

ORIGINAL ARTICLE

Sero-surveillance of *Mycobacterium avium* subspecies *paratuberculosis* infection in ruminants in Medina

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ABSTRACT

Objective: The present study aimed to assess for the first time, in Medina, the prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in ruminants due to its potential zoonotic importance.

Materials and methods: A total of 823 sera samples and 364 milk samples were used to determine the incidence of *Mycobacterium avium* subsp. *Paratuberculosis* (MAP) using the indirect Enzyme-Linked Immunosorbent Assay.

Results: The seroprevalence of MAP was 11.1% in sheep and 13.8% in goats, while no infection was recorded among camels. MAP infection was not influenced by the animal's gender, but it was influenced by its locality since the infection rate in local animals was higher than that in imported ones with a significant correlation ($p < 0.05$). MAP infection had a significant correlation ($p < 0.05$) with 2 years aged animals. On the other hand, the detection of MAP in milk revealed that 17 (13.8%) goats and 12 (4.9%) sheep were infected. The prevalence of MAP in milk samples was not influenced by either the animal's age or locality.

Conclusion: Sheep and goats may act as a reservoir for MAP to the Medina community. Since Medina is an active area of mass gatherings as a destination for pilgrims throughout the year, therefore, necessary control measures should be implemented to lower the economic losses, zoonotic infections, and the possibility of a global epidemic.

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Introduction

Animal's diseases pose a serious threat to human health, environment, and economics [1]. *Mycobacterium avium* subsp. *Paratuberculosis* (MAP) is an obligate intracellular, small, Gram-positive, rod-shaped, and acid-fast bacilli. MAP is one of the slow-growing mycobacteria, which can multiply only within the macrophage of the susceptible host [2].

Johne's disease is a chronic granulomatous intestinal disease caused by MAP affecting small ruminants, wild ruminants, and cattle [3]. Johne's disease is clinically presented as weight loss and diarrhea. It greatly affects the ruminant industries because of its impact on the global economy [1]. Infected animals become a source and excrete MAP in their feces and milk and spread the infection [3].

Ruminants as sheep, goats, and camels are considered an important source of meat and milk in Saudi Arabia. Sheep and goats constitute an integral part of the animal population in Saudi Arabia that is raised principally by private breeders for meat production [4] and their consumption increases during the pilgrimage season [5].

Crohn's disease is a chronic, debilitating disease affecting the human's gastrointestinal tract [6,7]. Due to the pathological and clinical similarities between Johne's and Crohn's disease, MAP has been considered to be a possible causative agent of Crohn's disease [8–10].

The zoonotic potential of MAP was suggested based on the detection of MAP in the blood or mucosal tissues of the Crohn's disease patients and the similarities between paratuberculosis in ruminants with the Crohn's disease (CD) in

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humans [9]. Public health issues have been raised about the transmission of MAP from animals to humans through animal products (dairy foods, meat, and contaminated surface water) and the potential for subsequent infection and perhaps disease [8]. Milk and milk products are the main sources of the transmission of MAP to humans since MAP is not inactivated during pasteurization [11,12]. The present study aimed to determine the prevalence of MAP in ruminants in the Medina region, compare our results with other MAP epidemiological studies, and give an understanding of the ruminant's role as a reservoir for MAP in Medina.

Materials and Methods

Ethical statement

Ethical approval for this study was conducted accordingly the Ethics Committee of Taibah University (No: 1438/12).

Study design

A prospective study was conducted from February to September 2018 to survey the incidence of MAP among ruminants in Medina, KSA. Sheep and goats reared under semi-extensive husbandry for their milk and/or meat. The sampled animals showed the clinical signs of Johne's disease mainly emaciation and/or persistent diarrhea. The imported sheep and goats were mainly from Sudan and Somalia.

Blood sampling

A total of 823 blood samples were collected from camels ($n = 107$), sheep ($n = 492$), and goats ($n = 224$). Blood samples were collected sterile syringe in Vacutainer tubes. The samples were collected from the slaughterhouse, veterinary clinics, and local herds in Medina. Animals were selected randomly and their species, age, locality, and gender were recorded. After blood collection, samples were left to clot at room temperature for 30–45 min. Then, the tubes were centrifuged at 5,000 rpm for 12 min. Sera were separated and transferred to sterile Eppendorf tubes and stored at -20°C .

Milk sampling

A total of 364 milk samples (123 sheep and 241 goats) were collected from dairy farms in Medina. Animals were selected randomly and their species, age, locality were recorded. The samples had different colors and densities because they were in different lactation stages. Milk samples were centrifuged at 5,000 rpm for 15 min. The lactosera were obtained by pipetting and transferred to sterile Eppendorf tubes and stored at -20°C .

Enzyme-linked immunosorbent assay (ELISA) assay

Serum and lactosera samples were tested for the presence of MAP-specific antibodies by using the ID

Screen® Paratuberculosis Indirect Screening test (ID.VET Diagnostics- France). The diagnostic kit was designed to detect anti-ruminant IgG antibodies directed against MAP. ELISA microplates were coated with a purified extract of MAP. To avoid cross-reactions, neutralizing buffer containing *Mycobacterium phlei* was used to dilute the samples in pre-incubated plates before being transferred to the coated plates. Anti-MAP only resulted from an antibody-antigen complex with MAP specific epitope. Briefly, 100 μl of diluted samples were added to the wells and incubated for 45 min at 21°C . After incubation, the wells were evacuated and washed three times by 300 μl of the wash solution. 100 μl pre-prepared conjugate (1 \times) was added to each well then incubated for 30 min at 21°C . After incubation, the wells were evacuated and washed three times by 300 μl of the wash solution. A hundred μl of the substrate solution was added to each well. In the dark, the ELISA plate was incubated for 15 min at 21°C . Finally, 100 μl of the stop solution was added to each well to stop the reaction. The optical density was measured at 450 nm with an automated ELISA reader (SIRIOS Elisa Reader, Indonesia).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 20.0 (SPSS Inc., Chicago, IL). The association between the incidence of MAP with animal species, age, gender, and/or locality was determined by Pearson's correlation coefficient. A p -value of 0.05 or less was considered as statistically significant.

Results

Demographic data of the examined animals (blood samples)

A total of 492 sheep, 224 goats, and 107 camels were examined for the presence of *Mycobacterium avium* subspecies *paratuberculosis* antibodies in their sera and milk. The demographic characteristics of the examined animals are listed in Table 1. According to the animal's gender, out of 224 goats, 153 were females and 71 were males, and out of 492 sheep, 290 were males and 202 were females, in addition to 107 female camels. Regarding the animal's locality, 248 sheep were local and 244 were imported, and 184 were local goats and 40 were imported, in addition to 107 local camels. Animals belonged to various sources, 181 from a slaughterhouse, and 343 from a veterinary clinic, in addition to 299 from local herds. Animals were categorized into three age groups as shown in Table 2.

Prevalence of MAP in animal's sera

As shown in Table 3 and Figure 1, MAP was more prevalent with significant correlation ($p < 0.05$) among sheep

Table 1. Demographic data of the examined animals (blood samples).

Animals species	Gender	Locality	Source of samples	Total
Sheep	Female (n = 202)	Local (n = 248)	Slaughterhouse (n = 109)	492
	Male (n = 290)	Imported (n = 244)	Veterinary clinic (n = 143) Local herds (n = 240)	
Goat	Female (n = 153)	Local (n = 184)	Slaughterhouse (n = 72)	224
	Male (n = 71)	Imported (n = 40)	Veterinary clinic (n = 93) Local herds (n = 59)	
Camel	Female (n = 107)	Local (n = 107)	Slaughterhouse (n = 0)	107
	Male (n = 0)	Imported (n = 0)	Veterinary clinic (n = 107) Local herds (n = 0)	
Total	Female (n = 462)	Local (n = 539)	Slaughterhouse (n = 181)	823
	Male (n = 361)	Imported (n = 284)	Veterinary clinic (n = 343) Local herds (n = 299)	

Table 2. Animal's age groups (blood samples).

Animals species	Age	N
Sheep (n = 492)	Group I (Less than - 2 year)	269
	Group II (3–5 years)	120
	Group III (6–7 years)	103
Goat (n = 224)	Group I (Less than - 2 year)	109
	Group II (3–5 years)	74
	Group III (6–7 years)	41
Total (n = 716)	Group I (Less than - 2 year)	378
	Group II (3–5 years)	194
	Group III (6–7 years)	144

at a rate of (55/492; 11.1%). The prevalence in goats was (31/224; 13.8%), with no significant variation ($p > 0.05$), while no infection was recorded among camels. Regarding the animal's gender, MAP was more frequent in male sheep (29/492; 5.8%) than in females (26/492; 5.2%). While in goats, the frequency rate among females (24/224; 10.7%) was higher than in males (7/224; 3.1%). Based on the locality of the animals, MAP was detected at higher and statistically significant frequencies among local animals. In sheep, the prevalence rate was (35/492; 7.1%) and (20/492; 4%) in local and imported ones, respectively. While in goats, no infection was reported among imported ones. The prevalence of MAP was significantly correlated with animals in the age group I ($p < 0.05$). In sheep, MAP prevalence was (5.8%), (2.2%), and (2.2%) in the age group I, II, and III, respectively. In goats, the infection was more common in age group II (4.4%) followed by age group III (3.9%) and then age group I (2.8%). Regarding the source of the animals, MAP infection was detected at higher, but not statistically significant, rates in animals

from the veterinary clinic (5.2% and 9.3% in sheep and goats, respectively). On the other hand, it had lower, but statistically significant rates ($p < 0.05$) in animals from the slaughterhouse (2.8% and 3.1% in sheep and goats, respectively).

Demographic data of examined animals (milk samples)

A total of 364 milk samples were collected from sheep and goats to be screened for the prevalence of MAP. Briefly, 123 (33.8%) samples were collected from sheep and 241 (66.2%) from goats. According to the animal's locality, a number of 353 (97%) animals were locally bred and 11 (3%) were imported. In sheep, 112 (91%) were local and 11 (9%) were imported. No imported goats were used in the present study. Animals belonged to different dairy farms in Medina. The animals were classified into three age groups, Group I (1–2 years), Group II (3–4 years), and Group III (5–6 years) (Table 4).

Prevalence of MAP in milk

As shown in Table 5 and Figure 2, the overall frequency of MAP was (29/364; 7.9%). MAP was detected at higher frequency in sheep (17/123; 13.8%), but not statistically significant ($p > 0.05$), while in goats, the infection rate was (12/241; 4.9%) with a highly significant correlation ($p < 0.01$). Based on the animal's origin, MAP infection was more frequent in local sheep (15/123; 12.1%) than in imported ones (2/123; 1.6%). None of the imported goats were infected. Although local animals had higher frequencies, they showed no significant correlation ($p > 0.05$). MAP prevailed among the different age groups; the highest prevalence was in age group II of sheep (17/123; 19.7%). Similarly, MAP prevalence in goats was also the most prevalent in the age of (3–4 years). The prevalence of MAP was not influenced by the age factor ($p = 0.066$).

Table 3. Prevalence of MAP in animal's sera.

Animals species	Demographic factor		Positive reactors
Sheep* (55, 11.1%)	Gender	Male	29 (5.8%)
		Female	26 (5.2%)
	Locality	Imported	20 (4%)
		Local*	35 (7.1%)
	Source	Slaughterhouse*	14 (2.8%)
		Veterinary clinic	26 (5.2%)
		Local herds	15 (3%)
	Age	Group I (Less than - 2 year)*	29 (5.8%)
		Group II (3–5 years)	10 (2.3%)
		Group III (6–7 years)	10 (2.3%)
Gender	Male	7 (3.1%)	
	Female	24 (10.7%)	
Locality	Imported	0 (0%)	
	Local*	31 (13.8%)	
Goat (31, 13.8%)	Source	Slaughterhouse*	7 (3.1%)
		Veterinary clinic	21 (9.3%)
	Age	Group I (Less than - 2 year)*	6 (2.8%)
		Group II (3–5 years)	9 (4.4%)
		Group III (6–7 years)	8 (3.9%)

*Correlation is significant at the 0.05 level (2 tailed).

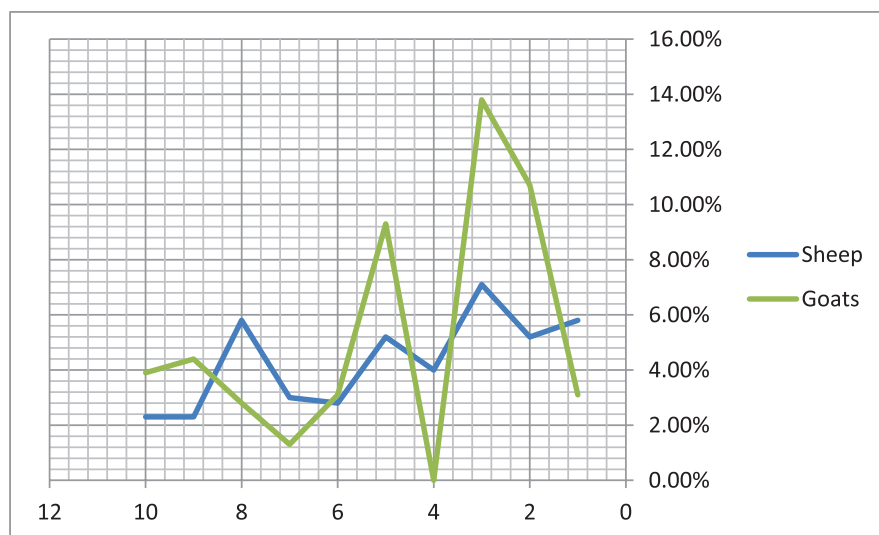


Figure 1. Prevalence of MAP in animal's sera.

Discussion

MAP, the cause of Johne's disease in ruminants, has long been thought to be the causative agent of Crohn's disease in humans [10]. Paratuberculosis or Johne's disease has

Public health importance due to its potential zoonosis [13,14]. The detection of MAP in pasteurized and raw milk suggests the possible exposure to MAP infection through milk consumption [7].

Table 4. Demographic data of examined animals (milk samples).

Animals species	Gender	Locality	Age
Sheep	Female (n = 123)	Local (n = 112)	Group I (1–2 years) (n = 19)
		Imported (n = 11)	Group II (3–4 years) (n = 86)
			Group III (5–6 years) (n = 18)
Goat	Female (n = 241)	Local (n = 241)	Group I (1–2 years) (n = 84)
		Imported (n = 0)	Group II (3–4 years) (n = 155)
			Group III (5–6 years) (n = 2)
Total	Female (n = 364)	Local (n = 353)	Group I (1–2 years) (n = 103)
		Imported (n = 11)	Group II (3–4 years) (n = 241)
			Group III (5–6 years) (n = 20)

Table 5. Prevalence of MAP in milk.

Animals species	Demographic factor	Positive reactors	
Sheep (12, 4.9%)	Gender	Male	29 (5.8%)
		Female	26 (5.2%)
	Locality	Imported	2 (1.6%)
		Local	15 (12.1%)
	Source	Slaughterhouse	14 (2.8%)
		Veterinary clinic	26 (5.2%)
		Local herds	15 (3%)
	Age	Group I (1–2 years)	0 (0%)
		Group II (3–4 years)	17 (19.7%)
		Group III (5–6 years)	0 (0%)
Goat** (17, 13.8%)	Gender	Male	7 (3.1%)
		Female	24 (10.7%)
	Locality	Imported	0 (0%)
		Local	12 (4.9%)
	Source	Slaughterhouse	7 (3.1%)
		Veterinary clinic	21 (9.3%)
		Local herds	3 (1.3%)
	Age	Group I (1–2 years)	3 (1.2%)
		Group II (3–4 years)	9 (3.7%)
		Group III (5–6 years)	0 (0%)

** Correlation is significant at the 0.01 level (2-tailed)

In Saudi Arabia, Johne's disease has been reported in sheep, goats, and camels since the last decade of the nineteenth of the last century [5,15]. However, paratuberculosis infection in the camel still suffers from a wide gap in knowledge and more studies are needed [15]. On the other hand, Crohn's disease prevalence is markedly increased over the last three decades in Saudi Arabia [16]; it was reported in Medina in the period between 2006 and 2013 [17]. Studying Johne's disease in ruminants in Medina was important for many reasons; the lack of studies in this

area, the similarity between Crohn's disease and Johne's disease, and the religious importance of Medina as a crucial destination for pilgrims.

There have been studies in several regions of Saudi Arabia, searching the spreading of Johne's disease in livestock; Al-Ahsa [4], Al-Ahsa and Riyadh regions [18], Eastern Province [19], Qassim region [20,21], Jeddah city [22], and in Makkah [23,24]. The present study as far as we know is the first report on Johne's disease in ruminants in Medina.

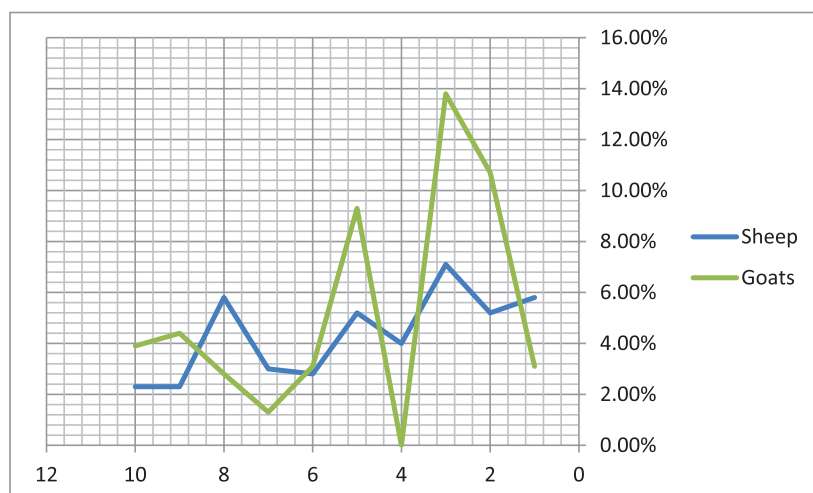


Figure 2. Prevalence of MAP in milk.

In the present study, the prevalence of *paratuberculosis* was investigated in different species of ruminants (sheep, goats, and camels) using the indirect ELISA for the detection of MAP antibodies in serum and milk samples.

MAP prevalence in sheep sera (11.1%) was lower than in goats (13.8%), with a significant correlation ($p < 0.05$). Our results almost agreed with Mathevon *et al.* [25], who confirmed MAP infection in sheep at a rate of 17.4%. MAP was frequent in both males (5.8%) and females (5.2%), this was consistent with other reports [26,27]. When it comes to the locality factor, it has been observed that the frequency of MAP among local animals (35/492; 7.1%) was higher than that of the imported ones (20/492; 4%) with a significant correlation ($p < 0.05$). This may be due to the restricted import terms since each animal examined to make sure of its health before release to the livestock market, while local animals need to be more examined and infected animals must be isolated to prevent the spreading of infection to the healthy ones [28]. With the age factor, MAP frequency in 1-year aged goats and sheep had a significant correlation ($p < 0.05$). Our results agreed with Vinodh Kumar *et al.* [27], who stated that all infected sheep were aged 1.5 years. Contrary, others reported that all infected sheep were 2 years or older [26,29,30]. In the current study, the screened animals were supplied from three sources: A veterinary clinic, local herds, and the central slaughterhouse. The highest prevalence rate was (26/492; 5.2%) in the veterinary clinic, followed by local herds (15/492; 3%), and slaughterhouse at a rate of (14/492; 2.8%) with a significant correlation ($p < 0.05$). Among the slaughtered animals, 168 were males and 13 were females, only one female was infected. This is a good indicator since females raised for reproduction and milk production.

In goats, MAP prevalence was 13.8% with no significant correlation ($p > 0.05$), following Mpenda and Buza [31], 10.9% of Tanzanian goats were MAP infected. The prevalence of MAP among female goats (10.7%) was higher than males (3.1%). Our results were contrary to what was reported by Singh *et al.* [32], where male goats had a higher frequency than females. Concerning the goat's locality, the prevalence of MAP in local goats was 13.8%, while no infection was recorded among the imported ones. Our results were following Al-Dubaib and Mahmoud [20], who reported that all of the infected goats (29/2610; 1.11%) in Al-Qassim were of the Najdi breed, indigenous to Saudi Arabia. Goats aged 3–5 years had the highest frequency (8/178; 4.4%), this was consistent with Al-Dubaib and Mahmoud [20], who reported that all of the infected goats in Qassim were aged more than 3 years. Also, Radad and Khalil [22] reported nine infected goats in Jeddah aged more than 2 years. When it comes to the source, the highest frequency was among goats from the veterinary clinic at a rate of 9.3%. This was following Radad and Khalil [22], who reported nine goats (13.6%) from a veterinary clinic were diagnosed with paratuberculosis.

Paratuberculosis infection in camels needs more study, where no seropositivity was recorded among the examined camels, unlike sheep and goats. This may be due to a different immune response in camels. Our results were contrasted with the seroprevalence results of Saudi studies [4,18]. ELISA is not effective at early detection in camel infection [4], may be for this reason; we did not record any positivity. However, camel age data were not available so we cannot be certain of it.

Concerning the prevalence of MAP in milk samples, our results revealed that the prevalence of MAP in goat's milk was 4.9% with a highly significant correlation ($p < 0.01$).

Salgado et al. [33] reported a similar rate in Chile but without any significant correlation ($p > 0.05$). Based on the origin of milk samples, the prevalence among local goats (12/241; 4.9%) was higher than the imported ones; this was following Singh et al. [12], who reported milk paratuberculosis infection in local goats (38.7%). Sheep milk samples had a higher frequency (13.8%) with no significant correlation ($p > 0.05$). Ngu Ngwa et al. [34] reported a similar frequency but with a highly significant correlation ($p < 0.01$). Local sheep had (12.1%) a higher frequency than that of the imported ones (1.6%).

Concerning the animal's age, a higher prevalence was in goats and sheep aged over 2 years. This was consistent with others who stated that the infected lactating sheep and goats were over 2 years [29,33]. Further investigation and evaluation of the public health risk associated with milk consumption relative to other routes of exposure, such as meat, water, and environment are urgently required.

Conclusion

MAP was prevalent in the sheep and goats' sera and milk. The animal's locality, age, and the source had an impact on the prevalence rates. Sheep and goats may act as reservoirs for MAP to the Medina community and the pilgrims throughout the year. MAP infection dynamics should be explored on a large scale using combined techniques. The necessary precautions should be taking during slaughtering, meat must be well cooked, and fresh milk must be boiled to reduce the possibility of infection.

Acknowledgments

Nothing to disclose.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

Shabana II contributed to the design and the establishment of experiment scheme, the performance of the ELISA assay, data modelling, analysis, and interpretation. She contributed also to the orientation of statistical graphics, the writing of the manuscript and important review contributions. Aljohani AB contributed to the design and the establishment of experiment scheme. She contributed also to the sample collection, serum separation, and the writing of the manuscript. All the authors read and approved the manuscript and no ethical issues involved.

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