BRIEF REPORT

REVISED A survey on the occurrence of *Brachyspira pilosicoli* and *Brachyspira hyodysenteriae* in growing-finishing pigs [version 3; peer review: 2 approved, 1 approved with reservations]

Arkadiusz Dors, Ewelina Czyżewska-Dors, Grzegorz Woźniakowski

Department of Swine Diseases, National Veterinary Research Institute, Puławy, 24-100, Poland

v3 **First published:** 30 Sep 2019, **8**:1702
https://doi.org/10.12688/f1000research.20639.1
Second version: 13 Mar 2020, **8**:1702
https://doi.org/10.12688/f1000research.20639.2
Latest published: 01 Apr 2021, **8**:1702
https://doi.org/10.12688/f1000research.20639.3

Abstract

Background: The major pathogenic intestinal spirochetes affecting pigs during the growing- finishing stage of production include *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli*. 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: *B. pilosicoli* was detected in 4.5% (95% CI, 2.5–7.0%) (18/401) of pig fecal samples. At the herd level 13.7% (95% CI, 7.5–22.3%) (13/95) of herds were positive for *B. pilosicoli. B. hyodysenteriae* was detected in 7.0% (95% CI, 4.7–9.9%) (28/401) of pig fecal samples and 18.9% (95% CI, 11.6–28.3%) (18/95) of pig herds were positive. Out of 18 *B. pilosicoli* positive samples, this pathogen was detected alone in 5 samples; simultaneously with *L. intracellularis* in 9 samples; simultaneously with *B. hyodysenteriae* in 1 sample and in 3 samples was detected simultaneously with both of these bacteria. 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.

Open Peer Review

Reviewer Status ? ✓ ✓

	Invited Reviewers		
	1	**2**	**3**
version 3 (revision) 01 Apr 2021			
version 2 (revision) 13 Mar 2020		✓ report	✓ report
version 1 30 Sep 2019	? report	? report	

1. **Matheus costa**, Utrecht University, Utrecht, The Netherlands
University of Minnesota, Minneapolis, USA
2. **Roberto M.C. Guedes**, Federal University of Minas Gerais, Belo Horizonte, Brazil
3. **Francesca Piras**, University of Sassari, Sassari, Italy

Any reports and responses or comments on the article can be found at the end of the article.

Keywords
Brachyspira pilosicoli, Brachyspira hyodysenteriae, Lawsonia intracellularis, pigs, intestinal pathogens, enterocolitis, diarrhea

Corresponding author: Arkadiusz Dors (adors@piwet.pulawy.pl)

Author roles: Dors A: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation; **Czyżewska-Dors E**: Conceptualization, Investigation, Supervision, Writing – Review & Editing; **Woźniakowski G**: Project Administration, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Dors A, Czyżewska-Dors E and Woźniakowski G. **A survey on the occurrence of *Brachyspira pilosicoli* and *Brachyspira hyodysenteriae* in growing-finishing pigs [version 3; peer review: 2 approved, 1 approved with reservations]** F1000Research 2021, **8**:1702 https://doi.org/10.12688/f1000research.20639.3

First published: 30 Sep 2019, **8**:1702 https://doi.org/10.12688/f1000research.20639.1

REVISED **Amendments from Version 2**

In the sentence "Out of 18 *B. pilosicoli* positive samples, this pathogen was detected alone in 5 samples" "this" has been replaced with "the".

In the sentence "The subclinical colonization of pigs, with *B. pilosicoli* is not uncommon and has been detected in several farms." Comma has been removed.

In the sentence "The second group was made up of samples (n=70) from pigs with clinical sings." Correction of "sings" to "signs" has been made.

The sentence "Besides, *B. pilosicoli* tests were performed in July and August 2019." has been rephrased.

Any further responses from the reviewers can be found at the end of the article

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 mucohemorrhagic 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* (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 (n=218) 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 (n=70) 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 (n=113) 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* and *L. intracellularis* immediately after samples submission according to the methods described previously (Zmudzki *et al.*, 2012). Detection of *B. pilosicoli* by real-time PCR was performed in July and August 2019 (Ståhl *et al.*, 2011). 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′-TATGAAGAAGGCAGCAGACGTTTAT-3′, reverse primer: 5′-GTAGGAAGAAGAAATCTGACAATGCA-3′, probe: 5′-FAM-ACACAATCATGCTGAAGC-TAMRA-3′) (Akase *et al.*, 2009); for *B. pilosicoli* (forward primer: 5′-GTAGTC-GATGGGAAACAGGT-3′, reverse primer: 5′-TTACTCAC-CACAAGTCTCGG-3′, probe: 5′-FAM-TATTCGACGAG-GATAACCATCACCT-BHQ-1-3′) (Ståhl *et al.*, 2011); for *L. intracellularis* (forward primer: 5′-GCGCGCGTAGGTGGT-TATAT-3′, reverse primer: 5′-GCCACCCTCTCCGATACTCA-3′, probe: 5′-FAM-CACCGCTTAACGGTGGAACAGCCTT-TAMRA-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 mins, 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 40 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 and association with *L. intracellularis* infection 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).

B. pilosicoli was detected in 4.5% (95% CI, 2.5–7.0%) (18/401) of pig fecal samples. At the herd level 13.7% (95% CI, 7.5–22.3%) (13/95) of herds were positive for *B. pilosicoli*. *B. hyodysenteriae* was detected in 7.0% (95% CI, 4.7–9.9%) (28/401) of pig fecal samples and 18.9% (95% CI, 11.6–28.3%) (18/95) of pig herds were positive.

Out of 18 *B. pilosicoli* positive samples, the pathogen was detected alone in 5 samples; simultaneously with *L. intracellularis* in 9 samples; simultaneously with *B. hyodysenteriae* in 1 sample and in 3 samples was detected simultaneously with both of these bacteria.

Differences in the presence of *B. hyodysenteriae* and *B. pilosicoli* in the fecal samples of various origin (pigs with diarrhea, herds with a history of diarrhea, routine monitoring) 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 7.3% (9/123), compared to 6.8% (19/278) in pigs negative for *L. intracellularis* with no statistical significance (p=0.861). 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* 9.8% (12/123) compared to *L. intracellularis*-negative pigs 2.2% (6/278) (p<0.001).

Table 1. The differences in the presence of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in fecal samples obtained from healthy pigs and pigs with diarrhea.

Group of fecal samples	Positive samples, %	
	B. hyodysenteriae	*B. pilosicoli*
Pig with diarrhea	22.8%*	2.9%
Herds with history of diarrhea	1.8%	1.8%
Routine monitoring	6.3%	6.9%

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

Discussion and conclusions

The results of this study show that *B. pilosicoli* infections occur in Polish pig herds more frequently than it has been thought so far. A previous study reported only one positive sample among 127 samples from 23 pig farms (Pławińska *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 (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 samples positive for *L. intracellularis* are more likely to be positive with

B. pilosicoli. 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.

In conclusion, our study shows 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. Secondly, *B. hyodysenteriae* is still a common cause of diarrhea among pigs from Polish herds. Moreover infection of *L. intracellularis* might be predisposing factor for *B. pilosicoli* occurrence in pigs.

Data availability

Figshare: A survey on the occurrence of *Brachyspira pilosicoli* and *Brachyspira hyodysenteriae* in growing-finishing pigs. https://doi.org/10.6084/m9.figshare.9878612.v1 (Dors *et al.*, 2019).

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

Acknowledgments

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

References

Akase S, Uchitani Y, Sohmura Y, *et al.*: **Application of real time PCR for diagnosis of Swine Dysentery.** *J Vet Med Sci.* 2009; **71**(3): 359–362.
PubMed Abstract | Publisher Full Text

Biksi I, Lőrincz M, Molnár B, *et al.*: **Prevalence of selected enteropathogenic bacteria in Hungarian finishing pigs.** *Acta Vet Hung.* 2007; **55**(2): 219–227.
PubMed Abstract | Publisher Full Text

Dors A, Czyżewska-Dors E, Woźniakowski G: **A survey on the occurrence of Brachyspira pilosicoli and Brachyspira hyodysenteriae in growing-finishing pigs.** *figshare.* Dataset. 2019.
http://www.doi.org/10.6084/m9.figshare.9878612.v1

Dors A, Pomorska-Mól M, Czyżewska E, *et al.*: **Prevalence and risk factors for *Lawsonia intracellularis*, *Brachyspira hyodysenteriae* and *Salmonella* spp. in finishing pigs in Polish farrow-to-finish swine herds.** *Pol J Vet Sci.* 2015; **18**(4): 825–831.
PubMed Abstract | Publisher Full Text

Duhamel GE: **Colonic spirochetosis caused by Serpulina pilosicoli.** *Vet Med Large Anim Prac.* 1998; **19**: 14–22.

Fellström C, Pettersson B, Johansson KE, *et al.*: **Prevalence of Serpulina species in relation to diarrhea and feed medication in pig-rearing herds in Sweden.** *Am J Vet Res.* 1996; **57**(6): 807–811.
PubMed Abstract

Hampson DJ: **The Spirochete *Brachyspira pilosicoli*, Enteric Pathogen of Animals and Humans.** *Clin Microbiol Rev.* 2018; **31**(1): pii: e00087-17.
PubMed Abstract | Publisher Full Text | Free Full Text

Hampson DJ, La T, Phillips ND: **Emergence of *Brachyspira* species and strains: reinforcing the need for surveillance.** *Porcine Health Manag.* 2015; **1**: 8.
PubMed Abstract | Publisher Full Text | Free Full Text

Hopwood DE, Pethick DW, Hampson DJ: **Increasing the viscosity of the intestinal contents stimulates proliferation of enterotoxigenic *Escherichia coli* and *Brachyspira pilosicoli* in weaner pigs.** *Br J Nutr.* 2002; **88**(5): 523–532.
PubMed Abstract | Publisher Full Text

Jacobson M, Gerth Löfstedt M, Holmgren N, *et al.*: **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.* 2005; **52**(9): 386–391.
PubMed Abstract | Publisher Full Text

Jacobson M, Hård af Segerstad C, Gunnarsson A, *et al.*: **Diarrhoea in the growing pig - a comparison of clinical, morphological and microbial findings between animals from good and poor performance herds.** *Res Vet Sci.* 2003; **74**(2): 163–169.
PubMed Abstract | Publisher Full Text | Free Full Text

Lindecrona RH, Jensen TK, Andersen PH, *et al.*: **Application of a 5' nuclease assay for detection of *Lawsonia intracellularis* in fecal samples from pigs.** *J Clin Microbiol.* 2002; **40**(3): 984–987.
PubMed Abstract | Publisher Full Text | Free Full Text

Merialdi G, Bonilauri P, Granelli F, *et al.*: **Bacterial pathogens in field cases of clinical colitis in growing and finishing pigs in Italy.** *Vet Rec.* 2003; **153**(17): 529–530.
PubMed Abstract | Publisher Full Text

Pławińska J, Jakubowski T, Rzewuska M, *et al.*: **Occurrence of *Lawsonia Intracellularis* and *Brachyspira* spp. infection in swine suffering from diarrhoea.** *Pol J Vet Sci.* 2004; **7**(3): 203–206.
PubMed Abstract

Reiner G, Hillen S, von Berg S, *et al.*: **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.* 2011; **124**(5–6): 236–241.
PubMed Abstract | Publisher Full Text

Ståhl M, Kokotovic B, Hjulsager CK, *et al.*: **The use of quantitative PCR for identification and quantification of *Brachyspira pilosicoli*, *Lawsonia intracellularis* and *Escherichia coli* fimbrial types F4 and F18 in pig feces.** *Vet Microbiol.* 2011; **151**(3–4): 307–314.
PubMed Abstract | Publisher Full Text

Stege H, Jensen TK, Møller K, *et al.*: **Risk factors for intestinal pathogens in Danish finishing pig herds.** *Prev Vet Med.* 2001; **50**(1–2): 153–164.
PubMed Abstract | Publisher Full Text

Stege H, Jensen TK, Møller K, *et al.*: **Prevalence of intestinal pathogens in Danish finishing pig herds.** *Prev Vet Med.* 2000; **46**(4): 279–292.
PubMed Abstract | Publisher Full Text

Weber N, Nielsen JP, Jakobsen AS, *et al.*: **Occurrence of diarrhoea and intestinal pathogens in non-medicated nursery pigs.** *Acta Vet Scand.* 2015; **57**: 64.
PubMed Abstract | Publisher Full Text | Free Full Text

Zmudzki J, Szczotka A, Podgórska K, *et al.*: **Application of real-time PCR for detection of *Lawsonia intracellularis* and *Brachyspira hyodysenteriae* in fecal samples from pigs.** *Pol J Vet Sci.* 2012; **15**(2): 267–273.
PubMed Abstract | Publisher Full Text

Open Peer Review

Current Peer Review Status: ? ✓ ✓

Version 2

Reviewer Report 23 March 2021

https://doi.org/10.5256/f1000research.25154.r81218

✓ **Francesca Piras**
Department of Veterinary Medicine, University of Sassari, Sassari, Italy

The paper “A survey on the occurrence of Brachyspira pilosicoli and Brachyspira hyodysenteriae in growing-finishing pigs” evaluate occurrence of two pathogenic intestinal spirochetes in finishing pigs herd.
The presented manuscript is well written and sound.
I have just a few comments:

- **Out of 18 B. pilosicoli positive samples, this pathogen was detected alone in 5 samples.** Change “this” with “the”.
- **The subclinical colonization of pigs, with B. pilosicoli is not uncommon and has been detected in several farms.** The sentence is not clear, delete comma.
- **The second group was made up of samples (n=70) from pigs with clinical sings.** Correct sings with signs.
- **Besides, B. pilosicoli tests were performed in July and August 2019.** The sentence is not clear.

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?
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: Microbiology, food safety, enteric pathogens

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.

Author Response 26 Mar 2021

arkadiusz dors,

I would like to thank the Reviewer for all the comments to our article. I included all the amendments and suggestions in the new version of the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Report 23 March 2020

https://doi.org/10.5256/f1000research.25154.r61312

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

I believe now the manuscript is adequate for indexing!

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?
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.

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.

Version 1

Reviewer Report 05 December 2019

https://doi.org/10.5256/f1000research.22699.r57051

?

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.

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.

Reviewer Report 26 November 2019

https://doi.org/10.5256/f1000research.22699.r55873

?

Matheus costa
[1] Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht University, Utrecht, The Netherlands
[2] University of Minnesota, Minneapolis, USA

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.

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.