First molecular detection of *Brachyspira suanatina* on pig farms in Poland

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Abstract

Introduction: Prior to the 2000s, swine dysentery was considered to be caused only by *Brachyspira hyodysenteriae* with contributing commensal intestinal anaerobes. Nowadays, it is known that the disease is caused by three strongly beta-haemolytic species of the anaerobic spirochaetal genus *Brachyspira*, *i.e. B. hyodysenteriae* and newly emerged *B. hampsonii* and *B. suanatina*. **Material and Methods:** The present investigation was carried out in November 2022 on nine Polish high-performing finisher pig farms. At every location one fresh pooled faecal sample was collected from 40 randomly selected pigs of between 60 and 110 kg live weight. Nucleic acid extracted from each pooled faecal sample was analysed by an in-house multiplex PCR for *Brachyspira* spp., which is capable of confirming the *Brachyspira* genus and detecting and differentiating *Brachyspira* species. **Results:** From a total of nine samples examined, the genetic material of *B. suanatina* was detected in seven. Non-pathogenic/questionably pathogenic *Brachyspira* spp. were found in six samples. **Conclusion:** To the best of our knowledge, this is the first report on the identification of *B. suanatina* in pigs outside Scandinavia, Germany and the United Kingdom. Our research not only provides valuable epidemiological data on *B. suanatina* infection in Europe but also highlights both the importance of modern laboratory diagnostics and the need for thorough investigation across regions, including retrospective studies.

Introduction

Swine dysentery (SD) is a severe mucohaemorrhagic diarrhoeal disease spreading *via* the faecal–oral route. The first experimental reproduction of SD was published in Indiana, USA in 1921 (11). Its causative agent, the bacterium *Brachyspira hyodysenteriae* (originally named *Treponema hyodysenteriae*) was identified almost simultaneously by two independent teams of researchers who successfully fulfilled Koch's postulates in conventional pigs in the early 1970s (11, 35). Regardless of the substantial effect of SD on the pig sector worldwide and the undertaking of several attempts to elucidate the complex mechanisms underlying the disease, its exact pathogenesis remains incompletely understood.

Traditionally, the infection has been thought to be transmitted mainly by direct contact between pigs (11); nevertheless, numerous research works have revealed that other species can also be involved in the carriage of the pathogen. Among others, the carriers are rodents (2), insects (3), feral pigs (27), and some species of domestic and wild birds (10, 18, 19). *Brachyspira hyodysenteriae* is able to persist for a long period of time in the environment (up to 112 days in porcine faeces at 10°C) (4). The length of the incubation period is variable, but usually ranges between 10 and 14 days. Infection provides a varying degree of protection. Moreover, untreated pigs that have recovered from SD can shed the pathogen asymptomatically for 70 to 90 days.

Swine dysentery is characterised by high morbidity and variable mortality. Once ingested, the bacterial agent of SD survives in the highly acidic gastric environment, passes through the small intestine, and eventually colonises the colonic and caecal crypts, which results in the development of mucohaemorrhagic colitis (11). The clinical manifestation of SD varies from moderate mucoid to bloody diarrhoea depending on the virulence of the isolate (36), the composition of the offered diet (9, 33), and environmental conditions in the wide sense (11).

Gross pathological changes typical of the disease are (except for signs accompanying dehydration) limited to the large intestine, and include mesocolonic oedema and rugose mucosa covered diffusely or in patches by fibrin, mucus, and flecks of blood (38). Microscopically, depending on the stage and severity of the disease, the most pronounced pathological lesions are mostly restricted to the large intestine, *i.e.* the caecum and/or colon, and involve erosion of the luminal epithelium, crypt hyperplasia, goblet cell hyperplasia, and blood congestion (17).

Uncontrolled development of profuse SD diarrhoea in affected herds results in substantial financial losses as a consequence of acutely depressed feed conversion and variable mortality (11). Besides which, the disease may ignite animal welfare concerns. Despite extensive research conducted on orally administered attenuated vaccines (34), bacterins (37), and recombinant vaccines (6), effective commercial prophylaxis against SD is not available yet. Consequently, *B. hyodysenteriae* infection needs to be controlled by antibiotic treatment (11). The escalating costs of high antimicrobial usage, tight restrictions on selling animals to some locations, and a considerable risk of violations of rules governing antimicrobial treatments collectively impel attempts towards complete eradication of the disease. Accordingly, a wide variety of measures applied in SD eradication plans have been thoroughly described in scientific and trade journals. Nevertheless, all the strategies, which are based on total or partial depopulation, heavy medication followed by outbreak-specific management and internal biosecurity measures, or a combination of all of these still have widely variable success rates in the long run (25).

Amongst all the virulence factors of *Brachyspira* spp. which have been characterised until now, those with haemolytic activity seem to be of the utmost importance in SD pathogenesis (11). Hence, historically, what sufficed for diagnosis was an appropriate clinical manifestation and characteristic necropsy findings followed by a successful blood agar anaerobic culture of a strongly beta-haemolytic, *i.e.* ring-phenomenon-positive, spirochete from affected intestine tissue or faeces excreted by diseased animals. More recently, a broad range of modern molecular methods have been successfully incorporated into the laboratory diagnosis of SD: bacteria species identification with PCR assays targeting the *nox* gene, 16S rRNA or 23S rDNA; other DNA-based typing methods such as restriction fragment length polymorphism analysis; or matrix-assisted laser desorption/ionisation–time-of-flight mass spectrometry.

Prior to the 2000s, SD was considered to be caused only by *B. hyodysenteriae*, with an essential, yet obscure, contribution made by commensal intestinal anaerobes (23). Nowadays, it is known that the disease is caused by the colonisation of the caecum and colonic epithelium by three Gram-negative, motile, strongly beta-haemolytic and fastidious species of the anaerobic spirochaetal genus *Brachyspira*. The most vital enteropathogenic spirochete of these three, and the one of which the biology and devastating impact on the economy of global swine production have been thoroughly studied, is *B. hyodysenteriae*, and *B. hampsonii* and *B. suanatina* are the two newly emerged other species. The newer species are thought to cause disease clinically indistinguishable from cases of SD in growing pigs (5, 11, 29).

Brachyspira suanatina was originally identified and characterised in wild ducks (*Anas platyrhynchos*) sampled in southern and south-eastern Sweden in the early 2000s (18). In the same period of time, three isolates were recovered from material collected from one Danish piglet-producing herd and on three Swedish farms comprising two which fattened pigs and one which produced piglets. Their enteropathogenic potential was successfully proven using a conventional porcine model in 2007 (29). Based on a thorough investigation involving DNA–DNA hybridisation and whole genome comparisons, *B. suanatina* was described as a unique species in 2015 (24) and validly published one year later (26). The first isolation of *B. suanatina* outside Scandinavia took place in 2017 in Germany. The samples were collected from diarrhoeic pigs reared in outdoor climate barns (31). The very first report demonstrating an occurrence of the pathogen in pigs farmed outside continental Europe was published by the Animal and Plant Health Agency in the UK in February 2022. The *B. suanatina*-positive samples were collected in England from 10-week-old diarrhoeic pigs (1).

Similar diagnostic investigation of another novel strongly haemolytic *Brachyspira* sp., *i.e. B. hampsonii*, started in the mid-2000s in Canadian and US American pig herds suffering from mucohaemorrhagic diarrhoea. Isolation of high levels of *Brachyspira* spp. not related to *B. hyodysenteriae* eventually resulted in a molecular and phenotypic characterisation of *B. hampsonii* in 2012 (5); however, recent epidemiological exploration of archived Canadian samples pushed the date of its first appearance on North American swine farms back to 2002 (15). To date in Europe, *B. hampsonii* has been reported in Belgium (in pigs imported from the Czech Republic) (21) and in Germany (in pigs transported from Belgium) (30).

The wide distribution and sharply varying prevalence of *B. hyodysenteriae* has been described in several studies from most pig-rearing regions of the world (8) and extensive data has been compiled on avirulent or weakly virulent strains (13) and those presenting markedly lower antimicrobial susceptibility (14). Reports of the prevalence of newly emerged strongly beta-haemolytic *Brachyspira* spp. are contrastingly scarce. Indeed, peer-reviewed publications on infections with *B. hampsonii* and *B. suanatina* in pigs are exceptionally rare. Therefore, this study aimed to indicate the presence of *B. suanatina* and *B. hampsonii* in faecal samples collected from diarrhoeic finishing pigs reared under farm conditions on modern high-performing operations located in Poland.

Material and Methods

Farm characteristics. The present investigation was carried out in November 2022 on nine Polish high-performing finisher farms (with 7,000–21,000 animals) belonging to the same pig producer. All the sampled pigs were born on one of six sow farms (1,500–5,000 DanBred sows in each), weaned after four weeks of lactation in weekly batches and transported to the assigned weaner farm. After another seven to eight weeks and after reaching an average weight of 28.5 kg, the pigs were moved to one of the finisher farms (Table 1). At all of the stages of the three-phase production system, pigs were reared on slats under conditions meeting the legal welfare requirements of Council Directive 2008/120/EC of 18 December 2008, laying down the minimum standards for the protection of pigs.

The finishers reared on farm F5 were fed with liquid feed based on maize silage offered by a wet feeding system three times a day. The levels of crude protein, fat and fibre in the wet feed were 13.8%, 2.3% and 2.8%, respectively. On the other farms the finishers were offered uninterrupted access to a cereal-based dry diet. The feed was dry-steam conditioned and formulated into 4×25 mm cylindrical pellets. The levels of crude protein, fat and fibre in the feed were 16.5%, 3.3% and 3.1%, respectively.

In all locations the all-in/all-out system and strict biosecurity rules were implemented, including compulsory shower in/shower out, dedicated staff, feed mills and transportation services, rodent control programmes, security cameras, fencing, inlets fitted with protective mesh, and purchase of animals from the same source.

Health status of the animals. All the farms enrolled in the study maintained pigs with similar health statuses. The finishers were *Actinobacillus pleuropneumoniae*-negative, *Mycoplasma hyopneumoniae*-positive, toxigenic *Pasteurella multocida*-negative, *B. hyodysenteriae*-negative, *B. hampsonii*-negative, transmissible gastroenteritis virus (TGEV)-negative, and porcine epidemic diarrhoea virus (PEDV)-negative.

Farm F8 was porcine reproductive and respiratory syndrome virus-positive and its stock was vaccinated on entry using Unistrain PRRS (Hipra, Amer, Spain).

All the sampled pigs were vaccinated orally against intestinal lesions caused by *Lawsonia intracellularis* infection using Enterisol Ileitis (Boehringer Ingelheim Vetmedica, Ingelheim am Rhein, Germany) at the age of eight weeks. Intradermal vaccination against porcine circovirus diseases and mycoplasmal pneumonia was given after weaning (Mhyosphere PCV ID; Hipra, Amer, Spain).

Neither oral nor parenteral antibiotic medication was administered to any sampled pigs except the finishers reared on farms F4 and F6, throughout the period between their entering the location (11th or 12th week of life) and the day of sampling. On every farm approximately 15% of the animals defecated abnormal stools without the cause of this having any substantial effect on the affected animals' body conditions.

Sample collection. At every location one fresh pooled faecal sample was collected by a veterinarian from 40 randomly selected pigs (between 60 and 110 kg live weight, 17 to 24 weeks of age) defecating abnormal, mushy stools only. Each sample containing approximately 120 mL of faecal matter was collected into a sterile screw-cap specimen jar using a plastic spoon and then allowed to cool. All the samples were transported overnight to IVD Gesellschaft für Innovative Veterinärdiagnostik mbH (IVD, Seelze-Letter, Germany) and processed on the following day using the laboratory methods specified in the following descriptions.

Parasitological examination. For the detection of parasite stages, approximately 20 g of pooled faeces sample was examined using the combined sedimentation and flotation method (7). For the detection of oocysts of *Cryptosporidia*, thin faecal smears were prepared, stained with carbolfuchsin according to Heine and finally examined microscopically (7).

Detection of *Salmonella*. The faecal samples were cultured for salmonellae according to DIN EN ISO 6579. For further differentiation of isolated *Salmonella* spp., a single colony was resuspended in 200 μL of phosphate-buffered saline and its DNA was extracted using a MagMAX system (MagMAX Pathogen RNA/DNA Kit and MagMAX Express-96 magnetic particle processor; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The nucleic acid was then analysed in an in-house multiplex PCR developed by the IVD. This reaction was carried out to confirm the species *Salmonella enterica* based on the *invA-targe* gene (22) with the enhanced green fluorescent protein plasmid (16) as an internal PCR control and to identify the serovars Choleraesuis (target gene: sopB; GenBank accession number AE017220) and Typhimurium (target gene: type II restriction enzyme; GenBank accession number KP763723.1).

Table 1. Farm descriptions

Finisher farm	Farm capacity (thousand)	Source of animals (weaner farm)	Type of feed	Finisher farm location (province)
F1	19	W1	pelleted	West Pomeranian
F2	9	W2	pelleted	Pomeranian
F3	13	W3	pelleted	West Pomeranian
F4	12	W3	pelleted	West Pomeranian
F5	14	W4	liquid	West Pomeranian
F6	11	W2	pelleted	West Pomeranian
F7	7	W5	pelleted	West Pomeranian
F8	21	W6	pelleted	Greater Poland
F9	10	W2	pelleted	West Pomeranian

Table 2. Targets for detection and differentiation of *Brachyspira* species by PCR

Detection of	Target gene	Reference
Brachyspira genus	50S ribosomal protein L4 (461 bp amplicon, mix 1)	CP0196*
B. hyodysenteriae	Bh100 (194 bp amplicon)	(32)
B. suanatina	hypothetic protein (248 bp amplicon; mix 2)	CVLB01000001*
B. hampsonii	oxidoreductase (313 bp amplicon; mix 2)	CP019914*
B. pilosicoli	methyl-accepting chemotaxis protein B (128 bp amplicon; mix 1)	CP002025*
B. murdochii	hypothetic protein (362 bp amplicon; mix 2)	CP001959*
B. intermedia	hypothetic protein (202 bp amplicon; mix 1)	CP002874*
B. innocens	ribulokinase (259 bp amplicon; mix 1)	ARQI01000130*

* – GenBank Database (www.ncbi.nlm.nih.gov/genbank/)

Table 3. Detection of different enteric pathogens in the study

Pathogen		Analysis	F1	F2	F3	F4	F5	F6	F7	F8	F9
Brachyspira spp.	*Brachyspira* genus	PCR	+	+	+	+	+	+	+	+	+
	B. hyodysenteriae	PCR	–	–	–	–	–	–	–	–	–
	B. suanatina	PCR	+	+	+	–	+	–	+	+	+
	B. hampsonii	PCR	–	–	–	–	–	–	–	–	–
	B. pilosicoli	PCR	–	+	+	+	–	–	–	–	+
	B. murdochii	PCR	–	+	–	–	+	+	–	+	+
	B. intermedia	PCR	+	–	–	–	–	–	–	–	–
	B. innocens	PCR	–	–	–	–	–	–	–	–	–
Salmonella spp.	*Salmonella* sp.	Culture	–	–	SE	–	–	SE	SE	SE	–
	Salmonella sp.	PCR	n/a	n/a	+	n/a	n/a	+	+	+	n/a
	Serovar Choleraesuis	PCR	n/a	n/a	–	n/a	n/a	–	+	–	n/a
	Serovar Typhimurium	PCR	n/a	n/a	–	n/a	n/a	–	–	–	n/a
Viruses	TGEV	PCR	–	–	–	–	–	–	–	–	–
	PEDV	PCR	–	–	–	–	–	–	–	–	–
Parasites	Helminth eggs, encysted protozoa	Sedimentation and flotation	–	–	–	–	–	–	–	–	–
	Cryptosporidia	Heine staining	–	–	–	–	–	–	–	–	–

F1 etc. – finishing farm 1 etc.; + – positive; − – negative; SE – *Salmonella enterica*; TGEV – transmissible gastroenteritis virus; PEDV – porcine epidemic diarrhoea virus; n/a – not applicable

Direct detection and differentiation of PEDV, TGEV and *Brachyspira* spp. by PCR. For the direct detection of further pathogens by PCR, nucleic acid was extracted from 0.5 g of each pooled faecal sample using the MagMAX system. Nucleic acid was analysed by in an in-house multiplex PCR developed by the IVD for PEDV and TGEV and also for *Brachyspira* spp. in an in-house PCR developed by the IVD which can confirm isolates' affiliation to the *Brachyspira* genus and detect and differentiate the *Brachyspira* species listed in Table 2.

Results

Out of the total of nine samples examined, seven were detected to contain the genetic material of *B. suanatina*. This bacterium was in material collected from farms F1–F3, F5 and F7–F9 (Table 3). *Brachyspira pilosicoli* was detected in four samples from farms F2–F4 and F9. The co-occurrence of these two bacteria was confirmed in three samples, farms F2, F3 and F9 being their places of origin. *Salmonella* Choleraesuis was identified in one sample from farm F7 out of four *S. enterica*-positive samples from farms F3 and F6–F8. Non-pathogenic/questionably pathogenic *Brachyspira* spp. were found in six samples: *B. murdochii* and *B. intermedia* were respectively detected in five samples taken on farms F2, F5, F6, F8 and F9 and one sample brought from farm F1. All the collected samples were *B. hyodysenteriae*-, *B. hampsonii*-, PEDV- and TGEV-negative. Helminth eggs, encysted protozoa and *Cryptosporidia* were also not detected.

Discussion

This publication is the very first report on *B. suanatina* detection on pig farms in Poland. To date, the isolation of the aforementioned bacterium from swine has been reported in samples collected in Sweden (18), Denmark (29), Germany (31), and the United Kingdom (1). The available research reported *B. suanatina* in waterfowl and indicated its role in the most proximate cases of infection reported in outdoor pigs reared in Germany (31); however, this particular type of link between free-living carriers and farm pigs can be entirely excluded from having had any significance in our study. There could have been no such transmission because the sampled animals originated from one integrated production system with strict biosecurity rules preventing all contact with other animals, including migratory birds, and/or contact with their natural habitats. Although in view of the strong environmental persistence of other pathogenic *Brachyspira* spp., transportation of *B. suanatina* from remote locations *via* other vectors could have been possible (4, 11), in the context of the biosecurity enforced on the farms sampled, both biological and mechanical carriage remain purely hypothetical.

Unfortunately, we are not able to definitely prove which route was the route of *B. suanatina* transmission to the Polish finisher farms; however, taking into account the clinical picture described and the history of *B. hampsonii* in North American swine herds (specified in an epidemiological study using archived samples) (15), *B. suanatina* was more likely to have entered the sampled locations with asymptomatic individuals. In other words, the pathogen was highly likely to have been circulating within some European pig herds and eventually spread to others as an undesirable effect of the globalisation of swine production. Even though asymptomatic passage reasonably explains its detection in Poland, further research aimed at identification of potential factors triggering disease outbreaks is of great importance.

Detailed assessment of the clinical relevance of *B. suanatina* infection in finishing pigs sampled during the investigation was beyond the scope of our study. Scientific reports describing its significance in disease development are infrequent and focused on experimental exposure only (29); neither the specific dose required in natural infection nor factors affecting its manifestation have been defined hitherto. With little knowledge of the disease course of *B. suanatina* infection contained in the literature and no determinants proved or disproved, it could be contended that some environmental and/or nutritional factors could have affected the clinical presentation, as this has already been described in *B. hyodysenteriae* infection (9, 11, 17, 28, 33). In a similar way, it might also be attributed to either the concurrence of infections with atypical strains or to abstruse interactions with other bacteria, both typical of *Brachyspira* spp. (11, 23). No peer-reviewed articles describing successful reproduction of clinical symptoms in gnotobiotic pigs after exposure to the three causative agents of SD separately have yet been published.

Furthermore, the diversity of the group comprising the bacterial genus *Brachyspira*, with its several unofficial and unrecognised species (13), may prompt an erroneous conclusion about the aetiology of the manifested disease. Even though a few weakly beta-haemolytic species colonising specialised niches in the digestive system of the pig have been identified so far (*B. innocens*, *B. intermedia* and *B. murdochii*) (11), none of those bacteria have been associated with the clinical presentation or pathological alterations typical of SD in the target species under field conditions (2, 12, 20).

The only weakly beta-haemolytic species detected in our study of which the role in the development of a disease in swine is certain is *B. pilosicoli*. This is an agent causing porcine intestinal spirochaetosis, a disease characterised by a milder colitis, loss of body condition, and low or no mortality (11). Nevertheless, the concurrence of the pathogens identified in each sample analysed in our study (including contagious agents which potentially could have exacerbated the outcomes of *B. suanatina* infection), is not diagnostically informative because the prevalence of diarrhoeic pigs and development of clinical symptoms were the same

in all the sampled herds, including *B. suanatina*-positive and *B. pilosicoli*-negative, *B. suanatina*- and *B. pilosicoli*-positive, and *B. suanatina*-negative but *B. pilosicoli*-positive cohorts. It must be borne in mind that the presented data were established applying qualitative methodology. The potential role of concurring intestinal bacteria deserves deeper examination using quantitative analysis amongst affected individuals.

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