Veterinary Microbiology 191 (2016) 15–19

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Brachyspira hyodysenteriae isolated from apparently healthy pig herds following an evaluation of a prototype commercial serological ELISA

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ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form 25 May 2016
Accepted 26 May 2016

Keywords:
Swine dysentery
Brachyspira hyodysenteriae
Diagnosis
ELISA
MLST

ABSTRACT

Swine dysentery (SD) is a disease mainly of grower/finisher pigs characterised by severe mucohaemorrhagic colitis. The classical aetiological agent is the anaerobic intestinal spirochaete *Brachyspira hyodysenteriae,* although "*Brachyspira hampsonii*" and *Brachyspira suanatina* also cause SD. This study reports on the unexpected isolation of *B. hyodysenteriae* from pigs in apparently healthy herds that gave positive reactions when tested with a prototype commercial serological ELISA for detecting herds infected with *B. hyodysenteriae* (Priocheck® *Brachyspira* porcine Ab ELISA). The ELISA was tested with sera collected at abattoirs from 1770 slaughtered pigs from 30 Australian herds, including 12 with a history of SD and18 that were considered by their consulting veterinarians to be healthy. The latter herds had no history of SD and did not routinely use antimicrobials that may have masked the disease. Based on the recommended ELISA cut-off value, 25 herds were recorded as showing evidence of infection, including 11 of 12 herds that were considered infected by the submitters and 14 of the 18 "healthy" herds. When faecal or colonic wall samples from 11 of the 14 "false positive" herds subsequently were culturing 6–24 months after the original ELISA testing was completed, different strains of *B. hyodysenteriae* were isolated from six herds, including a high-health status breeding herd. The existence of apparently healthy herds that are colonised by *B. hyodysenteriae* has major implications for the control of SD. Had the ELISA not been trialled it is unlikely that colonic samples from these herds would have been cultured and the colonisation identified.

1. Introduction

Swine dysentery (SD) is endemic in many regions of the world. The disease typically presents as a severe mucohaemorrhagic colitis mainly in grower and finisher pigs, although clinical signs may be ameliorated or prevented by the use of antimicrobial agents (Hampson, 2012). The classical agent of SD is the strongly haemolytic anaerobic intestinal spirochaete *Brachyspira hyodysenteriae,* but latterly the related strongly haemolytic species *Brachyspira suanatina* and "*Brachyspira hampsonii*" also have been shown to be able to cause SD (Råsbäck et al., 2007; Burrough et al., 2012; Chander et al., 2012; Rubin et al., 2013; Mushtaq et al., 2015).

Diagnosis of SD largely rests on observation of clinical signs and pathology, together with demonstration and identification of the aetiological spirochaetes in the colon or faeces. *Brachyspira* spp. grow slowly on specialised selective agar plates under anaerobic conditions, and their subsequent identification traditionally has been difficult and time consuming. The application of PCR technology to identify spirochaetes growing on plates or on DNA extracted from faecal or colonic samples represented an important diagnostic advance (Atyeo et al., 1998; La et al., 2003). Although culture and PCR can detect spirochaetes in the faeces of pigs that have diarrhoea and are excreting them in large numbers, they may not detect low numbers in healthy carrier animals (Fellström et al., 2001). Consequently these methods are not well suited for routine screening of apparently healthy herds to detect animals with low levels of colonisation, mainly because very large numbers of samples need to be tested and this is expensive and time consuming.

Enzyme-linked immunosorbent assays (ELISAs) have the potential to be used for quantifying serum antibodies to pathogens, and hence providing indirect evidence of past exposure to the pathogen. As a consequence they are very useful in routine screening of apparently healthy animals at the abattoir to assess

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http://dx.doi.org/10.1016/j.vetmic.2016.05.016
0378-1135/

the likely status of the herd of origin, particularly where the herd may have sub-clinical infection. ELISAs using either sonicated whole cells of *B. hyodysenteriae* or extracted lipooligosaccharide (LOS) as plate-coating antigens have been reported to be sensitive enough to allow detection of SD at a herd level, if sufficient numbers of pigs are tested (Joens et al., 1982; Wright et al., 1989; Smith et al., 1991; Mhoma et al., 1992; Song et al., 2012). Unfortunately as these antigens potentially could be shared by other *Brachyspira* species and generate false-positive cross-reactions this has hampered their further commercial development (La and Hampson, 2001). To overcome cross-reactivity, recombinant specific conserved surface proteins of *B. hyodysenteriae* have been trialled as ELISA antigens. One such antigen, Bhlp29.7 (formerly BmpB) has been used in a serological ELISA to identify infected herds (La et al., 2009a). Unfortunately the test also has potential for cross-reactivity with strains of the non-pathogenic *Brachyspira innocens* (La et al., 2005), and more importantly the *Bhlp29.7* gene was found to be absent in a significant number of *B. hyodysenteriae* strains (Barth et al., 2012). More recently an immunogenic surface protein named H114 was shown to be widespread amongst *B. hyodysenteriae* strains and to have potential to be used as an ELISA antigen to detect infected herds (Song et al., 2015). In the current study a prototype test using H114 developed by Prionics AG (Priocheck® *Brachyspira* porcine Ab ELISA) was tested on sera from pigs in 30 herds with or without a history of having SD. Fourteen of these herds gave apparently false positive results, but subsequent investigation by culture and PCR revealed that at least six were colonised with strains of *B. hyodysenteriae.*

2. Materials and methods

2.1. Permissions

This study was conducted with the approval of the Murdoch University Animal Ethics Committee under permit number R2292/09.

2.2. Herds and sample collection

Serum samples originating from 30 pig herds were collected by members of the Australian Pig Veterinarians (APV), a special interest group of the Australian Veterinary Association. The herds all had >500 sows, variously producing pure bred or commercial cross-bred progeny, and were located in the States of Western Australia (n = 10), Victoria (n = 9), New South Wales (n = 8) and South Australia (n = 3). The herds were not the same as those used in the original development of the ELISA (Song et al., 2015). The samples collected were from 12 herds where clinical SD had been recorded and where it was considered still to be present, even though controlled, and 18 where the consulting specialist veterinarian considered that the infection did not occur. Disease status was assessed based on clinical history, lack of use of routine medication in healthy herds, regular abattoir monitoring for disease signs and diagnostic testing of faecal samples from pigs with diarrhoea, including PCR for *Brachyspira* species. One of these herds was a breeding herd that was considered to be of very high health status. In most cases a minimum of 40 blood samples per herd was collected as a batch from healthy slaughter-aged pigs during exsanguination at the abattoir. Samples were collected into sterile screw cap plastic sample containers. Additional batches of sera were collected from seven herds where less than 40 samples were obtained at the initial sampling, or where consulting veterinarians questioned the initial results and sought additional testing. The blood samples were allowed to stand overnight at 4 °C and the serum was pipetted off and tested within 48 h.

2.3. ELISA testing

The testing was conducted blind to the origin of the samples. Serum samples were tested in a prototype commercial serological ELISA (Priocheck® *Brachyspira* porcine Ab ELISA; Prionics AG), as optimised by the manufacturer and following their detailed instructions. Recombinant antigen H114 was pre-coated on the plates by the manufacturer. Single strong positive, weak positive and negative control sera were supplied and included in each test run. Serum samples were diluted 1:20, added to wells on the plate, incubated at room temperature for 60 min, and after washing as recommended were reacted with a peroxidase labelled anti-pig antibody in a direct ELISA. Colour development using TMB substrate was measured optically at a wavelength of 450 nm. Percentage Positivity was calculated as the OD_{450nm} of the sample minus the OD_{450nm} of the negative control, divided by the OD_{450nm} of the positive control minus the OD_{450nm} of the negative control, multiplied by 100. Test samples with values equal to or above the cut-off of 30 Percent Positivity were considered positive in the test. Herds with one or more positive serum sample were considered to show evidence of infection with *B. hyodysenteriae.*

2.4. Culture and PCR

For 22 of the herds, a total of 350 colonic samples collected from pigs at the abattoir or faecal samples collected on farm subsequently were obtained for culture and PCR for *B. hyodysenteriae* (Table 1). Sampling occurred 6–24 months after the original ELISA testing, and was not possible in all cases as some producers did not want to be involved, or there had been changes in management, and one had closed down. The preferred samples were colonic mucosa obtained at the abattoir following slaughter, where minor thickening or irregularities in the otherwise apparently normal colonic wall were identified by external palpation. Between 1 and 20 colonic samples were obtained from 13 of the 22 herds. Faecal samples (between 1 and 50) were obtained from fattening pigs in 16 of the herds, including seven from herds where colon samples also were submitted. Loose faeces were requested, but in a number of cases the faeces received were of normal consistency and were considered unlikely to contain detectable levels of spirochaetes even if the animal was colonised.

Samples were plated onto selective Trypticase Soy Agar (BBL) plates containing 5% (vol/vol) defibrinated ovine blood, 400 mg of spectinomycin per ml, and 25 μg each of colistin and vancomycin (Sigma–Aldrich) per ml (Jenkinson and Wingar, 1981). The plates were incubated for 5–7 days at 37 °C in an anaerobic environment of 94% H_2 and 6% CO_2 generated with anaerobic Gaspak plus sachets (BBL). The plates were examined for the presence of a low, flat, spreading growth and associated haemolysis. Surface growth was re-suspending in phosphate-buffered saline and examined under a phase-contrast microscope. The harvested growth on plates suspected to have spirochaete growth were subjected to a PCR reaction for *B. hyodysenteriae*, as previously described (La et al., 2003).

2.5. Multilocus sequence typing (MLST)

Seven *B. hyodysenteriae* isolates obtained in pure culture were analysed by multilocus sequence typing (MLST), as previous described (La et al., 2009b). Two isolates came from herds where the veterinarian considered that SD was present and five were from apparently healthy herds. The isolate from the sixth of the healthy herds was not tested because it did not survive storage.