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Short communication

The value of Fluorescence Polarisation Assay in verification of problematic sera from pigs for brucellosis

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Abstract

The aim of the study was an evaluation of fluorescence polarisation assay (FPA) as a potential tool improving specificity of serological diagnosis of brucellosis in pigs. The evaluation was done by comparing the results of FPA with the results of rose bengal test (RBT), serum agglutination test (SAT), complement fixation test (CFT) and ELISA when false positive sera were tested. One hundred ninety porcine samples, reacting positively in at least one classical serological assay were used. We observed that among 198 sera, 104 were also positive in FPA. The studies confirmed that porcine FPA adds little as far as specificity in comparison to other methods is concerned.

Key words: *Brucella*, FPA, serology, classical methods

Introduction

Brucellosis, a bacterial disease caused by members of the genus *Brucella*, is an important zoonosis and significant cause of reproductive losses in animals. The laboratory diagnosis of brucellosis is mainly based on serological tests which are the rose bengal test (RBT), serum agglutination test (SAT), complement fixation test (CFT) and ELISA (OIE Terrestrial Manual 2009). The similarity of the O-antigenic side chain of *Brucella* LPS with other microbes as *Yersinia enterocolitica* O:9 and *Escherichia coli* O157:H7 has restricted the specificity of serological diagnosis resulting in false positive serological results (FPSR). The fluorescence polarisation assay (FPA) has a shorter history of use than classical serological assays and has been adopted to the OIE Terrestrial Manual in 2009 (OIE Terrestrial Manual 2009). The aim of this study was an evaluation of FPA as a potential tool improving specificity of serological diagnosis of brucellosis in pigs. The evaluation was done by comparing the results of FPA with the results of RBT, SAT, CFT and ELISA when problematic sera were tested.

Materials and Methods

One hundred ninety eight sera, positive at least in one serological test were used. The sera originated from confirmatory investigations conducted in years 2000-2010 by the National Reference Laboratory for Brucellosis in the National Veterinary Research Institute in Pulawy (NVRI, Poland) were finally classified as negative and observed reactions were regarded as false positive. The RBT, SAT, and CFT were done according to official instructions and protocols (OIE

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Terrestrial Manual 2009). The ELISA diagnostic kit elaborated in NVRI, described previously, was used (Szulowski et al. 1996). FPA was conducted as described previously (Weiner et al. 2010) with the major modification.

Results and Discussion

Among 198 porcine sera, 37 were positive in only one of classical serological assays and among them, n=30 were FPA positive. The RBT-positive only serum gave results greater than 20 mP of the mean negative control and was classified as FPA-positive. Among SAT-only positive samples (n=11), nine of them were also positive in FPA and among CFT-only positive samples (n=15), twelve of them were classified as positive in FPA. The ELISA-only positive sera (n=10) in 8 cases gave positive results in FPA. In our study we observed that among samples which were positive in two of the assays used: RBT and SAT-positive (n=4), or CFT and SAT-positive (n=10), the positive results in FPA were observed in 2 and 8 cases, respectively. Among sera which were positive in all classical tests – RBT, SAT, CFT and ELISA (n=147) 64 samples were FPA-positive.

No serological method is fully reliable in diagnosing brucellosis in animals. To improve diagnosis at least two tests should be used in parallel to avoid false positive or false negative results. One of the most important problems one should always bear in mind is the possibility of cross reactions caused in pigs primarily by *Y. enterocolitica* O:9 and in cattle by *E. coli* O157:H7, which are difficult to differentiate with specific anti-*Brucella* reactions. Those reactions between *Brucella* spp. and a number of other microorganisms have been well documented in the past and reviewed in a considerable detail (Garin-Bastuji et al. 1999).

FPA is increasingly used method in diagnosis of animal brucellosis and has many advantages: is very quick, does not require specialised staff, may be performed under field conditions, also with battery supply, and because data are obtained electronically, it is an objective test. The FPA has been validated for a number of species, including cattle, pigs and humans (Nielsen et al. 2002). As the method gained the status of prescribed OIE test for international trade both for cattle and pigs the aim of the studies was to evaluate usefulness of FPA in Polish conditions and possibility of adoption of the method for routine diagnosis of brucellosis in pigs. In Poland *B. abortus* has not been isolated for many years and *B. suis* infections are listed sporadically (Szulowski et al. 2011), while the serious problem are false-positive reactions. That is why the evaluation was done on false-positive porcine sera. The earlier studies concerning pigs (Paulo et al. 2000, Nielsen et al. 2002) showed a high specificity of the test. Our studies showed that in pigs the method adds little as far as specificity in comparison to other methods is concerned (104 positive results out of 198). In our opinion FPA has not the capability to differentiate antibodies to cross-reacting microorganisms, such as *Y. enterocolitica* O:9 from antibodies to *Brucella*, what was suggested by other authors (Paulo et al. 2000). This method does not constitute a tool for resolving all problems, particularly relevant to the presence of cross-reacting antibodies. Results obtained in FPA still do not allow making an unambiguous diagnosis regarding cross-reactions. Further evaluating studies especially on porcine brucellosis are needed.

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