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Chlamydia prevalence in Polish pig herds

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SUMMARY

Chlamydiae are frequently encountered intracellular Gram-negative bacteria. In pigs, these bacteria in combination with other pathogens contribute to the induction of a multi-aetiological syndrome. One of the major characteristics of *Chlamydia* spp. is their ability to cause prolonged, often subclinical infections. While the economic consequences of *Chlamydia* spp. infections in pig farms are not fully established, we know that reproductive disorders and other syndromes correlated with *Chlamydia* infection can lead to financial loss as a result of a reduction in pork production. Additionally, *Chlamydia* spp. presents a potential zoonotic hazard, therefore determining the prevalence of *Chlamydia* in pig populations is critical. In the present study 97 pig herds from Poland were involved. To determine the prevalence of *Chlamydia* PCR and CFT tests were used. In total 797 vaginal samples, 797 conjunctival samples, and 235 serum samples were collected and tested. The study took place from 2011 to 2014. We found *Chlamydia* spp. present in 71·2% of all tested farms. The percentage of animals testing positive on any given farm varied from 20% to 100%.

Key words: *Chlamydia*, PCR, prevalence, serological test, swine.

INTRODUCTION

Chlamydiae are a frequently encountered intracellular eubacteria with a unique biphasic developmental cycle, whose cell wall resembles that of Gram-negative bacteria [1–3]. Within the Chlamydiaceae family, nine distinct species have been found – *Chlamydia trachomatis, C. muridarum, C. suis, C. abortus, C. caviae, C. felis, C. pecorum, C. pneumoniae*, and *C. psittaci* [4]. Of these, the species commonly detected in pigs include *C. suis, C. abortus, C. psittaci, C. pecorum*, and *C. trachomatis* [5, 6]. In pigs, these bacteria in combination with *Mycoplasma* spp., porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV-2), *Pasteurella multocida,* or *Streptoccocus* spp. contribute to the induction of a multi-etiological syndrome [7–10]. One of the major characteristics of *Chlamydia* spp. is their ability to

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cause prolonged, often subclinical infections [11]. This syndrome can manifest with a wide range of symptoms, from a well-developed clinical infection to an asymptomatic infection, which occurs most often. Clinical symptoms manifest under the influence of certain conditions, such as stress, intensive pig production systems, or immunity deficiency. In pigs, *Chlamydia* contributes to disorders of the respiratory, digestive, and reproductive systems [12–14]. Epidemiological studies conducted in European countries have shown the importance of *Chlamydia* infection; however, in case of reproduction problems in pig farms, veterinarians rarely suspect *Chlamydia* infection. In such epidemiological studies the basic research panel includes PRRSV, PCV-2, parvovirosis, leptospirosis and swine influenza (SI) [8, 10, 12, 15].

The pig population in Poland amounts to almost 11 million animals, this includes almost 980 000 sows. The number of herds with sows/gilts on Polish territory is about 190 000 units. Regional distribution of pig herds is irregular but it can be concluded that the western part of the country is characterized by a greater number and density of pig production [16].

Pig production in Poland is a major branch of the economy. The large population of pigs, despite the irregular distribution of herds, promotes the spread of pathogens between farms and influences the pathogen prevalence in the general population. The presence of pathogens, even in the form of subclinical infection, has significant impact on the economic results of animal production.

Evaluation of the level of *Chlamydia* presence in pig herds will allow assessment of the scale of the problem and help in the implementation of preventive procedures.

Aim of the study

The aim of this study was to estimate the prevalence of *Chlamydia* spp. infection in Polish pig herds.

MATERIALS AND METHODS

Sample size

To determine the number of infected herds in our study population we used a list obtained from the Registration and Identification Animal System of the Agency for Restructuring and Modernization of Agriculture. This list includes approximately 380 000 pig farms [17]. The number of farms necessary for us to evaluate in order to accurately represent *Chlamydia* infection was determined based on equation (1) (with a confidence level of 0·95) [18]:

$$n = \frac{1{\cdot}96^2 \times P_{\text{exp}} \times (1 - P_{\text{exp}})}{d^2}, \quad (1)$$

where n is the required sample size, P_{exp} the expected morbidity, and d represents measurement accuracy.

Due to the lack of prevalence data for the pig population in Poland, the expected prevalence was arbitrarily set at 50% ($P_{\text{exp}} = 0{\cdot}5$) [18]. Measurement accuracy was arbitrarily set to 10% ($d = 0{\cdot}1$). Based on these parameters, the calculated sample size necessary to evaluate the epizootic situation of chlamydiosis in the Polish pig population was 97 herds. The number of sows/gilts examined in each herd was calculated according to equation (2):

$$\int n = [1 - (1 - p_1)^{1/d}] \times \left(N - \frac{d}{2}\right) + 1, \quad (2)$$

where n is the number of examined sows/gilts in the herd; p_1 is the probability of detecting at least one infected sow/gilt; N represents the total number of sows/gilts in the herd; and d represents the number of infected animals in the herd. The probability of detecting at least one infected sow in the herd was 95% ($p_1 = 0{\cdot}95$). Due to the lack of reference data regarding the prevalence of *Chlamydia* infection in pig herds in Poland, we arbitrarily set the herd prevalence at a low level of 30%. Based on these parameters we estimated the number of infected animals in each herd (d) and used that value to calculate the appropriate number of animals to examine (n). Samples were collected from randomly selected farms across the country. Polish pig production is mainly performed in closed indoor systems, therefore it was decided to analyse the results taking into account the farm size. The pig herds were considered separately based on the number of sows. A small herd was defined as a stock with $\leqslant$20 sows, a medium herd had 21–120 sows, and a large herd had >120 sows.

After random selection of herds and obtaining information from the owners regarding basic herd size, the number of samples to be collected from animals in order to detect *Chlamydia* was calculated using using the WinEpi program (www.winepi.net). In small herds ($\leqslant$20 sows) seven samples were collected, in medium herds (21–120 sows) nine samples were collected and in large herds (>120 sows) nine samples

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were collected. The total number of samples taken from 97 herds was 797.

In the following study, data of biosecurity levels of pig farms were considered. Information regarding biosecurity levels was obtained from records of Veterinary Inspection, which carries out the periodic control of animal farms. During every year a control veterinary inspector complete a survey regarding the conditions of animal maintenance, welfare and biosecurity procedures. Collecting and comparing annual data allows us to see if biosecurity procedures in animal farms are effective or not.

Sample collection

Animals were sampled at random according to the following schedule: all animals were marked sequentially using PAINT-FARM SPRAY (Vetos-Farma, Poland). The numbers were written on separate animal record cards which were drawn at random to determine which animal was sampled. The record cards were returned to the container after selection. In the case of selecting the same random number, the record card was returned to the container, and the draw continued until the selection of an untested animal.

We collected swabs from the vagina (P) and conjunctival sac (O) of sows and gilts. Swabs from the conjunctival sac were collected to determine whether the pathogen was present outside the reproductive system. Vaginal swabs from clinically healthy animals were collected during oestrus or up to 5 days after delivery, when the volume of vaginal secretions is high. Vaginal swabs from animals with clinical reproductive disorders were collected regardless of the phase of their cycle or the physiological state of the sow or gilt. In addition, when possible, serum samples were also collected to investigate the presence of antibodies against *Chlamydia* spp. In total, 797 vaginal samples, 797 conjunctival samples, and 235 serum samples were collected and tested.

Immediately after samples were collected swabs were placed in transport containers (Equimed, Poland) and transported to a diagnostic laboratory (Faculty of Veterinary Medicine, Wroclaw) at a temperature of 4–8 °C. The laboratory implements a quality management system (ISO/IEC 17025:2005 + API:2007 + AC:2007).

Sampling began in autumn 2011 and continued until 2014. The number of herds tested each year was similar and amounted to 25 surveyed units in 2011, and 24 in each subsequent year.

DNA isolation

Bacterial genetic material was isolated directly from the swab using a DNA Syngen Tissue Mini kit (Syngen, Germany). Isolated DNA (20 µl) was either immediately used for real-time polymerase chain reaction (PCR) or stored at −20 °C.

Real-time PCR

To amplify the gene coding the 23S ribosomal mRNA subunit we used the primers TQF (5′-GAAAAGAACCCTTGTTAAGGGAG-3′) and TQR (5′- CTTAACTCCCTGGCTCATCATG-3′). The sequence of the probe was FAM-CAAAAGGCACGCCGTCAAC-TAMRA. The primers and probe used were specific for all nine species of the Chlamydiae family. The 15 µl reaction mixture contained 10 µl KAPA PROBE FAST Bio-Rad iCycler 2× qPCR master mix (Kapa Biosystems, USA), 0·2 µl of probe (10 µM), 0·3 µl of each primer (10 µM), and 4·5 µl water. To this reaction mixture we added 5 µl of the test matrix. Amplification was performed using a thermal cycler iQ5 Bio-Rad (Bio-Rad, USA) according to the following protocol: initial denaturation, 3 min at 94 °C, amplification over 40 cycles of 15 s at 95 °C and 1 min at 60·5 °C. To assess the analytical sensitivity of our assay we used DNA isolated from *C. suis* VR-1474 (ATCC, USA) starting with a titre of 5×10^6 bacterial cells/ml and performed sequential tenfold dilutions. For all reactions the standard *C. suis* strain VR-1474 (ATCC, USA) was used as a positive control [K(+)] at a dilution of 10^{-3}.

Optimization and sensitivity evaluation of real-time PCR

In order to optimize our real-time PCR protocol, we used a standard *C. suis* strain VR-1474 (ATCC, USA) and a standard *C. felis* 905 (Merial, France) strain. Using these standard samples we performed real-time PCR with a gradient range of annealing temperatures from 55 °C to 66 °C, and determined that the optimal annealing temperature was 60·5 °C. Our assessment of sensitivity was performed by repeating the real-time PCR with tenfold dilutions of *C. suis* VR-1474 (ATCC, USA) with a titre of 5×10^6 cells. Using this method, our limit of detection was a 10^{-6} dilution, which equates to five copies of DNA.

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Table 1. *Distribution of farms surveyed by the presence of* Chlamydia suis *in the conjunctival sac (O) and vagina (P)*

Chlamydia presence					
Conjunctival sac	Vagina	Farm group	Number of farms	Percentage of farms	95% CI
+	+	O(+)P(+)	51	52·5	42·7–62·2
+	–	O(+)P(–)	9	9·3	5·0–16·7
–	+	O(–)P(+)	9	9·3	5·0–16·7
–	–	O(–)P(–)	28	28·9	20·8–38·6
Chlamydia in reproductive tract			60	61·9	51·9–70·9

CI, Confidence interval.
+, positive test result; –, negative test result.

DNA sequencing

The products obtained from samples positive in real-time PCR were sequenced (Genomed, Poland) and identified using BLAST (blast.ncbi.nlm.nih.gov).

Serological examination

To assess the prevalence of antibodies present in the serum we used the complement fixation test (CFT). The CFT is a quantitative test that detects the level of anti-*C. psittaci*, anti-*C. abortus* and anti-*C.suis* antibodies. Antibodies specific to *Chlamydia* spp. were detected using CFT according to EN ISO/IEC-17025:2005. The CFT was performed in the National Veterinary Research Institute (NVRI) in Puławy.

Statistical analysis

Statistical analysis was performed using Statistica v. 10 (StatSoft, USA) and Excel Microsoft (Microsoft Corp., USA) software. The nominal data collected (the number of farms according to certain criteria) are shown in Tables 1 and 2. The calculated relative risk (RR) of *Chlamydia* occurrence and the associated 95% confidence intervals (CI) are shown in Table 3, and were assessed using a χ^2 statistic with 1 D.F., at an arbitrary confidence level of $P < 0{\cdot}05$.

Ethics statement

The Ethical Committee for Animal Experiments, Wrocław, Poland approved this study, and all owners provided informed consent prior to initiation of the study.

RESULTS

Herds participating in the study

The study involved 97 pig herds in Poland. The herds were from the provinces of Wielkopolska (42 farms), Opole (12 farms), Podlaskie (10 farms), Małopolska (eight farms), the Lodz (five farms), Silesia (four farms), Lower Silesia (four farms), Mazowieckie (four farms), Lublin (four farms), and Kujawsko-Pomorskie (four farms). The studied farms comprised of 38·1% large herds, 22·7% medium herds, and 39·2% small herds.

Real-time PCR

For our analysis we divided the tested farms into groups depending on the presence of *Chlamydia* in the vagina and/or conjunctival sac (Table 1).

Without considering the origin of the swab, we found *Chlamydia* spp. in 71·2% of all tested farms. The percentage of animals testing positive on any given farm varied from 20% to 100%. Specifically, in the reproductive tract, we found *Chlamydia* spp. in 61·9% of the tested farms. In examining the prevalence of *Chlamydia* based on farm size, we found it in the vaginal swabs in 56·8% of large farms, 81·8% of medium farms, and 55·3% of small farms (Table 2).

While the proportion of vaginally infected pigs from medium-sized farms was higher than large and small farms, this difference was not statistically significant ($P = 0{\cdot}093$).

Results of multiple comparison of proportion taking into account the farm group and farm size is shown in Table 3.

The proportion of O(–)P(+) farms in the large farm group was significantly higher than in small farms (18·9% *vs.* 0·0%, $P = 0{\cdot}016$). The RR of *Chlamydia* occurrence in pigs kept in large farms is significantly

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Table 2. *Presence of* Chlamydia *in the conjunctival sac (O) and vagina (P) stratified by farm size (n = 97)*

	Farm size									χ^2 test		
	Large ($n = 37$)			Medium ($n = 22$)			Small ($n = 38$)					
Farm group	*n*	%	95% CI	*n*	%	95% CI	*n*	%	95% CI	χ^2	D.F.	*P*
O(+)P(+)	18	48·6	33·4–64·1	15	68·2	47·3–83·6	18	47·4	32·5–62·7	2·79	2	0·248
O(+)P(–)	3	8·1	2·8–21·3	3	13·6	4·7–33·3	3	7·9	2·7–20·8	0·64	2	0·725
O(–)P(+)	7	18·9	9·5–34·2	2	9·1	2·5–27·8	0	0·0	0·0–9·2	7·97	2	0·019*
O(–)P(–)	9	24·3	13·4–40·1	2	9·1	2·5–27·8	17	44·7	30·1–60·3	9·22	2	0·010*
ChRT	21	56·8	40·9–71·3	18	81·8	61·5–92·7	21	55·3	39·7–69·9	4·82	2	0·093

CI, Confidence interval; ChTR, *Chlamydia* in reproductive tract.
$P < 0·05$.
* Differences statistically significant.

Table 3. *Results of multiple comparisons of the presence of* Chlamydia suis *in different sized herds, and for different samples*

	Large *vs.* medium					Large *vs.* small				
Farm group	RR	95% CI	χ^2	D.F.	*P*	RR	95% CI	χ^2	D.F.	*P*
O(+)P(+)	0·71	0·46–1·10	1·42	1	0·234	1·03	0·64–1·65	0·01	1	0·904
O(+)P(–)	0·59	0·13–2·69	0·06	1	0·815	1·03	0·22–4·77	0·15	1	0·695
O(–)P(+)	2·08	0·47–9·14	0·41	1	0·522	—	—	5·85	1	0·016*
O(–)P(–)	2·68	0·63–11·3	1·23	1	0·268	0·54	0·28–1·06	2·61	1	0·106
ChRT	0·69	0·49–0·98	2·83	1	0·093	1·03	0·69–1·53	0·01	1	0·919
	Medium *vs.* small					Large *vs.* medium and small				
O(+)P(+)	1·44	0·93–2·24	1·67	1	0·196	0·88	0·59–1·32	0·16	1	0·690
O(+)P(–)	1·73	0·38–7·83	0·07	1	0·789	0·81	0·22–3·05	0·01	1	0·962
O(–)P(+)	—	—	1·31	1	0·252	5·68	1·24–25·9	4·88	1	0·027*
O(–)P(–)	0·20	0·05–0·80	6·62	1	0·010*	0·77	0·39–1·51	0·30	1	0·586
ChRT	1·48	1·05–2·10	3·23	1	0·072	0·87	0·62–1·22	0·36	1	0·551

RR, Relative risk; CI, confidence interval; ChTR, *Chlamydia* in reproductive tract.
$P < 0·05$.
* Differences statistically significant.

higher than in pigs from small- and medium-sized farms (RR 5·68, 95% CI 1·24–25·9, $P = 0·027$). The probability of lack of *Chlamydia* in medium farms is less than in small farms (9·1% *vs.* 44·7%, $P = 0·010$). The risk of obtaining the O(–)P(+) results in large farms is more than five times higher than in medium and small farms.

We sequenced 26 samples that were positive for *Chlamydia* spp. by real-time PCR. All samples were identified as the species *C. suis*, except for one vaginal sample that was identified as *C. pecorum*. Notably, the sow whose vaginal swab was identified as *C. pecorum* also had antibodies specific for *Chlamydia* spp. in the blood.

Thirty-two herds were randomly selected in the first stage of sampling from the high percentage of herds in which *Chlamydia* was present. The number of sows/gilts from which serum samples were taken in each herd was calculated according to equation (2). Those were the same animals from which swabs were taken – individual numbers identified all animals. A total of 235 serum samples were collected and tested.

From all of these serum samples only two contained antibodies against *Chlamydia* spp. In these two sows, which were from the same farm, chlamydiae were present in both the vaginal and conjunctival sac swabs. Otherwise, the CFT did not detect anti-*Chlamydia* antibodies in the serum even when the pathogen was present in the vaginal and/or conjunctival sac swabs.

To fully assess of the prevalence of *Chlamydia*, we divided Poland along the eastern borders of

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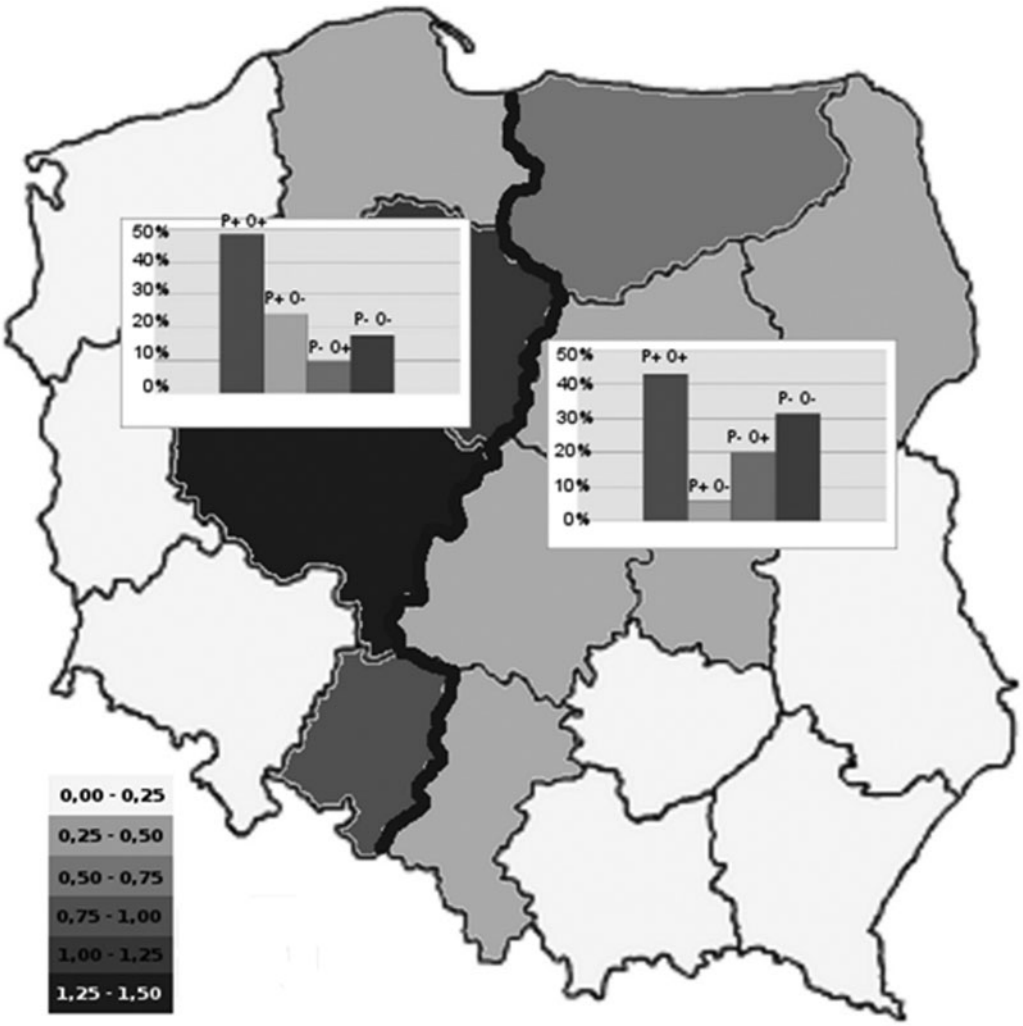

Fig. 1. Distribution of P(+)O(+), P(+)O(–), P(–)O(+) and P(–)O(–) herds in the Western and Eastern parts of Poland with a pig-herd density of >20 sows/1000 ha of agricultural land.

Pomerania, Kuyavian Pomerania, and the Łódź and Silesia provinces. This divided the country into its western and eastern parts. Western Poland is characterized by the presence of medium and large pig farms with a high or very high biosecurity level. Eastern Poland, on the other hand, typically has small and very small (backyard) pig stocks with low biosecurity level [16].

The studies found that the presence of P(+)O(+) farms in Western Poland was 48·4% and in Eastern Poland 42·9%. Presence of P(+)O(–) farms in Western Poland was 24·2% compared to 5·7% in Eastern Poland. In Western Poland presence of P(–)O(+) farms was 9·7% while in Eastern Poland it was 20%. Farms free of the presence of *Chlamydia* in vaginal and conjunctival sac swabs [P(–)O(–)] were found in 17·8% of farms in Western Poland and 31·5% in Eastern Poland. The obtained results are shown in Figure 1 regarding the division of the country into western and eastern parts. The results are reported on a background of pig-herd density of >20 sows/1000 ha of agricultural land in each province.

DISCUSSION

Chlamydia spp. infection often occurs without symptoms; however, even in these cases the infection still influences production indicators. Additionally, *Chlamydia* spp. present a potential zoonotic hazard [19, 20], therefore determining the prevalence of *Chlamydia* in pig populations is critical. The first study of *Chlamydia* prevalence in pigs was reported in 1966 in Great Britain. Capillary agglutination test was used to detect antibodies against *Chlamydia*

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spp. Antibodies were found in 23% of sera from tested pigs [21].

Some authors suggest that the type of pig farming may play a role in the prevalence of *Chlamydia* infection. Utilizing 16S RNA PCR, *C. suis* was detected in 1·5% of swabs from pigs with high rates of irregular return to oestrus and in 2·3% of swabs from pigs in control herds without reproductive problems. Moreover, *C. abortus* was detected in 33·3% of the examined fragments of genital tracts [22]. Another study on *Chlamydia* spp. infection was performed on 102 pigs from Germany (intensive farming) and 79 pigs from Switzerland (extensive farming). Both of these groups had a high prevalence of *C. suis* (90% in Germany, 79% in Switzerland). Additionally, Becker *et al*. suggest that intensively kept pigs are predisposed to ocular chlamydial infection [23]. Predisposition to ocular infection in intensively kept pigs might be explained among other things by the high density of animals in the farms, environmental conditions, reduction of costs of specific prevention and the pig's anatomy. A low-set head can result in increased emergence of ocular infections.

A similar observation was made in large pig production plants in Estonia where conjunctivitis and reproductive disorders associated with *Chlamydia* spp. presence were reported [8]. At present an assessment of the *Chlamydia* infection rate in pig herds has been reported for many countries throughout the world [5, 13, 24, 25].

The presence of *Chlamydia* spp. in pig herds was found in several European countries [26–32]. Belgium, where 96·5% of pigs are seropositive, is considered a country with endemic *Chlamydia*. In the study, 249 fattening pigs were examined with a recombinant enzyme-linked immunosorbent assay (ELISA), and 240 pigs had Chlamydiaceae family-specific antibodies [26]. Seroprevalence studies in Germany revealed the presence of *Chlamydia* in 33% of the tested herds. In these studies an ELISA was used to assess 1493 blood samples, and the rate of animals testing positive varied from 4·3% to 72·7% on individual farms. Therefore, the prevalence of chlamydial infections in breeding herds in Germany may be high and may play a role in reproductive disorders that indirectly affect pork production [24]. Furthermore, in 2004 a boar population from Thuringia province in Germany was tested, and *Chlamydia* spp. were detected in 57·1% of these animals. This high level of seroprevalence may suggest that wild fauna are an important reservoir of *Chlamydia* spp. [27]. A study conducted in Switzerland tested sows, older piglets (>4 weeks), and piglets (⩽4 weeks). The study used an LPS-based ELISA assay and reported a seroprevalence level of 6·9%, 48·1%, and 62% in piglets, older piglets, and sows respectively [22]. In pig populations in Italy the presence of *Chlamydia* was between 63·5% and 80·3% in tested herds [28]. A study utilizing the ELISA test conducted in the Murcia region of Spain revealed that the prevalence of *Chlamydia* reached a level of 47·1% [29], while in the Toledo region *Chlamydia* prevalence was reported at 36·4% [30]. In Lithuania 2502 blood samples from pigs from 24 farms were tested and only 7·7% tested positive by CFT. However, 87·5% of the regions in Lithuania are affected by *Chlamydia* infection. Similar to observations in Switzerland [22], the Lithuanian study also reported a correlation between *Chlamydia* infection and animals' age, where older animals are more frequently seropositive than young piglets [31], and there is an increase in *Chlamydia* infection in pigs aged >3–4 weeks [32].

Extensive studies regarding the prevalence of *Chlamydia* have also been carried out in Asia. In 2012 eleven administrative cities in Jiangxi province (southeastern China) were studied using an indirect haemagglutination assay (IHA) on 920 blood samples. The prevalence of *Chlamydia* ranged from 33·33% (Jingdezhen) to 90·91% (Pingxiang). The positive samples were distributed among all 11 administrative cities. In Guangdong province in southern China *Chlamydia* prevalence was reported as 63·38% in breeding boars, 41·10% in breeding sows and 36·25% in fattening piglets using an IHA. The differences in the prevalence in different age groups and gender is notable, and the authors suggest that breeding boars may be a source of the infection for other pigs on the farm [25]. In 2010, a study in Tibet examined the prevalence in different age groups and sexes using IHA. They reported an average seroprevalence level of 16·63%. However, sows had a higher seroprevalence (17·61%) than males (12·72%). Additionally, growing animals also had a higher incidence of *Chlamydia* [33].

There is insufficient data of *Chlamydia* prevalence in the American continent. Research conducted in the United States to evaluate whether *Chlamydia* spp. may be the cause of diarrhoea in pigs reported 15% prevalence in the intestinal tract of tested animals. They concluded that *Chlamydia* spp. are common in the intestinal tract but not always the cause of clinical symptoms [34].

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Until our study, in Poland the presence of *Chlamydia* spp. had been described in cattle and companion animals [35, 36]. Research on *Chlamydia* infections using CFT in Polish pigs during 2008–2013 revealed a low level of seroprevalence, i.e. 0·32%. While research conducted by real-time PCR samples collected from pig herds with and without reproductive disorders revealed a high percentage of infected animals [37].

Herein, we report a total prevalence of *Chlamydia* of 71·2%, without taking into account the origin of swabs. The percentage of positive animals on the farm varied from 20% to 100%.

In considering the obtained results we took into account only the farm size because most pig production in Poland is carried out in closed indoor systems with implemented procedures such as artificial insemination.

However, we noted a higher prevalence of *Chlamydia* in herds located in Eastern Poland. Compared to Western Poland, Eastern Poland is characterized by a lower degree of biosafety in pig production and often suboptimal zoo hygienic conditions. Further, intensive pig farming is more common in the western provinces. For example, ~36% of national pork production is located in Wielkopolska province [38]. In our study, DNA sequencing only identified *C. suis*, except for a single case of *C. pecorum* in a vaginal swab, which is supported by findings in a Swedish study [14].

Chlamydia infection affects the world's pork production [39]. While the economic consequences of *Chlamydia* spp. infections in pig farms are not fully established, we know that reproductive disorders and other syndromes correlated with *Chlamydia* infection can lead to financial loss as a result of a reduction in pork production or by the need to purchase antibiotic therapy. Therefore, it is critical to conduct research on the prevalence of *Chlamydia* in pig populations and to continuously monitor the scale of the problem and develop a strategy for treatment and prevention. However, this requires the standardization of diagnostic tests to determine the situation of infection and spread not only in Poland but in other countries. Improvement of the existing situation can be facilitated by sharing experiences and epidemiological information between countries.

The obtained results suggest the ineffectiveness of biosecurity procedures against chronic *Chlamydia* infections. An attempt to reduce the prevalence of *Chlamydia* should focus on the ongoing infectious agent monitoring and optional treatment. Constant monitoring will allow carriers to be identified and the deployment of individual approaches to infected pigs.

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DECLARATION OF INTEREST

None.

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