© CAB *International* 2001
ISSN 1466-2523

Animal Health Research Reviews **2**(1); 45–52
DOI: 10.1079/AHRR200115

Serologic detection of *Brachyspira* (*Serpulina*) *hyodysenteriae* infections

T. La and D. J. Hampson*

Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

Received and accepted 29 September 2000

Abstract

Swine dysentery (SD) caused by the intestinal spirochete *Brachyspira hyodysenteriae* is an economically important disease in pig-producing countries throughout the world. To date, no specific serologic assay is commercially available for the diagnosis of pigs with SD. Several serologic techniques have been identified in the past; however, these tests have all used either whole-cell proteins or lipopolysaccharide (LPS) as the antigen. Whole-cell antigens are plagued with false-positive reactions due to cross-reactivity with common proteins shared with other spirochetes. LPS antigens produce fewer false-positives; however, false-negatives may result due to LPS components being serogroup-specific. Generally, these techniques are useful for detecting infected herds, but are unreliable for the detection of individual infected pigs. In order to develop improved serologic tests it will be necessary to identify suitable diagnostic antigens, in particular immunogenic cell-surface structures which are specific to *B. hyodysenteriae* but common amongst different strains of the species. Recently, we identified and cloned a 30-kDa outer membrane lipoprotein (BmpB) which is specific to *B. hyodysenteriae* and is recognized by experimentally and naturally infected pigs. In this review we summarize the available serologic tests for SD, and speculate on the use of recombinant BmpB as an antigen for future development of an improved serologic test for SD diagnosis.

Introduction

Swine dysentery (SD) is a contagious mucohemorrhagic diarrheal disease of pigs, characterized by extensive inflammation and necrosis of the epithelial surface of the large intestine (Alexander and Taylor, 1969; Meyer, 1978; Harris *et al.*, 1999). The disease may occur in all ages of pigs, but is seen primarily in grower/finisher pigs. Economic losses due to SD result mainly from growth retardation, costs of medication, and mortality (Roncalli and Leaning, 1976; Hampson *et al.*, 1997; Harris *et al.*, 1999). The causative agent of SD was first identified and characterized in 1971 (Taylor and Alexander, 1971), and named *Treponema hyodysenteriae* (Glock and Harris, 1972; Harris *et al.*, 1972). This anaerobic spirochete was later assigned to the new genus *Serpula* (Stanton *et al.*, 1991), then to *Serpulina* (Stanton, 1992), and now has been reassigned as *Brachyspira hyodysenteriae* based on results of 16S rRNA gene sequence analysis (Ochiai *et al.*, 1997).

Diagnosis of swine dysentery

Swine dysentery is a relatively common disease which occurs in all major pig-producing countries (Roncalli and Leaning, 1976). When SD first enters a herd, the low exposure of the pigs to infectious doses initially results in disease in only one or two pigs. As infectious feces accumulate, the disease continues to spread to the remaining members of the herd. The rate of spread depends on the type of contact with the infected material. Once the infection becomes endemic within a piggery, the disease spectrum can vary from being mild, transient or inapparent, to severe and even fatal. Medication strategies on individual piggeries may mask clinical signs. Whether or not obvious disease is present,

* Corresponding author: Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia.
E-mail: hampson@numbat.murdoch.edu.au

B. hyodysenteriae may persist in infected pigs, or in other reservoir hosts such as mice, or the environment. All these sources pose potential for transmission of the disease between herds (Hampson *et al.*, 1997).

Various methods for the diagnosis of SD have been used over the years. In the first instance, observation of the clinical signs of the disease, including the presence of bloody and/or mucus-containing diarrhea, are commonly suggestive indications (Harris *et al.*, 1999). Similar clinical signs, however, may also be seen in salmonellosis, proliferative enteropathy, swine fever and intestinal spirochetosis (Hampson *et al.*, 1997). A definitive diagnosis of SD still involves the isolation and identification of *B. hyodysenteriae* from the feces or mucosa of the diseased pigs (Jensen, 1997; Harris *et al.*, 1999). Major problems involved in achieving this include the slow growth and fastidious nutritional requirements of the bacteria, and confusion due to the presence of morphologically similar spirochetes in the normal flora of the pig intestine (Hudson *et al.*, 1976; Joens and Harris, 1980). Significant improvements in diagnosis of individual pigs were achieved with the development of specific and sensitive nucleic acid-based assays, such as DNA probes (Jensen *et al.*, 1990; Sotiropoulos *et al.*, 1993) and polymerase chain reaction (PCR) assays (Elder *et al.*, 1994; Harel and Forget, 1995; Leser *et al.*, 1997; Atyeo *et al.*, 1998; Atyeo *et al.*, 1999) for the detection of spirochetes in feces. It has been suggested that PCR can detect as few as 10 cells per gram of feces; however, in practical applications the limit of detection of PCR consistently has been reported to be in the range of 10^3–10^4 cells per gram of feces. Although PCR can be a sensitive and rapid method for detection of *B. hyodysenteriae*, it requires the availability of a diagnostic laboratory with specialized equipment. The inability of PCR to detect low numbers of cells present in the feces also limits its application for the detection of carrier animals with subclinical infections.

Generally there is little difficulty in isolating and identifying *B. hyodysenteriae* in pigs with clinical SD. The main difficulty comes in detecting herds with subclinical infections, and individual healthy carrier animals which may be introduced into uninfected herds. As a consequence, there is a clear need to develop an effective diagnostic tool capable of detecting *B. hyodysenteriae* infection at the herd and individual pig level. This would allow appropriate treatment and quarantine procedures to be used. Follow-up testing also could provide an indication of the effectiveness of any treatment implemented.

Colonization by *B. hyodysenteriae* elicits a strong immunological response against the spirochete (Galvin *et al.*, 1997), hence indirect evidence of exposure to *B. hyodysenteriae* can be obtained by measuring circulating antibody titers in the blood of infected animals. These antibody titers have been reported to be maintained at lower levels, even in animals that have recovered from SD (Joens *et al.*, 1979, 1982). Serologic tests therefore have considerable potential for detecting carrier pigs that have undetectable numbers of spirochetes in their large intestines.

Serologic techniques presently available

A number of techniques have been developed to demonstrate the presence of circulating antibodies directed against *B. hyodysenteriae*. These have included indirect fluorescent antibody tests (IFAT), hemagglutination tests (HAT), microtitration agglutination tests (MAT), complement fixation tests (CFT) and enzyme-linked immunosorbent assays (ELISA) using either lipopolysaccharide (LPS) or whole sonicated spirochetes as antigen. All these tests can be useful for detecting infected herds, but they are less useful for identifying individual infected pigs. To date, no completely sensitive and specific assays are available for the detection of antibodies against *B. hyodysenteriae*.

Indirect fluorescent antibody test

Lee and Olson (1976) examined sera from 119 pigs that were exposed to *B. hyodysenteriae*, and then medicated with various drugs. They used an IFAT to detect serum antibodies raised against *B. hyodysenteriae* in these treated pigs and compared the results with those for 20 non-medicated pigs that had recovered naturally. Fluorescence was observed when the sera from 18 of the 20 non-medicated group were used, but none of the sera from the medicated pigs reacted at high titers. The more efficacious drugs apparently reduced the severity of the disease and resulted in the production of lower titers, which were still detectable in this simple assay. The specificity of the reaction for *B. hyodysenteriae* was not investigated.

Passive hemolysis test

Using sera from pigs experimentally infected with *B. hyodysenteriae*, Jenkins and co-workers (1976) were able to detect antibody against *B. hyodysenteriae* in a passive hemolysis test as early as 1 week after infection. Peak titers occurred 6 weeks after infection. In this test, sheep red blood cells (SRBC), sensitized with *B. hyodysenteriae* whole-cell antigens, were reacted with pig sera followed by treatment with guinea-pig complement. Antibody reactivity with the spirochete antigens on the SRBC would allow the activation of the complement cascade and consequently the lysis of the SRBC. Absorption of immune sera with 5% (v/v) sheep red blood cells reduced the hemolytic titers, indicating that some of the hemolysis of unabsorbed sera was probably due to het-

erogeneous antibodies. When hyperimmune sera were absorbed with organisms such as *Escherichia coli* and *Salmonella* spp. there was little or no effect on the resultant titer. Absorption of diseased pig sera with the homologous spirochete virtually abolished the hemolysis titers, although absorption with a heterologous spirochete did not significantly reduce the titers. It was therefore suggested that the passive hemolysis test could be a useful diagnostic tool as it was rapid to perform and was highly sensitive. However, the macroscopic scale of the reactions (2 ml final volume) would limit the number of samples which could be processed at one time. A small-scale variation of the assay in microtiter plates may overcome this problem. The specificity of this assay requires testing using sera from healthy pigs from a range of herds.

Complement fixation test

Adachi and co-workers (1984) compared the specificity of agglutinating and complement fixing antigens for the detection of antibody to *B. hyodysenteriae*. They found that agglutination reactions differed between strains. In contrast, the CFT showed reactions with all strains, although these were slightly weaker with heterogeneous strains than with the homologous strain. Furthermore, agglutination tests had the advantage of being about two- to eightfold more sensitive than the CFT at detecting antibody against *B. hyodysenteriae*. The CFT for SD has not been developed further.

Microtitration agglutination test

Joens and co-workers (1978) and later Joens and Harris (1980) developed a microtitration agglutination test to detect serum antibody to surface antigens of *B. hyodysenteriae*. This test was used to detect antibodies against *B. hyodysenteriae* in experimentally infected pigs, and was shown to have a high sensitivity and repeatability. Peak agglutinating titers occurred 3–4 weeks after the pigs were experimentally challenged with the organism. Only low cross-reacting antibody titers were produced against isolates of *Brachyspira innocens*, a non-pathogenic commensal spirochete commonly colonizing pigs, thus suggesting that the test was specific. The inactivated whole bacterial antigen used in this test remained stable for up to 10 days.

Diarra and co-workers (1995) evaluated the usefulness of the MAT, passive hemolysis test and indirect hemagglutination test for the detection of antibody against *B. hyodysenteriae* in pig sera. Among these tests, only the MAT was able to detect antibodies to *B. hyodysenteriae* in diseased pigs. The use of a boiled cell suspension as antigen in the MAT improved the reliability of the test for the diagnosis of SD on a herd basis. Boiling the antigen significantly reduced cross-reactions between *B. hyodysenteriae* and *B. innocens*.

Although this test appeared to be reasonably specific and sensitive, it lacked standardization to allow its use for routine diagnosis. The antigen used consisted of a complex mixture of inactivated whole bacteria, which would differ in composition depending on the culture conditions used. An incubation time of 18–36 hours was required, making this rather slow for routine testing. In general, pigs develop circulating antibody titers to *B. hyodysenteriae* from 1 to 2 weeks after inoculation (Joens *et al.*, 1979, 1982). The MAT detected agglutinating antibody titers 3–4 weeks after inoculation, suggesting that this test did not have sufficient sensitivity for detecting pigs with low titers of antibody. An overlying disadvantage of the MAT is that it can only be used to measure total antibody produced against the spirochete, thus subjecting it to variations depending on the strain of *B. hyodysenteriae* with which the pig is infected.

Enzyme-linked immunosorbent assay with lipopolysaccharide as antigen

An enzyme-linked immunosorbent assay (ELISA), using LPS extracted by the hot phenol–water method as a plate-coating antigen, has been used for the detection of antibody against *B. hyodysenteriae* infection in swine (Joens *et al.*, 1982). Infected pigs were detected within 1–2 weeks after inoculation, and circulating antibody could be demonstrated in convalescent pigs for as long as 19 weeks after inoculation (Joens *et al.*, 1982). Egan and co-workers (1983) compared the accuracy and sensitivity of MAT and ELISA, and concluded that the ELISA was more useful, but it still was not suitable for detection of individual infected pigs because false-positive and false-negative results occurred. *B. hyodysenteriae* isolates have been divided into nine or more serogroups based on their LPS components and cross-reactivities with absorbed antisera (Hampson *et al.*, 1989a, b, 1990, 1994; Hampson, 1991; Combs *et al.*, 1992). Knowing this, it is expected that no one extract of LPS can be used as a general antigen in the ELISA. Each serum must be tested with a range of different LPS antigen standards because of the serotype specificity of the LPS (Egan *et al.*, 1983). Therefore, knowledge of the serogroups of the *B. hyodysenteriae* present in a geographic area is necessary so that appropriate LPS extracts can be used (Mhoma *et al.*, 1992). Even then, false-negative reactions may arise from infections with members of new or undetermined serogroups. It is known that common LPS reactivities exist within serogroups (Wannemuehler *et al.*, 1988; Hampson *et al.*, 1989a, b; Lau and Hampson, 1992); however, there is no evidence showing common LPS reactivities across serogroups. For field conditions, the LPS-ELISA appears to be a robust tool for analysis of

multiple samples and can be completed with reasonably short incubation times. However, without a suitable general antigen specific for *B. hyodysenteriae*, this form of ELISA remains problematic for general use.

Enzyme-linked immunosorbent assay with sonicated bacteria as antigen

Swine dysentery has been detected in herds by the use of an ELISA in which serum antibodies are measured using whole sonicated cells of *B. hyodysenteriae* to coat the wells of the ELISA plate (Wright *et al.*, 1989; Smith *et al.*, 1991). This form of ELISA appears to be sensitive enough to detect the majority of infected herds. When tested at a herd level, it correctly identified 90% of individually infected pigs (Wright *et al.*, 1989). Occasionally, however, it can give false-negative results, but its most serious limitation is that it can give non-specific cross-reactivity (Burrows *et al.*, 1984; Wright *et al.*, 1989). However, the specificity and sensitivity of this form of ELISA for detection of antibody to *B. hyodysenteriae* in pigs can be improved when coupled with immunoblotting against outer envelope extracts of *B. hyodysenteriae* (Smith *et al.*, 1991). The use of sonicated spirochetes in ELISA may be a useful tool for screening herds; however, due to the many cross-reactivities between *B. hyodysenteriae* and other related spirochetes, it still cannot be used as a definitive test for SD. Even when coupled with immunoblotting, the possibility of cross-reactivity still exists. The increased complexity of the overall technique also makes this less suitable for applications in the field, and it does not appear to have been developed further.

Requirement for a specific antibody-based assay for SD

To date, techniques available for the detection of circulating antibodies against *B. hyodysenteriae* have been based on a complex mixture of cell surface antigens. Although this may provide preliminary information about the infection status of a pig herd where multiple samples are examined, it does not provide definite information regarding the exposure of individual animals to the spirochete. Such tests are also subject to uncertainties about their sensitivity and specificity.

Identification of a suitable antigen

Ideally, a suitable assay component would involve a single, membrane-associated protein, preferably recombinant, which is specific for *B. hyodysenteriae* and known to be highly immunogenic in experimentally and naturally infected pigs. In order to identify and characterize such antigens to which an immune response is generated, flagellar proteins and proteins associated with the outer membrane (OMP) of *B. hyodysenteriae* have been investigated.

Flagellar proteins

The periplasmic flagella of most spirochetes are complex structures consisting of several different polypeptides (Charon *et al.*, 1992). Many flagellar proteins of *B. hyodysenteriae* have been observed; however, most have cross-reacted with sera raised against *B. innocens* (Miller *et al.*, 1988; Kent *et al.*, 1989; Koopman *et al.*, 1992a, b, 1993; Li *et al.*, 1993). To date, three antigenically related flagella core proteins with masses of 37, 34 and 32 kDa have been identified and designated FlaB1, FlaB2 and FlaB3, respectively (Koopman *et al.*, 1993; Gabe *et al.*, 1995; Rosey *et al.*, 1995). Two flagella sheath proteins also have been identified and designated FlaA1 (44 kDa) and FlaA2 (35 kDa). Together, these flagellar proteins comprise approximately 10% of the total *B. hyodysenteriae* cell protein (Koopman *et al.*, 1992b), and their cell surface location makes these proteins potential candidates for a diagnostic reagent. However, flagellar proteins are highly conserved among spirochete species and genera, therefore false-positive results due to cross-reactivity are always a potential problem if these proteins are used for serologic tests (Jensen, 1997). This is consistent with polyclonal and monoclonal antibody reagents raised against *B. hyodysenteriae* cross-reacting with *B. pilosicoli* FlaA1 and FlaB by immunoblot (Fisher *et al.*, 1997).

Outer membrane proteins

The cell envelope of *B. hyodysenteriae* can be extracted based on its solubility in detergent, and the proteins associated with the envelope can then be identified (Chatfield *et al.*, 1988a, b; Sellwood *et al.*, 1989; Smith *et al.*, 1990; Thomas *et al.*, 1992; Joens *et al.*, 1993). Sarcosyl-insoluble fractions of *B. hyodysenteriae* were examined by Joens and co-workers (1993) using SDS-PAGE and immunoblotting with convalescent pig serum against *B. hyodysenteriae*. Seven major proteins were observed: six of these were periplasmic flagellar proteins in the range 32–42 kDa, and the other was a 16-kDa band. Serum obtained from pigs at the onset of clinical signs of SD contained antibodies to some of these proteins. Smith and co-workers (1990) also compared sarcosyl-insoluble fractions of *B. hyodysenteriae* and *B. innocens*, and found a distinct band in the molecular mass range of 39 kDa only in *B. hyodysenteriae*. This protein has since been identified as a major cell-surface protein, and the linked set of genes, designated *vsp*A, *vsp*B, *vsp*C and *vsp*D, encoding variable surface proteins homologous to it have been cloned and sequenced (Gabe *et al.*, 1998; McCaman *et al.*, 1999). Although this 39-kDa protein may contain regions of variability, conserved regions may be useful for detection of antibody

specific for *B. hyodysenteriae*. The cell-surface location of this protein also makes it potentially useful as the basis of a serologic assay.

SDS-soluble proteins were extracted from *B. hyodysenteriae* and analysed by Chatfield and co-workers (1988a, b). Porcine hyperimmune serum detected polypeptide antigens of molecular weights within the range 30–36 kDa. When the cell envelope from *B. hyodysenteriae* was extracted using Triton X-100, several major immunogenic polypeptides with molecular weights between 24 and 45 kDa were detected using serum from a pig vaccinated with whole cells of *B. hyodysenteriae* (Chatfield, 1988a). A 36-kDa antigen associated with the cell envelope was immunologically distinct in *B. hyodysenteriae*, and antibody against this antigen was not absorbed out by whole *B. innocens* cells. This 36-kDa protein may be related to the 39-kDa protein observed by Smith *et al.* (1990).

Thomas and co-workers (Thomas *et al.*, 1992; Thomas and Sellwood, 1993) identified a 16-kDa membrane-associated lipoprotein common to many strains of *B. hyodysenteriae*, which they designated SmpA (*Serpulina* membrane protein A). They identified and sequenced the gene encoding SmpA. When pigs were experimentally challenged with *B. hyodysenteriae*, SmpA was detected only during the initial postinoculation period and failed to be detected after the onset of clinical signs of SD. When the inoculated strain of *B. hyodysenteriae* was isolated and re-cultured *in vitro*, SmpA was detected. This loss of *in vivo* expression was proposed to be due to expression of the gene encoding SmpA being repressed (Sellwood *et al.*, 1995). The low antibody titers which developed against SmpA as a result of its lack of *in vivo* expression prevented its use as the basis of a serologic assay.

Li *et al.* (1995) reported that *B. hyodysenteriae* expressed at least three iron-regulated proteins with apparent molecular masses of 109, 134 and >200 kDa when grown under iron-restricted conditions. The 109-kDa major iron-regulated protein (IRP) was expressed *in vivo* and was conserved among all *B. hyodysenteriae* strains tested. However, presumably the IRPs are not antigenically specific for *B. hyodysenteriae* as they are also expressed in *B. innocens*.

Recently, a periplasmic ATP-binding cassette iron import system of *B. hyodysenteriae* was characterized (Dugourd *et al.*, 1999). This import system consisted of three periplasmic iron-binding proteins (BitABC), an ABC transporter protein (BitD) and two permeases (BitEF). Southern hybridization of the genes with genomic DNA of *B. hyodysenteriae* and *B. innocens* found that only *B. hyodysenteriae* strains hybridized with the probes. Affinity purified rabbit polyclonal antiserum prepared against *B. hyodysenteriae* whole cells, and enriched for anti-Bit antibody, was used in Western blotting with *B. hyodysenteriae* and *B. innocens* whole-cell extracts. Only *B. hyodysenteriae* strains reacted with the rabbit antiserum. Although it is believed that BitABCDEF is only expressed in *B. hyodysenteriae*, expression in the other *Brachyspira* spp. was not investigated (Dugourd *et al.*, 1999). The specificity of these proteins make them potential targets for serologic assay development.

More recently, Ochiai and co-workers (2000) demonstrated that sera from pigs which were not treated with spectinomycin prior to challenge with *B. hyodysenteriae* reacted strongly with 17- and 22-kDa proteins of *B. hyodysenteriae* by immunoblot. These sera did not recognize proteins of similar molecular weight from *B. innocens*; however, reactivity with the other *Brachyspira* spp. was not tested. Sera from pigs treated with spectinomycin and challenged with *B. hyodysenteriae* did not react with these proteins. In addition, *B. hyodysenteriae* MAT titers were very high in the untreated pigs but low in the spectinomycin-treated pigs. The cellular localization of these proteins was not determined; however, their apparent specificity to *B. hyodysenteriae* makes them potentially applicable for serologic tests for SD. Further analysis is required to determine whether the 17- and 22-kDa proteins are unique to *B. hyodysenteriae*.

Recent studies with a 30-kDa outer envelope lipoprotein

A 30-kDa outer membrane lipoprotein of *B. hyodysenteriae* has recently been identified in our laboratory, and the gene cloned and sequenced (Lee *et al.*, 2000). Lipoproteins of spirochetes are immunogenic, and potentially useful for use in serological assays (Haake, 2000). The gene for the 30-kDa lipoprotein, designated *bmp*B (*Brachyspira* membrane protein B), was found to be present in all of 79 strains of the spirochete tested by polymerase chain reaction (PCR) analysis. Similarly, a monoclonal antibody (BJL/SH1) directed against the lipoprotein was found to be specific for all 16 strains of *B. hyodysenteriae* tested, and did not cross-react with the other known species in the genus *Brachyspira* (Lee and Hampson, 1996). *Bmp*B has been cloned into *E. coli* cells and formalinized whole cells used to immunize mice and pigs. Sera from the immunized animals reacted with a range of *E. coli* proteins, including the 30-kDa recombinant BmpB lipoprotein, indicating that the animals recognized BmpB. Sera from pigs naturally infected with *B. hyodysenteriae* also reacted in an immunoblot with a 30-kDa band in whole-cell extracts of *E. coli* expressing the recombinant BmpB, confirming the *in vivo* expression of the gene. When *bmp*B was cloned into an *E. coli* expression system to produce a 34-kDa fusion protein with six histidine residues at the N-terminal, the purified recombinant BmpB reacted in Western blot assays with sera from 13 pigs naturally infected with *B. hyodysenteriae*. Unfortunately weak reactions also

were obtained when recombinant BmpB was tested with sera from seven pigs known to be free of SD (Lee *et al.*, 2000). It is possible that these cross-reactions may be removed in ELISA tests with suitable modifications to pH, ion concentrations and other reaction conditions.

Although the lack of specificity with recombinant BmpB was disappointing, it was not unexpected as the 30-kDa lipoprotein contains 271 amino acids and is likely to display multiple epitopes to which an antibody response can be elicited. It is highly likely that epitopes exist on other proteins that are similar to those on BmpB, and elicit cross-reactivity. The fact that monoclonal antibody BJL/SH1 reacts only with *B. hyodysenteriae* means that a section of BmpB may contain a specific epitope which will react only with sera from animals exposed to this spirochete. However, the reactivity of this epitope with sera from animals exposed to *B. hyodysenteriae* remains unknown.

Cloning of truncated versions of *bmp*B into *E. coli* expression vectors to map the location of the BJL/SH1 epitope is in progress. Once the peptide sequence for the epitope has been identified and confirmed to be specific, it can be expressed as a single unit, or as multiple repeating units, and serve as an antigen for ELISA. Sera from a range of healthy pigs as well as pigs experimentally and naturally infected with *B. hyodysenteriae* will be used to determine the specificity and sensitivity of this assay.

The assay might also be applicable for the detection of secretory IgA in the feces of pigs, as only a small volume of material is required. *B. hyodysenteriae* colonizes the intestinal mucosa, therefore the primary immunological response to the spirochete is likely to be a local one. If detectable, this response would permit an earlier diagnosis, and also may identify subclinically colonized carrier animals. Ultimately, a simple test which involves the incubation of a blood or fecal sample from the animal with a test strip, followed by the addition of a secondary reagent giving an immediate colorimetric result, would be most valuable for SD testing in the field.

Conclusion

A variety of techniques have been developed to detect circulating antibody against *B. hyodysenteriae*. Typically, these tests have used whole-cell proteins or LPS as the antigen. Whole-cell antigens are limited by the occurrence of false-positives due to cross-reactivity with common proteins shared with other spirochetes. LPS antigens produce fewer false-positive reactions; however, false-negatives may result due to LPS components being serogroup-specific. In general, these techniques are useful for detecting infected herds, but are unable to detect individual infected pigs that may act as carriers. Recently, a 30-kDa outer membrane lipoprotein (BmpB) which is specific to *B. hyodysenteriae* and is recognized by experimentally and naturally infected pigs was identified and the gene cloned and sequenced. Specific epitopes on BmpB are being identified, with the intention of producing these in recombinant form to use as the basis of a new serologic assay to detect pigs that have been exposed to *B. hyodysenteriae*. Such an assay could be used to help identify and treat herds with both clinical and subclinical infection, as well as to identify potential carrier pigs that might introduce infection into healthy herds.

Acknowledgments

We would like to thank Bong Joo Lee, Andrew Mikosza and Sophy Oxberry for their assistance in this project.

References

Adachi T, Tanaka T and Watase H (1984). A relationship between the outbreak of swine dysentery and antibody response to *Treponema hyodysenteriae*. In: Proceedings of the International Pig Veterinary Society Congress, Ghent, Belgium, P182.

Alexander TJ and Taylor DJ (1969). The clinical signs, diagnosis and control of swine dysentery. *Veterinary Record* **85**: 59–63.

Atyeo RF, Oxberry SL, Combs BG and Hampson DJ (1998). Development and evaluation of polymerase chain reaction tests as an aid to diagnosis of swine dysentery and intestinal spirochaetosis. *Letters in Applied Microbiology* **26**: 126–130.

Atyeo RF, Stanton TB, Jensen NS, Suriyaarachichi DS and Hampson DJ (1999). Differentiation of *Serpulina* species by NADH oxidase gene (nox) sequence comparisons and nox-based polymerase chain reaction tests. *Veterinary Microbiology* **67**: 47–60.

Burrows MR, Lysons RJ, Rowlands GJ and Lemcke RM (1984). An enzyme-linked immunosorbent assay for detecting serum antibody to *Treponema hyodysenteriae*. In: Proceedings of the International Pig Veterinary Society Congress, Ghent, Belgium, P186.

Charon NW, Greenberg EP, Koopman MB and Limberger RJ (1992). Spirochete chemotaxis, motility, and the structure of the spirochetal periplasmic flagella. *Research in Microbiology* **143**: 597–603.

Chatfield SN, Fernie DS, Beesley J, Penn C and Dougan G (1988a). Characterisation of the cell envelope of *Treponema hyodysenteriae*. *FEMS Microbiology* **55**: 303–308.

Chatfield SN, Fernie DS, Penn C and Dougan G (1988b). Identification of the major antigens of *Treponema hyodysenteriae* and comparison with those *of Treponema innocens*. *Infection and Immunity* **56**: 1070–1075.

Combs BG, Hampson DJ and Harders SJ (1992). Typing of Australian isolates of *Treponema hyodysenteriae* by serology and by DNA restriction endonuclease analysis. *Veterinary Microbiology* **31**: 273–285.

Diarra AT, Achacha M and Mittal KR (1995). Evaluation of different serological tests for detection of antibodies against *Serpulina hyodysenteriae* in pig sera. *Comparative Immunology Microbiology and Infectious Disease* **18**: 215–221.

Dugourd D, Martin C, Rioux CR, Jacques M and Harel J (1999). Characterization of a periplasmic ATP-binding cassette iron import system of *Brachyspira* (*Serpulina*) *hyodysenteriae*. *Journal of Bacteriology* **181**: 6948–6957.

Egan IT, Harris DL and Joens LA (1983). Comparison of the microtitration agglutination test and the enzyme-linked immunosorbent assay for the detection of herds affected with swine dysentery. *American Journal of Veterinary Research* **44**: 1323–1328.

Elder RO, Duhamel GE, Schafer RW, Mathiesen MR and Ramanathan M (1994). Rapid detection of *Serpulina hyodysenteriae* in diagnostic specimens by PCR. *Journal of Clinical Microbiology* **32**: 1497–1502.

Fisher LN, Duhamel GE, Westerman RB and Mathiesen MR (1997). Immunoblot reactivity of polyclonal and monoclonal antibodies with periplasmic flagellar proteins FlaA1 and FlaB of porcine *Serpulina* species. *Clinical and Diagnostic Laboratory Immunology* **4**: 400–404.

Gabe JD, Chang RJ, Slomiany R, Andrews WH and McCaman MT (1995). Isolation of extracytoplasmic proteins from *Serpulina hyodysenteriae* B204 and molecular cloning of the flaB1 gene encoding a 38-kilodalton flagellar protein. *Infection and Immunity* **63**: 142–148.

Gabe JD, Dragon E, Chang RJ and McCaman MT (1998). Identification of a linked set of genes in *Serpulina hyodysenteriae* (B204) predicted to encode closely related 39-kilodalton extracytoplasmic proteins. *Journal of Bacteriology* **180**: 444–448.

Galvin JE, Harris DL and Wannemuehler MJ (1997). Prevention and control of intestinal spirochaetal disease: immunological and pharmacological mechanisms. In: Hampson DJ and Stanton TB (eds), *Intestinal Spirochaetes in Domestic Animals and Humans*. Wallingford, UK: CAB International, pp. 343–374.

Glock RD and Harris DL (1972). Swine dysentery. II. Characterization of lesions in pigs inoculated with *Treponema hyodysenteriae* in pure and mixed culture. *Veterinary Medicine and Small Animal Clinician* **67**: 65–68.

Haake DA (2000). Spirochaetal lipoproteins and pathogenesis. *Microbiology* **146**: 1491–1504.

Hampson DJ (1991). Slide-agglutination for rapid serological typing *of Treponema hyodysenteriae*. *Epidemiology and Infection* **106**: 541–547.

Hampson DJ, Mhoma JR and Combs B (1989a). Analysis of lipopolysaccharide antigens of *Treponema hyodysenteriae*. *Epidemiology and Infection* **103**: 275–284.

Hampson DJ, Mhoma JR, Combs B and Buddle JR (1989b). Proposed revisions to the serological typing system for *Treponema hyodysenteriae* [published erratum appears in *Epidemiology and Infection* **103**: 401]. *Epidemiology and Infection* **102**: 75–84.

Hampson DJ, Mhoma JR, Combs BG and Lee JI (1990). Serological grouping of *Treponema hyodysenteriae*. *Epidemiology and Infection* **105**: 79–85.

Hampson DJ, Maltas CD, Stephens CP, McKechnie K and Buller NB (1994). Serogroups of Australian isolates of *Serpulina hyodysenteriae*. *Australian Veterinary Journal* **71**: 347.

Hampson DJ, Atyeo RF and Combs BG (1997). Swine dysentery. In: Hampson DJ and Stanton TB (eds), *Intestinal Spirochaetes in Domestic Animals and Humans*. Wallingford, UK: CAB International, pp. 175–209.

Harel J and Forget C (1995). DNA probe and polymerase chain reaction procedure for the specific detection of *Serpulina hyodysenteriae*. *Molecular and Cellular Probes* **9**: 111–119.

Harris DL, Glock RD, Christensen CR and Kinyon JM (1972). Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. *Veterinary Medicine and Small Animal Clinician* **67**: 61–64.

Harris DL, Hampson DJ and Glock RD (1999). Swine dysentery. In: Straw BE, D'Allaire S, Mengeling WL and Taylor DJ (eds), *Diseases of swine*. Ames, Iowa, USA: Iowa State University, pp. 579–600.

Hudson MJ, Alexander TJ, Lysons RJ and Prescott JF (1976). Swine dysentery: protection of pigs by oral and parenteral immunisation with attenuated *Treponema hyodysenteriae*. *Research in Veterinary Science* **21**: 366–367.

Jenkins EM, Sinha PP, Vance RT and Reese GL (1976). Passive hemolysis test for antibody *to Treponema hyodysenteriae*. *Infection and Immunity* **14**: 1106–1108.

Jensen NS (1997). Detection, identification and subspecific differentiation of intestinal spirochaetes. In: Hampson DJ and Stanton TB (eds), *Intestinal Spirochaetes in Domestic Animals and Humans*. Wallingford, UK: CAB International, pp. 323–341.

Jensen NS, Casey TA and Stanton TB (1990). Detection and identification of *Treponema hyodysenteriae* by using oligodeoxynucleotide probes complementary to 16S rRNA. *Journal of Clinical Microbiology* **28**: 2717–2721.

Joens LA and Harris DL (1980). Comparison of selective culture and serologic agglutination of *Treponema hyodysenteriae* for diagnosis of swine dysentery. *Veterinary Record* **106**: 245–246.

Joens LA, Harris DL, Kinyon JM and Kaeberle ML (1978). Microtitration agglutination for detection of *Treponema hyodysenteriae* antibody. *Journal of Clinical Microbiology* **8**: 293–298.

Joens LA, Harris DL and Baum DH (1979). Immunity to swine dysentery in recovered pigs. *American Journal of Veterinary Research* **40**: 1352–1354.

Joens LA, Nord NA, Kinyon JM and Egan IT (1982). Enzyme-linked immunosorbent assay for detection of antibody to *Treponema hyodysenteriae* antigens. *Journal of Clinical Microbiology* **15**: 249–252.

Joens LA, Marquez MR and Halter M (1993). Comparison of outer-membrane fractions of *Serpulina* (*Treponema*) *hyodysenteriae*. *Veterinary Microbiology* **35**: 119–132.

Kent KA, Sellwood R, Lemcke RM, Burrows MR and Lysons RJ (1989). Analysis of the axial filaments of *Treponema hyodysenteriae* by SDS-PAGE and immunoblotting. *Journal of General Microbiology* **135**: 1625–1632.

Koopman MB, Baats E, van Vorstenbosch CJ, van der Zeijst BA and Kusters JG (1992a). The periplasmic flagella of *Serpulina* (*Treponema*) *hyodysenteriae* are composed of two sheath proteins and three core proteins. *Journal of General Microbiology* **138**: 2697–2706.

Koopman MB, de Leeuw OS, van der Zeijst BM and Kusters JG (1992b). Cloning and DNA sequence analysis of a *Serpulina* (*Treponema*) *hyodysenteriae* gene encoding a periplasmic flagellar sheath protein. *Infection and Immunity* **60**: 2920–2925.

Koopman MB, Baats E, de Leeuw OS, van der Zeijst BA and Kusters JG (1993). Molecular analysis of a flagellar core protein gene of *Serpulina* (*Treponema*) *hyodysenteriae*. *Journal of General Microbiology* **139**: 1701–1706.

Lau TT and Hampson DJ (1992). The serological grouping system *for Serpulina* (*Treponema*) *hyodysenteriae*. *Epidemiology and Infection* **109**: 255–263.

Lee BJ and Hampson DJ (1996). Production and characterisation of a monoclonal antibody to *Serpulina hyodysenteriae*. *FEMS Microbiology Letters* **136**: 193–197.

Lee BJ, La T, Mikosza ASJ and Hampson DJ (2000). Identification of the gene encoding BmpB, a 30 kDa outer envelope lipoprotein of *Brachyspira* (*Serpulina*) *hyodysenteriae*, and immunogenicity of recombinant BmpB in mice and pigs. *Veterinary Microbiology* **76**: 245–257.

Lee CH and Olson LD (1976). Immunofluorescence of spirochetes with serum from swine recovered from swine dysentery using an indirect fluorescent antibody test. *Canadian Journal of Comparative Medicine* **40**: 404–407.

Leser TD, Moller K, Jensen TK and Jorsal SE (1997). Specific detection *of Serpulina hyodysenteriae* and potentially pathogenic weakly beta-haemolytic porcine intestinal spirochetes by polymerase chain reaction targeting 23S rDNA. *Molecular and Cellular Probes* **11**: 363–372.

Li Z, Dumas F, Dubreuil D and Jacques M (1993). A species-specific periplasmic flagellar protein of *Serpulina* (*Treponema*) *hyodysenteriae*. *Journal of Bacteriology* **175**: 8000–8007.

Li Z, Foiry B and Jacques M (1995). Growth of *Serpulina* (*Treponema*) *hyodysenteriae* under iron-restricted conditions. *Canadian Journal of Veterinary Research* **59**: 149–153.

McCaman MT, Auer K, Foley W and Gabe JD (1999). Sequence characterization of two new members of a multi-gene family in *Serpulina hyodysenteriae* (B204) with homology to a 39 kDa surface exposed protein: vspC and D. *Veterinary Microbiology* **68**: 273–283.

Meyer RC (1978). Swine dysentery: a perspective. *Advances in Veterinary Science and Comparative Medicine* **22**: 133–158.

Mhoma JR, Hampson DJ and Robertson ID (1992). A serological survey to determine the prevalence of infection with *Treponema hyodysenteriae* in Western Australia. *Australian Veterinary Journal* **69**: 81–84.

Miller DP, Toivio-Kinnucan M, Wu G and Wilt GR (1988). Ultrastructural and electrophoretic analysis of *Treponema hyodysenteriae* axial filaments. *American Journal of Veterinary Research* **49**: 786–789.

Ochiai S, Adachi Y and Mori K (1997). Unification of the genera *Serpulina* and *Brachyspira*, and proposals of *Brachyspira hyodysenteriae* comb. nov., *Brachyspira innocens* comb. nov. and *Brachyspira pilosicoli* comb. nov. *Microbiology and Immunology* **41**: 445–452.

Ochiai S, Adachi Y, Asano T, Prapasarakul N, Ogawa Y and Ochi K (2000). Presence of 22-kDa protein reacting with sera in piglets experimentally infected with *Brachyspira hyodysenteriae*. *FEMS Immunology and Medical Microbiology* **28**: 43–47.

Roncalli RA and Leaning WHD (1976). Geographical distribution of swine dysentery. In: Proceedings of the International Pig Veterinary Society Congress, Ames, Iowa, USA, L17.

Rosey EL, Kennedy MJ, Petrella DK, Ulrich RG and Yancey RJ Jr (1995). Inactivation of *Serpulina hyodysenteriae* flaA1 and flaB1 periplasmic flagellar genes by electroporation-mediated allelic exchange. *Journal of Bacteriology* **177**: 5959–5970.

Sellwood R, Kent KA, Burrows MR, Lysons RJ and Bland AP (1989). Antibodies to a common outer envelope antigen of *Treponema hyodysenteriae* with antibacterial activity. *Journal of General Microbiology* **135**: 2249–2257.

Sellwood R, Walton F, Thomas W, Burrows MR and Chesham J (1995). Expression of the SmpA outer membrane lipoprotein of *Serpulina hyodysenteriae* strain P18A in vivo. *Veterinary Microbiology* **44**: 25–35.

Smith SC, Roddick F, Ling S, Gerraty NL and Coloe PJ (1990). Biochemical and immunochemical characterisation of strains of *Treponema hyodysenteriae*. *Veterinary Microbiology* **24**: 29–41.

Smith SC, Barrett LM, Muir T, Christopher WL and Coloe PJ (1991). Application and evaluation of enzyme-linked immunosorbent assay and immunoblotting for detection of antibodies to *Treponema hyodysenteriae* in swine. *Epidemiology and Infection* **107**: 285–296.

Sotiropoulos C, Smith SC and Coloe PJ (1993). Characterization of two DNA probes specific for *Serpulina hyodysenteriae*. *Journal of Clinical Microbiology* **31**: 1746–1752.

Stanton TB (1992). Proposal to change the genus designation *Serpula* to *Serpulina* gen. nov. containing the species *Serpulina hyodysenteriae* comb. nov. and *Serpulina innocens* comb. nov. *International Journal of Systematic Bacteriology* **42**: 189–190.

Stanton TB, Jensen NS, Casey TA, Tordoff LA, Dewhirst FE and Paster BJ (1991). Reclassification of *Treponema hyodysenteriae* and *Treponema innocens* in a new genus, *Serpula* gen. nov., as *Serpula hyodysenteriae* comb. nov. and *Serpula innocens* comb. nov. *International Journal of Systematic Bacteriology* **41**: 50–58.

Taylor DJ and Alexander TJ (1971). The production of dysentery in swine by feeding cultures containing a spirochaete. *British Veterinary Journal* **127**: 58–61.

Thomas W and Sellwood R (1993). Molecular cloning, expression, and DNA sequence analysis of the gene that encodes the 16-kilodalton outer membrane lipoprotein of *Serpulina hyodysenteriae*. *Infection and Immunity* **61**: 1136–1140.

Thomas W, Sellwood R and Lysons RJ (1992). A 16-kilodalton lipoprotein of the outer membrane of *Serpulina* (*Treponema*) *hyodysenteriae*. *Infection and Immunity* **60**: 3111–3116.

Wannemuehler MJ, Hubbard RD and Greer JM (1988). Characterization of the major outer membrane antigens of *Treponema hyodysenteriae*. *Infection and Immunity* **56**: 3032–3039.

Wright JC, Wilt GR, Reed RB and Powe TA (1989). Use of an enzyme-linked immunosorbent assay for detection of *Treponema hyodysenteriae* infection in swine. *Journal of Clinical Microbiology* **27**: 411–416.