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

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(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 CN$ 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% 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.
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, 14]. 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 \( \chi^2 \) test was used to compare paired population proportions of the two ELISA results. Pearson’s correlation coefficient \( \gamma \) 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