

## Seroprevalence of bovine paratuberculosis in Arusha, Northern Tanzania

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### Abstract

Bovine paratuberculosis or Johne's disease is chronic, contagious granulomatous enteritis of cattle which is caused by *Mycobacterium avium* subspecies *Paratuberculosis* (MAP). The disease was first reported in Tanzania in two farms located in Kilimanjaro region in 1960. Despite of quarantine measures that were instituted in the area between 1960-1963, additional cases were reported in Arusha (1976), Mpwapwa (1984), Morogoro (1994). The disease was last reported in the country more than 14 years ago at Kitulo dairy farm in Mbeya region. The paucity of cases has created an impression that the disease is absent from the country. However, the well-known persistence of (MAP) once introduced in the area suggests that the disease may still be present and spreading unnoticed in the country. To obtain information on current state of bovine paratuberculosis, a seroprevalence of the disease in cattle was conducted on bovine serum samples kept in the repository at the Arusha Tanzania Veterinary Laboratory Agency. A total of 207 bovine sera that were collected from urban and peri-urban Arusha as part of contagious bovine pleuropneumonia surveillance in 2011 were tested for MAP-specific antibodies using the commercial ELISA. The overall seroprevalence was 5.3% (95% CI: 2.3 to 8.4%). These results confirm presence of bovine paratuberculosis in Arusha and indicate the disease may be prevalent in other parts of the country. This is the first seroprevalence report for paratuberculosis in Tanzania.

**Key words:** Bovine paratuberculosis, seroprevalence, ELISA, Arusha, Tanzania, LITA Tengeru

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## Introduction

While the prevalence and distribution of bovine paratuberculosis is well documented in many developed countries (Mullowney and Graham, 2012; Tiwari *et al.*, 2006), the situation is different in most African countries including Tanzania (Okuni, 2013). Lack of active surveillance of the disease in most African countries has led to underestimating the disease status in the continent (Okuni, 2013). It was however reported that in any African country where some surveillance has been done, the disease was detected. For example, in recent surveillances, bovine paratuberculosis has been confirmed in Uganda (Erume and Mutebi, 2012; Okuni *et al.*, 2012; Okuni *et al.*, 2013), Sudan (Mohammed *et al.*, 2010) and Egypt (Salem *et al.*, 2005; AbdEl-Malekand and Mohamed, 2011) suggesting that the disease is present and may be spreading undetected.

Bovine paratuberculosis has enormous impact on economies and animal welfare in cattle industry worldwide (Wolf *et al.*, 2014). The economic losses is due to reduced milk production, progressive weight loss, reduced slaughter value, premature culling, mastitis and increased possibility of infertility in infected herds (Ayele *et al.*, 2001; Eda *et al.*, 2012; Hasonova and Pavlik, 2006; Tiwari *et al.*, 2006). Economic losses due to bovine paratuberculosis specifically in dairy cattle has been estimated in several countries worldwide; for instance, in the United States the annual economic losses ranges from US\$ 200 to US\$250 million (Ott *et al.*, 1999). Importantly, MAP infection has been linked with inflammatory bowel disease or Crohn's disease in human, and hence public health concern (Chamberlin *et al.*, 2001; Tanaka *et al.*, 1991). Lack of information on occurrence and distribution of bovine paratuberculosis in most African countries including Tanzania means the economic and public health significance of the disease is undetermined.

Attempt has been done to control the disease despite the intractable nature. Strategies such as test and culling and herd status determination has been employed in bovine paratuberculosis control programs (Barker *et al.*, 2012). In many bovine paratuberculosis control programs, antibodies detection assays, specifically indirect enzyme linked immunosorbent assay (ELISA) has been substantial tool due to its cost-effectiveness (Wolf *et al.*, 2014). Indirect ELISA involves a pre-absorption step to eliminate antibodies against cross-reactive environmental mycobacteria (*Mycobacterium phlei*) which would otherwise produce false-positives results (Collins and Sockett, 1993). ELISA is cheap and cost-effective as compared to other bovine paratuberculosis diagnostic tests such as polymerase chain reaction (PCR) and fecal culture (Álvarez *et al.*, 2012; Aly *et al.*, 2012).

In Tanzania, bovine paratuberculosis was first reported in 1960 at Marangu and Lyamungu Coffee Research Station farms (reviewed by Nyange *et al.*, 1983). Three years later, the disease was reported in various government established livestock farms suggesting that the disease was wide spread in Tanzania (Tanganyika Veterinary Quarantine Notice on bovine paratuberculosis, 6<sup>th</sup> November 1963). Quarantine measures were imposed and later the disease was said to be

controlled. However, Nyange *et al.*, (1983) confirmed two-cases of bovine paratuberculosis among five clinically ill animals within Jersey cattle stock imported from New Zealand in 1976 at the Livestock training Institute Tengeru (LITI –Tengeru) Arusha, which is currently known as Livestock Training Agency Tengeru (LITA–Tengeru). Follow up studies reported occurrence of bovine paratuberculosis at LITA-Mpwapwa in Dodoma, Central Tanzania (Batamuzi *et al.*, 1984), and ten years later the disease was reported in one indigenous cattle at Sokoine University of Agriculture (SUA) farm (Matovelo *et al.*, 1994) that involved Boran cattle purchased from Kilimanjaro in Northern Tanzania in 1987. Almost 10 years later, Manjurano *et al.*, (2002) reported presence of MAP in feces after Ziehl Nielsen (ZN) staining of fecal samples from Kitulo dairy-farm in Mbeya, Southern Highlands of Tanzania. Put together, reports on bovine paratuberculosis in Tanzania are limited to isolated cases which are distributed in various parts of the country and interspaced by many years but without any epidemiological link or prevalence status.

Most Arusha urban and peri-urban households keep dairy cattle most of which are crosses between exotic and indigenous cattle. In Arusha, bovine paratuberculosis was reported at LITA-Tengeru (peri-urban) more than thirty-years ago (Nyange *et al.*, 1983). To date, it is unknown whether the disease was completely eliminated from the LITA-Tengeru farm and furthermore, no study has attempted to investigate the prevalence of the disease in Arusha. We hypothesized that bovine paratuberculosis is still present at LITA-Tengeru and within Arusha urban and peri-urban areas because the disease is persistent once introduced in the area and also to our knowledge, no significant efforts were instituted to control the disease. The purpose of the present study was to determine the seroprevalence of bovine paratuberculosis in urban and peri-urban Arusha using the commercially available ELISA.

## **Material and Methods**

### **Study design and area**

A retrospective cross-sectional study was conducted focusing on frozen bovine serum samples stored in serum bank at the Tanzania Veterinary Laboratory Agency–Arusha (TVLA-Arusha). Samples were collected in 2011 as part of surveillance on contagious bovine pleuropneumonia (CBPP) from Arusha urban and peri-urban areas of Arusha city, including LITA-Tengeru.

### **Study site and sample population**

Serum samples were from house-hold dairy cattle of Arusha municipal, which are exotic and hybrid between exotic and indigenous cows. Cattle aged >6months were eligible for inclusion in

the screening for CBPP. In this study, 207 serum samples frozen in storage tubes were selected randomly from over 1000 sera, and tested for presence of antibodies specific for MAP. The selected 207 serum samples included 99 samples from LITA-Tengeru, Arusha peri-urban, and 108 serum samples from Arusha urban. From each sample, 50 $\mu$ l was aliquoted into a clean and sterile Eppendorf tube, and frozen overnight before shipment. Next day, samples were shipped in cool box (PRINCE WARE; Dar es salaam, Tanzania) containing icepacks to Nelson Mandela African Institution of Science and Technology (NM-AIST) Serology Laboratory. At NM-AIST serology laboratory, serum samples were frozen at -20°C until screening for bovine paratuberculosis.

### Indirect enzyme linked immunosorbent assay

Serum samples were analyzed using the commercially available PARACHEK<sup>®</sup>2 ELISA (Prionics AG, Zurich, Switzerland). Briefly, 10 $\mu$ L of each serum sample and controls (positive and negative control) was diluted with 200 $\mu$ L of Green Diluent, containing *Mycobacteriu mplei* antigens, and incubated at room temperature for 30 minutes to remove cross-reactive non-specific antibodies. Then 100 $\mu$ L of each sample and 100  $\mu$ L of the manufacturer-provided positive and negative controls were added to microtitre plates coated with *M. paratuberculosis* antigens. The plates were shaken for one minute and incubated at room temperature for 30 minutes to allow reaction between antibodies specific for *M. paratuberculosis* from sample and antigens coated on the plate. Plates were then washed manually six-times with wash buffer at room temperature. Thereafter, 100 $\mu$ L of conjugate (Horseradish peroxidase labeled anti-bovine Ig), was added to each well. The plates were again shaken for one minute, incubated at room temperature for 30 minutes, and washed 6-times with wash buffer. Next, 100 $\mu$ L of enzyme substrate solution was added to each well and the plates were incubated and shaken at room temperature for another 30minutes. Finally, 50 $\mu$ L of enzyme stopping solution (0.5 M H<sub>2</sub>SO<sub>4</sub>) was added into each well. The absorbance was determined using iMark<sup>™</sup>MicroplateReader (BIO-RAD laboratories Inc., USA) with a 450 nm filter (reference filter 650nm) after 20 minutes of incubation.

### Results and Discussion

The last report on bovine paratuberculosis in Tanzania is more than 13 years old which creates an impression that the disease is not present in the country. However because many previous cases in the country were not followed by sustainable diseases control measures, it was hypothesized that the disease may still be present in the country because of the intractable nature of the disease. To obtain some information on the disease prevalence in the country, we analyzed bovine serum samples collected from urban and peri-urban Arusha for specific

antibodies to bovine paratuberculosis. Of the 207 serum samples tested by commercial indirect ELISA, 11 (5.26%; 95% CI: 2.3 to 8.4%) were seropositive (Table 1).

**Table 1. Seroprevalence of bovine paratuberculosis in peri-urban and urban Arusha**

Location	Number of samples tested	Number of seropositive samples	Prevalence (%)
Arusha Urban	108	7	6.48
Arusha peri-urban	99	4	4.04
	Total=207	Total=11	Mean (5.26%)

The seroprevalence reported in this study is higher compared to the last study reported prevalence of 1.9% within the Kitulo Farm herd in Southern highlands of Tanzania (Manjurano *et al.*, 2002). The discrepancy may be due to the fact that the Kitulo Farm prevalence was based on a single herd while results from the current study are based on multiple herds. Furthermore, differences may be due location (Northern vs Southern Tanzania) as well as the differences in the diagnostic methods used (ELISA vs ZN staining). However, the bovine paratuberculosis prevalence from the present study is comparable to other seroprevalence studies in other parts of the world including African countries. The reported seroprevalence was 4.3% in Uganda (Boniface Okuni *et al.*, 2013), 10.2% in Sudan (Mohammed *et al.*, 2010), 4.73% in Georgia, USA (Pence *et al.*, 2003), 5.5% in Southern Italy (Marchetti *et al.*, 2013) and 4.1% in Chile (Van Schaik *et al.*, 2007).

Bovine paratuberculosis was last reported at LITA-Tengeru, peri-urban of Arusha city more than 30 years ago (Nyange *et al.*, 1983). No other cases have been reported in the farm or within Arusha urban and periurban areas ever since, indicating the disease is no longer present. However, of the 99 serum samples from LITA-Tengeru which were tested, 4 (4.04%; 95% CI: 0.2 to 7.9%) were seropositive, confirming the presence of the disease in the farm. While it is difficult to tell whether the disease has persisted in the farm for 30 years or it is due to new infections, the absence of sustainable control measures after the confirmation of cases by Nyange *et al.*, 1983 strongly suggest the disease may have persisted in the farm.

Based on the nature of paratuberculosis, it is possible that the actual prevalence of the disease in Arusha may be higher than was established using the antibody ELISA. It is well established that *M. paratuberculosis*-infected animals start developing antibodies in advanced stage of disease (Stabel, 1998) therefore infected animals in early stages of the diseases will be missed when antibodies are targets for diagnosis. A study conducted in US dairy herds found that for every clinically infected animal that was born on the farm, a minimum of 25 other animals are

probably infected and less than 30% of those were detected by currently available tests (Whitlock and Buergelt, 1996)..

This is the first seroprevalence report of bovine paratuberculosis in Tanzania. The presence of reactors in the absence of reported cases is an issue of concern because the disease may be spreading unnoticed throughout the country. Furthermore, because of the possible link of bovine paratuberculosis with human Crohn's disease, consumers of animal products may be exposed to the disease. Active surveillance is therefore called upon to understand the extent of bovine paratuberculosis in the country.

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