2014-09

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International Journal of Science and Research (IJSR)

Seroprevalence of Paratuberculosis in Goats and Sheep in Arusha, Northern Tanzania

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Abstract: *A retrospective cross-sectional study was conducted to investigate the seroprevalence of paratuberculosis in small ruminants in the Arusha Municipality. A total of 383 samples comprising of 192 goat- and 191 sheep-sera from Arusha Municipality collected in the late 2010 and early 2011 by Tanzania Veterinary Investigation Agency – Arusha for surveillance on peste des petits ruminants were tested for antibodies specific to Mycobacterium avium subspecies paratuberculosis using the commercially available PARACHEK®2 indirect ELISA kit. Twenty one (21) of 192 (10.9%: 95% CI; 7.3 – 16.1%) goat sera were positive; however none of 191 sheep sera screened were sero-positive for paratuberculosis. This is the first report on seroprevalence of paratuberculosis in goats in Arusha and in Tanzania as a whole. The fact that seropositive cattle were previously detected in samples collected in the same flock at the same time indicates the disease is well established in the Arusha. Furthermore, the co-infection of cattle and goats may have implication on the epidemiology of the disease in the area. The sero-negativity of all sheep samples tested is yet to be established but may be due one or a combination of factors. Since the Parachek ELISA kit is capable of detecting responses in sheep, one of the possible reasons may be that the prevalent M.paratuberculosis strain(s) in Arusha is type II or C strain that preferentially infects cattle and goats as opposed to type I or S strain which preferentially infects sheep. Alternatively, the prevalence and or the antibody titres in sheep may have been too low to be detected within the sensitivity of the Parachek ELISA kit.*

Keywords: Paratuberculosis, Sheep, Goat, prevalence, seroprevalence, Arusha, Tanzania

1 Introduction

The first cases of paratuberculosis in Tanzania were reported in 1960 involving cattle in two farms in Kilimanjaro region [1]. Subsequently, cases of bovine paratuberculosis were reported in various farms in the country [1], [2], [3], [4]. More importantly, in our recent study in Arusha, seropositive samples were found in bovine serum samples collected in urban and peri-urban Arusha in year 2011 confirming presence of the diseases in the area [5]. However, to date there has never been any report of paratuberculosis in small ruminants in Tanzania.

Paratuberculosis is chronic, debilitating, enteritis disease affecting ruminants and non-ruminants animals of domestic and wild [6]. The disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (*M.paratuberculosis*), a small, acid-fast, facultative-intracellular, extremely slow growing mycobacterium [7]. *M.paratuberculosis* has been grouped into two strains infecting animal populations; the sheep type (S-type or type I) which is common in sheep, and cattle type (C-type or type II) which is common in cattle and goats [8], [9], [10].

In small ruminants, clinical signs characteristics to paratuberculosis are not as overt as in cattle [11], [12], [13]. In cattle, diarrhea unresponsive to treatment and emaciation are prominent characteristics of the disease [14]. In contrast, in *M.paratuberculosis* infected small ruminants, diarrhea is not a major manifestation instead, the dominant characteristic is body wasting which can be confused for other diseases [15], [16]. For example, it has been reported that only 10–20% of clinically infected small ruminants develop diarrhea at the end stage of the disease [17]. The non-specific signs makes it difficult to identify diseased small ruminants compared to cattle, and may lead to the spread of the disease in herds/flocks unnoticed.

Paratuberculosis has significant economic impact in the small ruminants industry. The economic losses are mainly due to decreased milk production, decreased slaughter value, early culling of infected animals, and eventually death [18]. However, there are few studies on paratuberculosis in small ruminants, particularly in goat compared to cattle; as a consequence, extrapolation of economic impact in small ruminants is not well established. *M.paratuberculosis* is also suggested to be a zoonoses because of linkage with inflammatory bowel disease or Crohn's disease in human, and hence of public health concern [19], [20].

In Africa, relatively recent reports on paratuberculosis in small ruminants are mainly limited to sheep in South Africa [21] and North African countries including Morocco and Egypt [22], [23]. Reports on goat paratubercolosis in Africa are old involving Sudan [24] and Kenya [25] probably due to lack of active surveillance of the disease as suggested previously by Okuni [26]. In the rest of the world, disease in goats and sheep has been reported in a number of countries including Australia [27], Germany [28], Portugal [29], Chile [30], Argentina, [31] Mexico [32] and Cyprus [33].

Lack of rapid and sensitive diagnostic tests capable of identifying infected animals before development of clinical signs is one of the major constraints in the control of paratuberculosis [34]. Currently, ELISA is the most sensitive diagnostic serological test for paratuberculosis [35]. The specificity of ELISA has been increased by including a pre-absorption step to remove cross-reactivity to environmental

Paper ID: SEP1435

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bacteria [36]. Currently, commercially available indirect ELISA kits for paratuberculosis involves *Mycobacteria phlei* preabsorption of sera before testing for specific antibodies to *M.paratuberculosis*.

In Arusha, Tanzania, paratuberculosis was first reported in cattle at livestock training institute Tengeru (LITI-Tengeru) more than thirty-years ago [1]. Interestingly, in our recent bovine seroprevalence study, seropositive samples was found [5] suggesting that *M.paratuberculosis* infection is present, and may be spreading in susceptible animals. Because mixed housing and mixed-grazing of small ruminants and cattle is common practice in Arusha region, we hypothesized that small ruminants will also be infected. The purpose of the present study was to determine the seroprevalence of paratuberculosis in small ruminants of Arusha municipal using the commercially available ELISA.

2 Materials and Methods

2.1 Study Samples

Retrospective cross-sectional study was conducted. Frozen goat and sheep serum samples stored at -80^oC in serum bank at the Tanzania Veterinary Laboratory Agency-Arusha (TVLA-Arusha) were used. The samples were collected in late 2010 and early 2011 by the TVLA-Arusha during surveillance on peste des petits ruminants (PPR). Serum samples were collected from small holder livestock farmers in Arusha municipality. Goat and sheep with >6months were eligible for inclusion in the screening for PPR. All serum samples of goat were from Arusha municipal, and that of sheep were from Arusha municipal and peri-urban Arusha. From each sample, 50μl was aliquoted and placed in clean and sterile Epperndorf tube, and frozen overnight before shipment. Following overnight freezing, samples were shipped in Cool Box containing icepacks to Nelson Mandela African Institution of Science and Technology (NM-AIST) Serology Laboratory where samples were tested for *M.paratuberculosis* antibodies.

2.2 Sample Size Calculation

A total of 192 goat and 191 sheep sera were randomly selected for testing. The sample size was calculated using the following formula: n = [Z^2 * p * (1-p)]/[d2] as previously described [37] [38]; where n = minimum number of animal sera to be sampled, Z = degree of confidence (Z = 1.96 for 95% of confidence), p = assumed apparent prevalence of paratuberculosis (i.e., ≥0.5), and d = maximum difference between observed and the true prevalence that we are willing to accept (0.077). On these grounds, a minimum of 162 samples were required but more samples were tested to increase precision.

2.3 Indirect Enzyme Linked Immunosorbent Assay

The commercially available PARACHEK®2 ELISA (Prionics AG, Zurich, Switzerland) was used to analyze serum samples based on manufacturer's instructions. Unless otherwise stated, all incubations were 30 minutes at room temperature

and plates were washed six-times using manufacturer provided washing buffer. Briefly, before testing, the samples were absolved of non-specific antibodies to environmental mycobacteria by incubation with *Mycobacterium phlei* antigens. Then 100μL of each diluted sample and manufacturer-provided positive and negative controls were added to microtitre plates pre-coated with *M. paratuberculosis* and incubated. The plates were then washed and 100μL of Horseradish peroxidase-labeled anti-bovine Ig conjugate was added to each well. After incubation step, the plates were washed and 100μL of enzyme substrate solution was added to each well and plates incubated. The reaction was stopped by adding 50μL of enzyme stopping solution (0.5 M H_2SO_4) and the absorbance was determined using Microplate Reader (BIO-RAD laboratories Inc., USA) with dual 450 nm and 650 nm filters.

2.4 Calculation of True Prevalence Estimation

True prevalence was calculated using the Rogan Gladen estimator [39]; and the exact 95% confidence (blaker's exact confidence) limits for the true prevalence estimate was calculated as previously described [40]. The apparent sensitivity of the Parachek, Johne's absorbed ELISA (Prionics AG, Zurich, Switzerland) for detecting paratuberculosis antibodies in goat and sheep sera were assumed to be 65% and 38% (range 65–88% and 38-44%), respectively, and specificity assumed to be 99% as stipulated by the ELISA kit manufacturers and collaborated by [41], [42].

3 Results and Discussion

Paratuberculosis is one of the neglected diseases in Tanzania and Africa as whole mainly because many countries do not practice active surveillance of the disease. In Tanzania, reports of paratuberculosis have been limited to bovine paratuberculosis and before our recent report on the disease in Arusha town [5], the previous report dated 14 years ago [4]. But upon testing of seropositive cattle in Arusha town, we hypothesized that because of mixed housing and grazing of cattle and sheep and goat, it was likely that the disease will be found in small ruminants. Indeed, out of 192 goat sera tested, 21 (10.9%: 95% CI 7.3 – 16.1%) were positive for *M.paratuberculosis* antibodies (Table 1) confirming paratuberculosis in goats for the first time in Arusha and Tanzania as a whole. However, none of the 191 sheep sera tested were positive for paratuberculosis.

Table 1: Seroprevalence of paratuberculosis in goat and sheep from Arusha Municipality

Animal species	Number of samples tested	Number of positive samples	Apparent prevalence (%)	Estimated true prevalence
Goat	192	21	10.9%	15.5%
Sheep	191	0	0	0

The prevalence in goats (10.9%) is two times more as compared to the prevalence in cattle (5.3%) sampled from the same herds and same time [5]. The difference between species remains to be established but may be due to species

difference in the susceptibility to *M.paratuberculosis*. These results indicate that *M.paratuberculosis* infection is well established in Arusha and suggest that a similar situation may be happening in other parts of Tanzania because of free movement of animals in the country.

The apparent disease prevalence (10.9%) reported from this study is comparable to what was reported by other studies different countries. The apparent paratuberculosis prevalence in Cyprian dairy goat was 7.9% [43], [33], 1.4% for Boer goats in Missouri, USA [38], 4.6-15.3% for black goats in Korea [44], 2.9% for dairy goats in France [43], 5.7% in Portugal [29], 7.2% in Ontario Canada [45].

Our results show that despite of the 10.9% prevalence of paratuberculosis in goats, none of the 191 sheep sera screened were seropositive for paratuberculosis. Considering that the Parachek ELISA kit used is capable of detecting antibodies in both cattle, sheep and goats, and that sheep are at the same risk of infection as goats and cattle because of mixed housing and grazing practiced in the study areas, one possible reason may be that the prevalent *M.paratuberculosis* strain in Arusha is the cattle strain (type II or C strain) that preferentially infects cattle and goats as opposed to type I or S strain which preferentially infects sheep [8, 10, 46]. This is consistent with the fact that earlier cases of paratuberculosis in Tanzania and in Tengeru Arusha (Nyange *et al*., 1983) involved exotic breeds of cattle which may have been the source of the prevalent *M.paratuberculosis* strain(s) in Arusha. The other possible reason may be the lower sensitivity of the Parachek ELISA kit for sheep (38%), as opposed to 65% for goats which may lead to negative results especially if the disease prevalence and or the antibody titres in sheep were very low.

4 Conclusion

We recently reported bovine paratuberculosis in samples from the same flocks where the goat and sheep samples for this study were obtained [5]. The two reports call for measures for nationwide surveillance of the disease in view of the economic impact of the disease and public health implications. Intestines and associated lymph nodes, which are sites where *M.paratuberculosis* localizes, are the major components of soups sold in bars and other recreational places in Arusha and other parts of Tanzania. Consumption of infected and improperly cooked intestinal soup will most likely expose consumers to high risks of contracting Chrohn's disease.

Further studies are still needed to further characterize the epidemiology of the disease including the molecular typing of the prevalent *M.paratuberculosis* strains in Arusha..

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Paper ID: SEP1435
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