A survey on the occurrence of *Brachyspira pilosicoli* and *Brachyspira hyodysenteriae* in growing-finishing pigs [version 1; peer review: 1 approved with reservations]

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**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**

*Brachyhsira pilosicoli*, *Brachyspira hyodysenteriae*, *Lawsonia intracellularis*, pigs, intestinal pathogens, enterocolitis, diarrhea
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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 mucus-hemorrhagic 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 was 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 (Pławińska 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 sings 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′-TATGAAGAGGCAGCAGACGTTAT-3′, reverse primer: 5′-GTAGGAAGAAAGAAATCTTGACAATGCA-3′, probe: 5′-FAM-ACACAATCATGTCTGAAGC-TAMRA-3′) (Akase et al., 2009); for B. pilosicoli (forward primer: 5′-GTAGTCGATGGGAAACAGGT-3′, reverse primer: 5′-TTACTCACCACAAGTCTCGG-3′, probe: 5′-FAM-TATTGCACGAGTAAACCATACCT-BHQ-1-3′) (Stähl et al., 2011); for L. intracellularis (forward primer: 5′-GCCCGGTAGGTGTATAT-3′, reverse primer: 5′-GCCACCCCTTCCGATACTC-3′, probe: 5′-FAM-CACCGTCTACCGGACCTT-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 1 sec 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 binomial 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.
Table 1. The differences in the presence of Brachyspira hyodysenteriae and Brachyspira pilosicoli in fecal samples obtained from healthy pigs and pigs with diarrhea.

<table>
<thead>
<tr>
<th>Group of fecal samples</th>
<th>Positive samples, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. hyodysenteriae</td>
</tr>
<tr>
<td>Pig with diarrhea</td>
<td>22.8%*</td>
</tr>
<tr>
<td>Herds with history of diarrhea</td>
<td>1.8%</td>
</tr>
<tr>
<td>Routine monitoring</td>
<td>6.3%</td>
</tr>
</tbody>
</table>

*Statistically significant difference (p<0.05) between sample groups.

(61.3%, Biksi et al., 2007). Therefore, the targeted sampling of pigs from age groups in which detection of this pathogen is most likely and random selection of Polish pig herds is necessary to assess the true prevalence of B. pilosicoli.

An association between B. pilosicoli infections in pigs and the occurrence of diarrhea in this study was not confirmed. Our results are in line with some previous reports (Biksi et al., 2007; Weber et al., 2015), but other authors have demonstrated positive associations between presence of diarrhea and B. pilosicoli detection (Fellström et al., 1996; Stege et al., 2001). It seems that the subclinical colonization of pigs by B. pilosicoli is predominant in pigs, in Poland. Considering the causality of PIS/PCS, other factors causing the development of diarrhea in pigs, besides the B. pilosicoli infection, should be considered. B. pilosicoli colonization and/or disease expression can be influenced by diet (Hopwood et al., 2002; Stege et al., 2001). Moreover, concurrent infection can influence B. pilosicoli colonization and disease manifestation.

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).

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References


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Matheus costa

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I read with great interest this article, that sought to investigate the presence of Bhyo and Bpilo in polish herds. As the authors observed, this is a somewhat challenging goal: both bacterium can survive in the healthy host, thus it's hard to evaluate the meaning of their presence.

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".

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.

Figure 1 - Please include actual n to the data shown. Also italicize scientific names.

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.

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

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...).

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.

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