Identification of *Mycobacterium avium* subspecies *paratuberculosis* in sheep farms in Bayannaoer, Inner Mongolia, China (short communication)

Yuandi Yu1,2, Suhui Zhang1,2, Guoyang Xu1,2, Dengfeng Xu1,2, Hua Zheng1,2, Bo Li1,2, Kefei Shen1,2* and Lizhi Fu1,2*

**Abstract**

**Background:** Paratuberculosis is a widespread chronic infection of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) that causes significant economic losses to the sheep industry. The current study investigated this disease, which causes diarrhea in sheep, particularly, in Bayannaoer, Inner Mongolia, China. Diagnosis was based on clinical symptoms, pathological autopsy, histopathological inspection, and serological and molecular methods.

**Results:** MAP was confirmed using polymerase chain reaction using DNA extracted from tissue and fecal samples. Serum samples from 472 individual sheep were obtained to detect antibodies against MAP using an enzyme-linked immunosorbent assay. MAP antibodies were separately detected in 17.86% (35/196) and 18.48% (51/276) of sheep herds at approximately 6 months and ≥ 1 year of age, respectively. The tissue lesion and pathological section results were consistent with *paratuberculosis* infection.

**Conclusions:** To our knowledge, this is the first report of *Mycobacterium avium* subspecies *paratuberculosis* seroprevalence in Bayannaoer sheep in Inner Mongolia. Our findings show that MAP is not only prevalent, but also a potential threat to this region. Further investigations, including long-term epidemiological surveillance and isolation are needed for the awareness and effective treatment of paratuberculosis in sheep of Inner Mongolia.

**Keywords:** Sheep, Paratuberculosis, ELISA, PCR, Histopathology

**Background**

Johne's disease (JD) or paratuberculosis (PTB) is a chronic, progressive granulomatous enteritis disease that is caused by the zoonotic bacterium, *Mycobacterium avium* subspecies *paratuberculosis* (MAP) [1]. The disease primarily affects ruminants, including cattle, sheep, and goats. *Paratuberculosis* is a public health threat, and it reduces animal productivity and causes significant economic losses in ruminant industries worldwide [2, 3]. The common clinical manifestations are chronic diarrhea or scours, decreased productivity, weight loss and eventually, death of the animal [4]. Many countries have reported that infection with JD in sheep and goats occurs in a more discrete and insidious manner than in other animals. In Europe, the infection rate in sheep and goat herds is approximately 4% [5, 6]. Furthermore, in some Australian flocks, mortality rate reached 20% per annum [7]. An economic study showed that MAP infection...
reduced the profit efficiency from 84% to 64% in Italian dairy sheep and goat farms [8]. Other countries and regions have also shown varying degrees of economic losses as a result of PTB [9].

In recent years, MAP infection of ruminants in China has occurred rapidly. Some studies have indicated that MAP has become a common pathogen in dairy farms. Notably, the herd-level prevalence of 57.9% in 19 dairy herds in the Shandong province of China was observed [10]. MAP infection in dairy cattle differs with farming modes at the animal and herd level, and farming density could be an important risk factor associated with the presence of MAP-infected cattle [11]. One study showed that the overall MAP seroprevalence in the tested Tibetan sheep was 11.29% [12]. Additionally, recent studies have shown that PTB is widely prevalent in dairy farms in Tai’an, in the Shandong province of China [13]. Therefore, it is evident that research should focus on the prevalence, prevention, and control of PTB.

Moreover, the mutton sheep industry plays an important role in Bayanaoer, which is in western Inner Mongolia in China. Every year, approximately 10% of sheep in this area have intermittent diarrhea, against which pharmaceutical treatments are ineffective. This results in the eventual death of these sick sheep due to exhaustion, which emphasizes the significant negative effect that PTB has on economic development. As such, we diagnosed several sheep herds with PTB through clinical inspection, molecular biological methods, histopathological examination, and serological detection, to understand the epidemiology of MAP in Bayanaoer.

Results

Clinical symptoms and necropsy findings

The sick sheep showed clinical signs of JD, including severe weight loss and chronic diarrhea (Fig. 1 A).

Necropsy findings in representative dead sheep showed ileal hyperplasia (Fig. 1 B), enlarged and edematous abomasum (Fig. 1C), in addition to mesenteric lymph node edema and liquefaction (Fig. 1D). Mesenteric congestion and intestinal mucosa exfoliation were also found in intestinal lesions (Fig. 1 E, and F).

Polymerase chain reaction (PCR) MAP detection

The deoxyribonucleic acids (DNA) from fecal and ileum samples of sheep with diarrhea were used as templates. The results showed that the fragment size amplified by the nested PCR (L/AV) targeting IS900 was 298 bp, consistent with the expected size (Fig. 2).

Incidence of MAP in sheep

A total of 472 serum samples collected from different sheep farms were tested using enzyme-linked immunosorbent assay (ELISA). The sheep were either approximately 6 months or ≥ 1 year old, and the detection rates of sheep in these two stages were similar. A total of 17.86% (35/196) and 18.48% (51/276) were separately tested positive for MAP in the two age groups (Table 1).
Histopathological assessment

The heart, spleen, lung, kidney, intestinal, and mesenteric lymph nodes of the sheep were stained with hematoxylin and eosin (H & E) to detect MAP infection. The myocardial fibers were swollen, thickened, and partially broken, and the transverse striations were not obvious. Moreover, there were scattered calcifications of different sizes (Fig. 3A). The endothelial cells of glomerular capillaries were partially necrotic, with red-stained silk reticular fibrin and a small amount of red blood cells distributed between them; a small amount of red blood cells was also seen in the renal capsule. In addition, the epithelial cells of the renal tubules were swollen and partially necrotic, and protein tubules appeared in most of the lumens (Fig. 3B). Many spleen lymphocytes were necrotic and significantly bleeding, especially around the splenic nodules, forming a red halo (Fig. 3C). The intestinal mucosa was thickened; many inflammatory cells were distributed in the lamina propria (Fig. 3D), and some lymphocytes in the mesenteric lymph nodes were necrotic and scattered in granulomas of different sizes. Moreover, epithelioid cells and lymphocytes were observed in these granulomas (Fig. 3E). The pulmonary interstitium widened, and many inflammatory cells were distributed. Most of the alveoli were dilated, and the alveolar wall ruptured (Fig. 3F).

Discussion

Johne's disease is one of the most economically important diseases affecting ruminants worldwide, and it is a growing concern in the sheep and goat industries [14]. The disease is caused by the zoonotic pathogen MAP, that poses a challenge to public health [3]. The sheep farming and industrial wool spinning industries are particularly developed in Bayannaoer. Therefore, MAP could act as a threat to workers in these industries and to public health security. In 2011, the flocks in this region suffered from intermittent diarrhea and eventually died of exhaustion. Most of them were adult sheep, and the main symptoms were similar to those reported in previous studies—elevated body temperature, dark green and thick feces [15, 16]. The course of the disease was 10–30

### Table 1

| Herds | Sampling Number | Number of MAP-positive samples | Positive MAP detection rate (%) |
|-------|-----------------|--------------------------------|---------------------------------|
|       | Approx. 6 months old | ≥ 1 year old | Approx. 6 months old | ≥ 1 year old | About 6 months old | ≥ 1 year old |
| A     | 11              | 16              | 3                  | 4              | 27.27            | 25            |
| B     | 18              | 25              | 4                  | 5              | 22.22            | 20            |
| C     | 20              | 27              | 5                  | 6              | 25               | 22.22         |
| D     | 12              | 17              | 3                  | 4              | 25               | 23.53         |
| E     | 16              | 22              | 1                  | 2              | 6.25             | 9.09          |
| F     | 19              | 24              | 4                  | 6              | 21.05            | 25            |
| G     | 14              | 20              | 2                  | 3              | 14.29            | 15            |
| H     | 13              | 19              | 4                  | 5              | 30.77            | 26.32         |
| I     | 9               | 14              | 2                  | 3              | 22.22            | 21.43         |
| J     | 15              | 23              | 0                  | 2              | 0                | 8.70          |
| K     | 21              | 29              | 3                  | 5              | 14.29            | 17.24         |
| L     | 10              | 15              | 0                  | 1              | 0                | 6.66          |
| M     | 18              | 25              | 4                  | 5              | 22.22            | 20            |
days, over which the sick sheep gradually lost weight and their eyelids became white. This brought huge economic losses to local herdsmen; therefore, it is essential to control the spread of this disease. However, clinical signs in sheep or goats are not a reliable indicator of the presence or absence of MAP infection [17]. Weight loss is the predominant clinical sign in infected sheep and goats. In sheep, the period of weight loss differs from one animal to another. Softening of the faeces or diarrhoea occurs only in 20% of the cases at the end stages of the disease [18]. Detailed epidemiological investigations are important to understand the regional prevalence and potential threats of MAP. MAP can also induce bacteremia, which was unevenly distributed in the filtering organs of the sheep [19]. Attention should be paid to MAP infection in young sheep. Two clinical syndromes have been described in red deer: sporadic disease with low morbidity and high mortality in adult populations, and severe outbreaks in young deer (8–15 months old) resulting in both high morbidity and high mortality [20]. Young animals, especially those under 6 months, have a higher risk of infecting MAP, but the risk decreases thereafter [21]. Research also shows that calves inoculated with MAP at an earlier age had more severe tissue lesions [22].

In this study, we provided a detailed description of the clinical inspection and pathological changes, as well as molecular biology techniques and ELISA to diagnose naturally occurring PTB cases. We found that approximately 10% of sheep were infected in February and March; notably, most infections occurred in August and fewer infections were observed in winter. This cycle is almost the same every year. The main symptoms were emaciation and general weakness with intermittent diarrhea in infected sheep, which is consistent with the infection of paratuberculosis [18].

Paratuberculosis was confirmed by PCR using DNA extracted from mesenteric lymph node tissues and feces. At the histopathological level, the tissues and organs of the sheep, in this study, showed different degrees of lesions at the late stage of the disease. Serum samples of different sheep flocks were subjected to a MAP ELISA, and the seroprevalence was found to be 17.86% (35/196) and 18.48% (51/276), respectively, for the two different age groups. This prevalence was lower than the 19.5% seroprevalence in sheep in the Eastern Province of Saudi Arabia [23]; however, it was higher than the 11.9% seroprevalence in the Tibetan sheep in China [12], and 3.25% in female sheep in Tunisia [24]. These data show that PTB may be a risk factor in Inner Mongolia; however, detailed statistical analysis is needed.

MAP causes chronic diarrheic intestinal infections, which are difficult to treat, in domestic animals, including ruminants [25]. Several aminoglycosides are active against several species of mycobacteria, including MAP [26]. In this study, gentamicin was used in combination with lincomycin to treat diseases associated with MAP. We found that the symptoms in sheep were relieved after treatment for the first time; however, diarrhea occurred approximately 30 days after the first treatment. At this time, the use of therapeutic drugs was ineffective, and
the sick sheep continued to suffer from diarrhea and sub-
sequently died of exhaustion. The results demonstrated
that some products effectively inhibited bacterial growth,
and since these findings are applicable to the veterinary
field, these products may become available for veterinary
use [27, 28]. Further research may substantiate the effi-
cacy of pharmaceuticals for the treatment and control of
PTB.

Conclusion
In conclusion, this study is the first to estimate the epide-
miology of PTB in sheep in Bayannaoer, Inner Mongolia,
China. Our results showed that MAP was prevalent in
this region. This information will provide a comprehen-
sive view for the prevention and control of MAP infec-
tion in Inner Mongolia sheep.

Methods
Sampled animals and Sample collection
This study was carried out during October 2020, a total
number of 472 sheep from 13 small to middle-sized
sheep farms were sampled. The sampling proportion
of the sheep was not less than 50% for the 6-month old
sheep, and the proportion of sheep over 1 year old was
not more than 5%. In addition, 196 sheep at approxi-
ately 6 months old and 276 sheep at ≥1 year old were
selected. Sheep owners were interviewed about all treat-
ments, therapeutic agents, and incidences of MAP in
their sheep flocks. In this study, aminoglycosides were
used to treat the disease, such as intramuscular injection
of gentamicin (4 mg/kg) combined with oral electrolytic
multidimensional preparation and lincomycin (500 mg
oral), twice a day for 3 consecutive days. Three sheep
that were extremely weak and were suspected of having
JD were euthanized. The study was in compliance with
ARRIVE guidelines. Serum and tissues samples, includ-
ing ileum and mesenteric lymph node samples, were
collected. Three milliliters of blood were collected from
the jugular vein of each animal using a vacutainer. Sera
were collected in Eppendorf tubes and stored at –20 °C
until use [24]. Necropsy findings of the carcasses showed
emaciated intestinal mucosa, enlarged lymph nodes, and
occasional serous fat atrophy. The samples were sepa-
rated into two parts, immediately placed in sterile plas-
tic bags, placed in cooling boxes, and transported to the
microbiology laboratory. Samples of the carcasses were
subjected to tissue staining and DNA extraction.

PCR detection of MAP
DNA was extracted from the feces and mesenteric lymph
node samples of sick sheep and subjected to PCR for
MAP detection. DNA was extracted from fecal samples
using the QIAamp Fast DNA Stool Mini Kit (Qiagen,
Hilden, Germany) and from tissue using the TIANamp
Genomic DNA Kit (Tiangen, Beijing, China). The prim-
ers targeting IS900 were designed as follows: L1 (5’-CTT
TCTTGAGGGTGTTCCG-3’) and L2 (5’-ACGTGA
CCTCGCCCTCAT-3’), AV1(5’-ATGTGGTTGCTGTTG
TGATGG-3’) and AV2 (5’-CCGCCCAATCACT
CCAG-3’) and were used for the first round of PCR and
nested PCR, respectively, as previously described [29].
For the first round of PCR, conditions were as follows:
94°C for 2 min, followed by 30 cycles at 94°C for 30 s,
58°C for 30 s, and 72°C for 30 s, with a final elongation
step at 72°C for 3 min. The second PCR round used a 10×
dilution of the products of the first round as templates,
and the reaction parameters were carried out according
to the primary reaction.

ELISA assays
Serum samples were collected from sheep farms in
Bayannaoer, with herd sizes ranging from 300 to 500.
The samples were analyzed using a commercial indi-
rect ELISA kit. The tests were performed according to
the manufacturer’s instructions (ID-VET, Montpellier,
France). Sera with sample to positive (S/P) ratios ≤75%
were scored as MAP-negative, while those with ratios
≥85% were considered MAP-positive. Moreover, 75%<
S/P% <85% were scored as “suspect” and treated as nega-
tive for data analysis.

Histopathological examination
Tissue samples from the heart, kidney, spleen, lungs,
ileum and mesenteric lymph nodes were fixed in 10%
neutral buffered formalin, embedded in paraffin, sec-
tioned at 5-μm thickness and stained with H & E.

Abbreviations
DNA: Deoxyribonucleic acid; ELISA: Enzyme linked immunosorbent assay; JD:
Johne’s disease; MAP: Mycobacterium avium subspecies paratuberculosis; PCR:
Polymerase Chain Reaction; PTB: Paratuberculosis.

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survey.

Authors’ contributions
Yu YD was primarily responsible for the writing of this manuscript, and
participated in detection and statistical analyses. Shen K F and Fu L Z were
responsible for study design. Shen K F and Zheng H carried out ELISA. Zhang
S H, Xu G Y, Xu D F, and Li B performed sample collection. All authors read and
approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was approved by the Committee on Animal Ethics of Chongqing Academy of Animal Sciences (permission number Cqaa2019004) and carried out in accordance with the approved guidelines. The animals sampled in this study are owned by private sheep farmers. The sheep owners consented to the use of their animals in the study under supervision of qualified veterinarians. Informed consent - Owners gave informed consent for their animals’ inclusion in the study. The study was in compliance with ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
1Chongqing Academy of Animal Sciences, Chongqing, China. 2Chongqing Research Center of Veterinary Biologicals Engineering and Technology, Chongqing Academy of Animal Sciences, 51 Changlong Avenue, Rongchang District, ChongQing 402460, China.

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References

1. Stevenson K. Genetic diversity of Mycobacterium avium subspecies paratuberculosis and the influence of strain type on infection and pathogenesis: a review. Vet Res. 2015;46(1):64.
2. Barkema HW, Orsel K, Nielsen SS, Koets AP, Rutten V, Bannantine JP, et al. Knowledge gaps that hamper prevention and control of Mycobacterium avium subspecies paratuberculosis infection. Transbound Emerg Dis. 2018;65(Suppl 1):125–48.
3. Kuenstner L, Kuenstner JT. Mycobacterium avium ssp. paratuberculosis in the Food Supply: A Public Health Issue. Front Public Health. 2021;9:647448.
4. Johansen MD, de Silva K, Plain KM, Whittington RJ, Purdie AC. Mycobacterium avium subspecies paratuberculosis is able to manipulate host lipid metabolism and accumulate cholesterol within macrophages. Microb Pathog. 2019;130:44–53.
5. Nielsen SS, Toft N. A review of prevalences of paratuberculosis in farmed animals in Europe. Prev Vet Med. 2009;88(1):1–14.
6. Whittington R, Donat K, Weber MF, Kelton D, Nielsen SS, Eisenberg S, et al. Control of paratuberculosis: who, why and how. A review of 48 countries. BMC Vet Res. 2019;15(1):198.
7. Windsor P. Managing control programs for ovine caseous lymphadenitis and paratuberculosis in Australia, and the need for persistent vaccination. Vet Med. 2011;45:11–22.
8. Sardaro R, Pieragostini E, Rubino G, Petazzi F. Impact of Mycobacterium avium subspecies paratuberculosis on profit efficiency in semi-extensive dairy sheep and goat farms of Apulia, southern Italy. Prev Vet Med. 2017;136:56–64.
9. Fiorentina P, Martino C, Mancini Y, De Iorio MG, Williams JL, Minozzi G. Using Omics Approaches in the Discovery of Biomarkers for Early Diagnosis of Johnes’ Disease in Sheep and Goats. Animals. 2021;11(7):1912.
10. Yue R, Liu C, Barrow P, Liu F, Cui Y, Yang L, et al. The isolation and molecular characterization of Mycobacterium avium ssp. paratuberculosis in Shaanxi province, China. Gut Pathog. 2016;8(1):9.
11. Liu X, Lu J, Yang X, Wang D, Wang J, Wu J. The seroprevalence of Mycobacterium avium subspecies paratuberculosis in dairy cattle in Xinjiang, Northwest China. Int J Vet Res. 2017;70(1).
12. Ma J, Tian A, Zheng W, Zou Y, Zhang Y, Yang Z. First report of bovine viral diarrhea virus and Mycobacterium avium subspecies paratuberculosis infection in Tibetan sheep (Ovis aries) in Tibetan Plateau, China. Trop Anim Health Prod. 2019;51(3):719–22.
13. Cheng Z, Liu M, Wang P, Liu P, Chen M, Zhang J, et al. Characteristics and Epidemiological Investigation of Paratuberculosis in Dairy Cattle in Tai’nan, China. BioMed Res Int. 2020;2020(11):1–7.
14. Windsor PA. Paratuberculosis in sheep and goats. Vet Microbiol. 2015;181(1–2):161–9.
15. Esilami M, Shafei M, Ghaseimian A, Valizadeh S, Al Marzooq AH, Shokouhi Mostafavi SK, et al. Mycobacterium avium ssp. paratuberculosis and Mycobacterium avium complex and related subspecies as causative agents of zoonotic and occupational diseases. J Cell Physiol. 2019;234(8):12415–21.
16. Aitken JM, Phan K, Bodman SE, Sharma S, Watt A, George PM, et al. A Mycobacterium species for Crohn’s disease? Pathology. 2021;53:818–23.
17. Whittington RJ, Sergeant E. Progress towards understanding the spread, detection and control of Mycobacterium avium subsp paratuberculosis in animal populations. Aust Vet J. 2001;79(4):267–78.
18. Carrigan MJ, Seaman JT. The pathology of Johnie’s disease in sheep. Aust Vet J. 2010;87(7):47–50.
19. Bower KL, Begg DJ, Whittington RJ. Tissue localisation of Mycobacterium avium subsp paratuberculosis following artificially induced intracellular and naked bacteraemia. Vet Microbiol. 2013;162(1):112–8.
20. Mackintosh CG, de Lisle GW, Collins DM, Griffin J. Mycobacterial diseases of deer. New Zeal Vet J. 2004;52(4):163–74.
21. Karuppumasy S, Kirby GM, Mutharia L, Tripathi BN. An update on Mycobacterium avium subspecies paratuberculosis antigens and their role in the diagnosis of Johnie’s disease. World J Microbiol Biotechnol. 2019;35(8):120.
22. Mortier RAR, Barkema HW, Byström JM, Illanes O, Orsel K, Wolf R, et al. Evaluation of age-dependent susceptibility in calves infected with two doses of Mycobacterium avium subspecies paratuberculosis using pathology and tissue culture. Vet Res. 2013;44(1):94.
23. Elsohaby I, Fayez M, Alkaify M, Refaat M, Al-Marri T, Alaql FA, et al. Serological and Molecular Characterization of Mycobacterium avium subsp. paratuberculosis (MAP) from Sheep, Goats, Cattle and Camels in the Eastern Province, Saudi Arabia. Animals. 2021;11(2):323.
24. Khamassi KM, Romdhane R, Sassi L, Amami A, Rekik M, Benzarti MH. Mycobacterium avium ssp. paratuberculosis infection in sheep and its role in the diagnosis of Johne’s disease. World J Microbiol Biotechnol. 2020;26(3):393–8.
25. Cirone KM, Lahiri P, Holani R, Tan YL, Arrazura R, De Buck J, et al. Synthetic cathelicidin LL-37 reduces Mycobacterium avium subsp. paratuberculosis internalization and pro-inflammatory cytokines in macrophages. Cell Tissue Res. 2020;379(1):207–17.
26. Fecteau M, Whitlock RH. Treatment and Chemoprophylaxis for Paratuberculosis. Vet Clin N Am-food Anim Pract. 2011;27(3):547–57.
27. Wong SY, Grant IR, Friedman M, Elliott CT, Skull A, Bull TJ, et al. Detection of Mycobacterium avium subsp. paratuberculosis in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn’s disease. J Clin Microbiol. 2003;41(7):2915–23.

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