PREVALENCE OF CHLAMYDIA SUIS IN POPULATION OF SWINE IN POLAND AND COMPARISON OF COMPLEMENT FIXATION TEST AND PCR USED IN THE DIAGNOSIS OF CHLAMYDIOSIS

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Abstract

The 61,020 samples of swine serum and vaginal swabs were collected in 2007-2009. The sera were examined by complement fixation test (CFT). Positive results were found in 277 pigs. The percentage of positive serological results in subsequent years was: 0.4%, 0.47%, and 1.65%, respectively. The vaginal swabs (n=277) selected from pigs diagnosed as positive in the CFT were tested by PCR. Occurrence of Chlamydia suis was confirmed in PCR in 200 cases. Statistical analysis ($\chi^2$ test, Person and Cramer correlation coefficient) demonstrated that both methods were coincident in the diagnosis of C. suis infection in swine.

Key words: swine, Chlamydia suis, complement fixation test, PCR, Poland.

Chlamydia suis, before 1999 referred to as porcine serovar of Chlamydia trachomatis because of ompA DNA sequence homology (11), was shown to be associated with disorders of the intestines and genital track, respiratory diseases, and conjunctivitis in pigs (5, 8, 12). The agent is believed to be widely spread in pig herds, often without causing clinical symptoms, but epidemiological data are scarce. Moreover, Chlamydia creates certain measurable risk to humans, and may be the cause of decline in profitability of pig production (4, 7). The economic importance of Chlamydia infections in pigs became clearer through the results of European Cooperation in Science and Technology (COST) Action 855 on Animal Chlamydiosis and the Zoonotic Implications (10). According to the literature, confirmed cases of the disease in pigs were reported in Italy, Belgium, Austria, Germany, Scotland, and Switzerland (2, 3, 6, 9). The Chlamydia/Chlamydophila sp. antibodies were also detected in Polish export sows in the National Reference Laboratory in Pulawy. However, the monitoring of the epidemiological status of chlamydial infection in pigs in Poland was not undertaken so far. A large turnover of these animals, especially relating to their import to Poland, justifies the undertaking of serological monitoring studies, aiming at defining the presence of Chlamydia antibodies in population of pigs in Poland. Therefore, the study was undertaken to evaluate the occurrence the Chlamydia suis infections in swine population in Poland and compare the usefulness of complement fixation test and PCR in the diagnosis of pig chlamydiosis.

Material and Methods

Materials. The 61,020 samples of swine sera and vaginal swabs were collected in 2007-2009. The samples were obtained from the companies involved in the export of pigs.

Complement fixation test. The serological examinations were performed using complement fixation test (CFT), a diagnostic technique recommended by the World Organisation for Animal Health (OIE). This technique was validated under the laboratory conditions and accredited by the Polish Centre for Accreditation (according to the PN-EN ISO/IEC 17025:2005). For the CFT, Institut Virion/Serion GmbH (Germany) and Sera and Vaccines Manufacturing (Biomed-Krakow, Poland) reagents were used. Before each examination, an intralaboratory evaluation, including antigen titration against a positive control serum and checking the activity of other reagents used in the reaction, were carried out to find the actual titre versus activity ratio in relation to that declared by the manufacturers. The specific reaction of the CFT, its consecutive steps, and interpretation of the results were performed according to the Manual of Standards for Diagnostic Tests and Vaccines (1).
Preparation of genomic DNA. DNA extraction from swabs was performed using the commercial QIAamp DNA mini kit (Qiagen), following the manufacturer’s instructions.

PCR. The vaginal swabs (n=277) selected from pigs diagnosed as positive in the CFT were tested by confirmatory nested PCR for *Chlamydia suis*. The first step was genus-specific amplification, in which primers: 191 CHOMP (5'-GGI GMI TTC CAA TAY GCI CAR TC) /TRACH269 (ACC ATT TAA CTC CAA TGT ARG GAG TG) were used. The primer set amplified a 576-597 base pair (bp). For the second amplification, we used 1 μl of the genus-specific product and primer combination: 201 CHOMP (5'-GGI GC W GMI TTC CAA TAY GCI CAR TC) /TRACH269 (ACC ATT TAA CTC CAA TGT ARG GAG TG), which is specific for *Chlamydia suis* and generates the amplicon of 250 bp size. The final volume of the reaction mixture amounted to 50 μl including: 5 μl of 10 x PCR buffer, 2 μl of 50 mM MgCl2, 0.2 μl of 10 mM dNTP, 1 μl of 20 pmol specific primer, 0.2 μl of 5 U/μl thermostable polymerase DNA, 39.6 μl of sterile water, and 1 μl of genomic DNA. The amplification was carried out in an Tepersonal termocycler (Whatman Biometra) using the following cycling parameters: 50 cycles, initial denaturation at 97°C - 60 s, denaturation at 97°C – 60 s, annealing at 50°C – 60 s, elongation at 72°C – 60 s, final elongation at 72°C – 60 s. PCR reactions were analysed by electrophoresis of 8 μl PCR product and 2 μl of Gel Loading Solution (Sigma) in a 1% agarose gel in 1 x TAE buffer and visualised by staining with ethidium bromide and ultraviolet transilumination. The molecular weight of the obtained product was determined on the basis of molecular weight marker, which was GeneRuler™100 bp DNA Ladder (Fermentas) and positive control of DNA (Genekam, Germany).

Statistical analysis. The χ² test was used to evaluate the reliability of results obtained by the CFT and PCR methods. In addition, a significant value of Person and Cramer correlation coefficient of P=0.00 were computed.

Results

The number of tested sera was 31,260 in 2007; 28,780 in 2008, and 980 in 2009. Positive CFT results were found in 277 pigs, which accounted for 0.46% of the tested population. The percentage of positive results in subsequent years was: 0.4%, 0.47%, and 1.65%, respectively. Moreover, in 2,520 pigs, which constituted 4.13% of tested animals, *Chlamydia* antibodies were found in the serum diluted from 1:8 to 1:16. In these cases, the degree of inhibition of haemolysis was interpreted as two plus (+++) and more, but finally it was interpreted as negative, in accordance with an accredited research procedure used in our laboratory. This percentage was 3.2%, 4.14%, and 3.26% in subsequent years (2007, 2008, 2009), respectively.

The results of PCR are presented in the Fig. 1. The presence of *Chlamydia suis* in the swabs was demonstrated in 200 cases, i.e. in 0.33% of all tested samples.

Statistical analysis of the results obtained using the CFT and PCR showed a significant coincidence. The statistical analysis (the χ² test, at P=0.00) revealed a high conformity of the results of the compared tests. The value x - 29.83 permitted stating that the compared methods are interrelated and give significantly unanimous results. Moreover, the values of Person linear correlation coefficient (0.678) ranged from 0 to 0.702 for a 2 x 2 chart and determined the dependency rate of the compared methods. It was found that the closer the value was to zero the weaker the relationship was, and in contrast, the closer the value was to 0.707 the stronger the dependence was. A similar relationship was found in the case of the Cramer coefficient, the values of which were also 0.682 in the range from 0 to 1.

![Fig.1. Electrophorical separation of amplification products.](image-url)

Lines: 1 - positive control, 2 - negative control, 4 – positive sample, 3, 5 - 8 – negative sample.
Discussion

Despite growing interest in infections caused by organisms of the genus *Chlamydia* in animals, and an increase in their zoonotic potential in recent years, there are no data on the prevalence of *Chlamydia suis* in swine in Poland. It should be stressed that the recent investigations are, at least in part, pioneering. *Chlamydia* infections in pigs have been reported mainly in Italy, Austria, Germany, Scotland, Switzerland and Belgium (2, 3, 6, 9). Most of the reports show only the research conducted in herds of pigs in which the disease affects the reproductive or respiratory systems (8, 10).

Comparing the results of serological and PCR tests published by research teams from neighbouring European countries with an intensive pig rearing, it can be concluded that the epizootic situation in the field of chlamydiosis in pigs in Poland is similar to that observed in other European countries (13, 14). However, it is difficult to compare our results with other studies because we used the CFT method, which is of lower sensitivity than ELISA technique used by other researchers.

Generally, in the available literature, there are no statistical data on the comparative assessment of the methods used in the diagnosis of *Chlamydia suis* infection. Positive serological results obtained in the presented study are important for assessing the epidemiological situation in pig chlamydiosis in Poland. The presented study demonstrated repeatability and reproducibility of results obtained in the CFT.

Summing up the results of research, it can be stated that the epidemiological situation of chlamydia infection in population of swine in Poland was evaluated. Moreover, the comparison of CFT with PCR showed that the results obtained with serological method (CFT) were significantly reproducible in the PCR and confirmed that the applied methods are useful in diagnosis of chlamydiosis in pigs.

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References