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Short communication

Rapid detection of *Chlamydia*/*Chlamydophila* group in samples collected from swine herds with and without reproductive disorders

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Abstract

The study was carried out in seven reproductive herds of pigs. In three of them reproductive disorders were observed. Three herds consisted of 10-50 and four consisted of 120-500 adult sows and they were called small and medium, respectively. Fifty-seven adult sows were randomly selected from herds. Serum samples were tested using the complement fixation test and swabs from both eyes and from the vaginal vestibule were examined using real-time PCR. All serum samples were negative. Infected sows were present in each of the study herds. In total, there were 28 positive samples (53%, 28/48) in real-time PCR in sows with reproductive disorders and 35 (53%, 35/66) in sows selected from herds without problems in reproduction. One isolate proved to be *Chlamydophila pecorum*, whereas all the remaining were *Chamydia suis*.

Key words: sows, *Chlamydiaceae*, Rt-PCR, complement fixation test

Introduction

Pathogens from *Chlamydia*/*Chlamydophila* group in the *Chlamydiaceae* family may be responsible for subclinical intestinal tract infection, pneumonia, polyarthritis, polyserositis, conjunctivitis as well as reproductive disorders such as late-term abortion in sows, an increased rate of perinatal and neonatal mortality, and epididymitis and vesiculitis in boars (Kaufold 2006, Schautteet and Vanrompay 2011). It was recently reported that chlamydiae were isolated from both clinically healthy animals and animals with clinical symptoms (Englund et al. 2012). Difficulties with cultivation of the pathogens cause that currently different methods, such as serology, immunohistochemistry, histology and PCR are preferred in diagnostics (Englund et al. 2012). The objective of the study was to determine prevalence of *Chlamydia*/*Chlamydophila* group infection in sows and identify the species of the pathogens infecting sows with, or without, reproductive disorders.

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Table 1. Results of CFT and Rt-PCR regard to size of the examined herds and clinical manifestation of disorders that may suggest *Chlamydia*/*Chlamydophila* group infection.

Examined samples	"M" farms (n=4)				"S" frams (n=3)		
	A	B	C	D	E	F	G
	positive CFT results/ total examined						
Serum	0/9	0/9	0/9	0/9	0/6	0/7	0/8
	positive Rt-PCR results/total examined						
Conjunctival swabs	6/9	4/9	8/9	5/9	4/6	4/7	4/8
Swabs from *vestibulum vaginae*	5/9	6/9	4/9	4/9	3/6	3/7	3/8

Explain: ■ present or □ absent of reproductive disorders

Materials and Methods

The study was carried out in seven (A-G) reproductive herds of pigs. In three of them reproductive disorders like low reproductive efficacy, repeated estrus and purulent, vaginal discharge were observed. The remaining four did not report any such symptoms. Tetracycline had not been used in any of the herds for at least 3 months before the onset of the study. The herds counted 10-500 adult sows, so they were divided into small (n=3, "S") and medium (n=4, "M"). Required sample size was computed in WinEpiscope 2.0 (EPIDECON) assuming expected prevalence of 30% and level of confidence of 95%. Fifty-seven adult sows were randomly selected – 24 from herds with reproductive disorders (6 and 18 from "S" and "M" herds, respectively) and 33 from clinically healthy herds (15 and 18 from "S" and "M" herds, respectively). From each sow serum sample, conjunctival swab from both eyes and swab from the vaginal vestibule were collected. Serum samples were tested using the complement fixation test (CFT) in the National Veterinary Research Institute in Pulawy. Real-time PCR on extracted genomic DNA (Thermo Scientific, Lithuania) was performed using *Chlamydiaceae* family-specific primers, fluorescent-labeled probe targeting the 23S ribosomal DNA and cycling conditions specified by Everett at al. (1999). Then, the products obtained in real-time PCR were sequenced (Genomed, Poland) and the species was identified using Basic Local Alignment Search Tool (BLAST; blast.ncbi.nlm.nih.gov) and Molecular Evolutionary Genetics Analysis (Tamura et al. 2011). Chi-square test was used to compare proportions (two-tailed of 0.05) and statistical analysis was performed in Statistica 10.0.0 (StatSoft).

Results and Discussion

All serum samples were negative in CFT. Infected sows were present in each of the examined herds (real-time PCR). Positivity regard to the size of examined herds, presence or absence of reproductive disorders and the place of sampling are shown provided in the Table 1. One isolate proved to be *Chlamydophila pecorum*, whereas all the remaining were *Chamydia suis*. The results of our study did not show the difference between ill diseased and asymptomatic pigs, however this may be attributed to the small number of pigs enrolled in the study. Laboratory tests allowing identification of the species of the pathogens from *Chlamydia*/*Chlamydophila* group in pigs are not routinely performed in Poland even though thes pathogens seem to be economically important (Rypuła et al. 2012). Seroprevalence in Europe ranges from 6.9% to 96.5% (Vanrompay et al. 2004, Camenish et al. 2005). In the study of Becker et al. (2007) prevalence of the infection ranged from 23% to 88% in clinically healthy pigs and from 79% to 90% in pigs manifesting clinical symptoms of the infection. The results of our study did not show the difference between clinically ill and asymptomatic pigs, however this may be attributed to the small number of pigs enrolled in the study. The present study shows that a high number of sows, regardless of the current clinical problems, may be carriers of the pathogen. Development of molecular methods allows quick identification of the isolated pathogenic species of *Chlamydia*/*Chlamydophila*. Given very low concordance between results of serological and molecular tests, the latter may be proposed as a routine diagnostic method.

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