Agreement between ELISA and complement fixation test used for diagnosing of paratuberculosis in goats

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Abstract

An ELISA with a lipoarabinomannan as an antigen, developed for diagnosis of bovine paratuberculosis, has been adapted for use in goats, and compared with complement fixation test. Kappa value of 0.62 indicated good agreement between CFT and the adapted ELISA and proved that the investigated ELISA may be helpful in diagnosis of \textit{Mycobacterium avium} subsp. \textit{paratuberculosis} infection in goats. The ELISA has been used to screen a randomly selected representative sample of Polish breeding goat population (21.78\% of herds, 21.33\% of goats). It has been demonstrated that only 2.42\% of animals coming from 15.79\% of herds were seropositive. Within-herd seroprevalence varied from 1.69\% to 38.10\%. Most of the infected animals (67.07\%) were 3-4 years old. No seropositive cases were found in group up to 1 year old animals.

Key words: ELISA, complement fixation test, paratuberculosis, goat, epidemiology

Introduction

Paratuberculosis (John’s disease) is a chronic infectious disease of ruminants, caused by \textit{Mycobacterium avium} subsp. \textit{paratuberculosis}. In small ruminants, especially goats, clinical diagnostics of paratuberculosis is relatively difficult. Quite frequently the disease has a subclinical course, without typical clinical symptoms like diarrhea (Storset et al. 2001, Stewart et al. 2006). In many cases, the only symptom of \textit{Mycobacterium avium} subsp. \textit{paratuberculosis} infection in goats is progressive cachexia. This symptom is not specific and occur within the course of many other goat diseases. Laboratory diagnostics of paratuberculosis in live animals is problematic, too (Eamens et al. 2007, Salgado et al. 2007). The infection can be confirmed by revealing the presence of typical acid-fast organisms in feces samples. However, this simple method features low sensitivity, and obtaining cultures from feces is very time-consuming (Eamens et al. 2007). For many years, the serological method widely used in diagnostics of paratuberculosis in goats

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was the complement fixation test (CFT) (Hilbink 1994). Recently it has been replaced by ELISA. These tests are currently most frequently used in clinical practice (Rajukumar et al. 2001, Salgado et al. 2007). There are more and more attempts to use new methods like PCR (Munjal et al. 2007), gamma interferon assay (Storset et al. 2005, Stewart et al. 2006) and lymphoproliferative test (Munjal et al. 2005). The objective of this study was to compare ELISA and CFT used in ante-mortem diagnostics of paratuberculosis in goats and evaluation of seroprevalence of the disease in the Polish breeding goat population using a set of serum samples collected in 1996.

Materials and Methods

Sera

Tests were carried out with the use of a collection of sera being a representative sample of goats submitted for breeding evaluation in Poland in 1996. Samples had been collected from 76 herds randomly selected from all 349 breeding herds (21.78% of all breeding herds in Poland). These herds were located in all 16 provinces of Poland. In each herd all animals were subject to tests. The collection of samples gathered in this manner included 1074 serum samples (21.33% of all breeding goats in Poland) from 1004 females from 1 to 11 years old and 70 males from 1 to 8 years old.

Positive control serum was taken from a four year old female goat with clinical symptoms of paratuberculosis (diarrhea, wasting). The diagnosis was confirmed by acid fast staining of fecal sample and cultivation of M. avium subsp. paratuberculosis. A negative control serum was obtained from a two years old female goat from the herd with no history (10 years) of clinical symptoms that might bring suspicion of infection with M. avium subsp. paratuberculosis.

ELISA

For study purposes an adapted ELISA (Jark et al. 1997, Munjal et al. 2004), originally designed for diagnosing John’s disease in cattle was used. This test is based on a lipoolarabinomannan containing preparation as solid phase antigen.

ELISA plates (Nunc, Germany) were coated as described previously (Jark et al. 1997). The optimal concentrations of antigen and biotinylated anti-goat-IgG heavy and light chain (Dianowa, Germany) and streptavidin peroxidase (Dianowa, Germany) were established by checkerboard titration with positive and negative control sera. ABTS (2,2’-azino-di-[3-ethylbenzthiazoline-6-sulfonic acid]) (Boehringer Mannheim, Germany) with 0.001% H2O2 was used as a substrate. All washings were performed using a plate washer (Dynatech AM85, Germany). The optical density was determined using a spectrophotometer (Dynatech MR700, Germany) and the results were analyzed by reference standard method. The ELISA activities of sera were expressed as ELISA units in relation to the positive control serum which was arbitrarily set to have an activity of 100 ELISA units (EU) (Jark et al. 1997).

After testing all sera, the herds with lowest levels in all animals were chosen. On the ground of population defined in this manner a provisional cut-off value for the test was established, as an average result expressed in EU, increased by a threefold value of standard deviation (Thrusfield 2007). The ultimate cut-off value was established on the basis of statistical comparison (kappa statistics) of ELISA and CFT results.

Complement fixation test

According to ELISA results based on provisional cut-off, all positive and questionable sera were chosen, as well as negative ones collected in 3 randomly selected negative herds and one randomly selected positive herd. The complement fixation test was carried out according to the methodology described by Gorrie (1959) as modified by Jark et al. (1997). Microplates U-bottom (Greiner, Germany) were coated with whole cells lysate from the same M. avium subsp. paratuberculosis strain used to prepare the ELISA antigen. Sera with hemolysis lower than 50% at the 1:10 dilution were considered positive.

Statistical analysis

Comparison of ELISA and CFT results was carried out by calculating the kappa value for several cut-off values of ELISA (provisional cut-off along with lower and higher values). In this analysis, sera giving doubtful and questionable results in CFT were omitted. The statistical significance of results was evaluated on the ground confidence intervals analysis, with a confidence level of 1-α = 0.95 (Thrusfield 2007). Calculations were performed with use of Win Episcope 2.0 software. For the final interpretation of ELISA results, the cut-off with highest kappa value was chosen.

Final interpretation of serological tests

Animals positive in both ELISA and CFT were considered as positive. A herd was considered positive if at least one animal proved to be positive.
Results

Agreement between ELISA and CFT

After testing all sera with ELISA, from among 76 herds 56 were chosen, where results in all animals were relatively low (≤ 30 EU). It was assumed that 653 goats from these herds constituted a seronegative population and on this basis a provisional cut-off was established for 20 EU (arithmetical mean 4.88 EU, standard deviation 4.78 EU). Using the cut-off calculated in this manner, among all tested sera 74 samples were found to be positive. These samples, along with 27 negative ones, collected from 3 negative herds and 32 negative samples obtained from positive herds, were tested with CFT. Test results for this set of samples are presented in Table 1. Agreement of ELISA and CFT was calculated for 4 different cut-off values of ELISA (Table 2). For cut-off 40 EU kappa statistics had highest values and this value was chosen for final interpretation of ELISA results.

Table 1. ELISA and CFT results of serological diagnosis of paratuberculosis in goats.

<table>
<thead>
<tr>
<th>ELISA results (EU)</th>
<th>Number of samples</th>
<th>CFT result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>≤ 10</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>11-20</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>21-30</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>31-40</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>41-50</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>51-60</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>61-70</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>≥ 71</td>
<td>27</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>32</td>
</tr>
</tbody>
</table>

Results of serological investigation

Among 1074 sera 26 (2.42%) were found to be positive in both tests applied. Goats from which these sera were collected were considered seropositive. These animals originated from 12 (15.79%) herds. The within-herd seroprevalence in particular herds varied from 1.69% to 38.10% (Fig. 1). The age structure of seropositive goats is presented on Fig. 2.

![Fig. 1. Seroprevalence of paratuberculosis in goat herds.](image1)

![Fig. 2. Age structure of seropositive goats.](image2)

Discussion

Establishing an appropriate cut-off value is an important stage in the elaboration and standardization of each diagnostic method. This is relatively easy if a validated and reliable test that could be considered a 'gold standard' is available. Otherwise, interpretation of the test results is usually carried out by comparison with other tests, hence evaluation of consistency of results obtained with both ELISA and CFT. Neither of these methods can be treated as the aforementioned 'golden standard' in the case of paratuberculosis in goats. CFT used to be a widely used diagnostic method for this disease. Currently it is gradually being replaced by ELISA. For this reason why the agreement of these two tests was assessed. The kappa statistics calculated for 4 cut-off values of ELISA varied from poor (approx. 0.3) to good...
(above 0.6) (Thrusfield 2007). For evaluation of the statistical significance of the results interval analysis, calculated for kappa, is essential. Only for cut-offs of 40 EU and 50 EU was the lower limit higher than 0.4. The agreement between ELISA and CFT was highest (0.615) for cut-off of 40 EU. The confidence interval in this case ranged from 0.444 to 0.787, thus confirming the statistical significance of the results. It is worth noting that among sera with ELISA results below 40 EU there were 4 of 5 sera with questionable CFT results. ELISA parameters (cut-off 0.615 and lower limit of confidence interval 0.444) are very close to the accepted minimal values (respectively 0.6 and 0.4) (Basu et al. 1995). These results demonstrate how difficult serological diagnostics of paratuberculosis in goats is. Apparently, establishing the cut-off at 40 EU evaluated ELISA sufficiently differentiates positive and negative sera, allowing this test to be treated as a useful tool in practice diagnostics. Testing randomly selected, representative samples of goat sera proved that infection with M. avium subsp. paratuberculosis occurred relatively rarely in goats bred in Poland in 1996. It affected at most 16% of herds. Paratuberculosis is a contagious disease, but it does not spread easily or fast (Stewart et al. 2006). Therefore, the number of infected animals in individual herds was very low and in most cases did not exceed 10%. Antibodies confirming infection were not found in young animals up to 1 year of age. For the infection to take place direct and long lasting contact with sick animals is necessary. The seroprevalence grew in proportion to the age of the animals and peaked in 3-year-old animals. As a result of the appearance and intensification of clinical symptoms of the disease and deterioration of their condition, such animals are removed from the herd. This can be an explanation for the drop in seroprevalence in older groups of animals.

The results of this study suggest that ELISA based on lipoolarabinomannan antigen could be useful for the detection of M. avium subsp. paratuberculosis infections in goats. Screening of the set of goat sera collected in 1996 showed that paratuberculosis was a rare disease of goats in Poland at that time.

References


