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Short communication

The porcine brucellosis – evidence of the role of *Yersinia enterocolitica* O:9 in occurrence of false positive serological reactions

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Abstract

Forty four pigs with typical characteristics for false positive serological reactions (FPSR) were examined for the presence of *Yersinia enterocolitica* O:9. The positive reactions were observed in rose bengal test (RBT, N=23 sera), serum agglutination test (SAT, N=16), complement fixation test (CFT, N=9), indirect ELISA (i-ELISA, N=11) in first, and in RBT (N=14), SAT (N=8), CFT (N=7) and i-ELISA (N=18) in second examination, respectively. In bacteriological examination *Y. enterocolitica* was confirmed in 12 cases. Six of these isolates were identified with PCR as *Y. enterocolitica* O:9.

Key words: brucellosis, pigs, cross-reactions, *Yersinia enterocolitica*

Introduction

Porcine brucellosis is an infectious and contagious disease caused by the bacteria *Brucella suis*. Main methods employed for serological diagnosing of the porcine brucellosis are RBT and i-ELISA. To explain dubious results, additional methods – complement fixation test (CFT), serum agglutination test (SAT) and 2-mercaptoethanol test (2-ME) are also involved. The reason of diagnostic problems is the similarity of the O-antigenic side chain of *Brucella* lipopolysaccharide (LPS) with that of other bacteria. Most commonly false positive serological reactions (FPSR) in cows are caused by infections with *Yersinia enterocolitica* O:9 (Weiner et al. 2010). The paper presents the results

of bacteriological and molecular research for the presence of *Y. enterocolitica* O:9 in material originated from pigs with typical picture of FPSR.

Materials and Methods

Forty four sows originated from quarantine station, intended for breeding purposes, were tested. The animals were tested twice with a 1 month interval. The swabs from the surface of the tonsils from all animals were taken for bacteriological examination for *Y. enterocolitica* O:9. The RBT, SAT, CFT were performed in accordance with OIE Manual 2012. The 2-ME test was performed in the same way as SAT except that

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2-ME was added to each test tube to a final concentration of 0.2 M. In i-ELISA the diagnostic kit described previously was used (Szulowski et al. 2001). Isolation of *Y. enterocolitica* O:9 was performed according to official manuals (ISO 10273:2003). The *Y. enterocolitica*-positive colonies with API-20E were tested for the presence of universal *Y. enterocolitica* *16S rRNA* gene (Wannet et al. 2001), and for the presence of *per* gene (Lubeck et al. 2003), characteristic for *Y. enterocolitica* O:9 serotype only. Each DNA amplification was performed in reaction mixture consisting of DNA template, 200 µM of dNTPs, 4 mM MgCl₂, 1 U of Taq DNA polymerase (Fermentas), nucleotide primers Y1 (AAT ACC GCA TAA CGT CTT CG), Y2 (CTT CTT CTG CGA GTA ACG TC), *per*-12 (GCA GAC GGGGGC AAA AGT A) and *per*-14 (CAC CTT GAT TTT TAA ATG GCA T) and water. PCRs were performed under the following conditions: 94°C for 5 min, followed by 36 cycles of 94°C for 1 min, 62°C for 1 min and 72°C for 1 min (*16S rRNA* gene) and 30 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min (*per* gene).

Results and Discussion

During the first testing 23 sera reacted positively in RBT, 16 in SAT, 9 in CFT and 11 in i-ELISA. Thirty days later 14 sera were positive in RBT, 8 in SAT, 7 in CFT and 18 in i-ELISA. There were no positive results in 2-ME, both in the first and the second examination. The range of values of positive results in SAT was 31.0-72.0 iu/ml in the first and 31.0-51.5 iu/ml in the second examination. In CFT the ranges of values were 20.0-26.5 icftu/m in both examinations. As regards serum from the pig subjected to diagnostic slaughter, it was positive in RBT, SAT, CFT and i-ELISA and doubtful in 2-ME. The titers in SAT and CFT were 51.5 iu/ml and 26.6 icftu/ml, respectively.

In bacteriological examination typical *Yersinia*-suspected "bull-eye" colonies were observed in 12 tonsils swabs on CIN *Yersinia*-selective solid medium. In all these cases affiliation to *Y. enterocolitica* was confirmed by using API-20E and PCR amplifying universal *Y. enterocolitica* *16S rRNA* gene. Six of these *Y. enterocolitica* isolates were identified as *Y. enterocolitica* O:9 serotype by confirmation of the presence of *per* gene.

The obtained results of serological examination of 44 porcine sera for anti-*Brucella* antibodies in our opinion might be regarded as a typical example of

FPSR. As the animals were examined twice with a 30 days interval we could gain enough information on dynamics of antibodies and conclude that observed reactions were typical for cross-reactions, probably due to *Y. enterocolitica* O:9. Detection of *Y. enterocolitica* O:9 in the herd with a high degree of probability confirms the reason of false positive reactions for brucellosis. Determining the presence of other serotypes of *Y. enterocolitica* in the herd, beside serotype O:9, is not surprising as there are no biological differences between the serotypes and their different distribution in the herd is possible (Jungersen et al. 2006). Implementation of bacteriological examination for the presence of *Y. enterocolitica* O:9 and isolation of this bacteria provides very important information enabling proper interpretation of the results. On one hand we have the confirmation that there is no *Brucella* infection, on the other hand we get information that *Y. enterocolitica* O:9 is present in the herd.

Taking into account the data from the literature and our current and previous results we conclude that false positive serological reactions in porcine brucellosis serology are not unusual (Jungersen et al. 2006, OIE Manual 2012) and clear guidelines for dealing with such cases should be created.

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