Polish Journal of Veterinary Sciences Vol. 18, No. 4 (2015), 825–831

DOI 10.1515/pjvs-2015-0107

Original article

Prevalence and risk factors for *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Salmonella* spp. in finishing pigs in Polish farrow-to-finish swine herds

A. Dors[1], M. Pomorska-Mól[1], E. Czyżewska[1], D. Wasyl[2], Z. Pejsak[1]

[1] Department of Swine Diseases, National Veterinary Research Institute, Partyzantów 57, 24-100 Puławy, Poland
[2] Department of Microbiology, National Veterinary Research Institute, Partyzantów 57, 24-100 Puławy, Poland

Abstract

The aim of the study was to estimate the herd-level, within-herd prevalence, the frequency of mixed infections and risk factors for *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. in selected farrow-to-finish Polish pig herds. A total of 254 pooled fecal samples were collected from 9 to 24 week-old pigs in 70 herds. Real time PCR for detection of *L. intracellularis* and *B. hyodysenteriae* was performed. For *Salmonella* spp. bacteriological examination was performed. The herd-level prevalences of *L. intracellularis, B. hyodysenteriae* and *Salmonella* spp. among examined herds were 65.7%, 1.4% and 8.6%, respectively. The within-herd prevalences (in positive herds) for *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. were 51.5%, 75.0% and 30.4%, respectively. All herds with diarrhea observed during sampling were infected with *L. intracellularis* and 60% of herds with no diarrhea at the moment of sampling were infected with *L. intracellularis* ($p=0.035$). In herds with more than 200 sows the prevalence of *Salmonella* spp. was significantly higher compared to herds with less than 200 sows ($p=0.027$). In herds where all-in/all-out (AIAO) was respected, prevalence of *L. intracellularis* was significantly lower than in herds where this rule was not kept ($p=0.024$). Obtained results confirm that *L. intracellularis* is the major cause of bacterial diarrhea in finishing pigs. The present study identified AIAO and herd size as a risk factor, at the herd level, for *L. intracellularis* and *Salmonella* spp., respectively.

Key words: pig, prevalence, risk factors, *Lawsonia intracellularis*, *Brachyspira hyodysenteriae*, *Salmonella*, diarrhea

Introduction

Intestinal diseases among growing and finishing pigs result in significant economic losses worldwide. Enteric infections can be caused by a number of pathogenic bacteria. The most important enteric pathogens during the fattening phase are *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Salmonella* spp. (McOrist 2005, Carlson et al. 2012, Hampson 2012). These pathogens are responsible for

Correspondence to: A. Dors, e-mail: arkadiusz.dors@piwet.pulawy.pl

porcine proliferative enteropathy (PPE), swine dysentery (SD) and porcine salmonellosis (PS), respectively. These diseases occur in growing and finishing pigs and are characterized by a fecal-oral route of transmission. Simultaneous presence of these bacteria is not uncommon in the case of diarrhea (Merialdi et al. 2003).

Porcine proliferative enteropathy is caused by *L. intracellularis*, an obligate intracellular pathogen. Proliferation of enterocytes and characteristic thickening of the intestinal mucosa are observed in pigs affected with PPE (Lawson and Gebhart 2000). Lesions in all parts of the intestine have been described but most commonly they are localized in the ileum (Jensen et al. 1997). The disease is distributed worldwide, and is an economic concern for the pig industry (McOrist 2005). Occurrence of *L. intracellularis* was reported in various countries throughout the world with prevalence ranging between 48% and 100% (Stege et al. 2000, Lee et al. 2001, Jacobson et al. 2005, Hands et al. 2010).

Swine dysentery, caused by *B. hyodysenteriae,* is characterized by severe mucohaemorrhagic diarrhea which primarily affects pigs during the growing and finishing phase. Swine dysentery is widespread around the world, although studies regarding epidemiology are scarce and the reported prevalence significantly varies among particular studies (from 0% to near 40%) (Fellström et al. 1997, Stege et al. 2000, Suh and Song 2005).

Salmonella spp. can also cause diarrhea in pigs and is a zoonotic agent. There are a number of studies regarding prevalence of *Salmonella* serovars isolated from humans and animals (Arguello et al. 2013, Bonardi et al. 2013, Li et al. 2013). Data from these studies reported that in pigs the most common serovar was Typhimurium, in cattle Typhimurium and Dublin serovars, and in chickens Enteritidis, Infantis and Typhimurium serovars. Prevalence of *Salmonella* varies depending on isolation and sampling methods (Jørgensen et al. 2002).

The latest data about prevalence of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. in Polish pig herds derived from the years 2007-2009 and require to be updated (Pejsak et al. 2007, Wasyl and Hoszowski 2007, Pejsak et al. 2009). Moreover, the results of these studies in terms of the prevalence of *L. intracellularis* were contradictory. These studies were focused only on herds with characteristic signs of PPE or SD, and only a small number of risk factors regarding these enteric pathogens were analyzed.

The aim of this study was to estimate the herd-level and within-herd prevalence of *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp. In addition, the frequency of mixed infections in selected Polish pig herds was studied. Also, the relationship between the prevalence of these pathogens and other factors (herd size, all-in/all-out (AIAO) implementation, diarrhea, type of herd and age of pigs) was investigated.

Materials and Methods

Herds

A total of 70 farrow-to-finish herds were selected. The selection criteria were as follows: sow herd size over or equal to 20 and no use of antibiotics in the fattening phase within 14 days prior to sampling. The herds differed in some factors such as symptoms of diarrhea of uncertain etiology in the fattening period, AIAO implementation, the introduction of pigs from other herds (open or closed status). The investigated herds were localized in all voivodships of Poland, proportionally to density of pig population.

Samples

A total of 254 pooled fecal samples form 70 pig herds were collected between March 2011 and April 2013. From 2 to 5 pooled fecal samples, representing different age groups (older than 9 weeks), were collected from each herd. Samples were pooled on the site (into sterile, 120 ml plastic containers) using feces from 6 to 10 pigs. Samples were collected from normal and diarrheic (if present) pigs, and transported within 24 h in cooling boxes to the laboratory for bacteriological culture and PCR examination.

Bacteriological method

Isolation of *Salmonella* involved buffered peptone water pre-enrichment, selective enrichment on Modified Semi-solid Rappaport-Vassiliadis (MSRV) medium, plating on XLD medium, followed by biochemical identification. *Salmonella* isolates were identified according to the White – Kauffmann – Le Minor scheme (Grimont and Weill 2007).

PCR techniques

Pooled fecal samples were homogenized in single-use plastic containers and total genomic DNA was extracted using a commercial isolation kit (Genomic Mini, A&A Biotechnology, Gdynia, Poland), according to the manufacturer's recommenda-

Table 1. Prevalence of *L. intracellularis, B. hyodysenteriae* and *Salmonella* spp. in pooled fecal samples (n=254) from fattening pigs in selected Polish pig herds (n=70).

Pathogens	Number of positive herds	%	95% CI	Number of positive samples	%	95% CI
L. intracellularis	46	65.7	53.4 – 76.7	88	34.7	28.8 – 40.8
B. hyodysenteriae	1	1.4	0.04 – 7.7	3	1.2	0.2 – 3.4
Salmonella spp.	6	8.6	3.2 – 17.7	7	2.8	1.1 – 5.6

Table 2. Simultaneous presence of *L. intracellularis, B. hyodysenteriae* and *Salmonella* spp. in pooled fecal samples (n=254) from fattening pigs in selected Polish pig herds (n=70).

Pathogens	Number of positive herds	%	95% CI	Number of positive samples	%	95% CI
L. intracellularis alone	39	55.7	43.3 – 67.6	82	32.3	26.6 – 38.4
B. hyodysenteriae alone	0	0.0	0.0 – 5.1	2	0.8	0.1 – 2.8
Salmonella spp. alone	0	0.0	0.0 – 5.1	2	0.8	0.1 – 2.8
L. intracellularis + *Salmonella* spp.	6	8.6	3.2 – 17.7	5	2.0	0.6 – 4.5
L. intracellularis + *B. hyodysenteriae*	1	1.4	0.04 – 7.7	1	0.4	0.01 – 2.2
None	24	34.3	23.3 – 46.6	162	63.8	57.5 – 69.7

tions. Extracted DNA samples were stored at -20°C until use in the PCRs for *L. intracellularis* and *B. hyodysenteriae* assays.

Real-time PCR amplification of total DNA extracted from fecal samples for detection of *L. intracellularis* and *B. hyodysenteriae* was carried out according to the method described previously (Zmudzki et al. 2012).

The assay was carried out using the Mx3005P QPCR System (Stratagene, La Jolla, California, USA). The amplification mixture consisted of 5 µl template DNA and a QuantiTect probe set (Qiagen, Hilden, Germany) containing 8 mM $MgCl_2$, dNTP mix and HotStarTaq DNA polymerase, in a total volume of 25 µl. The concentration of the primers and the probes were the same for both bacteria (20 µM). Amplification of *B. hyodysenteriae* was performed in a 96-well plate with optical caps at the following settings: 10 min at 95°C, 50 cycles of 15 s at 95°C and 1 min of annealing and extension at 52°C. For *L. intracellularis* the parameters were as follows: 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 1 min of annealing at 52°C and 1 min of extension at 62°C.

Epidemiological and statistical analysis

A herd was defined as positive for *L. intracellularis*, *B. hyodysenteriae* or *Salmonella* spp. when at least one fecal sample taken from the herd had a positive PCR or culture outcome. Within-herd prevalence was defined as the number of positive fecal samples divided by the total number of samples collected in positive herds. The bacteriological prevalence of all agents was reported as a percentage with a 95% two-sides exact binominal confidence interval (CI), using the equation described by Clopper and Pearson (1934) calculated in statcalc3 (Soper 2014). Differences in prevalence between herds of various sizes, with and without diarrhea of unknown etiology at the moment of sampling, usage or not of AIAO, and open or closed herd status were determined by chi-squared test with Yates's correction for continuity (statistically significant at $p<0.05$). Additionally, to determine differences in prevalence of pathogens among various age groups: <12, 12-15, 16-19 and ≥20 weeks of age, the chi-squared test was used.

Results

The mean herd size was 203 ± 339 sows (range: 22-2000) and ranged from 20 to 49 (N=21), 50 to 200 (N=33), and 16 herds with more than 200 sows. At the time of sampling, diarrhea in fatteners was observed in 10 herds. Nineteen herds used the AIAO system in all finishing sectors, while in 51 herds AIAO was partially implemented. Forty-three herds had closed status while the remaining purchased gilts from other farms.

Using the cut-off point of one positive sample for defining a herd as positive, the prevalence of *L. intracellularis* among examined herds was high (65.7%) and prevalence of *B. hyodysenteriae* and *Salmonella* spp. was significantly lower ($p<0.001$; 1.4% and 8.6%, respectively). The number and percentage of positive

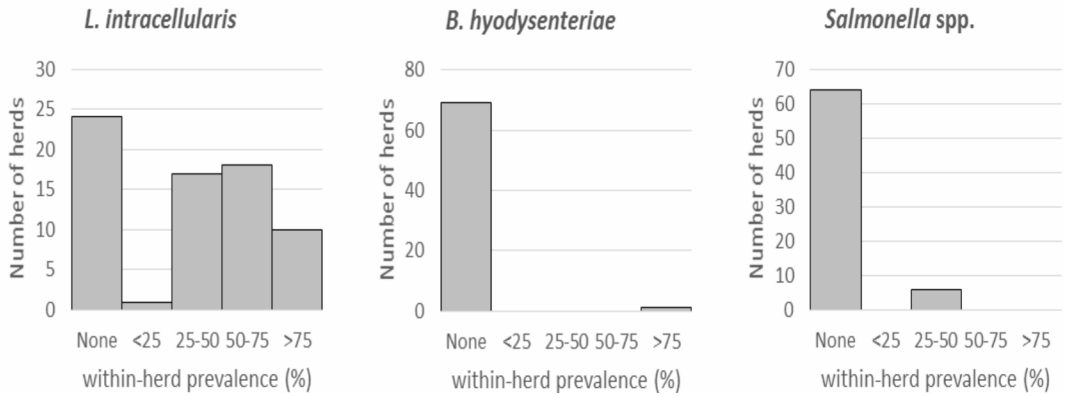

Fig. 1. Within-herd prevalence (%) of *L. intracellularis, B. hyodysenteriae* and *Salmonella* spp. in pooled fecal samples from fattening pigs in selected Polish pig herds (n=70)
Histograms illustrating the number of negative herds and the number of herds with within-herd prevalence less than 25%, from 25% to 50%, from 50% to 75% and more than 75%. Within-herd prevalence was calculated as a proportion: number of positive samples/total number of samples in each herd.

Table 3. Prevalence of *L. intracellularis, B. hyodysenteriae* and *Salmonella* spp. in herds varied in size, presence of diarrhea, AIAO and herd status (open/closed).

Variable group		Number of herds	*L. intracellularis*			*B. hyodysenteriae*			*Salmonella* spp.		
			No of positive	% of positive	95% CI	No of positive	% of positive	95% CI	No of positive	% of positive	95% CI
Herd size	20-49 sows	21	12	57.1	34.0-78.2	0	0.0	0.0-16.1	1	4.8	0.1-23.8
	50-200 sows	33	24	72.0	54.5-86.7	1	3.0	0.1-15.8	1	3.0	0.1-15.8
	> 200 sows	16	10	62.5	35.4-84.8	0	0.0	0.0-20.6	**4**	**25.0***	**7.3-52.4**
Diarrhea	yes	10	**10**	**100.0***	**69.2-100.0**	0	0.0	0.0-30.8	2	20.0	2.5-55.6
	no	60	36	60.0	39.8-64.4	1	1.7	0.04-7.8	4	6.7	1.6-14.2
AIAO	yes	19	8	42.1	20.2-66.5	0	0.0	0.0-17.6	1	5.3	0.1-26.0
	no	51	**38**	**74.5***	**60.4-85.7**	1	2.0	0.1-10.4	5	9.8	3.3-21.4
Herd statu	open	27	17	63.0	42.4-80.6	0	0.0	0.0-12.8	5	29.4	6.3-38.1
	closed	43	29	67.4	51.4-80.9	1	2.3	0.1-12.2	1	2.3	0.1-12.2

* – statistically significant differences calculated between rows in variable group ($p<0.05$)

Table 4. Distribution of positive samples in pigs from different age groups.

Age (weeks)	No of samples	*L. intracellularis*			*B. hyodysenteriae*			*Salmonella* spp.		
		No of positive	% of positive	95% CI	No of positive	% of positive	95% CI	No of positive	% of positive	95% CI
< 12	45	14	31.1	18.2-46.6	0	0.0	0.0-7.9	3	6.7	1.4-18.3
12-15	76	29	38.2	27.2-50.0	1	1.3	0.03-7.1	2	2.6	0.3-9.2
16-19	69	27	39.1	27.6-51.6	1	1.5	0.03-7.8	1	1.5	0.03-7.8
≥ 20	64	18	28.1	17.6-40.8	1	1.6	0.04-8.4	1	1.6	0.04-8.4

herds/samples with a 95% confidence interval is shown in Table 1.

Twenty-four herds were not infected with any of the examined pathogens, 39 herds were infected with one pathogen only (*L. intracellularis*), 7 herds with 2 pathogens, and none was infected with three pathogens. The simultaneous presence of *L. intracellularis, B. hyodysenteriae* and *Salmonella* spp. in herds and pooled samples is shown in Table 2.

The within-herd prevalence (in positive herds) for *L. intracellularis, B. hyodysenteriae* and *Salmonella* spp. were 51.5%, 75.0% and 30.4%, respectively. Only one herd was infected with *B. hyodysenteriae* and 6 with *Salmonella* spp., and 46 herds were positive for *L. intracellularis*. Distribution of within-herd prevalence is shown in Fig 1.

The prevalence of *L. intracellularis, B. hyodysenteriae* and *Salmonella* spp. in herds tested with differ-

ent size, presence or absence of diarrhea, AIAO procedure, and open or closed herd status are presented in Table 3. There was a significant difference between herd size and prevalence of *Salmonella* spp. (p=0.027). In herds with more than 200 sows the prevalence of the pathogen was significantly higher compared to herds with less than 200 sows. A statistically significant difference was also found with regard to symptoms of diarrhea and prevalence of *L. intracellularis* (p=0.035). All herds with diarrhea observed during sampling were infected with *L. intracellularis*. In contrast, in herds without diarrhea the prevalence of *L. intracellularis* was significantly lower (60.0%). In herds with the AIAO system the prevalence of *L. intracellularis* was significantly lower than in herds where this rule was not kept (p=0.024).

The highest percentage of *L. intracellularis* positive samples were found in pigs aged from 16 to 19 weeks, whereas *Salmonella* spp. were found mostly in samples taken from pigs younger than 12 weeks of age (Table 4). There were no other differences between pathogen prevalence in different age groups of pigs.

Salmonella spp. serotyping revealed 7 isolates representing 3 serovars (4 isolates of Typhimurium, 2 Derby and 1 Infantis).

Discussion

Results of the present study indicate that presence of *L. intracellularis* is quite common in medium and large size farrow-to-finish Polish swine herds, in contrast to the occurrence of *B. hyodysenteriae* and *Salmonella* spp.

The prevalence of *L. intracellularis* in Polish swine herds was comparable with data reported previously: 65.7% in the present study vs. 62.1% in the study of Pejsak et al. (2007). In the other study conducted by Pejsak et al. (2009) the mean prevalence of *L. intracellularis* was significantly lower (16.2%) than in the present study. These differences may be a consequence of the different types of herds that were investigated (breeding herds, farrow-to-finish herds and fattening units) as well as a smaller number of analyzed herds (8 breeding herds, 5 fattening units and 24 farrow-to-finish herds). It cannot be excluded that the sampling period also influenced the obtained results. In our study samples were collected from various age groups of pigs (<12, 12-15, 16-19, ≥20), while in the study of Pejsak et al. (2009) samples were collected once, from pigs 5-6 months (22-26 weeks). In the previous study (Pejsak et al. 2007) the percentage of positive samples was similar (33.6%) to that reported in the present study (32.3%). The current study confirmed that *L. intracellularis* is widely distributed among Polish medium and large size commercial swine herds. The herd-level prevalence of *L. intracellularis* in Poland is lower than the prevalence reported in Hungary – 93.6% (Biksi et al. 2007) and Denmark – 93.7% (Stege et al. 2000) but higher than reported in South Korea – 46.5% (Suh and Song 2005), Germany – 48.4% (Reiner et al. 2011), Italy – 44.6% (Merialdi et al. 2003) and Czech Republic – 31.8% (Cizek et al. 2006).

In contrast to data reported previously by Pejsak et al. (2007), the prevalence of *B. hyodysenteriae* in the present study seems to be very low. The higher prevalence reported previously by Pejsak et al. (2007) may be a result of a different study approach, enrolling only pigs with diarrhea or in bad condition, from herds with a history of hemorrhagic enteritis. Current results indicate that nowadays the relevance of *B. hyodysenteriae* infections in finishing pigs in Poland has decreased. This phenomenon was observed in previous years in several other countries (Jacobson et al. 2005, Viott et al. 2013) and is related to changes in production systems and better understanding of the role of biosecurity in the control of infectious diseases (Laanen et al. 2013). Our results are similar to those found in Denmark – 2.5% of positive herds (Stege et al. 2000) and Sweden, where a positive herd was not detected at all (Jacobson et al. 2005). In other European countries, including Czech Republic, Germany and Hungary, prevalence of *B. hyodysenteriae* was clearly higher 9.1%, 24.2% and 45.2%, respectively (Cizek et al. 2006, Reiner et al. 2011, Biksi et al. 2007).

Prevalence of *Salmonella* spp. was lower than those reported in Hungary – 54.8% (Biksi et al. 2007), South Korea – 51.1% (Suh and Song 2005), Portugal – 45.5% (Correia-Gomes et al. 2013) and Spain – 94.0% (Vico et al. 2011). Our results are similar to those reported by Stege et al. (2000) in Denmark – 10.1% and Nathues et al. (2013) in Germany – 11.2%. Prevalence of *Salmonella* spp. obtained in our study was also lower than those reported for the European Union by the European Food Safety Authority (EFSA), which demonstrated 33.3% of the production holdings positive for *Salmonella* spp. This prevalence varied from 0% to 55.7% among the Member States (EFSA, 2009).

The simultaneous presence of investigated pathogens was not common at either, sample or herd levels. Only 10.0% of herds were positive for two pathogens simultaneously. In the remaining herds a single (55.7%) or no (34.3%) pathogen was detected. Our results differ compared to those obtained in Denmark, where only 4% of herds were not infected at all and 32% of herds were infected with one pathogen only (Stege et al. 2000). According to Biksi et al.

(2007), in Hungary single infection of herds with the pathogens mentioned above was observed in only 9.7% of herds. In the above studies the prevalence of a higher number of pathogens was investigated.

Within-herd prevalence varied depending on the investigated pathogen. Since only one herd was positive to *B. hyodysenteriae* it was difficult to estimate the within-herd prevalence of this pathogen. *Salmonella* had the lowest within-herd prevalence. The within-herd prevalence of *L. intracellularis* at a level lower than 25% was observed in one herd only. These results indicate that the presence of *L. intracellularis* in herds results in high within-herd prevalence (more than 25%). Moreover, the pathogen is well distributed among different age groups of finishing pigs. Our results are consistent with those presented by Stege et al. (2000) in Danish pig herds.

In the present study the percentage of *Salmonella* spp. positive herds was different depending on the herd size. In herds with 20-49 and 50-200 sows, the percentage of positive herds were 4.8% and 3.0%, respectively. In contrast, in herds with more than 200 sows, the prevalence was as high as 25.0%, and differed significantly from smaller herds (<200 sows). More frequent occurrence of *Salmonella* spp. in larger herds was previously reported also by Suh and Song (2005). The reasons for such results are not clear, but herd size was previously reported as a risk factor for *Salmonella* spp. infections by Correia-Gomes et al. (2013). In our study, a significant percentage of isolated *Salmonella* strains were not specific for pigs (*S.* Derby, *S.* Infantis). Possibly, there is a higher risk for transmission of *Salmonella* spp. in large swine herds (i.e. with personnel, feed or with purchased animals). The lower prevalence of the infection in closed herds as compared to open herds may partially confirm this hypothesis (in large herds animals were purchased more frequently).

In herds where diarrhea was present at the moment of sampling, herd-level prevalence of *L. intracellularis* was significantly higher than in herds without diarrhea. A similar relationship did not occur for the other investigated pathogens. This result confirmed that *L. intracellularis* is the major cause of bacterial diarrhea in finishing pigs (Jacobson et al. 2003, Merialdi et al. 2003, Pedersen et al. 2012). The prevalence of the pathogen was significantly higher in herds where AIAO practice was not observed.

In summary, our results indicate that *L. intracellularis* is the major enteric bacterial pathogen in finishing pigs in Poland, which can cause both clinical problems or subclinical infections. The role of *B. hyodysenteriae* as a causative agent of enteric infection in Polish medium and large size swine herds seems to have diminished over recent years. Additionally, the study identified AIAO and herd size as a risk factor for *L. intracellularis* and *Salmonella* spp. at the herd level, respectively. Finally, in finishing pigs with diarrhea *L. intracellularis* should be considered as the most probable agent of clinical problems.

Acknowledgements

This work was supported by a grant from the National Science Centre, No. N N308 571740.

References

Arguello H, Sorensen G, Carvajal A, Baggesen D, Rubio P, Pedersen K (**2013**) Prevalence, serotypes and resistance patterns of *Salmonella* in Danish pig production. Res Vet Sci 95: 334-342.

Biksi I, Lorincz M, Molnár B, Kecskés T, Takács N, Mirt D, Cizek A, Pejsak Z, Martineau GP, Sevin JL, Szenci O (**2007**) Prevalence of selected enteropathogenic bacteria in Hungarian finishing pigs. Acta Vet Hung 55: 219-227.

Bonardi S, Bassi L, Brindani F, D'Incau M, Barco L, Carra E, Pongolini S (**2013**) Prevalence, characterization and antimicrobial susceptibility of *Salmonella enterica* and *Yersinia enterocolitica* in pigs at slaughter in Italy. Int J Food Microbiol 163: 248-257.

Carlson S, Branhill A, Griffith R (**2012**) Salmonellosis. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW (eds) Diseases of Swine, Wiley-Blackwell, Ames, Iowa, pp 821-833.

Cizek A, Sperling D, Bednar D, Pejsak Z, Biksi I, Martineau GP, Sevin JL, Hasman P, Mirt D (**2006**) *Lawsonia intracellularis* and *Brachyspira spp*. prevalence in fatteners in the Czech Republic, Hungary and Poland Czech large-scale farms without diarrhea. Proceedings of the 19th IPVS Congress, Copenhagen, Denmark, 2: 356.

Clopper CJ, Pearson ES (**1934**). The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 26: 404-413.

Correia-Gomes C, Mendonça D, Vieira-Pinto M, Niza-Ribeiro J (**2013**) Risk factors for *Salmonella spp.* in Portuguese breeding pigs using a multilevel analysis. Prev Vet Med 108: 159-166.

EFSA (**2009**) Analysis of the baseline survey on the prevalence of Salmonella in holdings with breeding pigs in the EU, 2008, Part A: Salmonella prevalence estimates, EFSA Journal 7(12): 1377 [p 93].

Fellström C, Pettersson B, Thomson J, Gunnarsson A, Persson M, Johansson KE (**1997**) Identification of *Serpulina* species associated with porcine colitis by biochemical analysis and PCR. J Clin Microbiol 35: 462-467.

Grimont PAD, Weill F-X (**2007**) Antigenic formulae of the *Salmonella* serovars https://www.pasteur.fr/sante/clre/cadrecnr/salmoms/WKLM-En.pdf

Hampson DJ (**2012**) Brachyspiral Colitis. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW (eds) Diseases of Swine, Wiley-Blackwell, Ames, Iowa, pp 680-696.

Hands I, McOrist S, Blunt R, Lawrence K (**2010**) Current

infection patterns of porcine proliferative enteropathy in Great Britain and the Republic of Ireland. Vet Rec 167: 343-344.

Jacobson M, Gerth Löfstedt M, Holmgren N, Lundeheim N, Fellström C (**2005**) The prevalences of *Brachyspira spp*. and *Lawsonia intracellularis* in Swedish piglet producing herds and wild boar population. J Vet Med B Infect Dis Vet Public Health 52: 386-391.

Jensen TK, Møller K, Leser TD, Jorsal SE (**1997**) Comparison of histology, immunohistochemistry and polymerase chain reaction for detection of *Lawsonia intracellularis* in natural porcine proliferative enteropathy. Eur J Vet Pathol 3: 115-123.

Jørgensen F, Bailey R, Williams S, Henderson P, Wareing DR, Bolton FJ, Frost JA, Ward L, Humphrey TJ (**2002**) Prevalence and numbers of *Salmonella* and *Campylobacter spp.* on raw, whole chickens in relation to sampling methods. Int J Food Microbiol 76: 151-164.

Laanen M, Persoons D, Ribbens S, de Jong E, Callens B, Strubbe M, Maes D, Dewulf J (**2013**) Relationship between biosecurity and production/antimicrobial treatment characteristics in pig herds. Vet J 198: 508-512.

Lawson GH, Gebhart CJ (**2000**) Proliferative enteropathy. J Comp Pathol 122: 77-100.

Lee SW, Kim TJ, Park SY, Song CS, Chang HK, Yeh JK, Park HI, Lee JB (**2001**) Prevalence of porcine proliferative enteropathy and its control with tylosin in Korea. J Vet Sci 2: 209-212.

Li R, Lai J, Wang Y, Liu S, Li Y, Liu K, Shen J, Wu C (**2013**) Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. Int J Food Microbiol 163: 14-18.

McOrist S (**2005**) Defining the full costs of endemic porcine proliferative enteropathy. Vet J 170: 8-9.

Merialdi G, Bonilauri P, Granelli F, Luppi A, Dottori M (**2003**) Bacterial pathogens in field cases of clinical colitis in growing and finishing pigs in Italy. Vet Rec 153: 529-530.

Nathues C, Grüning P, Fruth A, Verspohl J, Blaha T, Kreienbrock L, Merle R (**2013**) *Campylobacter spp.*, *Yersinia enterocolitica*, and *Salmonella enterica* and their simultaneous occurrence in German fattening pig herds and their environment. J Food Prot 76: 1704-1711.

Pedersen KS, Ståhl M, Guedes RM, Angen Ø, Nielsen JP, Jensen TK (**2012**) Association between faecal load of *Lawsonia intracellularis* and pathological findings of proliferative enteropathy in pigs with diarrhoea. BMC Vet Res 8: 198.

Pejsak Z, Żmudzki J, Wasak M, Porowski M, Wojciechowski J, Mirt D (**2007**) Prevalence of *Brachyspira hyodysenteriae* and *Lawsonia intracellularis* infections in Polish swine population. Med Weter 63: 994-996.

Pejsak Z, Żmudzki J, Wojciechowski J, Salwa A (**2009**) Prevalence and economic costs of proliferative enteropathy in the Polish swine herd population. Med Weter 65: 258-261.

Reiner G, Hillen S, von Berg S, Kixmoller M, Willems H (**2011**) Analysis of bacterial load and prevalence of mixed infections with *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and/or *Brachyspira pilosicoli* in German pigs with diarrhoea. Berl Munch Tierarztl Wochenschr 124: 236-241.

Soper DS (**2014**) Binomial Probability Confidence Interval Calculator. Available from http://www.danielsoper.com/statcalc

Stege H, Jensen TK, Møller K, Baekbo P, Jorsal SE (**2000**) Prevalence of intestinal pathogens in Danish finishing pig herds. Prev Vet Med 46: 279-292.

Suh DK, Song JC (**2005**) Prevalence of *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Salmonella* in swine herds. J Vet Sci 6: 289-293.

Vico JP, Rol I, Garrido V, San Román B, Grilló MJ, Mainar-Jaime RC (**2011**) Salmonellosis in finishing pigs in Spain: prevalence, antimicrobial agent susceptibilities, and risk factor analysis. J Food Prot 74: 1070-1078.

Viott AM, Lage AP, Cruz EC Jr, Guedes RM (**2013**) The prevalence of swine enteropathogens in Brazilian grower and finish herds. Braz J Microbiol 44: 145-151.

Wasyl D, Hoszowski A (**2007**) Occurrence of *Salmonella* in slaughter pigs in Poland. PIWet-PIB, Puławy, pp 1-26.

Zmudzki J, Szczotka A, Podgórska K, Nowak A, Grzesiak A, Dors A, Pejsak Z (**2012**) Application of real-time PCR for detection of *Lawsonia intracellularis* and *Brachyspira hyodysenteriae* in fecal samples from pigs. Pol J Vet Sci 15: 267-273.