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Occupational hazards associated with human brucellosis in abattoir settings: A case study of Dodoma abattoir in Tanzania

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Brucellosis is one of the most widespread zoonotic diseases posing a serious obstacle to public health, food safety and security and, socio-economic development in most African countries. A cross-sectional study was conducted to establish practices that may pose occupational risks of transmission of brucellosis to people working in abattoirs in Tanzania. A total of 452 serum samples; 190, 200 and 62 from cattle, goats and human, respectively were collected in animals and workers at Dodoma abattoir, Tanzania. The samples were screened for brucellosis using Rose Bengal Plate test (RBPT) and indirect enzyme-linked immunosorbent assay (iELISA). A semi-structured questionnaire was used to collect data for assessing the knowledge, awareness and practices related to brucellosis exposure. Data were analyzed to determine the association of brucellosis seropositivity with the knowledge, awareness and practices of the workers. The seroprevalence of brucellosis in cattle, goats and abattoir workers was 7.3, 1.5 and 1.6%, respectively based on Rose Bengal Plate Test. The seroprevalence was 4.7% in cattle, 1.6% in humans and none in goats when samples were tested by indirect enzyme-linked immunosorbent assay. The results of this study show that, there is a potential occupational risk of acquisition of brucellosis for abattoir workers and hence, the need for awareness campaigns and taking appropriate precautions to minimize the zoonotic risks is greatly required.

Key words: Brucellosis, abattoir, occupational risk, Rose Bengal plate test, indirect enzyme-linked immunosorbent assay.

INTRODUCTION

Brucellosis is one of the most widespread zoonotic diseases posing a serious obstacle to public health, food safety and security and, socio-economic development in most African countries (Mcdermott et al., 2013; Mcdermott and Arimi, 2002). Brucellosis affects many animal species, including such as cattle, sheep, and goats.

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Most human infections are acquired through direct contact with infected animals, placentas, foetal membranes, vaginal discharges or aborted foetuses, inhalation of infectious materials or via indirect transmission through untreated milk and milk products, undercooked meat or blood (Liu, 2015; Makita et al., 2008; Galińska and Zagórski, 2013). Brucellosis has wide-ranging impacts that include animal losses due to abortion, loss in milk production, death of infected animals and debilitating human illness causing reduced working capacity (Jergefa et al., 2009; Mcdermott et al., 2013).

Brucellosis in humans, also known as undulant fever, presents with intermittent fever, sweating, joint and low back pains, headache, fatigue, weight loss and general weakness persisting for a long time (Dean et al., 2012; Madut et al., 2018). These clinical signs resemble other febrile illnesses such as malaria, typhoid, rheumatic fever and viral diseases hence, the disease is often misdiagnosed and under-reported in most countries in sub-Saharan Africa (Bouley et al., 2012; Crump et al., 2013). Brucellosis is an occupational disease to farmers, herders, veterinarians, slaughterhouse workers, butchers and laboratory personnel who commonly get into contact with the animals or animal by-products in the course of their work (Gardner, 2005; Mirambo et al., 2018; Schneider et al., 2013). However, abattoir workers may be the more risky group compared to other occupations because they are overexposed to carcasses, viscera, body fluids and organs of infected animals (Mirambo et al., 2018; Mukhtar, 2010; Swai and Schoonman, 2009), and this is compounded by their proneness to knife cut wounds (Banjo et al., 2013). Consequently, abattoir workers are at greatest risk to get brucellosis through open wounds on bare hands, splashing of infected fluids in the conjunctiva and inhalation of aerosols in the slaughtering area (Aworh et al., 2013; Goncalves et al., 2006). The seroprevalence of human brucellosis in Tanzania has been reported to range from 14.1 to 28.2% (Chota et al., 2016; Mngumi et al., 2016) in pastoral and agropastoral settings.

In Tanzania, brucellosis in slaughterhouse personnel has been previously reported to be high, ranging from 19.5 to 42.1% in several slaughterhouses (Mirambo et al., 2018; Swai and Schoonman, 2009). The high prevalence may be attributed to lack of awareness on the disease among the workers and non-use of protective gears. It is well-known that a well-designed abattoir having separate sections from stunning to packaging with minimum exposure and coupled with strict adherence to biosafety measures and practices can reduce the chances of the occupational exposure to brucellosis (Zakaria et al., 2018). The objective of this study, therefore, was to determine the risk of occupational exposure to brucellosis among workers in Dodoma modern abattoir in Tanzania. The results of the study would be used to inform public health authorities on the magnitude of the problem so that they can design appropriate interventions for reducing the risk.

MATERIALS AND METHODS

Study area

The study was conducted at Dodoma abattoir, one of among the most modern abattoirs in Dodoma Region, Tanzania. Dodoma Region is located between latitudes 6° 57'S and 3° 82'S and between 36° 26' and 35° 26'E and, bordered by four regions, namely; Manyara, Iringa, Singida and Morogoro (Population and Housing Census of Tanzania [PHCT], 2013). Dodoma is the current capital city of the United Republic of Tanzania and is growing very fast in terms of both human population and economic activities resulting in increased demand for food supplies including meat.

Dodoma abattoir is the largest and most modern one in the central zone, currently the only in the country slaughtering animals for export. The animals slaughtered at the abattoir include cattle, goats and in a very few occasions sheep. Meat from goats is mainly exported to Comoro, Iraq, Vietnam, Oman, Qatar, Kuwait and United Arabs Emirates while beef is consumed locally. Dodoma abattoir has well established infrastructures from stunning area to packaging area which facilitates unidirectional flow of carcass. Animals slaughtered at Dodoma abattoir originate mainly from neighbouring regions of Iringa, Singida and Manyara as well as some from Lake and Western zone regions of Kigoma, Tabora, Mwanza, Shinyanga and Geita where brucellosis has been reported to be prevalent in livestock (Chitupila et al., 2015).

Study design

A cross-sectional study design was adopted. It was carried out from March to August 2018 to determine the occupational hazards and practices associated with risk of exposure of abattoir workers to brucellosis and assess their knowledge on the disease and other zoonotic diseases.

Determination of sample size

The sample size of animals was determined using the formula,

$N=1.96^2P\ (1-P)/d^2$ (Naing et al., 2006).

Where; N=Sample size, P=Expected prevalence, d=Precision level.

By using the expected prevalence (P) of 12%, precision level (d) of 0.05 and confidence level of 95%. Thus, 324 samples were to be collected from all animal species.

Sampling procedure

A systematic random sampling method was used to select cattle and goats. By using a sampling fraction of 20% every fifth animal was sampled (Cadmus et al., 2006). Before sample collection, all the animals to be slaughtered in that same day were arranged in the crush and the first animal was randomly selected followed by every fifth animal counted from the first selected in a row (Cadmus et al., 2006). Abattoir workers above 18 years who had worked in the facility for at least one year were included in the study upon their consent to participate in the study.

Collection of blood samples

A total of 390 animals destined for slaughter comprising 200 goats

and 190 cattle were bled for brucellosis screening. Furthermore, 62 human blood samples were collected from the abattoir workers who agreed and consented to participate in the study. The inclusion criteria of individuals to participate included age of at least 18 years and working in the abattoir for at least one year. However, prior to bleeding animals, verbal consent was sought from livestock traders. Prior to bleeding, individual animal information such as breed, age, sex and geographical origin were recorded. Sterile plain vacutainer tubes were used to collect approximately 5 ml of blood from the jugular vein in animals and brachial vein in humans. All blood samples were stored in a cool box with ice packs and transported to the Tanzania Veterinary Laboratory Agency station in Dodoma, where they were left at room temperature for six hours to allow separation of clear serum and blood clots.

A semi-structured questionnaire was used to assess the knowledge of abattoir workers about brucellosis as well as their attitude and practices with regard to the potential of occupational exposure to the disease. Social and demographic factors such as sex, age, marital status, education level, period of time worked at the abattoir, types of activities within and outside the abattoir were gathered. The questionnaire was administered in Swahili language because most of the workers were conversant with the same.

Laboratory analysis

Blood samples were centrifuged at 3000 g for 20 min for serum separation. Serum samples were collected in cryovials, well labelled and stored at -20°C until analysis.

Rose Bengal Plate Test (RBPT)

Four hundred and fifty-two serum samples were screened for anti-brucella antibodies using Rose Bengal buffered antigen (ID Vet, France). The test detects both the IgM and IgG. The test procedure was performed as recommended by (OIE, 2016). Briefly, 25 µl of buffered antigen and 25 µl of test serum were pipetted on the white tile plate and then mixed thoroughly. The white tile plate was rocked for four minutes while observing the degree of agglutination. The sample was classified as positive if agglutination was observed and negative if there was no agglutination. Sample with some degree of agglutinations whether weak or strong was considered positive (Figure 1).

Indirect Enzyme-Linked Immunosorbent Assay (SVANOVIR® Brucella-Ab I-ELISA) was used to further test positive samples from the Rose Bengal plate test. Indirect ELISA measures the binding of secondary antibodies to a primary antibody isotype bound onto the *Brucella* lipopolysaccharides antigen. The test detects only the Ig G. The test was performed according to the manufacturer's instructions. Briefly, 90 µl of sample dilution buffer was added to each well to be used for serum samples and controls followed by adding 10 µl of positive control serum and 10 µl negative control serum, respectively to selected wells coated with *Brucella abortus* antigen. For confirmation purposes, the control sera were run in duplicates.

In the remaining wells coated with *B. abortus* antigen, 10 µl of serum samples was added, shaken thoroughly, sealed and incubated at 37°C for one hour. The plate was rinsed with PBS-tween buffer by filling up the wells at each rinse, emptied and taps hard to remove all remains of fluid. After rinsing, 100 µl of Horseradish peroxidase(HRP) conjugate was added to each well, sealed the plate and incubated at 37°C for one hour, followed by adding 100 µl of substrate solution to each well and incubation for 10 min at room temperature. The reaction was stopped by adding 50 µl of stop solution to each well mixed thoroughly and the optical density of the controls and samples were measured at 450 nm in a microplate photometer within 15 min. The optical density (OD) values were read in a microplate reader (ELISA reader, Multiskan RC version 6.0, Thermo Labsystems, Helsinki). Strong positive (considered as 100% positivity) and negative standards were used. The results were expressed as per cent positivity (PP) of the sample tested in relation to the strong positive control. The criterion for considering a sample positive or negative was based on percent positivity calculated as follows:

$$\text{Percent Positivity (PP)} = \frac{\text{Mean ODD of tested sample}}{\text{Mean ODD of Positive control}} \times 100$$

A sample was considered positive or negative using the cut-off values recommended by the manufacturer. Samples with equal or above 60% positivity were considered positive.

Data analysis

Data were stored and cleaned in Microsoft Excel spreadsheet and analyzed using R software version 3.4.3 ("Kite-Eating Tree" Copyright (C) 2017 The R Foundation for Statistical Computing platform). Descriptive statistics, particularly frequencies were computed for proportions of *Brucella* positive animals and human. Categorical dichotomous variables were computed and compared using Chi-square test at a critical probability of $\alpha \leq 0.05$. Odds ratio, 95% confidence interval, Chi-square and Fisher's exact tests were computed to determine the degree of association between *Brucella* seropositivity with knowledge, awareness and practices of abattoir workers.

Ethical consideration

The ethical clearance for conducting this study was granted by the Institutional Review Board of Medical Research Coordinating Committee of the National Institute for Medical Research; reference number NIMR/HQ/R8a /Vol. IX /1627. Permission to conduct the study at the abattoir was sought and granted by the General Manager of the Tanzania Meat Company (TMC). Permission to conduct the study in Dodoma municipality was sought and granted by District Livestock Officer for animal studies and District Medical Officers for human studies, respectively. Additionally, the verbal consent was sought from the livestock traders after explaining to them the aim and benefit of the study. The research was conducted in compliance with the Animal Welfare Act 2008 and using guidelines and protocols stipulating how human subjects are used in research.

RESULTS

The seroprevalence of brucellosis in abattoir workers and animals slaughtered at Dodoma abattoir

The seroprevalence of brucellosis in cattle, goats and abattoir workers was 7.3, 1.5 and 1.6% based on Rose Bengal plate test, respectively. The positive sera by Rose Bengal plate test were further tested by enzyme-linked immunosorbent assay and only nine (4.7%) cattle and one (1.6%) human samples were positive. All samples from goats were seronegative (Table 1).

Demographic characteristics of participants

A total of 62 (51.7%) out of the 120 abattoir workers volunteered for blood sample collection and of these, 49

Table 1. The seroprevalence of brucellosis in animals and abattoir workers at Dodoma abattoir.

Sample source	Rose Bengal plate test (RBPT)		Enzyme-linked immunosorbent assay (ELISA)	
	No (Positive)	Prevalence (%)	No (Positive)	Prevalence (%)
Cattle	190 (14)	7.3	190 (9)	4.7
Goats	200 (3)	1.5	200 (0)	0.0
Human	62 (1)	1.6	62 (1)	1.6
Total	**452 (18)**	**3.98**	**452 (10)**	**2.1**

Table 2. Demographic characteristics of workers at Dodoma abattoir.

Variable	Levels	Frequency (N=49)	Percentage
Age	20-30	22	44.9
	31-40	19	38.8
	41-50	6	12.2
	51-60	2	4.1
Sex	Male	27	55.1
	Female	22	44.9
Level of education	Primary	20	40.8
	Secondary	23	46.9
	Certificate	1	2.0
	Diploma	4	8.2
	University	1	2.0
Marital status	Married	33	67.3
	Single	15	30.6
	Widow	1	2.0
Section at work	Carcass splitting	1	2.0
	Cleanliness	13	26.5
	Evisceration	7	14.3
	Meat inspection	6	12.2
	Skinning	16	32.7
	Slaughtering	1	2.0
Duration of work	1-3 years	9	18.4
	4 – 6 years	30	61.2
	7-9 years	9	18.4
	10-12 years	2	4.1
	13-15 years	3	6.1

(79%) also participated in semi-structured questionnaire survey. The remaining participants did not participate in the questionnaire survey because they were retrenched before the study was completed. The participants' age ranged from 21 to 59 years with the overall average age of 31 ± 8.087) years and the majority, 44.9% (n =22) were in the 20–30 years age group. Most of the participants were males 55.1% (n = 27) and 59.2% (n = 29) had post-primary education. The participants duration of work at the abattoir ranged from 1 to 14 years' duration of work at the abattoir ranged from 1 to 14 years with an average age of 5 ± 2.79) years (Table 2).

Table 3. Awareness of abattoir workers on brucellosis and other zoonotic diseases.

Variable	Level	No	Brucellosis (Yes) No (%)	χ^2 (df)	*P*-value	Zoonosis (Yes) No (%)	χ^2 (df)	*P*-value
Sex	Female	22	22 (100.0)			16 (72.73)		
	Male	27	15 (55.56)	10.56 (1)	**0.001**	14 (51.85)	1.43 (1)	0.231
Age	20-30	22	16 (72.73)			11(50.0)		
	31-40	19	13 (68.42)			11(57.89)		
	41-50	6	6 (100.0)			6 (100.0)		
	51-60	2	2 (100.0)	28.7 (23)	0.181	2 (100.0)		
Education	Primary	20	13 (65.0)			11 (55.0)		
	Secondary	23	18 (78.26)			13 (56.52)		
	Tertiary	6	6 (100.0)	3.23 (4)	0.519	6 (100.0)	4.34 (4)	0.362
Occupation	Carcass splitting	1	1 (100.0)			1 (100.0)		
	Cleanliness	13	12 (92.31)	23.7 (9)	**0.0048**	8 (61.54)	11.33(9)	0.254
	Evisceration	7	7 (100.0)			5 (71.43)		
	Meat inspection	6	6 (100.0)			6 (100.0)		
	Skinning	16	6 (37.5)			7 (43.75)		
	Slaughtering	1	1 (100.0)			1 (100.0)		
Duration of work (years)	≤3	9	4 (44.44)	18.8(10)	**0.05**	4 (44.44)	13.77(10)	0.179
	4-6	29	20 (68.97)			14 (48.28)		
	7-9	9	8 (88.89)			7 (77.78)		
	10-12	2	2 (100.0)			2 (100.0)		
	13-15	3	3 (100.0)			3 (100.0)		

Awareness of abattoir workers on brucellosis and other zoonotic diseases

In assessing the awareness level of abattoir workers on brucellosis and other zoonotic diseases, it was found that there is a significant difference in awareness on brucellosis among sex (p=0.001), occupation (p=0.0048), duration of work (p=0.05) groups while there was no significant difference in the education (p=0.519) and age categories (p=0.181). The participants were 14 times aware of brucellosis than other zoonotic diseases (OR 14, 95%CI 2.47,160.37, p=0.00046) (Table 3). However, there was no statistically significant difference in the knowledge of participants on other zoonotic diseases among gender, age, education, occupation and duration on the job categories (p > 0.05). Furthermore, 76% (n=37) of abattoir workers had knowledge of brucellosis and 61% (n=30) other zoonoses

DISCUSSION

Brucellosis is a zoonotic disease which affects both animals and humans who come in contact with infected animals and their products such as blood, milk, meat or foetus and placental fluids as well as other contaminated materials. Therefore, abattoir workers are at the greatest risk of acquiring brucellosis because they frequently come in contact with the animal and by-products during their routine activities.

The seroprevalence of 4.7% in slaughtered cattle in this study is an indication that brucellosis is prevalent in areas where the slaughtered animals originated. This poses a risk to abattoir workers who do not comply with basic biosafety principles in slaughter facilities. This prevalence recorded in this study is lower than those reported by other workers such as 12% in Tanga (Swai and Schoonman, 2012) and 21% in Karagwe (Kiputa et al., 2008). This finding may probably suggest that slaughter animals may have originated from areas with low infection rates.

Although the prevalence of brucellosis in humans was low (1.6%), it still indicates that workers at the abattoir were at risk of acquiring brucellosis in the course of their work, that is handling live animals and carcases. The observed prevalence was also low when compared to findings recorded in other studies in Tanzania (Mirambo et al., 2018; Swai and Schoonman, 2009) and elsewhere (Agada et al., 2018; Aworh et al., 2013; Cadmus et al., 2006; Mukhtar, 2010; Nabukenya et al., 2013; Osoro et

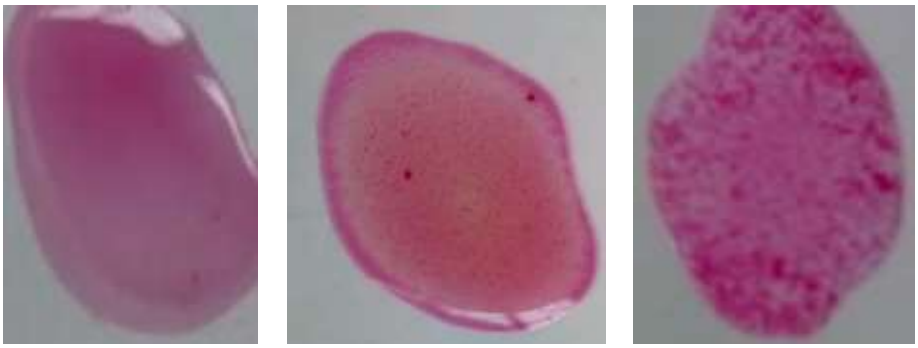

Figure 1. Rose Bengal plate test showing agglutinations. From left No agglutination, Moderate agglutinations, Strong agglutunations.

al., 2015). The difference may be attributed to wearing of protective gears, awareness through training and separation of various processes in the abattoir to enhance proper handling and cleanness. It was observed that all workers in the abattoir wear gloves, gumboots, overcoats and overhead covers, and a few also wore mouth covers and goggles. This was further supported by the fact that 59% of the workforce had post-primary education and 76% were aware of brucellosis. The interviewed participants were 14 times more aware of brucellosis than other zoonoses. The awareness on the occupational risk of brucellosis among the abattoir workers may have enhanced compliance with biosafety measures and practices leading to low prevalence found in this study. These findings concur with similar studies conducted elsewhere, which demonstrated that wearing personal protective gears and level of knowledge are protective factors for acquiring human brucellosis and other zoonoses (Ayoola et al., 2017; Islam et al., 2013; Madut et al., 2018).

In this study, it was observed that there was a statistical significant difference ($p<0.05$) in the knowledge of brucellosis among workers working in different sections. Sections having individuals with low awareness may be more prone to infection if the frequency of contact is high with susceptible infectious materials. The human seropositive case reported in this study was from the group of cleaners. Swai and Schoonman (2009) and Tsegay et al. (2017) argued that cleaners in the abattoir being the least educated group may lack knowledge on how brucellosis is transmitted. On the other hand, since they are most heavily involved in handling and disposing all condemned abattoir materials including aborted foetuses that usually have very high concentrations of *Brucella* pathogens; they are at a much higher risk of infection when compared to other groups.

Although the abattoir workers had some knowledge on brucellosis but it was observed that some of them had the tendency of opening the reproductive tract of slaughtered animals and aborted foetuses within the facility and sold them to vendors outside the abattoir premises. This practice may contribute to the contamination of the carcasses and environments resulting into increased risks of transmission of human brucellosis. Other studies have reported that consumption of gravid uteri increases the risks of humans to contract brucellosis (Adesokan et al., 2016). The reproductive tract is the predilection site for *Brucella* and it has to be removed from the animal without opening it in order to minimize the chances of dissemination of *Brucella* organisms thereby contaminating the meat and the surrounding environment. This increases the risk of transmission of the pathogen to the abattoir workers, meat vendors and the public in general. Therefore, training of all abattoir personnel on the proper practices, including proper disposal of reproductive organs should be emphasised. Despite the fact that the prevalence of human brucellosis was low in this study, the importance of human life underscores the need to undertake more systematic studies using the one-health approach in order to establish the magnitude of the problem country-wide as the basis for recommending practical control measures for the disease.

In conclusion, the present study has revealed that brucellosis is present both in animals slaughtered at the abattoir and humans working in the same facility. In view of this, there is a need for adopting biosafety measures such as wearing protective gear as well as educating the workers on the occupational risk of the disease in order to reduce its transmission. It is also recommended that integrated approaches be used in controlling the disease at the farm level in order to minimize transmission to abattoir workers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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SUPPLIMENTS

S1.docx: Questionnaire
S2:Rose Bengal plate test