Abstract

Background: The major pathogenic intestinal spirochetes affecting pigs during the growing-finishing stage of production include Brachyspira hyodysenteriae and Brachyspira pilosicoli. Infections by these pathogens, which affect the economics of pig production, can result in mortality, growth rate losses and substantial antibiotic costs. The aim of this study was to assess the current occurrence of B. hyodysenteriae and B. pilosicoli in Polish pig herds. Moreover, associations between the presence of diarrhea or other intestinal pathogens and occurrence of B. hyodysenteriae and B. pilosicoli in pigs were investigated.

Methods: Between January 2017 and August 2019, a total of 401 samples of pig feces from 95 different herds were submitted to the National Veterinary Research Institute of Poland. These samples were obtained from pigs older than 7 weeks. All the received fecal samples were examined for the presence of B. hyodysenteriae, B. pilosicoli and Lawsonia intracellularis by real-time PCR.

Results: For B. pilosicoli, 4.5% (95% CI, 2.5–7.0%) of samples and 13.7% (95% CI, 7.5–22.3%) of herds were positive. Out of 12 samples, B. pilosicoli was detected simultaneously with L. intracellularis, B. hyodysenteriae and B. pilosicoli were detected alone in two samples each. In terms of B. hyodysenteriae, 7.0% of samples (95% CI, 4.7–9.9%) from 18.9% of herds (95% CI, 11.6–28.3%) were positive in real time PCR. The presence of B. hyodysenteriae in fecal samples was associated with the presence of diarrhea in pigs.

Conclusions: This study confirmed that B. pilosicoli infections occur in Polish pig herds, but the prevalence is at a low level and the presence of B. pilosicoli is not associated with the development of diarrhea in pigs. B. hyodysenteriae is still a common cause of diarrhea among pigs from Polish herds.
Keywords
Brachyspira pilosicoli, Brachyspira hyodysenteriae, Lawsonia intracellularis, pigs, intestinal pathogens, enterocolitis, diarrhea
Introduction

The question of routine surveillance to monitor Brachyspira species infections in pigs at local, national and international levels is addressed by experts and authorities (Hampson et al., 2015). The major pathogenic intestinal spirochetes affecting pigs during the growing-finishing stage of production include Brachyspira hyodysenteriae and Brachyspira pilosicoli. B. hyodysenteriae is the cause of swine dysentery (SD) – a severe, enteric disease of pigs characterized by mucoschorrhagic diarrhea and inflammation in the large intestine. B. hyodysenteriae is present worldwide and affects the economics of pig production, resulting in mortality, growth rate losses and substantial antibiotic costs. Another brachyspiral disease with mild colitis and diarrhea is porcine intestinal spirochetosis or porcine colonic spirochetosis (PIS/PCS). B. pilosicoli is the causative agent of PIS/PCS, a disease that implies an important economic cost resulting from reduced growth performance and poor feed conversion (Duhamel, 1998). Most Brachyspira species have a restricted host range, whereas B. pilosicoli colonizes a wide range of hosts, including humans, and has the potential for interspecies transmission. There is also a potential for zoonotic transmission, especially in places where animals and humans live in close proximity, or in people working with intensively farmed pigs or chickens, due to the increased risk of exposure. Some species of the genus Brachyspira, including B. pilosicoli, can cause the disease in humans. There are a few reports on B. pilosicoli-associated human intestinal spirochetosis (HIS) (Hampson, 2018). The subclinical colonization of pigs, with B. pilosicoli is not uncommon and has been detected in several farms (Biksi et al., 2007). On other farms, B. pilosicoli were isolated from diseased pigs as the only causative agent or simultaneously with other enteric pathogens as part of a mixed infection (Reiner et al., 2011; Stege et al., 2000).

Recent changes in the management of pig farms and the movement of pigs within the EU have resulted in a shift in the relative prevalence of pathogenic Brachyspira species. There are very few studies addressing the prevalence of B. hyodysenteriae in pigs in Poland and only one concerning B. pilosicoli (Plawińska et al., 2004). The aim of the study was to assess the current occurrence of B. hyodysenteriae and B. pilosicoli in Polish pig herds. Moreover, associations between the presence of diarrhea or other intestinal pathogens and the occurrence of B. hyodysenteriae and B. pilosicoli in pigs were investigated.

Methods

Fecal samples

Fecal samples used in this study were submitted to the Department of Swine Diseases of the National Veterinary Research Institute (NVRI) for commercial laboratory diagnostics of selected porcine bacterial pathogens. Between January 2017 and August 2019, a total of 401 samples of pig feces were submitted to the NVRI. These samples originated from 95 different Polish pig herds, from pigs older than 7 weeks. All received fecal samples were submitted to the NVRI to be examined for the presence of B. hyodysenteriae and/or Lawsonia intracellularis. At that time, none of the diagnostic tools for B. pilosicoli identification were available for NVRI customers.

Owing to differing reasons for testing submitted fecal samples, three groups were distinguished. The first group of samples were obtained from pigs subjected to routine monitoring of herds free of one or both of the aforementioned pathogens (B. hyodysenteriae, L. intracellularis). The second group was made up of samples from pigs with clinical signs of diarrhea, where B. hyodysenteriae or L. intracellularis was suspected to be a cause of disease. The last group of samples was submitted to the laboratory due to unrecognized pathogen status and a history of diarrhea in the herd.

DNA extraction and PCR

Total genomic DNA was extracted from the fecal samples using a commercial isolation kit (Genomic Mini, A&A Biotechnology, Gdynia, Poland), according to manufacturer’s recommendations. Extracted DNA samples were stored at -20°C until examination. All samples were tested by separate singleplex real-time PCR assays for B. hyodysenteriae, B. pilosicoli and L. intracellularis according to the methods described previously (Stähl et al., 2011; Zmudzki et al., 2012). Primers were obtained from a commercial source (Genomed S.A., Poland).

The sequences of primers and probes are as follows: for B. hyodysenteriae (forward primer: 5'-TATGAAAGGCATGCAGGTTAT-3', reverse primer: 5'-GTGAGGAAGAAATCTGACAAATGCA-3', probe: 5'-FAM-ACACAATCATGCTGAA GC-TAMRA-3') (Akase et al., 2009); for B. pilosicoli (forward primer: 5'-GTAGTGAGGAAACAGCCTT-3', reverse primer: 5'-TTACTCACCACAAGGTTCTCAGG-3', probe: 5'-FAM-TATT CGACGAGGATAACCACATACCT-BHQ-1-3') (Stähl et al., 2011); for L. intracellularis (forward primer: 5'-GGCGCCTAGGTTGTTATAT-3', reverse primer: 5'-GCCACCTCTCCGATACTC A-3', probe: 5'-FAM-CACCGTTCACACCGTGACACCGACCTT-1A MRA-3') (Lindecrona et al., 2002). All assays were carried out using the Rotor-Gene Q real-time PCR system (Qiagen, Hilden, Germany).

Real-time PCR assays were run using a commercially available master mix Quantitect Probe PCR kit (Qiagen, Hilden, Germany). For B. hyodysenteriae, 12.5 μl of the master mix was combined with 0.5 μl of each primer, diluted to 20 μM and 0.5 μl of the probe, diluted to 20 μM and 6 μl of DNase-free water. The DNA template was added at 5 μl per reaction for a total reaction volume of 25 μl. PCR was run, as follows: 95°C for 15 min, followed by 50 cycles at 95°C for 15 secs and 52°C for 1 min. For B. pilosicoli, 12.5 μl of the master mix was combined with 0.5 μl of each primer, diluted to 20 μM and 0.5 μl of the probe, diluted to 10 μM and 8 μl of DNase-free water. The DNA template was added at 3 μl per reaction for a total reaction volume of 25 μl. PCR was run as follows: 95°C for 15 min, followed by 50 cycles of 95°C for 15 sec and 60°C for 1 min. For L. intracellularis, 12.5 μl of the master mix was combined with 0.5 μl of each primer, diluted to 20 μM and 0.5 μl of the probe, diluted to 20 μM and 6 μl of DNase-free water. The
DNA template was added at 5 μl per reaction for a total reaction volume of 25 μl. PCR was run as follows: 95°C for 15 min, followed by 40 cycles of 95°C for 15 sec and 62°C for 1 min.

**Statistical analysis**
A herd was defined as positive when at least one fecal sample taken from the herd had a positive PCR result. Percentages of positive samples/herds with a 95% two-sides exact binominal confidence interval (CI) were reported. Differences in the presence of pathogens between different fecal samples groups were established by a chi-square test (statistically significant at p < 0.05). Pairwise comparisons with Bonferroni corrections of the p-values were performed.

**Results**
Among a total of 401 samples 218 were submitted to the NVRI laboratory for the routine monitoring of pig herds. Of these, 70 samples originated from pigs with the clinical manifestation of diarrhea. The remaining 113 samples originated from herds with a history of diarrhea but of an unknown status, in terms of *Brachyspira* spp. occurrence. Underlying data are available on figshare (Dors et al., 2019).

The overall occurrence of *B. hyodysenteriae* and *B. pilosicoli* in assessed pig herds in Poland is presented in Figure 1. Real-time PCR detected *B. pilosicoli* in 18 samples from pigs in 13 different herds. This means that 4.5% (95% CI, 2.5–7.0%) of samples and 13.7% (95% CI, 7.5–22.3%) of herds were positive for *B. pilosicoli*. Out of 12 samples, *B. pilosicoli* was detected simultaneously with *L. intracellularis*, *B. hyodysenteriae* and *B. pilosicoli* were detected alone in two samples each. In terms of *B. hyodysenteriae*, 7.0% of samples (95% CI, 4.7–9.9%) from 18.9% herds (95% CI, 11.6–28.3%) were positive using real-time PCR.

Differences in the presence of *B. hyodysenteriae* and *B. pilosicoli* in the fecal samples obtained from pigs with diarrhea, from apparently healthy pigs, but originating from herds with a history of diarrhea and from pigs undergoing routine monitoring from herds free of *B. hyodysenteriae* and/or *L. intracellularis* are shown in Table 1.

Additional analyses were completed to compare the influence of *L. intracellularis* infection and the presence of *Brachyspira* spp., in fecal samples. The occurrence of *B. hyodysenteriae* in pigs whose feces was confirmed to be positive for *L. intracellularis* was 7.9%, compared to 7.3% in pigs negative for *L. intracellularis*. However, considering the simultaneous occurrence of *B. pilosicoli* and *L. intracellularis*, we found that the percentage of samples positive for *B. pilosicoli* was significantly higher in pigs simultaneously infected by *L. intracellularis* (10.8%) compared to *L. intracellularis*-negative pigs (2.2%).

**Discussion and conclusions**
The results of this study confirm that *B. pilosicoli* infections occur in Polish pig herds. A previous study reported only one positive sample among 127 samples from 23 pig farms (Plawińska et al., 2004). Our results show that *B. pilosicoli* is present in Polish pig herds, but that the prevalence is low, reaching 13.7% of herds and 4.5% of samples. Notably, considerably higher prevalence of *B. pilosicoli* infection has been detected in other countries, such as Germany (31.6%, Reiner et al., 2011), Denmark (19%, Stege et al., 2000) and Hungary.

![Figure 1](image)

**Figure 1.** The occurrence of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in 401 samples obtained from 95 Polish pig herds.
In our study, we have found that most of the positive samples came from pigs infected at the same time with *L. intracellularis*. Similar findings have been reported in previous studies (Biksi et al., 2007; Jacobson et al., 2003; Jacobson et al., 2005; Merialdi et al., 2003). Therefore, there is a need for further investigation to determine a risk factors and an association between the presence of *B. pilosicoli* in feces and the clinical signs or pig performance.

The occurrence of *B. hyodysenteriae* in our investigation was more common than *B. pilosicoli* and was higher than reported previously (Dors et al., 2015). Current results on the prevalence of *B. hyodysenteriae* could be biased, due to the large number of samples submitted to the NVRI with suspected clinical SD. Nonetheless, SD is still a common cause of diarrhea among pigs from Polish herds, despite improving biosecurity, management and disease control.

### Data availability


This project contains data on detection of infection with each pathogen studied for each sample. 1, yes; 0, no.

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

### Acknowledgments

We wish to express our gratitude to the veterinary practitioners who supplied us with fecal samples.

### References


Roberto M.C. Guedes
Department of Veterinary Clinic and Surgery, Veterinary School, Federal University of Minas Gerais, Belo Horizonte, Brazil

General comments:
This study describes the occurrence of pathogenic spirochetes for swine in Poland herds using routine diagnostic cases submitted to their reference laboratory. As a result, sampling was bias for a prevalence study, so the decision of just describe as occurrence was adequate. The data represents important information for Poland swine production, but not so relevant for the rest of the World. Figure 1 is illustrative but it is a repetition of the data that it is already stated in the text. It seems that qPCR for *B. pilosicoli* was not performed at the arrival of the sample, in contrast to qPCR for *B. hyodysenteriae* and *L. intracellularis*. So, when was the qPCR for *B. pilosicoli* performed?

Specific comments:
It was very difficult to list the modification required in the text as it does not have the lines numbered.

Abstract:
- Results: “…simultaneously with *L. intracellularis*. *B. hyodysenteriae* and *B. pilosicoli* were …”

Suggestion of Key-words: pathogenic spirochetes, Lawsonia intracellularis, swine, intestinal pathogens, enterocolitis, diarrhea.

Results:
- The 4th and 5th sentences of the second paragraph are confusing. Rewrite.
- The 3rd paragraph is too long and confusing. Rewrite.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Yes

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** enteropathogens of pigs

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Author Response 10 Mar 2020

arkadiusz dors,

I would like to begin by thanking the reviewer for the valuable comments. Below we have included our responses to specific comments.

**Figure 1 is illustrative but it is a repetition of the data that it is already stated in the text.**

Figure 1 was removed because of repetition of results described in the text.

**It seems that qPCR for B. pilosicoli was not performed at the arrival of the sample, in contrast to qPCR for B. hyodysenteriae and L. intracellularis. So, when was the qPCR for B. pilosicoli performed?**

Adequate explanation was added to Methods section.

**Abstract:**

Results: “...simultaneously with L. intracellularis. B. hyodysenteriae and B. pilosicoli were ...”

Abstract was adjusted to all changes that were made within the article.

**Suggestion of Key-words:** pathogenic spirochetes, Lawsonia intracellularis, swine, intestinal pathogens, enterocolitis, diarrhea.
We found it difficult to change.

**Results:**

*The 4th and 5th sentences of the second paragraph are confusing. Rewrite.*

*The 3rd paragraph is too long and confusing. Rewrite.*

These sentences was rephrased according to reviewer suggestions.

**Competing Interests:** No competing interests were disclosed.
Please include a conclusion statement, and clearly lay out your main findings.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Veterinary medicine, swine medicine, molecular diagnostic tests, microbiome, transcriptome.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 10 Mar 2020

arkadiusz dors,

I would like to begin by thanking the reviewer for the valuable comments. Below we have included our responses to specific comments.

Methods - Fecal samples - Please include a numeric description of each group. You mention the total number of fecal samples, but not how many came from X many herds, and how many are part of each "submission group".

Number of fecal samples in "submission group" was provided in results section but according to your suggestion we have number also in methods.

Results - How many samples were tested out of the 18 that were found positive for Bpilo? Please include the actual numbers in all your descriptions.

Changed as suggested by reviewer.
Figure 1 - Please include actual n to the data shown. Also italicize scientific names.

Figure 1 was removed because of repetition of results described in the text.

Lawsonia comparison - Please include actual numbers in all the descriptions (n=?). A statistical test to show that samples positive for LI are more likely to be positive with Bpilo would be interesting here, besides the simple description.

Corrected as suggested by reviewer. Necessary explanation has been added to Methods section and p-values were added in the Results.

Discussion - Was is thought that Poland was free of Bpilo? The first sentence is odd.

Sentence was rephrased

Please acknowledge that this data set is inherently biased (at least partially, except for the ones that are routine surveillance but we don’t know how many samples were part of the group...).

In discussion we have mentioned that: “Current results on the prevalence of B. hyodysenteriae could be biased, due to the large number of samples submitted to the NVRI with suspected clinical SD.”

Please include a conclusion statement, and clearly lay out your main findings.

Final conclusions was added at the end of discussion

Competing Interests: No competing interests were disclosed.
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