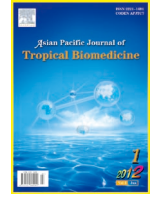




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A survey of zoonotic diseases in trade cattle slaughtered at Tanga city abattoir: a cause of public health concern

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ABSTRACT

Objective: To estimate the prevalence of hydatidosis, cysticercosis, tuberculosis, leptospirosis, brucellosis and toxoplasmosis in slaughtered bovine stock (aged ≥ 3 years) at Tanga city abattoir, Tanzania. **Methods:** Prevalence estimation of the five zoonotic diseases was undertaken through an active abattoir and sero-survey was carried out in Tanga city, during the period of January 2002 and March 2004. Serum samples collected from a sub-sample ($n=51$) of the slaughter stock were serologically screened for antibodies against brucellosis, leptospirosis and toxoplasmosis using Rose Bengal plate test, microscopic agglutination test (for 5 serovars of *Leptospira interrogans*) and Eiken latex agglutination test, respectively. The same animals were tested for tuberculosis using the single intradermal tuberculin test. **Results:** Post mortem examination of 12 444 slaughter cattle (10 790 short horn zebu and 1 654 graded) over a period of twenty two months, showed a prevalence of 1.56% (194) for hydatidosis, 1.49% (185) for cysticercosis and 0.32% (40) for tuberculosis. In all three zoonoses, a statistically significant difference in infection rates was noted between the short horn zebu and graded breeds ($P<0.05$). The overall seroprevalences of animals with brucellosis, toxoplasmosis and leptospirosis antibodies were found to be 12%, 12% and 51%, respectively. The most common leptospiral antibodies detected were those against antigens of serovars *Leptospira hardjo* (29%), *Leptospira tarassovi* (18%), *Leptospira bataviae* (4%) and *Leptospira pomona* (0%). With regard to tuberculosis, 10% ($n=5$) of the animals tested were classified as non-specific reactors or inconclusive. **Conclusions:** The study findings suggest that brucellosis, toxoplasmosis and leptospirosis are prevalent in Tanga and provide definitive evidence of slaughtered stock exposure to these zoonotic agents with concurrent public health consequences.

1. Introduction

Infections that are naturally transmitted from vertebrates animals to humans and vice versa are classified as zoonoses[1,2]. In the livestock sector the different types of farm animals are capable of carrying a wide range of zoonotic pathogens. In the beef sector, zoonotic pathogens are normally present in slaughtered stock, raw hides/skin, blood, meat and the farm environments, but are often difficult to diagnose. Moreover, animals brought for slaughter into urban areas come from villages where disease

control regimens are weak, uncoordinated and very often not available. Animal health delivery services in rural setting are hampered by remoteness, poor infrastructure and lack of qualified veterinary staff, inadequate transport, and insufficient funds to support surveillance operations or purchase reagents and drugs. The lack of veterinary services to these livestock-rearing areas poses a substantial risk of widespread occurrence of disease in the livestock population and concurrent human exposure to zoonotic disease agents. There is a further risk that many of the slaughtered animals brought to the abattoir may be harbouring chronic or sub clinical infections which are rarely detected during routine ante-mortem examination.

Zoonoses can be transmitted to humans by several routes that include: consumption of infected raw blood, milk and meat; by direct contact with infected animals through handling abortions, slaughters, dystocia and parturitions;

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and indirectly from infected farm environments^[3,4]. However, most meat-borne zoonoses are acquired through the consumption of infected and under cooked blood and meat^[5].

Currently in Tanzania, there is limited documentation of zoonoses in slaughtered stock^[6,7]. Lack of awareness of meat-borne zoonoses can put the lives of livestock producers, abattoir workers and the general public at risk from infection. Considering that most backyard slaughter slabs and abattoirs are not adequately regulated and given that there is a higher level of contact with raw meat, it can be argued that there is an even greater risk of meat-borne zoonoses in this type of facility. Therefore, it is imperative that cattle owners, traders, butchers and policy makers are made aware of the risks posed by meat-borne zoonoses that are prevalent in their areas. The information provided should also explain how zoonoses are transmitted in order to enable those at risk to make informed decisions as to how they might best protect themselves^[8,9]. Frequently detected and reported abattoir diseases or conditions include fascioliasis, cysticercus of *Taenia saginata* (*Cysticercus bovis*), tuberculosis and hydatidosis^[10,11]. This paper focuses on these zoonotic diseases and others such as toxoplasmosis, leptospirosis and brucellosis that are relevant to human and livestock health. The above diseases are of long-standing public health concerns, and are the most widely reported in the Tanzanian dairy and traditional cattle sectors^[6,7]. This study was conducted to generate epidemiological data to better understand the public health implication of zoonoses in slaughtered cattle in Tanga.

2. Materials and methods

2.1. Study area

The study was conducted at Tanga city abattoir located 330 km north east of Dar-es-Salaam, the main capital of Tanzania. This abattoir provides the daily beef requirements of the inhabitants of Tanga and neighbouring areas. Geographically the city is located between latitude 4° 21' and 6° 14' S and longitude 36° 11' and 38° 26' E. It experiences tropical climatic conditions, typified by hot and humid weather throughout the year. Annual rainfall is approximately 1 100 mm/year with two distinct rainy seasons: the long rain season, which fall between April and May, and the short rain season between October and November. The mean annual temperature and humidity on average range are from 23 °C to 33 °C and 60% to 70%, respectively. Smallholder mixed farming dominates 80% and livestock is an integral part of the farming system^[7].

2.2. Study animal and design

The study animals were cattle brought for slaughter from all districts of Tanga region and nearby districts of Kilimanjaro, Arusha and Morogoro. Some animals were

transported to the abattoir using vehicles and others were trekked in. The study design employed in this work was an active abattoir survey, carried out from June 2002 to March 2004.

2.3. Animal selection and data collection

Sampled slaughter cattle (for seroprevalence estimates) were selected on two randomly selected days. After arrival to the abattoir, age, sex, breed, number and origin of the animals were recorded in a purposively designed recording form. The age was determined based on dentition and owner's information^[12,13]. For quality control purpose of the data, these forms were collected regularly and discussed with the meat inspector in charge. It was not possible to get the exact records on owner, origin for each slaughter animal during the period due to the lack of reliable animal identification methods and poor recording systems at the farm and marketing points making it difficult to relate the findings to a particular locality. In addition to the collection of abattoir data, serum samples were collected from a sub-sample of slaughtered animals to assess the level of exposure to some of the zoonotic diseases like brucellosis, toxoplasmosis and leptospirosis. The same animals were also skin-tested for bovine tuberculosis.

2.4. Meat inspection protocol

Post mortem examinations were carried out by para-veterinarians using standard procedures recommended by FAO/UNEP/WHO^[14] as well as described in the meat hygiene (meat, abattoir and butcheries) regulations under CAP 16 & 17 of the laws of Tanzania^[15] and as described by Gracey *et al*^[16]. Post mortem examination procedure employed visual inspection, palpation, and systematic incision of each carcass, visceral organs particularly the lung, liver, spleen, kidney, and heart and targeted disease lesions were consistent with cysticercus of *Taenia saginata* (*Cysticercus bovis*), tuberculosis and hydatidosis.

2.5. Sample collection, handling and screening

Approximately 10 mL of blood was collected from the jugular vein of each selected animal using a plain vacutainer tube (Becton Dickson, UK). Each sample was labelled using codes describing the specific animal and owner. The tube was set tilted on a table over night at a room temperature to allow clotting. Next morning, the clotted blood in the tubes was centrifuged at 3 000 g for 20 min to obtain clear serum. The obtained serum was stored at -20 °C until tested by Rose Bengal plate test (RBPT), microscopic agglutination test (MAT) and Eiken latex agglutination test (LAT).

2.6. Rose Bengal plate test

All sera samples were screened using RBPT antigen (VLA Weybridge, UK). The test procedure recommended by Alton

et al^[17] was followed. Briefly, 30 μ L of RBPT antigen and 30 μ L of the test serum were placed alongside each other on the plate, and then mixed thoroughly. The plate was shaken for 4 min and the degree of agglutination reaction was recorded. The sample was classified positive if any agglutination was observed and negative if no agglutination. The RBPT, when compared with blood culture and complement fixation test (CFT), has shown a sensitivity of 94.2% and a specificity of 62% on field sera and has been described by other researchers^[18,19]. Confirmation of positive samples with tests of higher sensitivities and specificities such as a CFT or enzyme linked immunosorbent assay (ELISA) was not done due to the lack of resources (funds) to buy the required kits.

2.7. Microscopic agglutination test

Serum samples were checked for anti-leptospira antibodies in MAT in which the test antigens were provided by cultures of the six serovars of *Leptospira interrogans* (*L. interrogans*) considered to be those most likely to be found in Tanzania^[20]. The reference *L. interrogans* serovars included in the antigen panel were *Ballum* (serogroup *Ballum*, strain *Mus 127*), *Bataviae* (serogroup *Bataviae*, strain *Bataviae*), *Icterohaemorrhagiae* (serogroup *Icterohaemorrhagiae*, strain *RM1*), *Pomona* (serogroup *Pomona*, strain *Pomona*), *Hardjo* (serogroup *Sejroe*, strain *Hardjoprajitno*) and *Tarassovi* (serogroup *Tarassovi*, strain *Tarassovi*). The MAT was carried out in microtitre plates using dilutions (1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1: 640) of a test serum in phosphate-buffered saline at pH 7.2 (PBS), with 100 μ L of a diluted serum in each well. After the addition of an equal volume of one of the six *Leptospira* cultures to each well, the plates were incubated at 30 °C for 2 h. The results were read using a dark-field microscope, a test serum being considered seropositive, for the serovar used as antigen, if agglutination of at least 50% of the leptospirae was detected at a final serum dilution of 1: 160^[21].

2.8. Eiken latex agglutination test

A modified Eiken LAT as described by Lee *et al*^[22] was used for testing the sera. The original test is modified by diluting the latex 1 in 5 in AMP buffer, 0.2 M 2-amino-2-methyl-1-propanol-HCl (Sigma Ltd, UK). It was shown that this modification produced a 4.2 fold increase in antibody titre. The samples were therefore tested in only two screening dilutions, 1:4 and 1:8. A positive control serum was tested alongside the samples. Tests were performed according to the manufacturer's instructions but in V well microtitre plates. Following overnight incubation at room temperature the plates were placed at a sloped angle of 70 ° and the results were read after 10 min. Antibody titres of 1:8 were considered positive (analyzed statistically) and titres of 1:4 weak positive.

2.9. Single intradermal tuberculin test

The single intradermal tuberculin test was applied to all selected cattle as a screening test. The skin thickness of the injection site on the cervical fold was measured with special calipers, then 0.1 mL of bovine PPD tuberculin (ID-Lelystad, the Netherlands) was injected intradermally with a tuberculin preset syringe (McLintock). The skin reaction was measured after 72 h. The interpretation of the test was done on the basis of >4 mm being a positive reaction and 3–4 mm being a doubtful reaction. All thickness \leq 2mm was considered negative. Differentiation of true *Mycobacterium bovis* infection or reactors from exposure to non-tuberculous mycobacterium with the single intradermal tuberculin test could not be undertaken before slaughter.

2.10. Data analysis

Data were entered, stored and analysed using both Microsoft Excel and Epi-info version 6 statistical software version 6.04b (Centre for Disease Control, 1996). Descriptive statistics to generate frequency distributions of cysticercosis, hydatidosis and tuberculosis infections in examined bovines were performed and further compared using *Chi*-square test at a critical probability of $P < 0.05$. Cross-tabulation was performed to assess the strength of association between infected animals and important variables such as the breed, *etc.*

3. Results

3.1. Meat inspection data

Over 85% of the cattle presented to the abattoir for slaughters were males, local breed (Tanzania shorthorn zebu) and above 3 years of age. Graded or crossbred cattle represented only 13% of all slaughters. A total of 12 444 cattle were slaughtered between January 2002 and March 2004 and during the period 40 (0.32%), 194 (1.56%) and 185 (1.49%) were found to have lesions suggestive of tuberculosis, hydatidosis and cysticercosis, respectively (Table 1). The majority of the tuberculosis lesions were found in the pluck. The prevalences of the three zoonoses were significantly higher in zebu as compared with dairy cattle ($P < 0.05$).

3.2. Tuberculin testing and laboratory analysis

Overall, 51 animals were tested for tuberculosis and sera were collected. The average age of the animals screened and tested was 78 months and majority (>95%) were males and of local Tanzanian shorthorn zebu breeds. Most (>95%) of the animals originated from outside Tanga region. The prevalence of tuberculosis reactors (SIT >2 mm) and the seroprevalence of brucellosis, leptospirosis and toxoplasmosis in a sub-sample of slaughter animals at the Tanga abattoir were shown in Table 2.

Table 1Reported tuberculosis, hydatidosis and cysticercosis lesions in slaughtered cattle at Tanga abattoir (January 2002–March 2004) [*n* (%)].

Breed	Tuberculosis	Hydatidosis	Cysticercosis
Zebu (<i>n</i> =10 790)	38 (0.35)	188 (1.74)	175 (1.62)
Dairy (<i>n</i> =1 654)	2 (0.12)	6 (0.36)	10 (0.60)
Overall (<i>n</i> =12 444)	40 (0.32)	194 (1.56)	185 (1.49)

Table 2Brucellosis, leptospirosis, tuberculosis and toxoplasmosis prevalence in sample of slaughtered cattle at Tanga abattoir (*n*=51) [*n* (%)].

Disease	Positive prevalence	95% CI
Brucellosis	6 (12)	9.1–14.9
Toxoplasmosis	6 (12)	9.1–14.9
Tuberculosis	5 (10)	7.2–12.8
Leptospirosis	26 (51)	44.1–57.9
<i>Leptospira hardjo</i>	15 (29)	17.4–43.8
<i>Leptospira tarassovi</i>	9 (18)	8.4–30.8
<i>Leptospira bataviae</i>	2 (4)	0.5–13.4
<i>Leptospira pomona</i>	0 (0)	0.0–6.9

4. Discussion

This study revealed that the prevalence of hydatidosis in cattle slaughtered at Tanga city abattoir was 1.56%. This finding is lower than the reports from other places in Tanzania and neighboring countries with 4.2% in Arusha^[23], 47% in Ngorongoro^[10] and 31% in South West Ethiopia^[24]. Factors such as differences in culture, social activity, animal husbandry systems, lack of proper removal of infectious carcasses, abundance of infective definitive host and attitude to dogs in different regions might have contributed to the variation in prevalence in different areas of a country^[25,26]. The rates of infection between breeds showed that local breeds were found to harbor a significantly higher infection than graded or crossbred. That is attributed to the nature of pasture–grazing patterns of animals^[25,27]. The crossbreeds were most commonly kept indoors, and there was less chance of exposure to parasite ova than pasture grazing animals. Importantly, hydatidosis is a potential threat to human in Tanzania due to the large population of stray dogs and the close association between dogs and humans.

The overall *Cysticercus bovis* prevalence of 1.49% obtained in this study is higher than the reports from the retrospective study of 0.051% recorded by Mellau *et al*^[11] in Arusha municipal abattoir in north Tanzania. Slaughter inspection for *Cysticercus bovis*, based on ‘eye and knife’ meat inspection, finds at most between 10% to 20% of the cases if proper inspection is done. Macpherson *et al*^[28,29] when doing proper inspection of predilection sites, found that cysticercosis was detected in 10.5% of slaughtered cattle in different abattoirs in Tanzania, with Arusha having the highest proportion of 16.7% and Morogoro the lowest with 6.5%^[30]. Heart muscle and musculus triceps brachii harbored the highest numbers of cysts^[31]. It is likely that the reported cases in Tanga are significantly under–reported, as all predilection sites are not always properly inspected. Humans are the definite hosts for *Taenia saginata* whose faeces can contaminate cow pastures. People, especially in

Tanga, often do not use proper latrines to defecate. Under these circumstances, just one human can be a source of infection for hundreds of cattle. Humans acquire *Taenia saginata* taeniasis by consuming raw or undercooked meat containing cysticerci.

Only 0.35% and 0.12% of the carcasses of zebu and dairy cattle, respectively showed macroscopic lesions suggestive of tuberculosis. Most of the lesions were of the pulmonary form. The overall detected prevalence of infection in the cattle was generally lower than observed in other studies in Tanzania *e.g.* up to 0.7% reported in indigenous zebu in Arusha^[32]. Also the overall prevalence of doubtful or inconclusive reactors (10%) was high and not comparable to the findings reported from eastern zone of Tanzania (from 6.0% to 7.5%)^[33,34]. The proportion of carcasses showing lesions is much lower compared with the 10% doubtful reactors in the small sample of slaughtered cattle. It is possible that some of the doubtful reactors were in fact negative or reactors to atypical *Mycobacterium*. It is also possible that a proportion of animals with tuberculous lesions are not detected during the normal routine meat inspection. Shirima^[33] reported that in another region in Tanzania, 24% of the carcasses were found with tuberculous lesions, by applying a more intensive inspection procedure which involved multiple slicing and close examination of selected lymph nodes. Although the doubtful reactor cases were not retested, there was an indication of exposure to *Mycobacterium bovis* in the slaughtered cattle sampled. It is also possible that these doubtful reactors were poor producers and therefore culled for slaughter, causing a higher prevalence in this group. Some of the animals, which showed tuberculous lesions at slaughter originated from Tanga, confirmed the presence of tuberculosis in cattle in the area.

Both leptospirosis and brucellosis antibody prevalences in the abattoir sample did not differ significantly as compared with the recent reports on leptospirosis and brucellosis in traditional cattle in Tanga^[35,36]. As most of the areas where the cattle originating from had a lower average rainfall,

it suggests that leptospirosis also has a high prevalence in these drier areas. The prevalence of brucellosis is apparently quite common in traditional herds. Kanuya *et al*[37] reported an animal prevalence of 15.6% in zebu cattle in pastoral herds in a semi arid area of Tanzania. The overall brucellosis seroprevalence of 12% recorded from this study is higher than 5% and 0.014% recorded in slaughter stock in Nigeria[38] and United State of America[39], respectively, but significantly lower than 18% recorded in trade slaughter stock in Karagwe north western Tanzania[40]. RBPT was employed in both studies. On the contrary, toxoplasmosis seroprevalence in the abattoir sample showed a similar, but slightly higher prevalence when compared with the recent studies on toxoplasmosis in traditional and dairy herds in Tanga[41]. Higher *Brucella*, *Leptospira*, *Mycobacterium bovis* and *Toxoplasma gondii* seroprevalences in trade slaughtered stock coupled with sub-standard slaughter premises and negligence in safety precaution during meat inspection can be potent sources of diseases transmission and persistence. This implies greater occupational hazard to butchers and abattoir attendants. These occupational groups are exposed to materials such blood, vaginal discharges, foetus, urine, placentas from infected animals. They are therefore at a higher risk of acquiring infection through broken skin and aerosol[38].

The apparent high spectrum of zoonotic diseases investigated and detected in this study is of epidemiological and public health significance. Apart from its veterinary and economic importance throughout the world, bovine tuberculosis, leptospirosis, brucellosis, cystercercosis are listed and classified by WHO as the zoonoses of world concern[42]. The risk of transmission to human is exacerbated by the growth of livestock production in close proximity to humans, the rising rate of HIV, inadequate hygienic practices, and cultural customs and beliefs. Inadequate disease reporting systems and insufficient collaboration and communication between human health and veterinary services further compounds the problem[43]. The unhygienic conditions of slaughter slabs and the presence of zoonotic diseases pose a health risk to both meat consumers and the general public. This suggests a need for immediate abattoir or slaughter slab sanitary measures, regulation enforcement and a rigorous meat inspection procedure in order to reduce exposure and to minimize the associated public health risks. Consistently, enforcement of legislation and establishment of policy on dog keeping and handling including registration, and treatment and elimination of stray dogs are essential. Moreover, community based public health education in handling of beef and beef products, coupled with high standard of hygienic practices should be put in place to reduce cases of zoonotic meat-borne disease in cattle and man.

The finding of this study was based on gross pathology lesions and on serological evidence of exposure to diseases pathogen, which is required to be critically reviewed to

identify and allow for inherent bias. However, abattoir and sero-surveys are known to provide valuable disease information, a key component toward designing disease monitoring, control and eradication programmes. However, the data obtained from this survey cannot be wholly relied upon, it can be used as an indicator and baseline for more extensive epidemiological investigations.

Conflict of interest statement

We declare that we have no conflict of interest.

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