The Veterinary Journal 214 (2016) 10–13

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Short Communication

Experimental infection of conventional pigs with a '*Brachyspira hampsonii*' isolate recovered from a migrating waterfowl in Spain

Luis Miguel Aller-Morán *, Francisco Javier Martínez-Lobo, Pedro Rubio, Ana Carvajal

Department of Animal Health, Veterinary Faculty, University of León, León 24071, Spain

ARTICLE INFO

Article history:
Accepted 3 February 2016

Keywords:
'*Brachyspira hampsonii*'
Migrating waterfowl
Pigs
Swine dysentery

ABSTRACT

'Brachyspira hampsonii' is a recently proposed new species within the *Brachyspira* genus, which produces a dysentery-like disease in pigs. This study aims at investigating whether a '*B. hampsonii*' isolate recovered from a migrating waterfowl was capable of colonizing pig intestines, inducing clinical signs of dysentery and being transmitted among pigs. Eleven 7-week-old pigs were randomly assigned into two separate groups which were orally administered an avian isolate of '*B. hampsonii*' (inoculated group, $n = 5$) or BHI broth (control group, $n = 6$). After inoculation, three pigs from the control group were placed in the inoculated pen and served as sentinel pigs. Our results show the capacity of this avian '*B. hampsonii*' isolate to colonize the large intestine of pigs and to be transmitted among pigs. According to this, migrating birds could play a role in the epidemiology of '*B. hampsonii*' as a possible source of infection in swine populations.

'*Brachyspira hampsonii*' was identified for the first time in pig faeces collected on farms with swine dysentery (SD)-like disease in the USA and has emerged as an important swine pathogen in the USA and Canada, replacing *B. hyodysenteriae* as the predominant aetiological agent of SD (Rubin et al., 2013a). Recently, '*B. hampsonii*' was identified in European pigs for the first time (Mahu et al., 2014; Rohde et al., 2014). Apart from pigs, '*B. hampsonii*' isolates have also been recovered from wild waterfowl, from lesser snow geese in the Canadian Arctic (Rubin et al., 2013b) and wild mallards and Greylag geese in Spain (Martínez-Lobo et al., 2013).

Although two genetically distinct clades of '*B. hampsonii*' isolates were initially recognized (Chander et al., 2012), recent research using multilocus sequence typing has revealed a heterogeneous population with a total of 20 sequence types and three clonal complexes (Mirajkar et al., 2015). The pathogenicity of '*B. hampsonii*' isolates recovered from pigs has been demonstrated (Burrough et al., 2012; Rubin et al., 2013a; Costa et al., 2014; Wilberts et al., 2014). The ability of a Canadian avian '*B. hampsonii*' isolate to colonize pigs was also demonstrated in an experimental study, although no clinical signs were observed in the infected pigs. The aim of this pilot inoculation study was to evaluate a European '*B. hampsonii*' isolate, AIS50 (Genbank accession no: KF386054), recovered from faecal samples of a Greylag goose (Martínez-Lobo et al., 2013) to determine whether this isolate was capable of infecting pigs, inducing pathological outcomes and being transmitted between pigs.

This study was approved by the University of León Committee on Animal Care and Supply (No. 7–2010; 3th February 2010). No positive control group inoculated with *B. hyodysenteriae* was included due to limited space, but this model has been successfully used previously in our laboratory to reproduce SD.

Eleven 7-week-old commercially obtained cross-breed female pigs from a farm with no history of SD or porcine spirochaetosis were randomly assigned into two groups, inoculated ($n = 5$; no. 1–5) and control ($n = 6$; no. 6–11), which were kept in two separate rooms throughout the experiment. All pigs were fed ad libitum with a commercial pelleted starter diet with no added antibiotics and they all had free access to water. During a 10-day acclimation period, the pigs were confirmed negative for *B. hyodysenteriae, B. pilosicoli* and *Salmonella* spp. by culture of rectal swabs and free of *Lawsonia intracellularis* by PCR testing. On post-inoculation days (PID) 0, 1 and 2, pigs from the inoculated group were orally administered 100 mL of AIS50 culture in brain heart infusion broth (BHI) supplemented with 10% foetal bovine serum (FBS) containing between 2.4 and 8.0×10^8 of spirochaetes per mL using a feeding syringe. Pigs in the control group received 100 mL of BHI-10% FBS. A diet containing a high proportion of soybean meal (50%) was used between PID 0 and PID 21 to encourage spirochaete colonization and the development of brachyspiral colitis (Hampson et al., 2000; Jacobson et al., 2004). On PID 4, three pigs from the control group (no. 6–8) were placed in the inoculated group and served as sentinel pigs.

All pigs were examined daily to assess faecal consistency (scored 0–4/4) as previously described (Rubin et al., 2013a) and systemic clinical signs. A masked analysis of *Brachyspira* spp. shedding in faecal samples by culture-PCR combination (Martínez-Lobo et al., 2013) and a masked assessment of gross and microscopic lesions in the

* Corresponding author. Tel.: +34987291306.
E-mail address: lallm@unileon.es (L.M. Aller-Morán).

http://dx.doi.org/10.1016/j.tvjl.2016.02.001
1090-0233/

Table 1
Faecal consistency score[a] and *Brachyspira* culture and identification results after experimental inoculation with an avian isolate of '*B. hampsonii*' (AIS 50).

PID	Inoculated pigs[b]					Sentinel pigs[c]			Control pigs[d]		
	1	2	3	4	5	6	7	8	9	10	11
0	0	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0 (Bm)	0
2	1	1	1	2	0	0	0	0	0	0 (UI)	0
3	1	1	1	2	0	0[e]	0[e]	0[e]	0	1	0
4	1	1	1	2	0	1	1	0	0	1 (Bm)	0
5	2	1	1	2	0	1	2	0	0	1	0 (Bm)
6	2 (Bh)	1	2	1	1 (Bh)	4	2	0	0	0	0 (UI)
7	0 (Bh)	0	0	2	0 (Bm)	4	0	0	1	1	1 (UI)
8	3 (Bh)	0 (Bh)	0	1	0		0	0	2	0	1
9	3 (Bh)	0	0	1	0 (Bh)		0	0	2 (UI)	1	0
10	3 (Bh)	0	0	1	0 (Bh)		0	0	1	0	0
11	4 (Bh)	0 (Bh)	0	1	0 (Bh)		0	0	1	1	0
12	1 (Bh)	0	0	1	0		0	0	1	0	1
13		1	0	1	1		0	0	1	1	1
14		0	0 (Bh)	0	0		0	0	0	1	1
15		1	0	0	0		0	0	0	0	0
16		1	0	0	0		0	0	0	0	0
17		0	0	0	0		0	0	0	0	0
18		0	0	0	0		0	0	0	0	0
19		0 (Bh)	0	0 (Bh)	0		0	0	0	0	0
20		0	0	0	0		0	0	0	0	0
21		0	0	0	0 (Bh)		0	0	0	0	0
22		0	0	0	0		0	0	0	0	0
23		0 (Bh)	0	0 (Bh)	0		0 (Bh)	0	0	0	0
24		1	0	1	0		2 (Bh)	1	0	0	0
25		1	0	1	0		2 (Bh)	1	0	0	0
26		1	0	1	0		2 (Bh)	1	0	0	0
27		0	0 (Bh)	1	0 (Bh)		1	1	0	0	0
28		0	0	0	0		0	0	0	0	0
29		0	0	0	0		0	0	0	0	0
30		0	0	0	0		0 (Bh)	0	0	0	0
31		0	0	0	0		0	0	0	0	0
32		0	0	0	0		0	0	0	0	0
33		0 (Bh)	0	0	0		0	0	0	0	0
34		0	0	0	0		0	0	0	0	0
35		0	0	0	0		0	0	0	0	0
36		0	0	0	0		0	0	0	0	0
37		0 (Bh)	0 (Bh)	0	0		0	0 (UI)	0	0	0
38		0 (Bh)	0	0	0		0	0 (UI)	0	0	0
39		0	0	0	0		0	0	0	0	0
40		0	0	0	0		0	0 (UI)	0	0	0

PID, postinoculation days.
[a] Faecal consistency was scored as follows: 0, formed-normal; 1, soft-wet cement consistency; 2, runny-watery; 3, mucoid diarrhoea; 4, bloody diarrhoea. Days with positive culture of spirochates are coloured and identified as Bh, isolation of '*B. hampsonii*'; Bm, isolation of *B. murdochii*; or UI, unidentified spirochaete.
[b] Inoculated pigs were orally administered on three consecutive days (PID 0, 1 and 2) 100 mL of a 10^8 CFU/mL culture of AIS 50.
[c, d] Control pigs and sentinel pigs were orally administered 100 mL of BHI broth on the same days.
[e] Sentinel pigs were placed in the inoculated pen on PID 3.

large intestine, including evaluation for the presence of *Brachyspira*-like organisms, inflammation grade, number of inflammatory and plasma cells and depth of crypts was also carried out according to Burrough et al. (2012). Differences between groups were evaluated for significance using non-parametric tests; Kruskal–Wallis for data on a continuous scale and Mann–Whitney U tests for ordinal variables (scored parameters). Results were considered statistically significant when $P < 0.05$.

Intermittent shedding of strongly haemolytic spirochaetes was shown in all inoculated animals; only weakly haemolytic spirochaetes were sporadically isolated from faeces of control pigs (Table 1). All the isolates were further characterized by the amplification and sequencing of *Brachyspira* spp. partial *nox* gene (Martínez-Lobo et al., 2013). Twenty five of 26 strongly haemolytic isolates obtained from inoculated pigs were identified as '*B. hampsonii*' (100% of nucleotide homology with the isolate AIS 50 used as inoculum), showing the ability of a European '*B. hampsonii*' isolate recovered from a wild waterfowl to colonize porcine intestine and be shed into the environment. A total of seven weakly haemolytic spirochaete isolates were recovered from control pigs. Three were identified as *B. murdochii* but we were not able to amplify the *nox* gene of the remaining four weakly haemolytic isolates, so they remained unidentified. Notably, shedding of '*B. hampsonii*' by inoculated animals led to the infection of one sentinel pig and probably to a second one, although in the latter sequence confirmation was not achieved.

In agreement with the results previously reported by Rubin et al. (2013b) using a Canadian avian isolate of '*B. hampsonii*', diarrhea was mild in 4/5 inoculated pigs, ranging from normal to wet cement consistency at PID 0–14. Control pigs also exhibited loose stools at PID 7–14 that might have been associated with the high levels of soybean meal in the pigs' diet to encourage the development of SD (Hampson et al., 2000; Rubin et al., 2013b). No statistically significant differences were observed when mean accumulative faecal scores among inoculated pigs (12.4; standard deviation = 8.62) and control pigs (7.3; standard deviation = 2.08) were compared ($P = 0.571$). However, one inoculated pig (no. 1) developed clinical signs and lesions typical for SD, including systemic clinical signs