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The herd-level prevalence of antibodies against *Mycobacterium avium subspecies paratuberculosis* in the Gyeonggi and Chungbuk of Korea, as detected by bulk tank milk ELISA

Yunho Jang, Haet-Nim Lee, Narae Kim, Yu Sin Ok,
Tae-woon Kim, Suk Chan Jung, Jae-Myung Kim*

Animal and Plant Quarantine Agency, MIFAFF, Gimcheon 39660, Korea

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Abstract

Mycobacterium avium subspecies paratuberculosis (MAP) causes Johne's disease (JD) in ruminants. This is the first large scale report to estimate the herd-level prevalence of antibodies against MAP by using an ELISA to detect antibodies in bulk tank milk (BTM) samples. The samples were collected from January 2011 to November 2011, from 636 herds of the dairy farms in the Gyeonggi and Chungbuk areas of Korea. The overall apparent prevalence of MAP antibody-positive herds was 8.5%, and regional prevalence were 32/440 (7.3%) and 22/196 (11.2%) of dairy farms in the Gyeonggi and Chungbuk areas, respectively. The results did not differ significantly by region. While we have determined the prevalence rate of MAP in the Gyenoggi and Chungbuk areas in this study, there is a continuing need for well-designed studies to calculate the prevalence of MAP in dairy herds based on culture and molecular findings.

Key words : Bulk tank milk, Dairy herd, *Mycobacterium avium subspecies paratuberculosis*, Prevalence, Johne's disease

INTRODUCTION

Paratuberculosis, or Johne's disease (JD), is chronic infectious disease of ruminants and mammals caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). Infected animals show symptoms of diarrhea, decreased milk production, and weight loss, and infection may lead to death (Herthnek et al, 2008). Various species of domestic and wild ruminants are susceptible to JD and the treatment of sick animals is not recommended due to cost and transmission. JD is one of the most costly infectious disease of dairy cattle, resulting in annual losses of $250 million in the USA alone (Ott et al, 1999). The major reasons for controlling MAP infections are that they cause economic losses to farmers because of loss of milk production and the necessary for early culling, and they may also possibly play a role in Crohn's disease in human beings (Mendoza et al, 2009).

Milk has been implicated as a potential source of MAP infection in humans since viable organisms have been recovered from commercial pasteurized milk (Grant et al, 2002). Because of this risk, it is important to identify the route of transmission through which humans become infected, and to be investigated the prevalence of MAP in milk sample. The control of this infection within cattle herds require both herd management changes to limit fecal-oral transmission and diagnostic testing to identify infectious adult cattle for segregation or removal (Kennedy and Benedictus, 2001). Although the detection of MAP in clinical sample by culture or PCR affords a definitive diagnosis, these methods are

*Corresponding author: Jae-Myung Kim, Tel. +82-54-912-0729, Fax. +82-54-912-0759, E-mail. kimjm88@korea.kr

slow, laborious, and expensive.

As the antibody prevalence of MAP in dairy herds from the Gyeonggi and Chungbuk provinces of Korea are unknown, and control programs are not currently being conducted. These areas are very important in terms of the dairy industry of Korea, with a 43.6% (175,684) of total estimated population to agricultural statistical data (Statistics Korea, 2011). The purpose of the current study is to describe the antibody prevalence of MAP in milk samples of dairy cattle in Korea.

MATERIALS AND METHODS

Study area and study population

Of the dairy farms in the study area, 636/2,677 (23.7%) were participated. A total of 636 BTM samples were collected from herds in the areas of the Gyeonggi and Chungbuk between January and November 2011. All samples collected by 2 milk buyers were stored at 4°C. Using portable 4°C containers, all samples were transported to the Animal and Plant Quarantine Agency (QIA).

ELISA

All milk samples were tested in duplicate for antibodies to MAP by using an indirect ELISA-kit (IDEXX MAP Ab test, Westbrook, USA) following manufacturer's manual and samples with a sample-to-positive ratio >0.15 were defined as positive.

Data analysis

The parameter used in statistical analyses was herd, and chi-square tests were performed to compare the MAP prevalence between the two geographical areas, as well as between herd sizes. *P*-values of less than 0.05 were considered statistically significant. The antibody prevalence of MAP was estimated as 10% by serum ELISA from previous sets of unpublished data from the QIA. Based on this estimated prevalence, the sample had to consist of at least 138 herds in order to obtain a relative of 5 percent with 95% confidence (Wilson et al, 2010).

RESULTS

Using a sensitivity of 52% and a specificity of 98%, as claimed by the manufacturer, the overall antibody prevalence of MAP in herds was determined to be 8.5% in the study areas, 7.3% of dairy farms in the Gyeonggi and 11.2% in the Chungbuk areas, respectively. The true prevalence for Gyeonggi was 10.5% (95% CI 5.2 to 10.1%) and for Chungbuk was 18.4% (95% CI 7.5 to 16.4%), respectively. The differences in the number of positive herds were not statistically significant between regions ($x^2=2.727$, $P>0.05$, Table 1). And table 2 shows the distribution of positive herds according to the herd size. The percentage of positive herds were not statistically between herds size sizes ($x^2=6.244$, $P>0.05$, Table 2).

DISCUSSION

This is the first large scale report of MAP antibody prevalence by using BTM samples in Korean dairy herds. In this study, we investigated the presence of antibodies to MAP by using a specific ELISA, and determined that herd-level prevalence of MAP infection

Table 1. Apparent antibody prevalence (AP), with 95 percent confidence intervals (CI), of MAP in dairy herds in Gyeonggi and Chungbuk areas of Korea

Area	Number of herds tested	Number Positive	Number Negative	Apparent Prevalence (%)	True Prevalence (%)	95% CI
Gyeonggi	440	32	408	7.3	10.5	5.2～10.1
Chungbuk	196	22	174	11.2	18.4	7.5～16.4
Total	636	54	582	8.5	13.0	6.6～11.0

$x^2=2.727$, $P>0.05$ for regions.

Table 2. Herd level prevalence (%) of MAP in dairy herds, as determined by ELISA testing of bulk tank milk samples

Herd size	No. of herds	No. of positive herds	No. of negative herds
15～49	305	39	266
50～99	300	14	286
100～200	31	1	30
Total	636	54	582

x^2=6.244, P>0.05 for herd levels.

was 8.5% in the Gyeonggi and Chungbuk areas of Korea. This result is similar to other recent estimates of herd-level MAP prevalence in other countries. For instance, ELISA studies of BTM samples collected in Spain showed that 9.2% of the samples were positive (Boadella et al, 2010), while studies in Mexico that employed indirect ELISAs found that MAP prevalence ranged between 8.0% and 24.07% in the dairy herds and 8.29% and 9.67% in the caprine flocks examined in the study (Favila-Humara et al, 2010). Sero-prevalence of MAP has been detected in dairy cattle throughout South Korea (Lee and Jung, 2009; Park et al, 2006). Most previous reports were based on serum samples and the individual-level prevalence was from 5.8% to 6.2%. Actually, there are no other comparative data on the detection of MAP in milk samples for estimation of herd-level prevalence. The present study represents the first attempt to collect baseline MAP prevalence data from Gyeonggi and Chungbuk areas in South Korea, which can be used when considering the implementation of specific preventive measures against John's disease.

The utilization of BTM testing is also a practical way to screen dairy herds for the presence of JD, as milk sampling is noticeably more convenient than testing fecal or blood samples for diseases in dairy cattle. Therefore, additional studies to identify the accuracy and the practicality of ELISA using BTM samples are needed along with the elucidation of management strategies to control the JD in dairy herds. The analysis of BTM samples is also a relatively inexpensive approach that can be used for the detection and estimation of prevalence of different pathogens in dairy herds (Garcia-Perez et al, 2009).

Although JD is a significant problem in dairy herds and is required forceful and effective control efforts, no comparative data has been published with regard to the detection of MAP in milk samples for the estimation of herd-level prevalence to data. In Korea, the estimate of herd prevalence obtained in this study could be used in the future by the animal health services at regional level as reference values to support decision making with regard to the implementation of control strategies. Moreover, it is critical to conduct in-depth epidemiologic studies to identify cow-level prevalence and economic impacts of MAP infection throughout the country. In conclusion, data provided in this BTM survey using ELISA support the need of a careful study of MAP prevalence based on culture and molecular tools.

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