Correlation between seropositive reactors to paratuberculosis and to pseudotuberculosis in sheep

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SUMMARY

Paratuberculosis and pseudotuberculosis are chronic diseases occurring often simultaneously in the same flock. Since the causative agents, Mycobacterium avium subsp. paratuberculosis (Map) and Corynebacterium pseudotuberculosis (C. pseudotuberculosis) are genetically related, serological cross-reactions may occur. In our study serological reactors for paratuberculosis and pseudotuberculosis in the flock with 519 animals were compared, using ELISA method. Among 42 pseudotuberculosis positive sera 42.9 % were positive and 14.3 % were doubtful in paratuberculosis test. The percentage of paratuberculosis positive animals was increased to 71.4 % in cases of pseudotuberculosis highly positive sera. In pseudotuberculosis doubtful sera, there were 10 % of paratuberculosis positive, whereas in pseudotuberculosis negative sera, only 3.4 % were paratuberculosis positive. Significantly higher percent of paratuberculosis positive animals among pseudotuberculosis positive animals suggests that special care should be taken when interpreting paratuberculosis positive serology in the flocks infected with C. pseudotuberculosis.

INTRODUCTION

Paratuberculosis and pseudotuberculosis are chronic diseases. Sheep flocks are often infected simultaneously with both diseases. The causative agents, Mycobacterium avium subsp. paratuberculosis (Map) and Corynebacterium pseudotuberculosis (C. pseudotuberculosis) are members of the same order Actinomycetales. It is known that bacteria from Corynebacterium and Mycobacterium genus share some common antigenic determinants and therefore serological cross-reactions may occur (1-5,7,12). It was found out that sheep, experimentally infected with C. pseudotuberculosis, were positive in CFT and ELISA for paratuberculosis (6).

In this study, serological reactions in paratuberculosis and pseudotuberculosis infected flock were compared with respect to potential cross reactivity in diagnostic approaches.

MATERIALS AND METHODS

Sera. Blood samples were taken from 519 animals in the flock where paratuberculosis and pseudotuberculosis were confirmed bacteriologically. Two sera of rabbits, immunised with C. pseudotuberculosis, were used for the positive control in pseudotuberculosis ELISA. Sera from flock known to be free of pseudotuberculosis were used as negative control.

Paratuberculosis serology. For the detection of antibodies against Map, commercial ELISA kit (IDEXX, Sweden) was used. The test was performed according to the manufacturer’s instructions.

Pseudotuberculosis serology. For the detection of antibodies against C. pseudotuberculosis ELISA was performed as previously described (10), with some modifications. The mixture of the bacterial cells and toxins in carbonate-bicarbonate buffer was used for coating the microplates (Polysorp, NUNC, Denmark) overnight at 4 ºC. Sera were diluted 1:50 in PBS-T (phosphate buffered saline with 0.05 % of Tween 20) containing 1 % of bovine serum albumin. After one hour of incubation at room temperature, protein G horseradish peroxidase conjugate (SIGMA, USA) was added. The plates were incubated as in the previous step. After 10 minutes incubation of TMB (Tetramethylbenzidine) in the dark, followed by stopping the reaction with sulphuric acid, the optical densities (OD) were read at dual wavelength (450/630 nm). Mean OD of negative sera (mODneg) was calculated. Samples with OD lower than mODneg were considered negative. Samples having OD between 100 % and 200 % of mODneg were considered doubtful whereas those with OD between 200 % and 400 % of mODneg were scored
as positive. Sera with OD higher than 400 % of mOD$_{neg}$ were considered as highly positive.

RESULTS

In paratuberculosis test 38 samples were positive and 17 were doubtful. 42 samples were positive in pseudotuberculosis test (7 of them highly positive) whereas 60 samples were doubtful. Results are shown in Figure 1.

In total, among 42 pseudotuberculosis positive sera 42.9 % were positive and 14.3 % were doubtful in paratuberculosis test.

DISCUSSION

Concluding from the results, it is obvious that the rate of paratuberculosis positive sera was much higher in pseudotuberculosis positive animals. Cross-reactions in paratuberculosis ELISA without preabsorption probably occur due to the presence of nonspecific (or C. pseudotuberculosis specific) antibodies in sheep, infected with pseudotuberculosis. These antibodies bind to Map antigens (2,5,11). In our case, commercial ELISA with preabsorption with M. phlei was used. This preabsorption is obviously not sufficient to avoid cross-reactions with C. pseudotuberculosis specific antibodies. Some authors suggested to use AGID test to avoid cross-reactions (8), whereas others described some problems with cross reactions in AGID test (9). However, further studies regarding adequate preabsorption would be necessary in order to overcome this problem.

CONCLUSIONS

Our results suggest that special care should be taken when interpreting paratuberculosis positive serology in the flocks infected with C. pseudotuberculosis.

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REFERENCES


