Identification of weakly haemolytic *Brachyspira* isolates recovered from pigs with diarrhoea in Spain and Portugal and comparison with results from other countries

J. Osorio[a], A. Carvajal[a], G. Naharro[a], P. Rubio[a], T. La[b] , N.D. Phillips[b], D.J. Hampson[b]

[a]Animal Health Department, Faculty of Veterinary Sciences, University of León, León 24071, Spain

[b]School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

Abstract

Weakly haemolytic anaerobic intestinal spirochaetes of the genus *Brachyspira* are commonly identified based on species-specific gene sequences. Apart from the pathogenic *Brachyspira pilosicoli*, the distribution and disease associations of the other weakly haemolytic *Brachyspira* species in pigs have not been comprehensively investigated. In this study weakly haemolytic *Brachyspira* isolates (*n* = 67) from Spanish and Portuguese pigs with diarrhoea, negative in a routine diagnostic PCR for *B. pilosicoli*, were identified by sequencing their NADH oxidase genes (*nox*). Nearly half the isolates were identified as *Brachyspira murdochii* (*n* = 31; 46.3%). The others were *Brachyspira innocens* (*n* = 26; 38.8%), *Brachyspira intermedia*(*n* = 7; 10.4%), "*Brachyspira pulli*" (*n* = 1; 1.5%) and a potentially novel *Brachyspira* species (*n* = 2; 3%). Multilocus sequence typing (MLST) on a subset of 18 isolates confirmed their species designations, including the potential new species, and identified similarities to strains from other countries.

Keywords; Pig; *Brachyspira; Nox;* MLST; Spain; Portugal

1. Introduction

The genus *Brachyspira* comprises seven official and several provisionally named species of anaerobic intestinal spirochaetes. The three strongly haemolytic species *Brachyspira hyodysenteriae*, "*Brachyspira suanatina*" (Råsbäck et al., 2007a) and "*Brachyspira hampsonii*" (Chander et al., 2012) are porcine pathogens, causing colitis, whilst the weakly haemolytic species vary in their pathogenic potential. *Brachyspira pilosicoli* is a pathogen, being the agent of porcine intestinal spirochaetosis (Trott et al., 1996), *Brachyspira murdochii* has been associated with mild colitis and diarrhoea (Weissenböck et al., 2005; Komarek et al., 2009; Jensen et al., 2010; Hammer and Gebhart, 2013), *Brachyspira intermedia* is of uncertain clinical significance in pigs, and *Brachyspira innocens* generally has been considered to be non-pathogenic (Hampson, 2012). Recently, pigs colonized with one or more of any of these four weakly haemolytic species were shown to have a 5% increase in colonic thickness, and impairment of the colonic mucosa (Fjelkner et al., 2012) and hence interest is refocusing on weakly haemolytic species as potential pathogens.

Biochemical schemes have been used for identifying *Brachyspira*isolates (Fellström et al., 1995), but there are limited species-specific phenotypic markers available and "atypical" isolates occur (Thomson et al., 2001; Hampson, 2012). Consequently other identification methods have been developed based on the sequence of conserved genes, notably those encoding 16S rRNA, 23S rRNA

and NADH oxidase (*nox*). Analysis of *nox* sequences has emerged as a robust method for identification of *Brachyspira*species as the gene is relatively conserved but also shows species-specific variation (Atyeo et al., 1999; Burrough et al., 2012; Chander et al., 2012; Rubin et al., 2013). A more discriminatory multilocus sequence typing (MLST) scheme analyzing the sequence of seven genes encoding "housekeeping" enzymes has been described for identification and typing of *Brachyspira* isolates (Råsbäck et al., 2007b), but it is laborious and has not been widely used apart from analysis of *B. hyodysenteriae* (La et al., 2009; Osorio et al., 2012) and other indole-positive species (Phillips et al., 2010).

B. hyodysenteriae and *B. pilosicoli* have been reported infecting pigs in Spain (Carvajal et al., 2006), but no data is available about other *Brachyspira* species. This study aimed to improve knowledge about the identity and diversity of weakly haemolytic *Brachyspira* species in pig farms in the Iberian Peninsula.

2. Materials and methods

2.1. Spirochaete isolates

Sixty-seven unidentified weakly haemolytic *Brachyspira* isolates, five *B. hyodysenteriae* and three *B. pilosicoli* isolates were obtained from the University of León culture collection. They originated from diagnostic porcine faecal samples that had been subjected to selective anaerobic culture for *Brachyspira* species, and had been tested by PCR for *B. hyodysenteriae* and *B. pilosicoli* (Carvajal et al., 2006). The unidentified weakly haemolytic isolates were chosen based on their availability as representatives from throughout the Iberian Peninsula, being obtained from nine of 15 Spanish autonomous regions (60%) and from 23 of 48 provinces (48%). Eight isolates (12%) were from Portugal. The isolates came from pigs affected with diarrhoea on 57 farms between 2001 and 2009. The isolate names, origins and isolation dates are presented in Supplementary Table 1. Most came from commercial white pigs, but eight (12%) were from Iberian pigs, an indigenous rustic breed traditionally reared in extensive units. Single isolates were used from most farms, but two were analysed for each of eight farms, and three from one farm (Table 1). The isolates were cultured and DNA was extracted as previously reported (Osorio et al., 2012).

All isolates were used for *nox* sequencing. For comparative analysis, *nox* sequences for 39 other *Brachyspira* isolates with a minimum of 855 base pairs (bp) of sequence available were obtained from GenBank (Table 2). They belonged to each available *Brachyspira* species, except "*B. corvi*", and included others currently not assigned to any *Brachyspira* species.

For comparative purposes 18 randomly selected weakly haemolytic isolates were subjected to MLST (Table 3). The sequences were compared with data from PubMLST (http://pubmlst.org/brachyspira/) for 66 other *Brachyspira* isolates analysed by Råsbäck et al. (2007b). Data for these 84 isolates/strains were used for the global analysis. Sequence data for a subset of 36 of the 66 isolates, together with more recently deposited data for 14 isolates belonging to each of the 14 groups of spirochaetes positive in a PCR for *B. intermedia* (Phillips et al., 2010), including P280/1, a representative of the proposed "*B. hampsonii*" (Chander et al., 2012), the weakly haemolytic Spanish and Portuguese isolates and three Spanish *B. hyodysenteriae*isolates were used for drawing a dendrogram (71 isolates in total; see Supplementary Table 2). These included type/reference strains and at least two well characterized isolates for the six officially named species (and "*B. suanatina*") colonizing pigs. Moreover, all isolates not designed to any described *Brachyspira*species by MLST, without sequences missing, were included.

2.2. Nox sequencing

PCR amplification was in 60 µl reaction mixtures with *Taq* DNA polymerase (Invitrogen) using the previously described primers and protocol (Atyeo et al., 1999; Townsend et al., 2005). Positive and negative controls were *B. hyodysenteriae* WA1^R and ultrapure water respectively. Conditions were 95 °C for 2 min, then 33 cycles at 95 °C for 30 s, 50ºC for 15 s, and 72 °C for 1 min, an extension period of 5 min at 72 °C and cooling to 10 °C. Products were purified with the AxyPrep™ PCR Clean-up Kit (Axygen Scientific) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit with the 3730 sequencing system (Applied Biosystems) using the same primers. Results were analysed and assembled using the ContigExpress component of VectorNTI Advanced 10 (Invitrogen).

Nox sequences were aligned over 855 bp from position 368 to 1222 (*B. hyodysenteriae* strain B204^R numbering), and used to generate a tree with the neighbour-joining method in MEGA version 4.0 (Tamura et al., 2007). Nucleotide sequences were translated to predicted amino acid sequences using BioEdit version 7.0.9.0 (Hall, 1999).

2.3. Multilocus sequence typing (MLST)

MLST was conducted as previously described (Råsbäck et al., 2007b). The genes analysed encoded alcohol dehydrogenase (*adh*), alkaline phosphatase (*alp*), esterase (*est*), glutamate dehydrogenase (*gdh*), glucose kinase (*glp*K), phosphoglucomutase (*pgm*), and acetyl-CoA acetyltransferase (*thi*).

The initial analysis was on the data from the selected Spanish and Portuguese isolates, and subsequently combined with the data from PubMLST. Alignments for each locus identified identical sequences, and unique sequences were assigned a unique allele number. The allelic profile for each isolate was a line listing the allele number for each locus in turn. Isolates were assigned a sequence type (ST) according to their allelic profiles. Unique amino acid types (AATs) predicted from the sequences were recorded. A dendrogram was constructed from the data matrix of allelic mismatches using START2 (Jolley et al., 2001), applying the unweighted pair group method with averages. Dendrograms for each of the amplified loci were independently constructed, to compare topographies.

Allele sequences for each ST also were concatenated using Geneious Pro version 3.8.5 (http://www.geneious.com/) in the order *pgm*, *est*, *glp*K, *gdh* and *thi* (2930 nucleotides). Nucleic acid and deduced amino acid sequences were concatenated in the same order. The sequences were aligned using ClustalW2 (Larkin et al., 2007) and converted to the MEGA format. Dendrograms were constructed using the neighbour joining method in MEGA version 4.0. The maximum likelihood model was used for the nucleic acid sequences and the Poisson correction model for the amino acid sequences, both with 1000 bootstrap replicates (Felsenstein, 1985).

3. Results

3.1. Analysis of nox sequences

Sixty-four of the Spanish and Portuguese isolates clustered with the three species *B. murdochii* (*n* = 31; 46.3%), *B. innocens*(*n* = 26; 38.8%) and *B. intermedia* (*n* = 7; 10.4%) (Fig. 1). These species were recovered from farms throughout the Iberian peninsular, except for *B. intermedia* which was only isolated in the north of Spain (Fig. 2). Of the remaining isolates, H48 was identified as "*Brachyspira pulli*", with an identical *nox* sequence to "*B. pulli*" isolate AN6052:2/1/00 obtained from a Swedish mallard (Jansson et al., 2011), while isolates H29 and H51 had the same sequence and were separate from other species. The latter two were recovered from Iberian pigs from two different herds in different provinces, and their *nox* sequence had 63 nucleotide substitutions (7.4%) compared with the sequence of their closest neighbour *B. hyodysenteriae* strain B204^R. All

sequences were submitted to GenBank (accession numbers listed in Table 1: protein identifiers fielded as /protein_id.).

Of the farms where more than one isolate was examined (Table 1), three sets of isolates were obtained at different times: *B. murdochii* was isolated both times in two cases (farms C and H), and two isolates of *B. innocens* were obtained at the first sampling and an isolate of *B. murdochii* at the second sampling (Farm F). For the farms where isolates were obtained at the same visit, H51 and *B. innocens* were obtained from farm B (Iberian pigs), *B. murdochii* and *B. innocens* from farm D (Iberian pigs), two *B. murdochii* from farm E, two *B. innocens* from farms G and J, and "*B. pulli*" and *B. murdochii* from farm I. Isolates of *B. hyodysenteriae* were obtained at the same sampling time from farms D, I and J, as well as concurrently with isolates X26, X27 and X30 from other farms where single weakly haemolytic isolates were obtained. Of the eight isolates from Iberian pigs, two each were *B. murdochii*, *B. innocens*, *B. intermedia* and "*Brachyspira*spp.".

For the pairs of isolates of the same species from the same farms, in three cases the two *B. murdochii* on the same farms had the same *nox* sequence (farms C, E and H). In the case of the two farms with pairs of *B. innocens* isolates, on one the sequence was identical for the two isolates (farm J) and on the other they were different (farm F).

As can be seen from Fig. 1, the *nox* sequences for the 114 isolates were more heterogeneous amongst the *B. intermedia*, *B. alvinipulli* and "*B. pulli*" isolates than amongst the other species. *Brachyspira* isolates recorded as unidentified in GenBank (24916/1 and AN3617:2/1/02; 10363/4; AN6043:2/1/00; 9757/1 and AN3649/2b/02) did not cluster with any of the new unidentified isolates.

Generally, the use of peptide sequences helped to unify isolates from the same species or groups, with 33 different sequences rather than 51 using nucleotide information (data not shown). It did not change the deduced species identity based on the nucleotide sequences. The peptide sequence of "*B. pulli*" isolate H48 was identical to the sequence of "*B. pulli*" isolates AN3651/3b/02 and AN6052:2/1/00, and differed in only one substitution to the sequence of AN6045/1/00, all of which were recovered from Swedish mallards (Jansson et al., 2011). Most *B. murdochii*isolates only had two substitutions in their peptide sequences. Novel isolates H29 and H51 had a minimum of 20 substitutions (7%) from other isolates compared to 16 differences between the *B. hyodysenteriae* and *B. murdochii* reference strains.

3.2. MLST analysis of Spanish and Portuguese isolates

Five genes were successfully amplified and sequenced for all 18 isolates (*pgm*, *est*, *glp*K, *gdh* and *thi*) (Table 3). The raw sequences are recorded at the PubMLST site. The isolates were allocated to 17 STs and 16 AATs. Allelic frequency ranged from 10 (*est*) to 15 (*pgm* and *thi*) per locus, with a mean of 13.2. Most alleles were newly described, but one for *glp*K and two each for *pgm*, *est*, *gdh* and *thi* were in the PubMLST database. At the amino acid level the number of previously described variants varied from three (*pgm*, *glp*K and *gdh*) to five (*est*). All STs had at least one new allele.

3.3. Global MLST analysis

The number of alleles per locus for the 84 isolates ranged from 43 (*est*) to 58 (*glp*K), with a mean of 51.6. There were 67 STs and 62 AATs. Most isolates were in clusters, although some were arranged separately, with a high percentage of nucleotide substitutions compared with neighbours. There were 1 or 2 isolates per ST, and 1–4 per AAT. The percentage of nucleotide substitutions between species was high, being 11.1% between reference strains of *B. hyodysenteriae* and *B. pilosicoli*, 10.8% between reference strains of *B. hyodysenteriae* and *B. murdochii*,and 9.8% between reference strains of *B. hyodysenteriae* and *B. innocens*. The percentage of nucleotide substitutions

between *B. hyodysenteriae* and "*B. suanatina*" was 4.7, very similar to the value obtained from *nox* (4.8%).

The 18 isolates from Spain and Portugal did not share STs with isolates from other countries. The identities of the *B. murdochii*and *B. innocens* isolates were confirmed by their clustering with international strains. Isolate H48, identified as "*B. pulli*" by *nox*sequencing, was closely related to unidentified isolate AN2929/1/03 from a Swedish chicken (Råsbäck et al., 2007b), and shared peptide sequence number three for *adh* with AN2929/1/03 and unidentified *Brachyspira* Swedish chicken isolate AN1268/7/04, which therefore may be previously unrecognized "*B. pulli*" isolates. Isolates H29 and H51 were distinct from other *Brachyspira* isolates in PubMLST and did not share any of their nucleotide/amino acid sequences with other *Brachyspira* isolates. Their percentage of nucleotide substitutions with respect to *B. hyodysenteriae* was 6.7%, even higher than the 4.7% for "*B. suanatina*" isolates. The isolates were arranged into *Brachyspira* species with bootstrap values of 100, except for the *B. intermedia* isolates which showed a high genetic heterogeneity separated by large genetic distances.

Dendrograms constructed using the sequences of individual genes had similar topologies, with a few exceptions focused mainly on *B. intermedia* isolates (data not shown). One *B. pilosicoli* and seven *B. intermedia* isolates had inconsistencies in their positions at one or more loci on the trees, with 1–3 alleles belonged to a different *Brachyspira* species (Table 4). Depending on the number of alleles with similarities to other *Brachyspira*species, these isolates had intermediate positions in the dendrogram based on concatenated sequences (Fig. 3). These findings suggest that alleles of all five genes may have been transferred between species, especially for the *glp*K and *est* loci, and particularly for strains originally thought to be *B. intermedia* (Phillips et al., 2010).

4. Discussion and conclusions

The current study improves understanding of the occurrence of weakly haemolytic *Brachyspira* isolates in the Spanish and Portuguese pig industries. Based on their *nox* sequences, 46.3% of 67 isolates from pigs with diarrhoea were *B. murdochii*, 38.8% *B. innocens*, and 10.4% *B. intermedia*. One isolate was "*B. pulli*", and two (3%) were representatives of a new potential species. These results are comparable with findings elsewhere. For example, an Austrian study found that of 27 weakly haemolytic *Brachyspira* spp. recovered from diseased pigs, *B. murdochii*occurred alone in 11 (41%), and with other weakly haemolytic spirochaetes in nine (33%) (Komarek et al., 2009). *B. innocens*, *B. intermedia* and *B. pilosicoli* were less common, and 37% of pigs were colonized by two or more weakly haemolytic species. In Germany, of 2050 *Brachyspira* isolates collected from pigs between 2009 and 2011, 25% were *B. hyodysenteriae*, 5% *B. pilosicoli*, 24% *B. innocens*, 21% *B. murdochii*, 18% *B. intermedia*, and 7% "atypical" (Rohde and Habighorst-Blome, 2012). Analysis of 79 *Brachyspira* isolates from pigs with diarrhoea in the U.S. identified 30.4% as *B. hyodysenteriae*, 7.6% as *B. pilosicoli*, 25.3% as *B. murdochii*, 12.7% as *B. intermedia*, 3.8% as *B. innocens*, 13.9% as "unidentified", and 6.3% as mixed *Brachyspira*species (Clothier et al., 2011). A more recent study from the U.S. confirmed the predominance of *B. murdochii* amongst *Brachyspira*isolates from pigs with mild to moderate mucoid diarrhoea (Hammer and Gebhart, 2013). Hence *B. murdochii* generally is the most commonly identified weakly haemolytic species in pigs with diarrhoea, including in Spain and Portugal, and this is important given that there is histopathological and experimental infection data supporting its likely role as a pathogen (Weissenböck et al., 2005; Komarek et al., 2009; Jensen et al., 2010). The significance of the other weakly haemolytic species is less clear.

In this as in previous studies, isolates from different *Brachyspira*species were identified in animals from the same diarrhoeal outbreak, and different strains of the same species were identified on the

same farm. This is important for disease control, and emphasizes the importance of identifying several isolates from an outbreak. Besides helping to clarify the aetiology of a clinical problem, where different *Brachyspira* species or strains co-exist on a farm this increases the opportunity for exchange of genetic information between them. Indeed, the MLST data suggested the occurrence of interspecies gene transfer of alleles. Interspecies recombination involving genes used for MLST has been reported for other bacteria (McMillan et al., 2010), but not previously for *Brachyspira* species. Analysis of the genome sequence of *B. intermedia* strain PWS/A^T provided evidence for bacteriophage-mediated gene transfer events occurring between *Brachyspira*species and other genera (Håfström et al., 2011), and such activity, as well as that of *Brachyspira* prophage-like gene transfer agents (Matson et al., 2005; Motro et al., 2009), may account for the deduced recombinations.

All but two (2.9%) of the 67 weakly haemolytic isolates could be identified based on their *nox* sequences. The identification of an isolate as "*B. pulli*" was interesting, as previously this species has only been described in chickens (Stephens and Hampson, 2001), mallards (Jansson et al., 2011) and dogs (Prapasarakul et al., 2011). The two isolates from Iberian pigs that could not be assigned may be representatives of a new species, and their identity and pathogenic potential require investigation.

Only five of the seven MLST loci could be routinely amplified for the subset of 18 isolates. Råsbäck et al. (2007b) had similar difficulties with some species, and lack of amplification of *adh* and *alp* may be attributed to selection of suitable primers to cover the range of *Brachyspira* species investigated. Nevertheless, the five loci were useful for differentiating species, and gave enhanced strain discrimination compared to *nox* sequencing.

The Spanish and Portuguese isolates were diverse, and none shared STs with isolates from other countries. Nevertheless, most of the isolates had 2–4 previously described alleles and were related to isolates from other countries. Isolates H29 and H51 were the only two not sharing alleles with isolates from other countries, and they may have emerged independently in Iberian pigs.

In conclusion, *B. murdochii* was the most common species of weakly haemolytic *Brachyspira* species recovered from Spanish and Portuguese pigs with diarrhoea, as it has been in other countries. This finding supports the contention that *B. murdochii*may have pathogenic potential. Identification of *Brachyspira*species by *nox* sequencing was straightforward, although greater strain discrimination could be achieved using MLST at five loci. The current data justifies further epidemiological and pathological studies investigating the occurrence and pathogenic potential of weakly haemolytic *Brachyspira* species in healthy and diseased pigs.

Conflict of interest

There are no conflicts of interest.

Acknowledgements

This study was the result of an international collaboration between staff at the University of León, Spain, and Murdoch University, Perth, Australia. Jesús Osorio was in receipt of an FPU Research Fellowship AP 2003-3438 from the Ministerio de Ciencia e Innovación (Spanish Ministry of Science and Innovation).

References

Atyeo, R.F., Stanton, T.B., Jensen, N.S., Suriyaarachichi, D.S., Hampson, D.J., 1999. Differentiation of *Serpulina* species by NADH oxidase gene (*nox*) sequence comparisons and *nox*-based polymerase chain reaction tests. Veterinary Microbiology 67, 47–60.

Burrough, E.R., Strait, E.L., Kinyon, J.M., Bower, L.P., Madson, D.M., Wilberts, B.L., Schwartz, K.J., Frana, T.S., Songer, J.G., 2012. Comparative virulence of clinical Brachyspira spp. isolates in inoculated pigs. Journal of Veterinary Diagnostic Investigation 6, 1025–1034.

Carvajal, A., de Arriba, M.L., Rodríguez, H., Vidal, A.B., Duhamel, G.E., Rubio, P., 2006. Prevalence of *Brachyspira* species in pigs with diarrhoea in Spain. Veterinary Record 158, 700–701.

Chander, Y., Primus, A., Olivera, S., Gebhart, C., 2012. Phenotypic and molecular characterization of a novel strongly haemolytic *Brachyspira* species provisionally designated ‘‘*Brachyspira hampsonii*’’. Journal of Veterinary Diagnostic Investigation 5, 903–910.

Clothier, K.A., Kinyon, J.M., Frana, T.S., Naberhaus, N., Bower, L., Strait, E.L., Schwartz, K., 2011. Species characterization and minimum inhibitory concentration patterns of *Brachyspira* species isolates from swine with clinical disease. Journal of Veterinary Diagnostic Investigation 23, 1140–1145.

Fellström, C., Pettersson, B., Uhlen, M., Gunnarsson, A., Johansson, K.E., 1995. Phylogeny of *Serpulina* based on sequence analyses of the 16S rRNA gene and comparison with a scheme involving biochemical classification. Research in Veterinary Science 59, 5–9.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Fjelkner, J., Pedersen, K.S., Nielsen, J.P., Jensen, T., 2012. Infection with *Brachyspira* spp. is associated with increased mucosal thickness. In: Proceedings of the 22nd International Pig Veterinary Society Congress, Korea. p 236.

Håfström, T., Jansson, D.S., Segerman, B., 2011. Complete genome sequence of *Brachyspira* intermedia reveals unique genomic features in *Brachyspira* species and phage-mediated horizontal gene transfer. BMC Genomics 12, 395.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

Hammer, J.M., Gebhart, C., 2013. The evolving world of *Brachyspira*-associated colitis. Proceedings of the 2013 American Association of Swine Veterinarians Annual Meeting. San Diego, USA. pp. 229–230.

Hampson, D.J., 2012. *Brachyspiral* colitis. In: Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds.), Diseases of Swine, 10th ed. Wiley-Blackwell, Iowa, USA, pp. 680–696.

Jansson, D.S., Persson, M., Zimmerman, U., Johansson, K.E., 2011. Phenotypic and genetic diversity among intestinal spirochaetes (genus *Brachyspira*) in freeliving wild mallards (*Anas platyrhynchos*) sampled in southern Sweden. Systematic and Applied Microbiology 34, 566–575.

Jensen, T.K., Christensen, A.S., Boye, M., 2010. *Brachyspira murdochii* colitis in pigs. Veterinary Pathology 47, 334–338.

Jolley, K.A., Feil, E.J., Chan, M.S., Maiden, M.C., 2001. Sequence type analysis and recombinational tests (START). Bioinformatics 17, 1230–1231.

Komarek, V., Maderner, A., Spergser, J., Weissenböck, H., 2009. Infections with weakly haemolytic *Brachyspira* species in pigs with miscellaneous chronic diseases. Veterinary Microbiology 134, 311–317.

La, T., Phillips, N.D., Harland, B.L., Wanchanthuek, P., Bellgard, M.I., Hampson, D.J., 2009. Multilocus sequence typing as a tool for studying the molecular epidemiology and population structure of *Brachyspira hyodysenteriae*. Veterinary Microbiology 138, 330–338.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Matson, E.G., Thompson, M.G., Humphrey, S.B., Zuerner, R.L., Stanton, T.B., 2005. Identification of genes of VSH-1, a prophage-like gene transfer agent of *Brachyspira hyodysenteriae*. Journal of Bacteriology 187, 5885–5892.

McMillan, D.J., Bessen, D.E., Pinho, M., Ford, C., Hall, G.S., Melo-Cristino, J., Ramirez, M., 2010. Population genetics of *Streptococcus dysgalactiae* subspecies *equisimilis* reveals widely dispersed clones and extensive recombination. PLoS One 5, e11741.

Motro, Y., La, T., Bellgard, M.I., Dunn, D.S., Phillips, N.D., Hampson, D.J., 2009. Identification of genes associated with prophage-like gene transfer agents in the pathogenic intestinal spirochaetes *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli* and *Brachyspira intermedia*. Veterinary Microbiology 134, 340–345.

Osorio, J., Carvajal, A., Naharro, G., La, T., Phillips, N.D., Rubio, P., Hampson, D.J., 2012. Dissemination of clonal groups of *Brachyspira hyodysenteriae* amongst pig farms in Spain, and their relationships to isolates from other countries. PLoS One 7, e39082.

Phillips, N.D., La, T., Amin, M.M., Hampson, D.J., 2010. *Brachyspira intermedia* strain diversity and relationships to the other indole-positive *Brachyspira* species. Veterinary Microbiology 143, 246–254.

Prapasarakul, N., Lugsomya, K., Disatian, S., Lekdumrongsak, T., Banlunara, W., Chetanachan, P., Hampson, D.J., 2011. Faecal excretion of intestinal spirochaetes by urban dogs, and their pathogenicity in a chick model of intestinal spirochaetosis. Research in Veterinary Science 91 (3), e38–e43.

Råsbäck, T., Jansson, D.S., Johansson, K.E., Fellström, C., 2007a. A novel enteropathogenic, strongly haemolytic spirochaete isolated from pig and mallard, provisionally designated ‘‘*Brachyspira suanatina*’’ sp. nov. Environmental Microbiology 9, 983–991.

Råsbäck, T., Johansson, K.-E., Jansson, D.S., Fellström, C., Alikhani, M.Y., La, T., Dunn, D.S., Hampson, D.J., 2007b. Development of a multilocus sequence typing scheme for intestinal spirochaetes of the genus *Brachyspira*. Microbiology 153, 4074–4087.

Rohde, J., Habighorst-Blome, K., 2012. An up-date on the differentiation of *Brachyspira* species from pigs with nox-PCR-based restriction fragment length polymorphism. Veterinary Microbiology 158, 211–215.

Rubin, J.E., Costa, M.O., Hill1, J.E., Kittrell, H.E., Fernando, C., Huang, Y., O’Connor, B., Harding, J.C.S., 2013. Reproduction of mucohaemorrhagic diarrhea and colitis indistinguishable from swine dysentery following experimental inoculation with ‘‘*Brachyspira hampsonii*’’ strain 30446. PLoS One 8 (2), e57146.

Stephens, C.P., Hampson, D.J., 2001. Intestinal spirochete infections of chickens: a review of disease associations, epidemiology and control. Animal Health Research Reviews 2, 83–91.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24, 1596–1599.

Thomson, J.R., Smith, W.J., Murray, B.P., Murray, D., Dick, J.E., Sumption, K.J., 2001. Porcine enteric spirochete infections in the UK: surveillance data and preliminary investigation of atypical isolates. Animal Health Research Reviews 2, 31–36.

Townsend, K.M., Giang, V.N., Stephens, C., Scott, P.T., Trott, D.J., 2005. Application of nox-restriction fragment length polymorphism for the differentiation of *Brachyspira* intestinal spirochetes isolated from pigs and poultry in Australia. Journal of Veterinary Diagnostic Investigation 17, 103–109.

Trott, D.J., Stanton, T.B., Jensen, N.S., Duhamel, G.E., Johnson, J.L., Hampson, D.J., 1996. *Serpulina pilosicoli* sp. nov., the agent of porcine intestinal spirochetosis. International Journal of Systematic Bacteriology 46, 206–215.

Weissenböck, H., Maderner, A., Herzog, A.M., Lussy, H., Nowotny, N., 2005. Amplification and sequencing of *Brachyspira* spp. specific portions of *nox* using paraffin-embedded tissue samples from clinical colitis in Austrian pigs shows frequent solitary presence of *Brachyspira murdochii*. Veterinary Microbiology 111, 67–75.

Fig. 1. Neighbour-joining dendrogram based on *nox* sequences from a population of 114 *Brachyspira* isolates. The length on the scale indicates a distance of 17 substitutions in the sequence (855 nucleotides). Isolates from countries other than Spain and Portugal are marked in bold. The Spanish and Portuguese isolates that were also used for MLST are marked with an asterisk. The unidentified and "*B. pulli*" isolates are indicated in red/grey. Previously characterized Spanish isolates of *B. hyodysenteriae* are marked in bold with two asterisks. Type and reference strains are noted. Bootstrap values greater than 50 are shown in the nodes. Branch lengths are proportional to genetic distance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

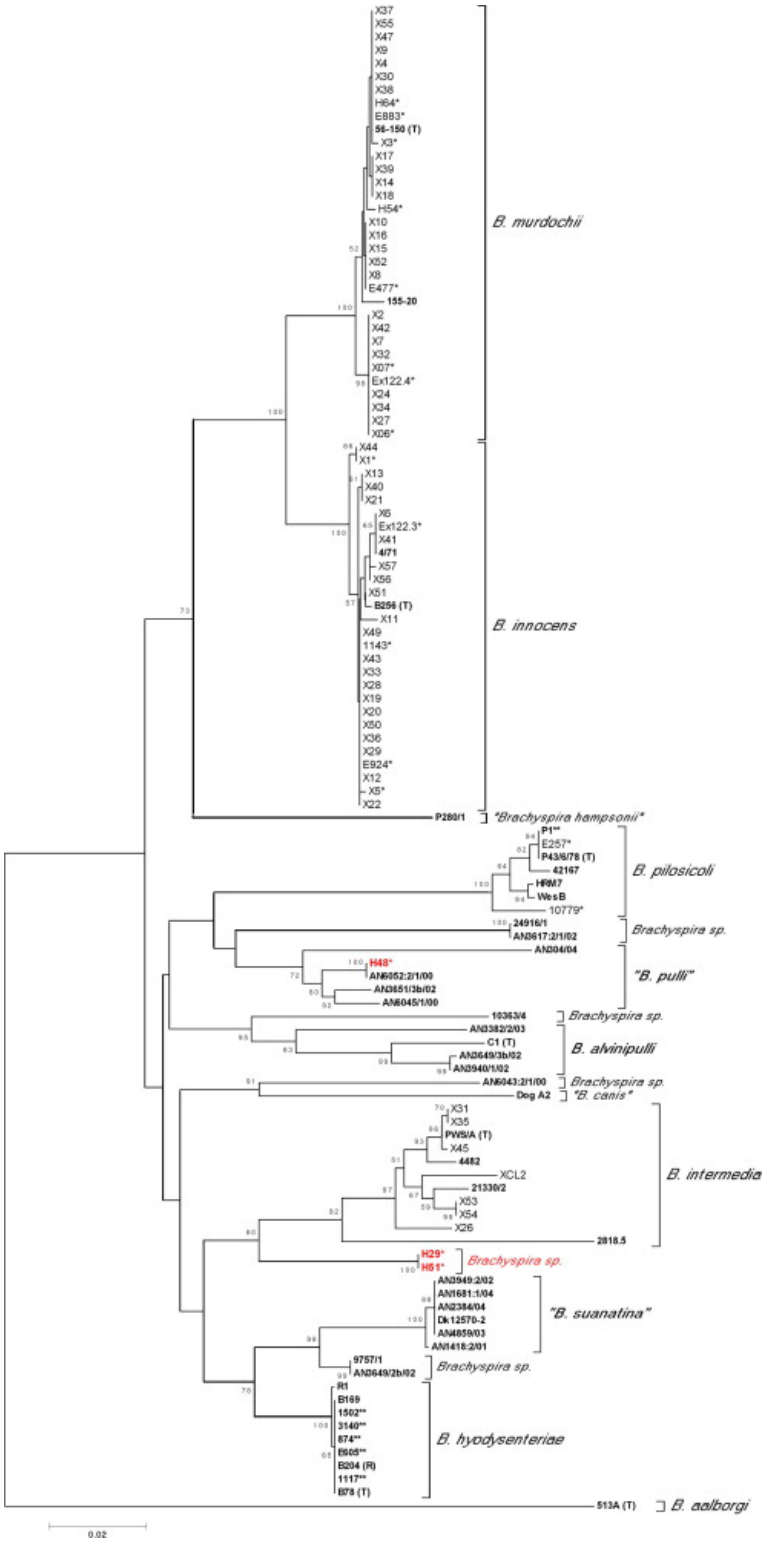

Fig. 2. Map showing administrative regions where the farms were located. For each region, the numbers of isolates are indicated as well as the species they belong to (*B. murdochii, B. innocens, B. intermedia*, respectively). The locations of the farms from which the "*B. pulli*" isolate (H48) and unidentified isolates (H29, H51) were obtained are marked. The number of Portuguese isolates and their affiliation to *Brachyspira* species are also noted.

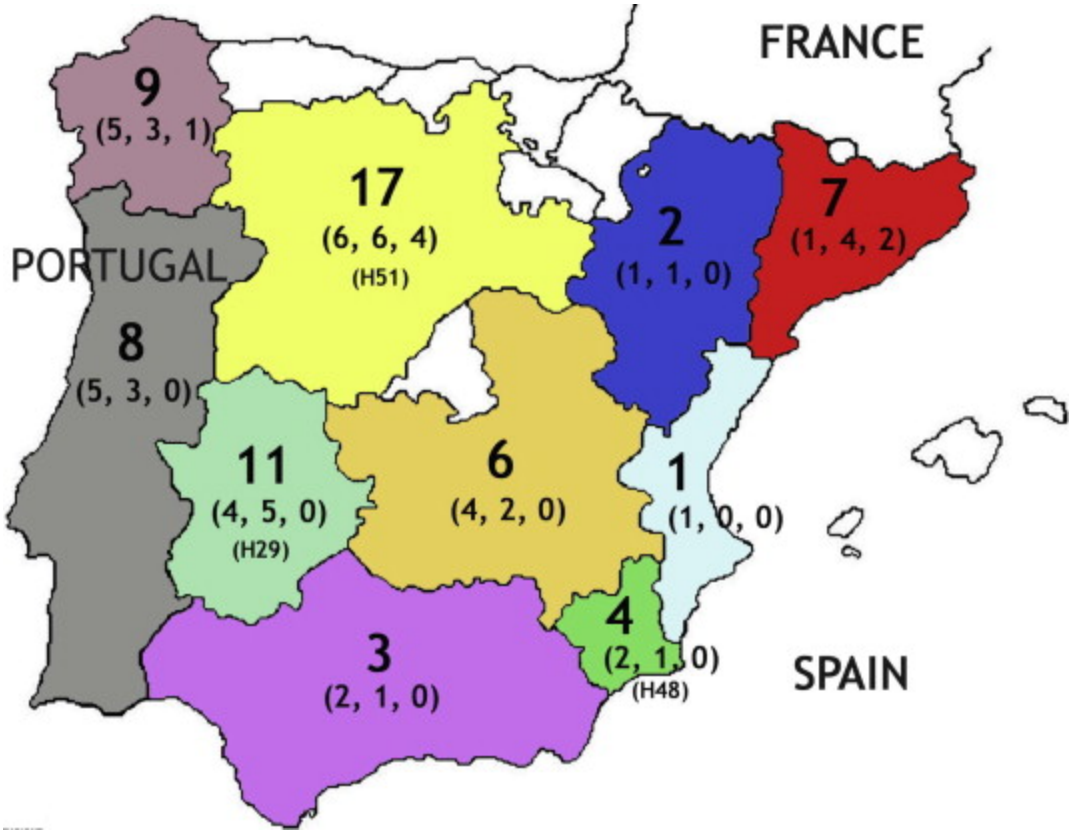

Fig. 3. Neighbour-joining dendrogram based on concatenated nucleotide sequences from the five loci from a population of 71 *Brachyspira* isolates. The length on the scale indicates a distance of 60 substitutions in the sequence (*pgm*, *est*, *glpK*, *gdh* and *thi*: 2930 nucleotides). The Spanish and Portuguese isolates are indicated in blue, and type and reference strains in red. Isolates that were positive in a *B. intermedia* PCR, as described by Phillips et al. (2010) are indicated with an asterisk. Isolates of *B. hyodysenteriae* characterized by Osorio et al. (2012) are indicated with two asterisks. Bootstrap values greater than 50 are shown in the nodes. Branch lengths are proportional to genetic distance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

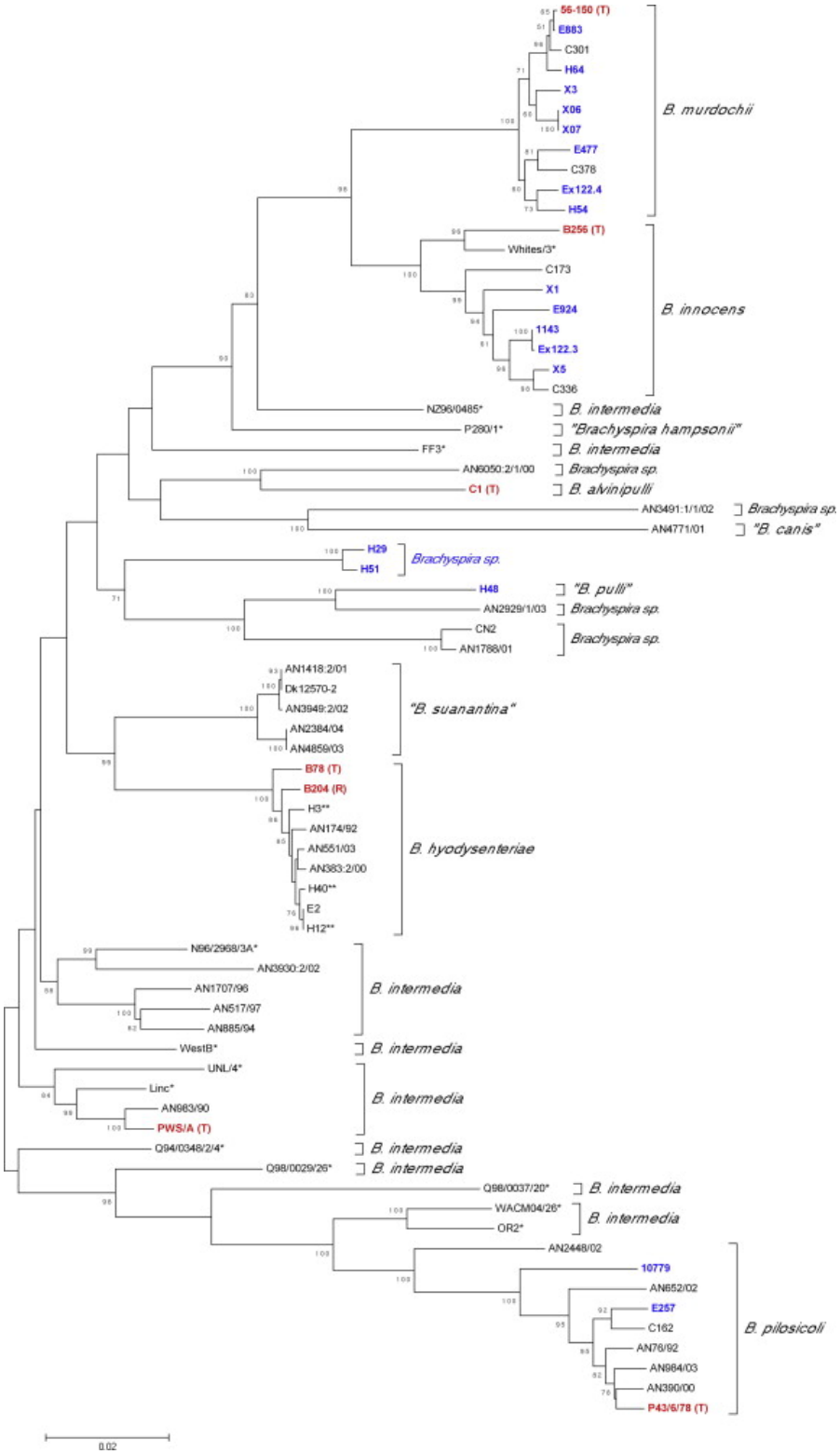

Table 1. Designation of multiple isolates from pigs on 10 farms.

Strain/isolate	Farm[a]	Species name[b]	Autonomous region[c]	Province	Isolation[d]
10779	A	*B. pilosicoli***	Extremadura	Badajoz	Oct-06
H29	A	*Brachyspira*sp.**	Extremadura	Badajoz	Feb-07
H51	B	*Brachyspira*sp.**	Castilla-León	León	Apr-07
X5	B	*B. innocens***	Castilla-León	León	Apr-07
X06	C	*B. murdochii*	Castilla-León	León	Feb-06
X07	C	*B. murdochii*	Castilla-León	León	Mar-07
Ex122.4	D*	*B. murdochii***	Extremadura	Badajoz	Sep-07
Ex122.3	D*	*B. innocens***	Extremadura	Badajoz	Sep-07
X16	E	*B. murdochii*	Galicia	Unknown	Jan-08
X15	E	*B. murdochii*	Galicia	Unknown	Jan-08
X11	F1	*B. innocens*	Castilla-León	Segovia	Nov-07
X12	F1	*B. innocens*	Castilla-León	Segovia	Nov-07
X32	F2	*B. murdochii*	Castilla-León	Segovia	May-08
X28	G	*B. innocens*	Rio Maior (P)	Unknown	May-08
X29	G	*B. innocens*	Rio Maior (P)	Unknown	May-08
X24	H	*B. murdochii*	Extremadura	Badajoz	Mar-08
X34	H	*B. murdochii*	Extremadura	Badajoz	Jul-08
H48	I*	"*B. pulli*"	Murcia	Murcia	Apr-07
X4	I*	*B. murdochii*	Murcia	Murcia	Apr-07
X19	J*	*B. innocens*	Extremadura	Cáceres	Feb-08
X20	J*	*B. innocens*	Extremadura	Cáceres	Feb-08

[a]Isolates from the same ten farms are marked with letters A through to J. Farm F is marked F1 and F2 as it was sampled at two different times, with two isolates recovered at the first visit. An asterisk indicates that isolates of *B. hyodysenteriae* were obtained in the same outbreak of diarrhoea.

[b]Weakly haemolytic isolates identified on the basis of their *nox* sequence. Isolates marked with two asterisks were from Iberian pigs, with unmarked isolates coming from commercial white pigs.

[c]For all isolates the administrative region is specified. Abbreviation used for two regions are: C-La Mancha, Castilla-La Mancha; Comunidad Valenciana, C. Valenciana. Regions in Portugal are marked (P), the rest are in Spain.

[d]The month/year of isolation is marked.

Table 2. Species names and origin of 39 reference strains of *Brachyspira*spp., the *nox* sequences of which were downloaded from GenBank and included in the *nox* trees.

Strain/isolate	Species name	Species of origin	Country of origin	GenBank accession no.
B78^{T}	*B. hyodysenteriae*	Pig	USA	AF060800
B204^{R}	*B. hyodysenteriae*	Pig	USA	U19610
R1	*B. hyodysenteriae*	Rhea	USA	AF060802
B169	*B. hyodysenteriae*	Pig	Canada	AF060801
AN3949:2/02	"*B. suanatina*"	Mallard	Sweden	DQ487123
AN1418:2/01	"*B. suanatina*"	Mallard	Sweden	DQ487124
AN2384/04	"*B. suanatina*"	Pig	Sweden	DQ487121
Dk12570-2	"*B. suanatina*"	Pig	Denmark	DQ487122
AN4859/03	"*B. suanatina*"	Pig	Sweden	DQ487119
AN1681:1/04	"*B. suanatina*"	Pig	Sweden	DQ487121
P43/6/78^{T}	*B. pilosicoli*	Pig	UK	AF060807
42167	*B. pilosicoli*	Chicken	USA	AF060809
HRM7	*B. pilosicoli*	Human	Italy	AF060806
WesB	*B. pilosicoli*	Human	Australia	AF060808
Apr-71	*B. innocens*	Pig	UK	AF060805
B256^{T}	*B. innocens*	Pig	USA	AF060804
56-150^{T}	*B. murdochii*	Pig	Canada	AF060813
155-20	*B. murdochii*	Pig	Australia	AF060803
C1^{T}	*B. alvinipulli*	Chicken	USA	AF060814
AN3382/2/03	*B. alvinipulli*	Chicken	Sweden	JF430770
AN3649/3b/02	*B. alvinipulli*	Mallard	Sweden	JF430746
AN3940/1/02	*B. alvinipulli*	Mallard	Sweden	JF430747
AN6052:2/1//00	"*B. pulli*"	Mallard	Sweden	JF430740
AN304//04	"*B. pulli*"	Chicken	Sweden	JF430769
AN3651/3b/02	"*B. pulli*"	Mallard	Sweden	JF430741
AN6045/1/00	"*B. pulli*"	Mallard	Sweden	JF430739
Dog A2^{R}	"*B. canis*"	Dog	Australia	EU819071
513A^{T}	*B. aalborgi*	Human	Denmark	AF060816
P280/1	"*B. hampsonii*"	Pig	UK	AF060815
24916/1	*Brachyspira*sp.	Duck	Hungary	HM462462
AN3617:2/1/02	*Brachyspira*sp.	Mallard	Sweden	JF430758
10363/4	*Brachyspira*sp.	Duck	Hungary	HM462458
AN6043:2/1/00	*Brachyspira*sp.	Mallard	Sweden	JF430734
9757/1	*Brachyspira*sp.	Duck	Hungary	HM462456
AN3649/2b/02	*Brachyspira*sp.	Mallard	Sweden	JF430735
PWS/A^{T}	*B. intermedia*	Pig	UK	AF060811
4482	*B. intermedia*	Pig	USA	AF060812
21330/2	*B. intermedia*	Duck	Hungary	HM462459
2818.5	*B. intermedia*	Pig	Australia	AF060810

Table 3. Names of the Spanish and Portuguese weakly haemolytic *Brachyspira* isolates included in the global MLST study. Sequence types (STs) and amino acid types (AATs) are indicated, and the allelic profiles (nucleotide/amino acid) are shown for each locus. A zero indicates that no amplification was obtained.

Isolatea	**ST/AAT**	***adh***	***pgm***	***est***	***glp*K**	***gdh***	***thi***	***alp***
H48	58/54	31-Mar	46/32	40/34	48/32	42/23	39/32	0
H29 (A)	59/55	0	47/33	41/35	49/33	43/24	40/33	0
H51 (B)	60/56	0	47/33	42/35	49/33	44/25	41/33	0
E257	61/57	32/1	48/34	43/3	50/34	45/26	42/34	34/31
10779 (A)	62/58	33/1	49/35	44/2	51/35	46/2	43/35	0
H54	63/59	0	50/10	45/9	52/36	47/9	44/36	0
H64	64/45	0	24-Sep	22-Jul	25-Nov	21-Sep	45/15	0
E477	65/60	0	23-Oct	22-Jul	53/37	21-Sep	37/16	0
E883	66/45	0	24-Sep	22-Jul	25-Nov	47/9	38/15	0
X3	67/61	0	51/10	22-Jul	54/36	21-Sep	46/15	0
X06 (C)	68/62	0	52/36	46/36	55/36	48/27	47/15	0
X07 (C)	68/62	0	52/36	46/36	55/36	48/27	47/15	0
Ex122.4 (D)	69/63	0	53/36	45/9	55/36	49/27	48/15	0
Ex122.3 (D)	70/64	0	54/37	24-Dec	56/14	50/10	49/20	0
E924	71/65	0	55/38	24-Dec	57/15	24-Oct	50/19	0
1143	72/66	0	56/39	24-Dec	56/14	50/10	49/20	0
X1	73/67	0	57/38	47/37	58/14	51/9	51/19	0
X5 (B)	74/68	0	58/16	24-Dec	59/14	52/10	50/19	0
	17/16	–	15-Nov	10-Sep	13-Sep	13-Aug	15-Sep	–

[a]Isolates from the same farm are marked with letters A, B, C and D in brackets.

Table 4. Eight isolates with non-congruent positions on individual genes trees. The position of each isolate on the five dendrograms generated for individual genes is showed.

Species	**Isolates**	**MLST genes**				
		***glp*K**	***est***	***pgm***	***thi***	***gdh***
B. intermedia	OR2	*B. pilosicoli*	*B. intermedia*	*B. pilosicoli*	*B. intermedia*	*B. pilosicoli*
	WACM04/26	*B. pilosicoli*	*B. intermedia*	*B. pilosicoli*	*B. intermedia*	*B. pilosicoli*
	NZ96/0485	*B. innocens*	*B. innocens*	*B. innocens*	*B. intermedia*	*B. intermedia*
	UNL/4	*B. innocens*	*B. intermedia*	*B. intermedia*	*B. intermedia*	*B. intermedia*
	Q98/0037/20	*B. intermedia*	*B. innocens*	*B. intermedia*	*B. intermedia*	*B. pilosicoli*
	Q98/0348/2/4	*B. intermedia*	*B. innocens*	*B. intermedia*	*B. intermedia*	*B. intermedia*
B. innocens[a]	Whites/3	*B. innocens*	*B. innocens*	*B. innocens*	*B. innocens*	*B. intermedia*
B. pilosicoli	AN2448/02	*B. pilosicoli*	*B. murdochii*	*B. pilosicoli*	*B. pilosicoli*	*B. pilosicoli*

[a]This was labelled as *B. intermedia* in the publication of Phillips et al. (2010) but is likely to have been misidentified.

506 **Supplementary Table 1**

507 Strain and isolate designation, species name and origin of the 75 *Brachyspira* spp. from

508 pigs in the Iberian Peninsula that were included in the *nox* trees.

Strain/isolate[a]	Species name[b]	Autonomous region[c]	Province	Isolation[d]	GenBank accession no.
1502	*B. hyodysenteriae*	Andalucía	Málaga	Jan-02	JX428806
3140	*B. hyodysenteriae*	Navarra	Navarra	Oct-02	JX428806
874	*B. hyodysenteriae*	Andalucía	Málaga	May-01	JX428806
E605	*B. hyodysenteriae*	La Rioja	La Rioja	Feb-01	JX428806
1117	*B. hyodysenteriae*	Aragón	Zaragoza	Sep-01	JX428806
P1	*B. pilosicoli**	Murcia	Murcia	Sep-07	JX428822
E257	*B. pilosicoli*	C-La Mancha	Ciudad Real	Oct-02	JX428822
10779	*B. pilosicoli***	Extremadura	Badajoz	Oct-06	JX428823
H48*	"*B. pulli*"	Murcia	Murcia	Apr-07	JX428824
H29	*Brachyspira* sp.**	Extremadura	Badajoz	Feb-07	JX428805
H51	*Brachyspira* sp.**	Castilla-León	León	Apr-07	JX428805
H54	*B. murdochii*	Alentejo (P)	Unknown	May-07	JX428810
H64	*B. murdochii*	Murcia	Murcia	Jun-07	JX428808
E477	*B. murdochii*	Cataluña	Lérida	Jan-04	JX428811
E883	*B. murdochii*	Galicia	La Coruña	May-06	JX428808
X3	*B. murdochii*	Galicia	Unknown	Apr-07	JX428807
X06	*B. murdochii*	Castilla-León	León	Feb-06	JX428812
X07	*B. murdochii*	Castilla-León	León	Mar-07	JX428812
Ex122.4*	*B. murdochii***	Extremadura	Badajoz	Sep-07	JX428812
X37	*B. murdochii*	Andalucía	Córdoba	Nov-08	JX428808
X55	*B. murdochii*	Montijo (P)	Unknown	Jun-09	JX428808
X47	*B. murdochii*	Castilla-León	Valladolid	Jan-09	JX428808
X9	*B. murdochii*	C-La Mancha	Ciudad Real	Oct-07	JX428808
X4*	*B. murdochii*	Murcia	Murcia	Apr-07	JX428808
X30*	*B. murdochii*	Castilla-León	Zamora	May-08	JX428808
X38	*B. murdochii*	Aragón	Huesca	Dec-08	JX428808
X17	*B. murdochii*	Montijo (P)	Unknown	Feb-08	JX428809
X39	*B. murdochii*	Castilla-León	Zamora	Dec-08	JX428809
X14	*B. murdochii*	C. Valenciana	Valencia	Dec-07	JX428809
X18	*B. murdochii*	Leiria (P)	Unknown	Feb-08	JX428809

X10	*B. murdochii*	Galicia	Lugo	Oct-07	JX428811
X16	*B. murdochii*	Galicia	Unknown	Jan-08	JX428811
X15	*B. murdochii*	Galicia	Unknown	Jan-08	JX428811
X52	*B. murdochii*	Tomar (P)	Unknown	Apr-09	JX428811
X8	*B. murdochii***	C-La Mancha	Toledo	Sep-07	JX428811
X2	*B. murdochii*	Málaga	Andalucía	Jan-09	JX428812
X42	*B. murdochii*	C-La Mancha	Albacete	Jan-09	JX428812
X7	*B. murdochii*	Extremadura	Badajoz	Sep-07	JX428812
X32	*B. murdochii*	Castilla-León	Segovia	May-08	JX428812
X24	*B. murdochii*	Extremadura	Badajoz	Mar-08	JX428812
X34	*B. murdochii*	Extremadura	Badajoz	Jul-08	JX428812
X27*	*B. murdochii*	C-La Mancha	Toledo	May-08	JX428812
Ex122.3*	*B. innocens***	Extremadura	Badajoz	Sep-07	JX428815
E924	*B. innocens*	Cataluña	Barcelona	Jun-01	JX428820
1143	*B. innocens*	Castilla-León	Palencia	Sep-01	JX428820
X1	*B. innocens*	Cataluña	Lérida	Feb-07	JX428813
X5 (B)	*B. innocens***	Castilla-León	León	Apr-07	JX428821
X44	*B. innocens*	Cataluña	Lérida	Jan-09	JX428813
X13	*B. innocens*	Andalucía	Sevilla	Dec-07	JX428814
X40	*B. innocens*	Castilla-León	Valladolid	Jan-09	JX428814
X21	*B. innocens*	Murcia	Murcia	Feb-08	JX428814
X6	*B. innocens*	Extremadura	Badajoz	Sep-07	JX428815
X41	*B. innocens*	Castilla-León	Burgos	Jan-09	JX428815
X57	*B. innocens*	C-La Mancha	Toledo	Jun-09	JX428816
X56	*B. innocens*	Cataluña	Lérida	Jun-09	JX428817
X51	*B. innocens*	Aragón	Huesca	Feb-09	JX428818
X11	*B. innocens*	Castilla-León	Segovia	Nov-07	JX428819
X49	*B. innocens*	Leiria (P)	Unknown	Feb-09	JX428820
X43	*B. innocens*	Galicia	Lugo	Jan-09	JX428820
X33	*B. innocens*	C-La Mancha	Toledo	Jul-08	JX428820
X28	*B. innocens*	Rio Maior (P)	Unknown	May-08	JX428820
X19 *	*B. innocens*	Extremadura	Cáceres	Feb-08	JX428820
X20 *	*B. innocens*	Extremadura	Cáceres	Feb-08	JX428820
X50	*B. innocens*	Galicia	Lugo	Feb-09	JX428820
X36	*B. innocens*	Galicia	Orense	Nov-08	JX428820
X29	*B. innocens*	Rio Maior (P)	Unknown	May-08	JX428820
X12	*B. innocens*	Castilla-León	Segovia	Nov-07	JX428820

X22	*B. innocens*	Extremadura	Badajoz	Mar-08	JX428820
X31	*B. intermedia*	Castilla-León	Valladolid	May-08	JX428825
X35	*B. intermedia*	Galicia	Orense	Jul-08	JX428825
X45	*B. intermedia***	Castilla-León	Valladolid	Jan-09	JX428826
XCL2	*B. intermedia*	Castilla-León	Unkonwn	Unknown	JX428827
X53	*B. intermedia*	Cataluña	Lérida	May-09	JX428828
X54	*B. intermedia*	Cataluña	Lérida	Jun-09	JX428828
X26*	*B. intermedia***	Castilla-León	Salamanca	Apr-08	JX428829

509 [a] An asterisk indicates that isolates of *B. hyodysenteriae* were obtained in the same
510 outbreak of diarrhoea.
511 [b] Weakly haemolytic isolates identified on the basis of their *nox* sequence. Isolates
512 marked with two asterisks were from Iberian pigs, with unmarked isolates coming
513 from commercial white pigs.
514 [c] For all isolates the administrative region is specified. Abbreviation used for two
515 regions are: C-La Mancha, Castilla-La Mancha; Comunidad Valenciana, C.
516 Valenciana. Regions in Portugal are marked (P), the rest are in Spain.
517 [d] The month/year of isolation is marked.

Supplementary Table 2. Strain and isolate designation, species name and origin of the 71 *Brachyspira* species included in the MLST dendrogram. Sequences for 50 isolates belonging to the *Brachyspira* genus that previously have been analysed by MLST were obtained from PubMLST (Råsbäck et al., 2007b; Phillips et al., 2010). The 21 isolates from Spain and Portugal are shown in the bottom portion of the table, and are named according to their *nox* sequence.

Strain/isolate	Species name	Species of origin	Country of origin
AN174/92	*B. hyodysenteriae*	Pig	Sweden
AN383:2/00	*B. hyodysenteriae*	Mallard	Sweden
B78^{T}	*B. hyodysenteriae*	Pig	USA
AN551/03	*B. hyodysenteriae*	Mouse	Sweden
E2	*B. hyodysenteriae*	Pig	UK
B204^{R}	*B. hyodysenteriae*	Pig	USA
AN3949:2/02	*"B. suanatina"*	Mallard	Sweden
AN1418:2/01	*"B. suanatina"*	Mallard	Sweden
AN2384/04	*"B. suanatina"*	Pig	Sweden
Dk12570-2	*"B. suanatina"*	Pig	Denmark
AN4859/03	*"B. suanatina"*	Pig	Sweden
AN652/02	*B. pilosicoli*	Pig	Sweden
C162	*B. pilosicoli*	Pig	Sweden
AN984/03	*B. pilosicoli*	Pig	Sweden
P43/6/78^{T}	*B. pilosicoli*	Pig	UK

AN390/00	*B. pilosicoli*	Mallard	Sweden
AN76/92	*B. pilosicoli*	Pig	Sweden
AN2448/02	*B. pilosicoli*	Pig	Sweden
C173	*B. innocens*	Pig	Sweden
C336	*B. innocens*	Pig	Sweden
B256^{T}	*B. innocens*	Pig	USA
56-150^{T}	*B. murdochii*	Pig	Canada
C378	*B. murdochii*	Pig	Sweden
C301	*B. murdochii*	Pig	Sweden
C1^{T}	*B. alvinipulli*	Chicken	USA
AN4771/01	*"B. canis"*	Dog	Sweden
AN1788/01	*Brachyspira* sp.	Dog	Sweden
CN2	*Brachyspira* sp.	Dog	Norway
AN3491:1/1/02	*Brachyspira* sp.	Eider	Sweden
AN2929/1/03	*Brachyspira* sp.	Chicken	Sweden
AN6050:2/1/00	*Brachyspira* sp.	Mallard	Sweden
AN1707/96	*B. intermedia*	Pig	Sweden
AN517/97	*B. intermedia*	Pig	Finland
AN3930:2/02	*B. intermedia*	Mallard	Sweden
AN983/90	*B. intermedia*	Pig	Sweden
AN885/94	*B. intermedia*	Pig	Sweden
PWS/A^{T}	*B. intermedia*	Pig	UK
N96/2968/3A	*B. intermedia*	Pig	Australia

UNL/4	*B. intermedia*	Pig	USA
WestB	*B. intermedia*	Pig	Australia
Q94/0348/2/4	*B. intermedia*	Chicken	Australia
Linc	*B. intermedia*	Pig	UK
Q98/0029/26	*B. intermedia*	Chicken	Australia
Q98/0037/20	*B. intermedia*	Chicken	Australia
OR2	*B. intermedia*	Pig	Australia
WACM04/26	*B. intermedia*	Chicken	Australia
Whites/3	*B. intermedia*	Pig	Australia
NZ96/0485	*B. intermedia*	Pig	New Zealand
FF3	*B. intermedia*	Chicken	Australia
P280/1	*"B. hampsonii"*	Pig	UK
H3	*B. hyodysenteriae*	Pig	Spain
H12	*B. hyodysenteriae*	Pig	Spain
H40	*B. hyodysenteriae*	Pig	Spain
H48	"*B. pulli*"	Pig	Spain
H29(A)	*Brachyspira* sp.	Iberian pig	Spain
H51(B)	*Brachyspira* sp.	Iberian pig	Spain
E257	*B. pilosicoli*	White pig	Spain
10779(A)	*B. pilosicoli*	Iberian pig	Spain
H54	*B. murdochii*	Pig	Portugal

H64	*B. murdochii*	Pig	Spain
E477	*B. murdochii*	Pig	Spain
E883	*B. murdochii*	Pig	Spain
X3	*B. murdochii*	Pig	Spain
X06(C)	*B. murdochii*	Pig	Spain
X07(C)	*B. murdochii*	Pig	Spain
Ex122.4(D)	*B. murdochii*	Pig	Spain
Ex122.3(D)	*B. innocens*	Iberian pig	Spain
E924	*B. innocens*	Pig	Spain
1143	*B. innocens*	Pig	Spain
X1	*B. innocens*	Pig	Spain
X5(B)	*B. innocens*	Iberian pig	Spain

Spanish isolates from the same farm are marked with letters A, B, C and D in brackets.

Type and reference strains are indicated are marked with superscripts.