Seroprevalence of Mycobacterium avium subsp. paratuberculosis in Dairy Cattle in Khartoum State, Sudan.

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Research article

**Keywords:** Mycobacterium avium paratuberculosis, Seroprevalence, Sudan

**DOI:** https://doi.org/10.21203/rs.3.rs-44169/v1

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Abstract

Background: Paratuberculosis, caused by Mycobacterium avium subspecies paratuberculosis (MAP), is a chronic wasting disease of mainly domestic and wild ruminants (mammals). It occurs worldwide causing significant economic losses through decreased productivity, low fertility, increased cull rates and mortality. It is an OIE listed disease of concern to trade in animals. The aim of this study was to investigate the current prevalence of MAP infection in cattle in Khartoum State.

Results: The overall apparent prevalence of MAP infection was found to be 6.3% and 18.9% (95% CL) at animal and herd levels, respectively. All seropositive animals were cross-bred females of good body condition; most of them (>90%) were >3 years old and >50% were from medium sized herds in Omdurman. No significant association ($X^2 > 0.05$) was found between seropositivity and animal herd data.

Conclusion: The prevalence of MAP infection in Khartoum State is still low to medium compared to other parts of the world, but it is comparable to that reported from other African countries. Further studies with the view of designing wide national surveys in domestic ruminants and camels in other states of the country are needed for establishing control programmes.

Background

Paratuberculosis (PTB) also known as Johnne's disease, is a chronic enteritis caused by Mycobacterium avium subsp. paratuberculosis (MAP) affecting ruminants and wild mammals worldwide [1, 2]. It is an OIE listed disease of concern to trade in animals. The disease affects animals of all ages manifesting different clinical stages [3–5] causing decreased productivity, low fertility and increased susceptibility to infections [6]. Moreover, MAP is suspected to be involved in the aetiology of Crohn's disease, a chronic diarrhoeal inflammatory bowel disease of humans, sharing some features with PTB [3, 7]. Due to the serious economic impact of PTB on livestock production, control of MAP is very important wherever it occurs. This requires early detection of infected livestock and sufficient information of the rate of infection and associated risk factors in the population.

Diagnosis of PTB is based on one or more of the following: detection of MAP or its DNA in tissues or faeces, detection of MAP antibodies and culture to isolate the organism. The suitability and sensitivity of the tests depend on the clinical stage of the disease [8, 9]. Silent infection can be detected only by histopathology or MAP tissue culture, which takes a long time [10, 11]. Enzyme-Linked Immuno-Sorbent Assay (ELISA), is a useful and commonly used serological test to detect MAP antibodies in serum and milk in the subclinical stage of MAP infection and afterwards [3, 10]. ELISA sensitivity may reach maximum of 50% [8] and the specificity can be improved by pre-treatment of samples with Mycobacterium phlei to absorb non-specific antibodies [12] and minimize possible false positives.

Knowledge of the global distribution of PTB is important for establishing control programmes. The prevalence of PTB has been reported from different countries, mainly for bovine PTB. Herd level prevalence of up to 75.8% was reported in the Caribbean and Latin America [13]; >50% in Europe and North America [14]; 20.35%-41.7% in Asia [15, 16]. Animal level prevalence ranging from 2.31–29.8% was also reported in Asia [15–18]. In Africa, reports on MAP infections in animals are rather scanty. Available published data show herd prevalence of 45.2% and 13% in Egypt and Uganda, respectively [19, 20], and animal level prevalence of 9% in Uganda [20] and 5.26% in Tanzania [21]. Although PTB was first diagnosed in livestock in the Sudan more than 50 years ago [22], there has been little research and few reports about its incidences and prevalence. The first report on bovine PTB in the Sudan was from Khartoum with 53% prevalence based mainly on clinical disease [23]. A more recent study on bovine PTB in Khartoum State by Mohammed et al. (2010) revealed 66.7% and 10.2% at the herd and animal levels, respectively [24], but it was limited since it involved only few farms. The present study aimed at evaluating the current situation of PTB in dairy farms in Khartoum State by increasing the number of herds in a wider area.

Results And Discussion

In this study, antibodies against MAP have been demonstrated by ELISA in 7 out of 37 dairy herds tested in Khartoum State with an overall herd prevalence of 18.9% and animal level prevalence of 6.3%. Three of the positive herds were in Omdurman, which also had the highest prevalence at the animal level (33.3%); two positive herds were found in each of the other two localities. Summary of the results are presented in Table 1. As the maximum sensitivity of ELISA is about 50%, the true estimation of seroprevalence could be higher than the obtained results. However, ELISA positive animals may or may not be MAP shedders, but precautions should be taken to minimize transmission of infection within the herd.
than that reported here, but our study seems to be more representative by involving greater number of herds distributed over a wider area of

The only reliable report from the Sudan on seroprevalence of bovine PTB to compare the current results with is that of Mohammed and others [24] who reported 66.7% and 10.2% prevalence at the herd and individual animal levels, respectively in Khartoum State. These rates are higher than that reported here, but our study seems to be more representative by involving greater number of herds distributed over a wider area of

### Table 1

| Area           | Animal level | Herd level |
|----------------|--------------|------------|
|                | Total        |            |
| Local          | Cross        |            |
| No. Tested     | + ve         | + ve       | No. Tested     | + ve         | No. Tested | + ve |
|                | No. | %     | No. | %     | No. | %     | No. | %     |
| Khartoum       | 7   | 0     | 44  | 2     | 3.9 | 51  | 2     | 11  | 2     | 18.2 |
| Khartoum North | 0   | 0     | 78  | 4     | 5.1 | 78  | 4     | 15  | 2     | 13.3 |
| Omdurman       | 0   | 0     | 45  | 5     | 11.1| 45  | 5     | 9   | 3     | 22.2 |
| Total          | 7   | 0     | 168 | 11    | 6.3| 175 | 11    | 37  | 6     | 17.1 |

All seropositive animals were cross-bred females of good body condition, most (>90%) of them were above three years old and >50% were from medium herd size (50–100 head). The chronic nature of MAP infection and the long incubation period render the infected animals apparently healthy for years before showing overt symptoms of the disease [25] which interpret our findings. The numbers of local cattle in the farms investigated was small and none of the tested animals was seropositive. Local breeds are poor milkers compared to exotic breeds or their crosses and, therefore, limited numbers of selected cows are included. However, PTB surveys should also include pastoral local herds in the Sudan. It is interesting that Chi-Square showed no significant association between these descriptive animal data and serostatus (Table 2).

### Table 2

Chi square results for association between cattle age, sex, herd size and body condition and prevalence of MAP antibodies in Khartoum State

| Result | Age | Body condition | Herd size | Sex |
|--------|-----|----------------|-----------|-----|
|        |     |                |           |     |
|        | > 3 | < 3            | Total     |     |
|        |     | Emaciated      | Good      |     |
|        |     | Total          | L         | M   | S   |
|        |     | % of total     |            |     |
| -ve    |     | % within result |           |     |
| Count  | 107 | 42             | 149       | 24  | 117 | 141 | 42  | 78  | 38  | 158 | 126 | 25  | 152 |
| % within result | 71.8 | 28.2 | 100.0 | 17.0 | 83.0 | 100.0 | 26.6 | 49.4 | 24.1 | 100.0 | 83.6 | 16.4 | 100.0 |
| % within category | 91.5 | 97.7 | 93.1 | 100.0 | 91.4 | 92.8 | 95.5 | 92.9 | 92.7 | 93.5 | 92.6 | 96.2 | 93.3 |
| % of total | 66.9 | 26.3 | 93.1 | 15.8 | 77.0 | 92.8 | 24.9 | 46.2 | 22.5 | 93.5 | 77.9 | 15.3 | 93.3 |
| +ve    |     |                |           |     |
| Count  | 10  | 1              | 0         | 11  | 11  | 2   | 6   | 3   | 11  | 10  | 1   | 11  |
| % within result | 90.9 | 9.1 | 100.0 | 0.0 | 100.0 | 100.0 | 18.2 | 54.5 | 27.3 | 100.0 | 90.9 | 9.1 | 100.0 |
| % within category | 8.5 | 2.3 | 6.9 | 0.0 | 8.6 | 7.2 | 4.5 | 7.1 | 7.3 | 6.5 | 7.4 | 3.8 | 6.7 |
| % of total | 6.3 | 0.6 | 6.9 | 0.0 | 7.2 | 7.2 | 1.2 | 3.6 | 1.8 | 6.5 | 6.1 | 0.6 | 6.7 |
| Total  |     |                |           |     |
| Count  | 117 | 43             | 160       | 24  | 128 | 152 | 44  | 84  | 41  | 169 | 136 | 26  | 163 |
| % within result | 73.1 | 26.9 | 100.0 | 15.8 | 84.2 | 100.0 | 26.0 | 49.7 | 24.3 | 100.0 | 83.4 | 16.0 | 100.0 |
| % within category | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| % of total | 73.1 | 26.9 | 100.0 | 15.8 | 84.2 | 100.0 | 26.0 | 49.7 | 24.3 | 100.0 | 83.4 | 16.0 | 100.0 |

*The chi square results showed that the association between cattle age and the prevalence of MAP antibodies was not significant (chi-value = 1.9, \( p > 0.05 \)); similarly the association between the cattle body condition and the prevalence of such antibodies was insignificant (chi-value = 2.2, \( p > 0.05 \)). Neither sex nor the herd size was significantly associated with the prevalence of MAP antibodies (chi-value = 0.5, \( p > 0.05 \)) and (chi-value = 0.38, \( p > 0.05 \)), respectively.
Khartoum State (Fig. 1). Moreover, they involved clinical cases, which when excluded, the apparently healthy animals would reveal a comparable animal-level prevalence (6.9%). Contrary to the current results they reported the lowest seropositivity in Omdurman. It is obvious that PTB in Sudan is poorly documented. Similarly, reports from African countries on MAP infections in animals are rather scanty [26]. However, the prevalence rates, herd or individual animal levels, reported from Egypt [19], Uganda [20], and Tanzania [21] are reasonably comparable with the present results. Global prevalence of PTB is high and the disease is well documented in many developed countries, where it causes tremendous economic losses. The prevalence rates of PTB obtained in this study seem to be low compared to that mentioned globally [13, 15–18].

Generally, variation between results obtained by various authors may be influenced by stage of infection, animal age, shedding level of the organism, lactation, antibody concentration and varying sensitivity among different ELISAs [5, 8, 25, 27]. These should be considered when comparing results on PTB prevalence.

It has been stated that ELISA is more precise in determining herd level prevalence [8], however, combination of ELISA with culture or molecular assays for screening of MAP infection would be more reliable in investigating both animal and herd level prevalence.

**Conclusion**

The present study confirms that MAP infection occurs in dairy cattle in Khartoum State by using more representative samples. However, more studies with the view of designing wide national surveys in domestic ruminants and camels in other states are needed for establishing control programmes.

**Methods**

**Study population**

Animals included in this study were local (Butana/ Kenana) and cross-bred (local x Friesian) cattle of both sexes and different ages selected through stratified random sampling from different dairy herds in three major areas in Khartoum State (Fig. 1): 11 herds in Khartoum, 17 in Khartoum North and 9 in Omdurman. Most of the animals were of good body condition.

**Blood collection**

A total of 174 animals (7 local and 167 cross-bred) selected from herds in the three major localities in the State; Khartoum (N = 51), Khartoum North (N = 78) and Omdurman (N = 45) were bled for serum. After disinfecting the site, blood was collected by jugular venipuncture in sterile vacutainers. The harvested sera were kept at -20° C till being tested. Breed of animals, sex, age, body condition and other herd information related to the sampled animals were noted. The Sudanese regulations of animal welfare have been followed during handling of animals and blood collection.

**ELISA procedure**

The obtained sera were tested for anti-MAP antibodies using IDEXX ELISA kits (IDEXX laboratories Inc. Westbrook, USA) according to manufacturer's instructions. Briefly, the test samples and the provided controls were diluted 1:20 with the provided buffer containing *Mycobacterium phlei* and left (as pre-incubation) for 35 min. The plate coated with MAP antigen was incubated for 45 min with 100 µl of the diluted test samples and controls. The plate was then washed thrice, 100 µl of conjugate were added, left for 30 min and washed as before; 100 µl of TMB solution were added and left for 10 min followed by 100 µl of stop solution. The optical density of the plates was read at 450 nm in plate reader. According to manufacturer's guidelines, the results were validated and then interpreted as positive and negative.

**Statistical analysis**

The results were analysed using SAS software version 9 (SAS Institute Inc., Cary, NC, USA). The data obtained were compared using the Pearson Chi-Square test.

**Abbreviations**

MAP *Mycobacterium avium* subspecies *paratuberculosis*

PTB Paratuberculosis

OIE World Organization for Animal Health (Office International des Epizooties)

ELISA Enzyme-Linked Immunosorbent Assay
Declarations

Ethics approval and consent to participate

Ethics approval for the study was waived by Research Board of the Faculty of Veterinary Medicine and the Scientific Committee of the Institute for Studies and Promotion of Animal Exports, University of Khartoum. The samples were taken by authorized veterinarians according to the national veterinary and animal welfare regulations. Animal owners had given oral consent according to the national ethical regulations.

Consent to publish

Not applicable

Availability of data and materials

All data supporting our findings are contained within the manuscript.

Competing interests

All authors have no reported conflicts of interest.

Funding

This study is part of a research project (404935781) funded by the German Research Foundation (DFG). The funding agency has no role in the design of the study, collection, analysis, and interpretation of data and in writing the manuscript.

Authors’ contributions

Study design: NMA, SAA, MEM, KHE; samples and data collection: NMA; conducting ELISA, data preparation and analysis: WAE, SMI; statistical analysis: MEM; writing the first draft: WAE; preparing the final manuscript: WAE, AAG, KHE; critical revisions and editing: JBO, LO, MEM, AEW, EE. All authors read and agreed on the final draft.

Acknowledgments

The Dr. Mojahed Haroun provided assistance during sample collection; Dr. Islam Kamaleldin, Dr. Nehal Mohamed and Dr. Eiman Hassab Elrasoul of Detasi Company provided equipment facility.

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28. Legend. to Fig. 1.

29. Locations of dairy farms sampled for seroprevalence of Mycobacterium aviums subsp. paratuberculosis in Khartoum State. The map was created from google maps® using google's account of KHE

Figures
Sampled Dairy Farms in Khartoum State for Seroprevalence of Paratuberculosis

Locations with seropositive farms
- Al Jazeera Islanj
- Alrodwan Dairy Complex
- Ass-Shigalab
- Mahlab 2
- Soba Gharb

Locations with seronegative farms
- Al Kadare
- Al-Silali Shamal
- As-Saigh Dairy Complex
- As-Silali Ganoob
- As-Surub
- Al-Haq Younis
- Mahlab Carwala
- Nepta
- Shambat
- Soba Al-Hilla
- Tayba Al-Hasnab
- Um Dawan Ban
- Um El-Gura Ganoob
- El-Faki Hashim

Figure 1

Locations of dairy farms sampled for seroprevalence of Mycobacterium avium subsp. paratuberculosis in Khartoum State. The map was created from google maps® using google's account of KHE
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