

## Seroprevalence of *Mycobacterium avium* Subspecies *Paratuberculosis* Antibodies in Cattle from Wakiso, Mpigi and Luwero Districts in Uganda

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**Abstract:** This study was carried out to determine the seroprevalence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection of cattle in selected districts in the central region of Uganda. Until recently MAP infection was unknown in any domestic or wild life species in Uganda and the neighbouring regions. To determine the extent of the challenge posed by this new threat, 943 heads of cattle from 62 herds were tested using a commercial absorbed ELISA. Of these, 3.7% were serologically positive. The estimated true prevalence was 8.8% and the proportion of herds with at least one positive reactor was 33.8%, while 11.3% of the herds had at least two positive reactors. The mean within-herd prevalence was 13% with a range of 2 to 37.5%. The prevalence of MAP antibodies among cattle of different breeds was 8.9% in Holstein Friesian, 3.7% in Zebu, 1.4% in Ankole longhorn, 9% in Guernsey and 0% in Ayrshire cattle. Cattle of three and a half years of age and above had significantly higher prevalence than younger cattle. This study has provided the first seroprevalence information on paratuberculosis in the country and to our knowledge in Africa. From this study it can be concluded that paratuberculosis is one of the important threats facing the livestock industry in Uganda and immediate action should be taken for its control. Further studies will be required in order to institute appropriate control measures.

**Key words:** Cattle, Johne's disease, *Mycobacterium avium* subsp. *paratuberculosis*, seroprevalence, Uganda

### INTRODUCTION

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the cause of paratuberculosis or Johne's disease, a granulomatous enteritis that affects a wide range of ruminants and wild life. Paratuberculosis is characterised by diarrhoea, emaciation, reduced production and ultimately death (Clarke, 1997; Harris and Barletta, 2001). The disease is considered as a serious threat to the livestock industry in all the countries where it occurs (Collins, 2003). In the USA, it has been estimated that the losses due to paratuberculosis amount to United States dollars 1.5 million annually or approximately 227 US dollars per cow (Manning and Collins, 2001). MAP has also been implicated in Crohn's disease, an inflammatory bowel disease of humans (El-Zataari *et al.*, 2001; Naser *et al.*, 2004), thus paratuberculosis is also considered as a potential zoonosis.

Bovine paratuberculosis is a problem of both dairy cattle and to less extent beef cattle (Waldner *et al.*, 2002). The disease has been found to have a genetic

predisposition in some cattle (Pinedo *et al.*, 2009). Previously, some breeds of cattle, namely Channel island breeds, were believed to be more susceptible to MAP than others (Chiodini *et al.*, 1984). The susceptibility of African cattle breeds is unknown. The prevalence of MAP infection in cattle populations in the world is on the increase (Cashman *et al.*, 2008). The herd prevalence of paratuberculosis infection in western Europe ranges from 7 to 55%, while in Australia and USA it ranges from 9-22% (Manning and Collins, 2001; Woodbine *et al.*, 2009), although other reports indicate that in the USA, herd prevalence might vary from 21-93% (Pillars *et al.*, 2009b). In many European countries paratuberculosis has been described as endemic (Dreier *et al.*, 2006; Hayton, 2007; Woodbine *et al.*, 2009). In contrast to these industrialised countries, in most African countries, the occurrence, distribution and prevalence of the disease is unknown (OIE, 2008). To our knowledge, only a single study on the prevalence of the disease in Egypt has been published in recent years (Salem *et al.*, 2005). Prior to the commencement of this study, the existence of MAP

infection in Ugandan cattle was only based on a small abattoir study in which paratuberculosis-incriminating lesions were found in 12/100 tissue samples from a local abattoir (Kirabo, 2002). Unfortunately, these were not confirmed by a specific method. In the course of this study, confirmation of the infection was made and a report has been published to this effect (Okuni and Ojok, 2010). There is concern that, since cases of paratuberculosis may take up to 10 years to become clinically evident (Clarke, 1997), the disease might insidiously spread within the livestock population. Control of the disease is an expensive process that can only be embarked on after ascertaining the infection status within the herd or population (Benjamin *et al.*, 2009). It is now a requirement of the *Organisation Internationale des epizooties* (Organisation for World Animal Health) that freedom of the disease be declared only after testing the population using an acceptable testing method (Martin, 2008). The aim of this study was to estimate the current burden of the infection among the different categories of cattle in the central region of Uganda.

## MATERIALS AND METHODS

**Study area and sampling criteria:** The study was conducted in Wakiso, Luwero and Mpigi districts of Uganda from April to September 2007. These districts were chosen because they lie on what is called the 'cattle corridor' which is a strip of woodland savannah running from the South-South Western part of the country to the North-Eastern part. The three districts have all the cattle breeds kept in Uganda as well as the main husbandry practices in the country. Wakiso district is a peri-urban area. Most of the cattle are kept in small numbers up to five, under stall feeding, locally known as 'zero grazing'. There are also commercial farms on paddocks. The cattle population consists of mainly exotic breeds and their crosses. Mpigi and Luwero districts are rural with mainly local cattle breeds and few exotic dairy breeds or their crosses. The predominant husbandry system in these two districts is the open communal grazing or range grazing system, with very few stall fed or closed paddocked herds.

A multi-stage sampling method was used to select five sub-counties each from Mpigi and Wakiso and four sub counties in Luwero. Sixty-two herds of cattle ranging from 10 to 600 heads of cattle were selected, 13 of which were from Luwero, 19 from Mpigi and 30 from Wakiso.

Only cattle which were two years and above were chosen (Johnson-Ifearulundu and Kaneene, 1998). Sampling was done as described by Hill *et al.* (2003). A total of 943 heads of cattle were selected from the 3 districts. The sex, breed and district were recorded for each sample. In addition, a specific identity number, name and tag number of each animal sampled were recorded, together with the names of the farm or farmer, village and sub county.

About 4 mL of blood were drawn from the jugular or caudal vein of each animal into plain vacutainer tubes (Becton Dickinson). Serum was harvested 24 h later into 1.5 mL cryovials and stored at -20°C until tested. A commercial ELISA kit consisting of 96 well plates (Institut Pourquier) was used to test the sera against MAP antibodies according to the manufacturer's instructions. The optical density readings (OD) of the plates were determined using a Multiskan mcc/340 version 2.20 plate reader (Lab systems, Vanta) at 450 nm. The OD of each sample was subtracted from the OD of the Negative Control (NC) and divided by the difference between the mean OD of the Positive Control (PCM) and NC to get a sample to positive ratio (S/P). Thus:

$$S/P = (\text{OD of sample} - \text{NC}) / (\text{PCM} - \text{NC})$$

Based on the manufacturer's instruction we considered an S/P value of 70% and above as positive.

Data for each animal were entered into a Microsoft Excel spreadsheet and exported into STATA 8.0 (StataCorp. 2003. Stata Statistical Software: Release 8) for analysis. Using frequency tabulations, the number of serologically positive animals was determined for every herd, district breed and husbandry practices, then used to calculate the overall apparent prevalence, mean within-herd prevalence and herd prevalence. Differences in prevalence among age-groups, breeds, districts and husbandry practices were analysed using Pearson's chi-square test. The apparent (test) prevalence of the herds was used to calculate the test prevalence using the formula:

$$tP = N_1p_1 + N_2p_2 + \dots + N_n p_n / N_1 + N_2 \dots + N_n$$

where tP is the test prevalence,  $N_1$  is the herd size of herd 1,  $p_1$  is the proportion of MAP positive cattle in herd 1, etc. (Hill *et al.*, 2003). The test prevalence was used to estimate the true prevalence of the disease in the population using the formula:

$$TP = tP + (Sp-1)/Se + (Sp-1)$$

where TP is the calculated true prevalence, tP is the test prevalence; Sp is the specificity of the test and Se is the sensitivity of the test. The Sp and Se of Pourquier ELISA was estimated to be 99.3 and 51% respectively (van Shaik *et al.*, 2005).

## RESULTS

The total number of serologically positive cattle was 35 (n = 943) giving an overall prevalence of 3.7% (95% CI: 2.6-5.2). The true prevalence was estimated to be 8.8% (95% CI: 0-66%). The number of herds with at least one positive animal was 21 (n = 62), giving a herd

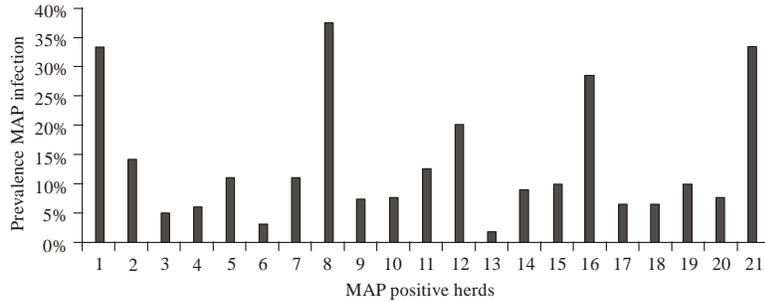


Fig. 1: A bar graph showing the prevalence of ELISA positive cattle within each of the 21 herds that were positive for MAP infection

Table 1: Prevalence of MAP infection among the five breeds of cattle

Breed	No. of animals sampled	No. of positive	Percentage of positive animals
Friesian	259	23	8.9 <sup>1</sup>
Ankole	573	8	1.4
Zebu	81	3	3.7
Jersey	19	0	0
Guernsey	11	1	9
Total	943	35	3.7

<sup>1</sup>: There was significant difference in prevalence between Friesian and Ankole cattle but not other breeds. Jersey cattle were not included in the analysis because they had an expected frequency of less than 1.

herd prevalence for each herd is shown in Fig. 1. Table 1 shows the prevalence of MAP antibodies among cattle of different breeds; Table 2 shows the prevalence among cattle under different husbandry practices, while Table 3 shows the prevalence within age-groups. The proportion of MAP-infected cattle within the three districts under the study is shown in Table 4. Pair wise comparison of the prevalence of the infection among the different breeds showed a significant difference between Friesian and Ankole cattle (Pearson's chi = 23.291,

Table 2: Prevalence of MAP antibodies among cattle under different husbandry practices in the three districts of study

Serostatus	Type of husbandry			Total
	Paddocked	Communal grazing	Range grazed	
No. of sampled	636	270	37	943
No. of negatives	613	258	37	908
No. of positives	23	12	0	35
Percentage positive	3.75	4.7	0	3.7

Table 3: Seroprevalence of MAP infection among cattle of different age groups

Serostatus	Age of cattle in years				Total
	2-3.5	3.6-4.5	4.6-6.5	6.6-13	
Positive	6	7	14	8	35
Positive (%)	1.9	4.1 <sup>a*</sup>	4.8 <sup>*</sup>	4.6 <sup>*</sup>	3.7
Negative	304	162	275	67	908
Total	310	169	289	75	943

<sup>a</sup>: There was significant prevalence among cattle older than three and a half years (asterisk) (p<0.05, CI = 95%) compared to those less than three and a half years

Table 4: Seroprevalence of MAP infection among cattle sampled from the three districts

District	No. of sampled	No. of positive	Positive (%)	95% CI
Luwero	182	6	3.3	0.69-5.9
Mpigi	261	6	2.3	0.48-4.1
Wakiso	500	23	4.6 <sup>a</sup>	2.7-6.4
Totals	943	35	3.7	

<sup>a</sup>: Pearson's Chi-square analysis showed a lack of statistical significance in the differences in prevalence in the three districts (p>0.05)

prevalence of 33.8% (95% CI: 22.7-47.10). When at least two animals were considered for a herd to be counted as positive, 7/62 herds were positive, thus resulting in a herd prevalence of 11.3% (95% CI: 5.0-22.5). The mean within-herd prevalence was 13% (95% CI: 8 -17%), the range being 0 to 37.5%. The within-herd or intra-

4df; p = 0.000) and no significant difference between Friesians and other breeds. Differences observed in prevalence between husbandry practices were not statistically significant (p>0.05). There was a significantly higher prevalence among animals older than 3½ years compared to younger animals (p<0.05; Table 3). The

prevalence of MAP infection in Wakiso was 4.6% compared to 3.3% in Luwero and 2.3% in Mpigi. These differences were not statistically significant ( $p>0.05$ ).

## DISCUSSION

In this study, 3.7% of the cattle were serologically positive, while 33.8% of the herds had at least one positive animal. This is comparable to the figures in USA where it is reported that 21-93% of the dairy herds and at least 2.9% of the dairy cattle are infected (Hill *et al.*, 2003; Pillars *et al.*, 2009a, b). This finding should be taken seriously since Ugandan cattle were previously thought to be free from the disease. For the first time we found serological evidence of MAP infection in Ankole and Zebu cattle, some of which were later confirmed by culture (data not presented here). Both breeds are *Bos indicus* and are considered native to Uganda and some of the neighbouring countries. All the breeds sampled but one (in which only 19 cattle were tested) had a positive reactor. The implication is that most if not all the breeds of cattle kept in Uganda are susceptible to paratuberculosis. Although Guernsey and Friesian cattle had higher prevalence than their two local counterparts namely: - Zebu and Ankole; Jersey cattle did not show a higher prevalence. The reason for the lack of significance in this comparison is likely due to the fact that the number of cattle sampled among the Zebu, Jersey and Guernsey were considerably smaller than the other two. This skew resulted from the fact that the sampling units were herds which tended to have unpredictable mix of breeds. This might also explain the lack of statistical significance among cattle kept under different grazing types, since those kept under range grazing were also comparably smaller in number. It is interesting though, to note that cattle on paddocks and communal grazing had almost comparable prevalence figures. The absence of a serologically positive animal under range grazing must not be taken to preclude cattle under that grazing system from infection, since infection of cattle under this grazing management has been confirmed in a related study (Okuni and Ojok, 2010). Communal grazing and rangeland grazing comprise the pastoral husbandry practice common in many areas of Uganda. One risk posed by these two management practices is the potential for a single infected animal to spread the disease to a large number of herds. Another risk that might be posed by the presence of MAP infection in local cattle is that frequent movements of stock due to rustling, migration, cultural exchanges and restocking in areas that were affected by war, could lead to accelerated spread of the infection to all parts of the country and even to neighbouring countries. There is currently no information about the status of MAP infection in cattle in the neighbouring countries.

Salem *et al.* (2005) found a higher prevalence of paratuberculosis in exotic breeds in Egypt (87.5%),

compared to local Egyptian cattle (16.7%). Our study has shown a very small difference between Friesians, Guernsey, Zebu and Ankole Breeds. The overall prevalence of MAP infection is also lower than was found in Egypt but it has to be borne in mind that Salem *et al.* (2005) used a combination of culture, PCR and direct microscopy.

According to our findings, MAP infection is also present in all the three districts of the study and there was no significant difference in prevalence between the districts though the prevalence was highest in Wakiso followed by Luwero then Mpigi. As mentioned earlier, this is the first seroprevalence study of paratuberculosis in Uganda and only the second prevalence study in Africa; and was designed mainly to detect the presence of MAP infection in the cattle population. Future studies should be carried out to determine the disease prevalence, its impact on cattle production and the epidemiology in the entire livestock population in the country.

In this present study, we have used a single ELISA test due to logistical constraints; however, it is documented that a single serological test might give either false negative or false positive results, because of its comparatively low sensitivity and specificity (Muskens *et al.*, 2003). It could also be due to the variation in immune response of the host that is very well documented in paratuberculosis (Stabel and Goff, 2004). We tried to minimise this weakness by using Pourquier ELISA (Institut Pourquier) which was found by previous studies to have higher sensitivity and specificity than other commercial ELISA tests (Collins *et al.*, 2005). We recommend that in subsequent studies, consideration be made for the use of multiple serological tests and multiple testing of the same herd. Despite this limitation, our findings provide an important landmark for the understanding of the epidemiology of bovine paratuberculosis in the region.

## ACKNOWLEDGMENT

We wish to acknowledge the Carnegie Corporations and Makerere University School of Graduate studies for the financial support. We also thank all the District Veterinary staff of Wakiso, Mpigi and Luwero for the technical assistance rendered during sample collection. Thanks also go to Mr. Watoya Charles and Mr. Kisseka Magid for assistance during serological testing. We thank Mr. Omala Kizito for the statistical analysis.

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