egyptians, where 83% of Egyptian farmers have heard about the brucellosis and 98% of them were confident that cow can have brucellosis (Holt 2011). Nevertheless, the majority of these farmers (91%) drink milk from their animals and only 57% boil milk before drinking and 73% of farmers sell milk from their animals, 96% of them sell raw milk for public consumption unlike in Egypt where only 32.7% of households regularly sell their raw milk for public consumption (Holt 2011) and 7% of these farmers eat raw meat. Studies in Egypt shows that, 78% of interviewee never sell suspected or infected animal to the neighbors (Holt 2011) unlike in Tanzania where animals or their products are sold to the public without knowing the disease status of their animals. These statistics reveals the complex epidemiology of acquisitioning milk-borne zoonosis in human being not only in Mpwapwa district but also throughout the country.

Animal health extension services delivery in Mpwapwa is facing a lot of challenges, forty five percent (45%) of the village do not have extension staff, unreliability of the extension officers due to lack of reliable transport as only 13% of extension staff have motorcycle, some live in town but working in the village, this have been a serious hindrance in development of livestock sector in Mpwapwa District as there is limited time to plan and decide with the farmers on how livestock sector can be developed right from the village or ward level. In Mpwapwa district, there are three reviving veterinary centers which are under rehabilitation. They are found in Kibakwe, Chipogolo and Matomondo wards. These veterinary centers do not have capacity of diagnosing a single disease so far due to lack of diagnostic equipments and skilled persons, apart from this challenge; extension officers do not make regular use of laboratory facility at Veterinary Investigation Center for their daily work. This is supported by the fact that 97% of interviewed have never seen their animals taken samples for further laboratory investigation to confirm the disease and 45% of farmers were not revealed the cause of death of their animals. This existing situation cause significant losses to farmers and profession disrespects amongst farmers.

CONCLUSION AND RECOMMENDATIONS

This study reports the seroprevalence of brucellosis at a prevalence of 0.9 % in Mpwapwa District, Dodoma region in Tanzania. Even though the prevalence is relatively low, the presence of anti-*Brucella* antibodies suggests the exposure status to *Brucella* spp. Therefore due to presence of *Brucella*spp and the complex nature of the brucellosis epidemiology brings an alarming situation in the district. The disease is highly contagious and of economic importance therefore silently kills the farm and adversely causes food insecurity, financial insecurity of farmers and health of the farmer, household and general public. The disease needs to be controlled by ensuring that selected breeding cows are free from the disease, first by test and voluntary slaughter of positive animals and vaccination using reliable and affordable vaccine produced in the country. Since majority farmers are not aware of the disease and its zoonotic nature, the authorities at the district level can provide informal education and create public awareness campaigns by taking the advantage of sedentary farmers using committed extension staffs. Animal health extensions staffs need to be given first priority by being provided with transport particularly motorcycles for them to be productive and bring impact to the community they serve. It is also call to the central government, private sector and development partners to put resources together to resolve the existing problems in Mpwapwa district, region and country at large.

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