大韓獸醫學會誌(2009) 第49卷 第2號
Korean J Vet Res(2009) 49(2) : 121~125

Agreement of two ELISAs for *Mycobacterium avium* subspecies *paratuberculosis* in cattle in Korea

Kyung Woo Lee[1], Byeong Yeal Jung[1,*], In Yeong Hwang[2], Su Hwa Lee[1], Ji Yeon Kim[1], Young Hoan Kim[3], Seong Hyo Lee[4], Oun Kyoung Moon[1], O Soo Lee[1]

[1]Animal Disease Diagnostic Center, and [2]Bacteriology and Parasitology Division, National Veterinary Research and Quarantine Service, Anyang 430-824, Korea
[3]Gyeongbuk Veterinary Service Laboratory, Daegu 702-701, Korea
[4]Institute of Livestock and Veterinary Research, Jeonju 560-860, Korea
(Accepted: June 12, 2009)

Abstract : Paratuberculosis caused by *Mycobacterium avium* subspecies *paratuberculosis* (Mpt) is a chronic infectious enteric disease with deleterious impact on the performance in ruminants. In Korea, ELISA has been introduced to detect antibodies to Mpt in individual cattle. However, comparison study with ELISA has not been studied until now. In total, a panel of 899 serum samples obtained from dairy cattle was analyzed with two commercial ELISAs for Mpt to assess the performance. Two ELISAs employed in this study were both licensed worldwide. Two ELISAs applied onto same serum samples showed the moderate agreement (kappa value = 0.60). There was non-significant McNemar test (p = 0.0614) between two ELISA results indicating that each proportion detected by two kits did not differ. In addition, the percent agreement between two ELISA results was turned out to be 96.8% which interpreted excellent reproducibility. It was shown from this study that two ELISAs revealed moderate kappa agreement performance. The implication raised is that when ELISAs as diagnostics are used to detect Mpt in individual cattle, positive reaction by either ELISA should be interpreted as serologically Mpt positive due to presumed low sensitivity of ELISAs and their test agreement being less than 100%.

Keywords : agreement, dairy cattle, ELISA, kappa value, *Mycobacterium avium* subspecies *paratuberculosis*

Introduction

Paratuberculosis (Johne's disease) is a chronic infectious enteric disease of ruminants. It is seen primarily in cattle, sheep, and goat and is caused by *Mycobacterium avium* subspecies *paratuberculosis* (Mpt). Mpt deleteriously affects the performance of dairy and beef cattle. Direct losses due to infection by Mpt were estimated to be CND$ 2,462 annually per 50-cow herd [1]. Mpt prevalence studies have been reported in many cattle-raising countries with the rates being more than 50 percent at the herd level [17]. It is also believed that Mpt infection is associated with Crohn's disease in humans, but this association remains controversial [19]. It is thus considered important to have information on seroprevalence of Mpt before any control measures are established.

Since first isolation and identification of the pathogen was reported in 1984 in Korea [7], serological monitoring on Mpt has been conducted by several researchers. First, the seroprevalence of 18.7% and 11.7% for dairy and beef cattle that measured by in-house ELISA was reported in 1994 [10]. Three years later, the prevalence of 10.9% for cattle was reported although the cattle's species was not clearly specified [11]. In addition, the cattle and herd level prevalence was reported to be 16.4% and 67.3%, respectively [9]. Very recently, Park *et al.* [18] showed that the cattle-level prevalence in Korea was estimated to be 0.7% and 5.8% for beef and dairy cattle, respectively. Also, the prevalence of Mpt in black goats was reported to be 25.0% and 8.2% at the herd- and goat-levels, respectively [13]. Because of the various sensitivity of the ELISA used, the seroprevalence of Mpt would be dramatically different

*Corresponding author: Byeong Yeal Jung
Animal Disease Diagnostic Center, National Veterinary Research and Quarantine Service, Anyang 430-824, Korea
[Tel: +82-31-467-1756, Fax: +82-31-467-1868, E-mail: jungby@nvrqs.go.kr]

at each time points measured.

In general, bacteria isolation from tissues or feces is the gold standard test for Mpt detection. However, the ability to cultivate the pathogen differs between laboratories due to the lack of standardized cultivation techniques [2]. Colonies can be seen after four weeks, but more often after 10 to 16 weeks. In addition, shedding of the bacteria at levels detectable by fecal culture is not regular and does not occur during the early stages of infection, thus diminishing the sensitivity of this methodology [15].

The most common immunological tests to detect Mpt infection are among the complement fixation test (CFT), agarose gel immunodiffusion (AGID), and ELISA, respectively. AGID can reach 100% of specificity, but it is low in sensitivity as compared to the ELISA [5]. According to Kim *et al.* [10], ELISA was the most sensitive method to detect antibodies to Mpt as compared to either CFT or AGID. ELISA-based methods show the highest sensitivity of serological tests for Mpt since these assays are capable to detect small amounts of antibodies. Also, serological survey on Mpt by commercial ELISAs is widely used due to high throughputs, quick availability of the results and low test costs [2].

Comparison study of commercially available ELISAs has been reported in Netherlands [8], Germany [12], Spain [3], Denmark [16], Canada [14], USA [2], and United Kingdom [6]. In addition, comparison between in-house developed ELISA and commercial ELISAs was reported in Korea [18] and Brazil [4]. And, the inconsistent results have been shown in earlier studies [2, 3, 13]. Until now, comparison study of commercial Mpt ELISAs has not been tested yet in Korea. In this study, we performed to assess the test agreement between two ELISAs that were in use around the world. The results obtained in this study were also compared with those reported previously [2, 3, 14].

Materials and Methods

ELISAs and serum samples

The ELISAs tested were as follows: ELISA A (HerdChek; IDEXX Laboratories, USA) and ELISA B (Parachek; CSL, Australia). They both are licensed for use in North America. The two ELISAs are based on detection of antibodies to protoplasmic antigens for Mpt and are an absorbed ELISAs with the use of *Mycobacterium phlei* in the absorption step. However, the nature of putative antigen differences in kit components is not known due to company secrets. The test was performed according to the manufacturer's instructions. The ELISA A reports the analyzed optical densities (OD) as an s/p ratio (sample OD to positive control OD ratio). The ELISA B reports as a score value, which is assessed in relation to the cut-off that is determined by the mean of the negative controls plus 0.100. The diagnostic specificities ranged from 84.0% to 100.0% and 98.5% to 100.0% for ELISA A and B, respectively [2]. Assay sensitivities ranged from 9.61% to 76.83% and from 9.61% to 73.2% for ELISA A and B, respectively [2]. The serum samples were collected from June to August in 2006 for the national sero-monitoring on the mosquito-borne viral diseases. Among them, 899 samples were randomly selected from dairy cows of more than two years old from 97 dairy herds throughout Korea. We assume that the sample size employed in this study should be sufficient as based on the earlier studies [2, 3, 14]. The serum samples then were analyzed with two commercial ELISAs. No attempts were done to identify the cattle being either positive or negative by fecal culture.

Statistical analysis

The test results were calculated for kappa values (Win Episcope 2.0; CLIVE, Scotland). The McNemar χ^2 test was used to compare paired population proportions of the two ELISA results. Pearson's correlation coefficient (γ) was also calculated to observe the linear relationship between the test results. Differences were considered significant at $p < 0.05$.

Results

Of 899 sera tested, 43 and 32 sera were reacted by ELISA A and B, respectively (Table 1). There were 23 sera which were positive by both ELISAs. Comparison of two ELISAs had the moderate agreement ($\kappa = 0.60$; 95% confidence interval, 0.46 to 0.73). There was non-significant McNemar test ($p = 0.0614$) between two ELISA results indicating that the proportion detected by two kits did not differ. When the test results were visualized on the scatter plot using the recommended cut-off values for each ELISA by the manufacturer, positive association ($\gamma = +0.680$) between two ELISA results was observed (Fig. 1). The percent of agreement between two ELISA results was turned out to be 96.8% which could be interpreted excellent reproducibility (Table

Table 1. Comparison of two ELISAs for *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle

ELISA test*	ELISA A		Total
	Positive	Negative	
ELISA B			
Positive	23	9	32
Negative	20	847	867
Total	43	856	899

*κ = 0.60 (95% confidence interval, 0.46 to 0.73), *p*-value for McNemar test = 0.0614 (χ^2 value = 3.448). Percent of agreement between two ELISA results is 96.8%.

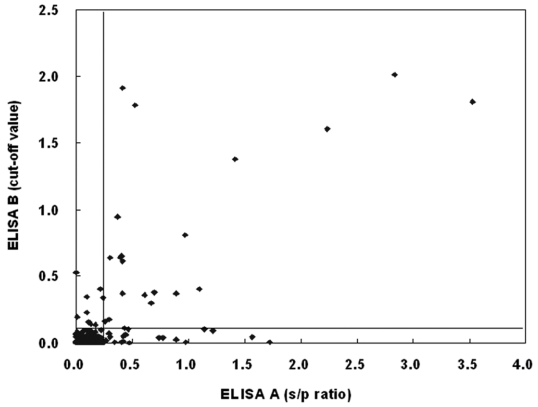

Fig. 1. Scatter plot of s/p ratios from ELISA A compared to cut-off values from ELISA B. Horizontal and vertical lines indicate the recommended cut-off values for each ELISA by the manufacturer. Coefficient (*r*) of correlation between two ELISA results was calculated to be 0.680 ($p < 0.001$).

1). In other words, 29 sera (3.2%) were only reacted by either ELISA, leading to discordant results on same sera. Twenty sera that were positive by ELISA A were negative by ELISA B, and 9 sera that were positive by ELISA B were negative by ELISA A.

Discussion

The present study was conducted to compare the test agreement between two commercial ELISAs for Mpt. It was considered of relevance to study the performance of diagnostic tools for Mpt in the light of the possibility of zoonotic disease [19] and the widespread prevalence in animal population [13] in Korea. Although in-house Mpt diagnostic tools have been developed in Korea [9-11, 18], they are not commercially available at present. In addition, CFT that used for Mpt screening on the serum samples of the imported cattle has been replaced by commercially manufactured ELISAs. Thus, the results raised from this study will provide the valuable information on establishing national Mpt control program as proposed earlier [18].

Until now, there are several studies in different countries on test agreement of the same ELISAs that used in this study. Those results are summarized in Table 2. McKenna *et al.* [14] showed the relatively low agreement between two ELISAs (κ = 0.45). On the contrary, Collins *et al.* [2] observed higher agreement (κ = 0.77) between two ELISAs without having statistical significance. Thus, our result is similar to that of Collins *et al.* [2], but different from that of McKenna *et al.* [14]. Very recent study that conducted in Spain [3] reported markedly low disagreement between the ELISAs, having percent agreement (21.0%), kappa value (κ = 0.19) and correlation coefficient (γ = +0.26). These results by Diéguez *et al.* [3] are considerably lower than those obtained by Collins *et al.* [2] in the US, by McKenna *et al.* [14] in Canada and also in this

Table 2. Summary of test agreements of two ELISAs for *Mycobacterium avium* subspecies *paratuberculosis* that conducted in USA, Canada, Spain and Korea

Species	No. samples	% agreement	*p*-value for McNemar test	Kappa value	Coefficients (γ)	References; Country
Dairy	415	90.6	NS*	0.77	+0.76†	Collins *et al.* 2005; USA
Dairy	160	NA*	S*	0.45	NA	McKenna *et al.* 2006; Canada
Dairy	326	21.0	NS	0.19	+0.26	Diéguez *et al.* 2009; Spain
Dairy	899	96.8	NS	0.60	+0.68	Lee *et al.* this study; Korea

*NA: not available, NS: non-significant, S: significant. †This coefficient (γ) of correlation was obtained from regression coefficient (γ^2).

study. The explanation on the differences in the test agreements of the ELISAs between studies is not well understood. However, as Collins *et al.* [2] postulated, the discrepancy in test results would in part relate to differences in the microbial population of the animals qualitatively or quantitatively, or notably the presence of other mycobacteria. Many researchers [2, 8, 18] agreed upon that ELISA is useful to estimate Mpt seroprevalence rather than using as a definitive diagnostic tool for individual cattle. However, currently, ELISA has been used to diagnose Mpt infection in individual cattle in Korea and elsewhere [14]. Further studies are warranted to reveal the different test agreement between ELISAs on a molecular basis.

Recently, we estimated that the Mpt-seropositive herds were 4.8 and 41.7% for beef and dairy herds, respectively (data not shown). Similarly, cattle-level seroprevalence was higher in dairy cattle (apparent prevalence 6.1%) than in beef cattle (apparent prevalence 1.2%). Earlier, cattle-level seroprevalence of Mpt was reported to be 18.7, 16.4 and 5.8% when measured in 1994, 2002 and 2006, respectively [9-11]. It is thus assumed that Mpt has been prevalent in cattle industry in Korea although the prevalence has been tended to decrease by year. However, the latter statement would be elusive due to the differences in diagnostic kits used as well as sensitivities of the assays, and thus needs careful interpretation. At this stage, national Mpt control program has not been implemented in Korea except for Mpt monitoring on pure-bred breeding dairy and beef cattle. Due to recent zoonotic association with respect to Mpt [19] and its economic impact on cattle industry [1], numerous studies on seroprevalence, risk factors and diagnostics of Mpt have been conducted in many cattle-producing countries. However, relatively little information on Mpt is available in Korea that needs certain measures to control the disease. At beginning, nationwide prevalence and risk factors of Mpt in cattle herds are needed that can be explored by serological survey. In many countries, herd Mpt infection rate is assessed by ELISA that has advantage of high throughputs, quick availability of the results and low test costs [2].

In this study, we tested two commercial ELISA A and B, both used worldwide. Our results showed a moderate agreement between two ELISAs when applied onto same serum samples. Furthermore, when ELISAs as diagnostics are used to detect Mpt in individual cattle, positive reaction by either ELISA should be interpreted as serologically Mpt positive due to presumed low sensitivity of ELISAs and their test agreement being less than 100%.

Acknowledgments

This study was financially supported by the National Veterinary Research and Quarantine Services, Ministry for Food, Agriculture, Forestry and Fisheries, Korea.

References

1. **Chi J, VanLeeuwen JA, Weersink A, Keefe GP.** Direct production losses and treatment costs from bovine viral diarrhoea virus, bovine leukosis virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum*. Prev Vet Med 2002, **55**, 137-153.
2. **Collins MT, Wells SJ, Petrini KR, Collins JE, Schultz RD, Whitlock RH.** Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. Clin Diagn Lab Immunol 2005, **12**, 685-692.
3. **Diéguez FJ, González AM, Menéndez S, Vilar MJ, Sanjuán ML, Yus E, Arnaiz I.** Evaluation of four commercial serum ELISAs for detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cows. Vet J 2009, **180**, 231-235.
4. **Ferreira R, Fonseca L, Lilenbaum W.** Comparison between a commercial and an in-house ELISA for anti-*M. avium paratuberculosis* antibodies detection in dairy herds in Rio de Janeiro, Brazil. Rev Latinoam Microbiol 2002, **44**, 129-132.
5. **Harris NB, Barletta RG.** *Mycobacterium avium* subsp. *paratuberculosis* in veterinary medicine. Clin Microbiol Rev 2001, **14**, 489-512.
6. **Horwood P, McGowan M, Orpin P, Thompson I, Brownlie J.** Evaluation of two ELISA's for the detection of *Mycobacterium avium paratuberculosis* antibodies in milk samples from dairy cattle. Cattle Practice 2003, **11**, 147-149.
7. **Jeon YS, Lee BW, Kim JB, Choi CS, Kim JK.** Isolation and identification of mycobactin dependent acid-fast bacteria (*M. paratuberculosis*) from bovine fecal material. Korean J Vet Res 1984, **24**, 58-63.
8. **Kalis CHJ, Barkema HW, Hesselink JW, van Maanen C, Collins MT.** Evaluation of two absorbed enzyme-linked immunosorbent assays and a complement fixation test as replacements for fecal culture in the

detection of cows shedding *Mycobacterium avium* subspecies *paratuberculosis*. J Vet Diagn Invest 2002, **14**, 219-224.

9. **Kim D, Jeon KJ, Kim JT, Shin KS, Shin MK, Chang GH, Kim JK, Kim OS, Jung JY.** Prevalence of paratuberculosis of dairy cattle in Kangwon area. Korean J Vet Res 2002, **42**, 81-88.
10. **Kim JM, Ahn JS, Woo SR, Jo DH, Jo YS, Park JM, Yoon YD, Chang GH.** A survey of paratuberculosis by immunological methods in dairy and Korean native cattle. Korean J Vet Res 1994, **34**, 93-97.
11. **Kim TJ, Kim YS, Kim JC, Yoon WJ, Lee WC, Shin SJ, Chang YF.** Studies on molecular biological and immunological diagnosis of Johne's disease. Korean J Vet Res 1997, **37**, 349-358.
12. **Köhler H, Burkert B, Pavlik I, Diller R, Geue L, Conraths FJ, Martin G.** Evaluation of five ELISA test kits for the measurement of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* in bovine serum. Berl Munch Tierarztl Wochenschr 2008, **121**, 203-210.
13. **Lee KW, Jung BY, Moon OK, Yang DK, Lee SH, Kim JY, Kweon CH.** Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Korean black goats (*Capra hircus aegagrus*). J Vet Med Sci 2006, **68**, 1379-1381.
14. **McKenna SLB, Barkema HW, Keefe GP, Sockett DC.** Agreement between three ELISAs for *Mycobacterium avium* subsp. *paratuberculosis* in dairy cattle. Vet Microbiol 2006, **114**, 285-291.
15. **McNab WB, Meek AH, Duncan JR, Brooks BW, Van Dreumel AA, Martin SW, Nielsen KH, Sugden EA, Turcotte C.** An evaluation of selected screening tests for bovine paratuberculosis. Can J Vet Res 1991, **55**, 252-259.
16. **Nielsen SS, Houe H, Thamsborg SM, Bitsch V.** Comparison of two enzyme-linked immunosorbent assays for serologic diagnosis of paratuberculosis (Johne's disease) in cattle using different subspecies strains of *Mycobacterium avium*. J Vet Diagn Invest 2001, **13**, 164-166.
17. **Nielsen SS, Toft N.** A review of prevalences of paratuberculosis in farmed animals in Europe. Prev Vet Med 2009, **88**, 1-14.
18. **Park KT, Ahn J, Davis WC, Koo HC, Kwon NH, Jung WK, Kim JM, Hong SK, Park YH.** Analysis of the seroprevalence of bovine paratuberculosis and the application of modified absorbed ELISA to field sample testing in Korea. J Vet Sci 2006, **7**, 349-354.
19. **Turenne CY, Wallace R Jr, Behr MA.** *Mycobacterium avium* in the postgenomic era. Clin Microbiol Rev 2007, **20**, 205-229.