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Citation	Japanese Journal of Veterinary Research, 63(4), 191-194
Issue Date	2015-11
DOI	10.14943/jjvr.63.4.191
Doc URL	<a href="http://hdl.handle.net/2115/60301">http://hdl.handle.net/2115/60301</a>
Type	bulletin (article)
File Information	JJVR63-4 p.191-194.pdf



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# A preliminary survey of the seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Mongolian cattle

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Received for publication, June 4, 2015; accepted, August 10, 2015

## Abstract

Johne's disease is a chronic infection with *Mycobacterium avium* susp. *paratuberculosis* (MAP), which causes huge economic losses to cattle industry. The seroprevalence of MAP in cattle of Mongolian was estimated by an ELISA assay using 356 serum samples which were collected from eleven provinces and Ulaanbaatar city. Out of these samples, 3 (0.84%) were found to be seropositive for MAP, originating from Tsenkher sum of Arkhangai province, Murun sum of Khuvsgul province, and Bornuur sum of Tuv province in Mongolia. This study represents first conformation of Johne's disease in Mongolian cattle. These findings provide vital information that can be used for the planning and execution of control measures for Johne's disease in the Mongolian cattle industry.

Key Words: Johne's disease, *Mycobacterium avium* susp. *paratuberculosis*, cattle, Mongolia.

Johne's disease is caused by the infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The disease has mainly been reported in common domestic agricultural species (e.g., cattle, goats, sheep, deer, bison)<sup>10)</sup>. In addition, this infection can circulate among wildlife hosts, including various species of deer and rabbits<sup>2)</sup>. Although younger animals are more susceptible, adult cattle can be infected if exposed to large doses of MAP. The infection begins in early age

animals, however, the symptoms of the illness do not appear until they are adults. Clinical signs of the disease begin to appear 1 and 10 years after dairy cattle are infected with MAP<sup>3)</sup>. MAP infections have been extensively studied in dairy cattle than in other domestic animals because the disease can cause significant economic losses in the affected dairy cattle, and there is still no effective treatment<sup>7)</sup>. These economic losses are associated with decreased milk production

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doi: 10.14943/jjvr.63.4.191

concurrent with an increased incidence of mastitis, changes in milk parameters, increased somatic cell counts, reproductive dysfunction, and increased predisposition to other diseases<sup>10</sup>. Main clinical symptoms of Johne's disease are persistent diarrhea and progressive weight loss. The primary source of MAP transmission is fecal contamination of a calf's environment, including contamination of milk and feed, resulting in ingestion of the agent. Furthermore, calves may become infected from the mother in utero<sup>8</sup>. Cattle or sheep may also acquire infection through contaminated pastures shared with wildlife<sup>2</sup>. MAP is widely distributed all over the world and has been recognized as one of the most costly infectious disease of dairy cattle. The reported prevalence of MAP infection in cattle herd in Ireland was 21.4%<sup>7</sup>, the USA was 70.4%<sup>9</sup>, Canada was 9.8–43.1%, Denmark was 80–86%, the Netherlands was 20–71%, and the UK was 27.6–42.5%<sup>6</sup>. Overall the reported prevalence in Latin American and Caribbean countries was 16.9%, with 75.8% in cattle, 16% in sheep, and 3.7–4.3% in goats<sup>5</sup>. In the past, several countries, including France, Iceland, Norway, the USA, Britain, and Australia, have initiated programs to control MAP infections in sheep, goats, and cattle. Based on the results of these various eradication programmes, it is evident that successful eradication programmes for sheep, goats, and cattle will only be possible with the use of various diagnostic tests, vaccination, restriction of animal movement, and preventive management measures<sup>1</sup>.

The Mongolians have been engaged in animal husbandry for centuries. In 2014, there were more than 51.9 million livestock, including sheep, goats, cattle, horses, and camels. Animal husbandry is a special, traditional sector of the national economy and an important source of employment and export income for Mongolia. Today the agriculture sector employs approximately 35% of the total labor force and produces 20% of all the gross domestic product<sup>11</sup>. During the last 10 years, a number of transboundary diseases

have been detected in Mongolian livestock, such as foot-and-mouth disease, high pathogenic avian influenza, sheep and goat pox, equine influenza, and several newly diagnosed viral infections, such as an ovine pulmonary adenomatosis and maedivisna<sup>4</sup>. Conversely, several bacterial diseases occur in cattle population, such as brucellosis, anthrax, leptospirosis, tuberculosis, pasteurellosis, listeriosis, and blackleg<sup>12</sup>. However, there is no information about MAP infection in the livestock of Mongolia. The main purpose of the present study was to detect the prevalence and distribution of MAP infection in Mongolian cattle.

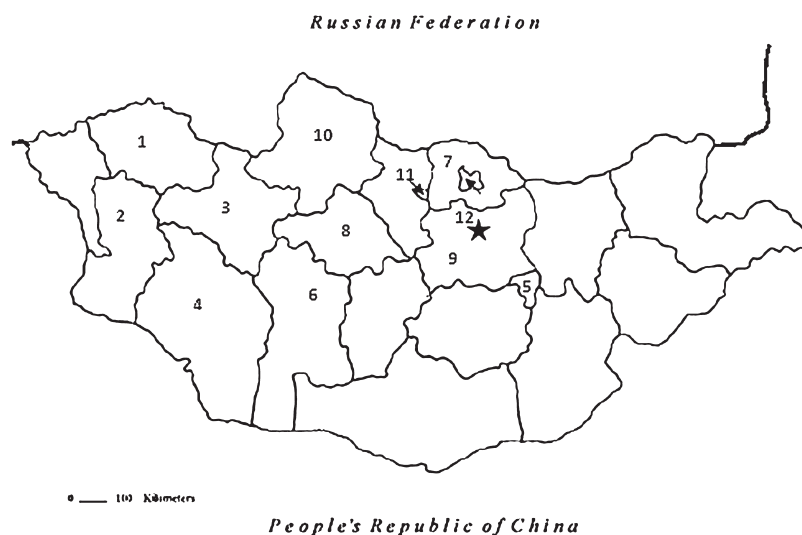
A total of 356 of bovine blood samples (Mongolian native cattle, yaks, Holstein, Simmental and Alatau breed cattle) were collected in 2011–2014 in Ulaanbaatar city and eleven provinces, including Uvs, Khovd, Zavkhan, Govisumber, Govi-Altai, Bayankhongor, Selenge, Arkhangai, Tuv, Khuvsgul and Orkhon (Fig. 1). Most of the sampled cattle were 3–8 years old, but several calves which had diarrhea also included in the sampling cattle. Blood was collected from the jugular vein of each animal using vacutainer tube with serum clot activator (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Tubes were kept in upright position at room temperature for about 3 hrs after collection, followed by separation of serum into 1.5 ml plastic tubes (Watson, Tokyo, Japan). Blood serum was tested for specific antibodies against MAP using a commercial ELISA test kit (Kyoritsu Seiyaku Corporation, Tokyo, Japan) according to the manufacturer's protocol. The optical densities (OD) at 450 nm were read by Titertek Multiskan PLUS MK II Microplate Reader Typ 314 (Labsystems, Finland). The required OD values for test validity were confirmed as followed, OD negative is less than 0.25 and OD positive is between 0.8 and 1.6 in successful test.

ELISA value calculation method

$$= \frac{OD (sample) - OD (negative control)}{OD (positive control) - OD (negative control)}$$

Out of the 356 samples tested 3 (0.84%) were found positive for MAP. The seropositive bovine samples were derived from 3 independent sampling sites, cattle from Tsenkher sum of Arkhangai province, Murun sum of Khuvsgul

province were belonged pasture based cattle herds and the cattle from Bornuur sum of Tuv province was belonged to intensive farming cattle herd (Table 1). Distances between the 3 positive cattle were approximately five hundred km far each



**Fig. 1. A map of the sampling area in Mongolia.** (1) Uvs, (2) Khovd, (3) Zavkhan, (4) Govisumber, (5) Govi-Altai, (6) Bayankhongor, (7) Selenge, (8) Arkhangai, (9) Tuv, (10) Ulaanbaatar, (11) Khuvsgul, and (12) Orkhon.

**Table 1. Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Mongolian cattle in 2011 and 2014**

Name of province	Number of tested samples	Number of positive (Mean seroprevalence)	Note of positive animals	SP-value
Uvs	50	0		
Khovd	20	0		
Zavkhan	30	0		
Govisumber	30	0		
Govi-Altai	20	0		
Bayankhongor	20	0		
Selenge	22	0		
Arkhangai	20	1 (5.00%)	MNC <sup>2)</sup> , F <sup>3)</sup> , 8 years old	1.2
Tuv	60	1 (1.67%)	HS <sup>4)</sup> , F, 5 years old	0.6
Ulaanbaatar	40	0		
Khuvsgul	20	1 (5.00%)	MNC, F, 6 months old	0.3
Orkhon	24	0		
Total (12 provinces)	356	3 (0.84%) <sup>5)</sup>		

1) All treated cattle were serologically tested using ELISA.

2) Mongolian native cattle

3) Female

4) Holstein breed

5) 95%CI: -0.26%, 2.21%

other, all sampling areas belonged to the forest area of the country.

In this survey, the prevalence of MAP infection among Mongolian cattle was very low compared with previous reports in other countries. The positive cattle were six months, five and eight years old, and all of them were female. Interestingly, the youngest cow had diarrhea. Further studies with larger number of samples are necessary to confirm whether this low rate of infection is consistent in the Mongolian cattle population. Furthermore, detailed studies are required to determine the incidence of MAP infection in other animal species and to perform molecular characterization of the Mongolian isolate. This is the first report about MAP infection in the Mongolian cattle population. The implementation of systematic control measures against infectious agents, including MAP, in livestock should still be considered important in Mongolia. However, the epidemiology of infectious agents requires further in-depth investigation to provide information that is vital for the planning and execution of effective control measures in the Mongolian dairy and beef industries.

### Acknowledgements

This study was supported by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry, Japan Society for the Promotion of Science and the Program for Leading Graduate Schools (Hokkaido University) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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