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Application and evaluation of enzyme-linked immunosorbent assay and immunoblotting for detection of antibodies to *Treponema hyodysenteriae* in swine

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SUMMARY

An enzyme-linked immunoassay (ELISA) has been developed to detect serum Immunoglobulin antibodies G and M to *Treponema hyodysenteriae* in vaccinated, experimentally infected and naturally infected swine. Naturally infected swine gave ELISA titres that were similar to experimentally infected swine, but were significantly less than the titres of vaccinated swine. When serum from naturally infected swine was used to probe nitrocellulose blots of sodium dodecyl sulphate–polyacrylamide gel electrophoresed whole cell proteins of *T. hyodysenteriae*, the immunoblotting patterns showed IgG antibodies were produced against many *T. hyodysenteriae* protein antigens and against lipopolysaccharide (LPS). The IgG antibodies directed against LPS were serotype-specific for that LPS and could be used to identify the serotype involved in the *T. hyodysenteriae* infection in that herd. IgM immunoblots also reacted with the many protein antigens but were less specific for LPS antigen, with a substantial degree of cross-reaction between the LPS of all serotypes.

The data demonstrate that a microplate enzyme-linked immunosorbent assay, coupled with immunoblotting, is a very specific and sensitive test for detection of antibody to *Treponema hyodysenteriae* in swine.

INTRODUCTION

Treponema hyodysenteriae, an anaerobic spirochaete, is the causative agent of swine dysentery (SD). *T. hyodysenteriae* infection is confined to the large intestine in swine and causes severe enteritis, presenting as mucohaemorrhagic diarrhoea with consequent damage to the large intestine [1–4]. The detection and control of swine dysentery is important to the swine industry to enable proper management of infected swine, to facilitate the detection of asymptomatic carriers [5] and to assist in monitoring control mechanisms for the disease [4].

Humoral antibody titres to *T. hyodysenteriae* infection in swine have been detected by a number of different serological assays and techniques. These include plate agglutination and microtitration agglutination tests [6, 7]. However, these

tests are not effective in the detection of individual swine infections nor are they sensitive methods to evaluate post-infection antibody titres [8]. More recently, Joens and co-workers [8] described the use of an enzyme-linked immunosorbent assay (ELISA) to detect antibody to lipopolysaccharide (LPS) extracted from cells of *T. hyodysenteriae* serotypes 1 and 2 [9]. However, this assay is LPS specific and so will not detect antibodies to other serotypes of *T. hyodysenteriae*.

There is a need within the swine industry for a specific, sensitive assay to detect *T. hyodysenteriae* infection. However, because avirulent *Treponema* spp. (including *T. innocens*) exist as normal enteric flora in swine [10], an assay must be able to differentiate antibodies to *T. hyodysenteriae* from those against normal flora.

In this study we report on the use of a sonicated *T. hyodysenteriae* bacterial-extract as antigen in an ELISA assay to measure humoral antibodies in experimentally infected and naturally infected swine, and we report on the use of a Western blotting procedure to detect *T. hyodysenteriae* specific antigens to complement the ELISA assay and confirm the specificity of the assay. The techniques have been used in tandem to detect field outbreaks of SD and to identify the serotype of *T. hyodysenteriae* causing infection.

MATERIALS AND METHODS

Bacterial strains and antigen preparation

T. hyodysenteriae reference strains B78 (serotype 1), B204 (serotype 2), B169 (serotype 3), A1 (serotype 4) and *T. innocens* reference strain B256 used in this study were provided from the A.F.R.C. Institute for Research on Animal Disease, Compton, England by Dr R. J. Lysons and *T. hyodysenteriae* strain 8929 (serotype 1) was provided by Professor Synkiewicz at the Warsaw Agricultural University, Poland. Two *T. hyodysenteriae* strains, 5380 and 70A, isolated from colonic lesions from swine suffering swine dysentery in Australia and non-pathogenic spirochaete strain 594 were also included in this study.

All cultures were grown at 37 °C for 72 h on solid media containing Trypticase soy agar (BBL) supplemented with 5% defibrinated horse blood and spectinomycin at 400 μg/ml under anaerobic conditions (94% H_2: 6% CO_2).

For sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), whole cells were removed from blood agar plates, washed twice by repeated centrifugation (8000 g, 10 min, 4 °C) and resuspended in 10 mM Tris-HCl (pH 7·4) and solubilized in SDS buffer as previously described [12].

T. hyodysenteriae strain 5380 was used for antigen preparation for enzyme-linked immunosorbent assay (ELISA). Cells were harvested by centrifugation at 10000 g at 4 °C for 10 min, washed three times in 0·01 M phosphate – buffered saline (PBS), pH 7·4 and then suspended in 10 ml of ice-cold PBS for sonification. Cells were then sonicated on ice, using a Branson sonifier, twice for 30 seconds with a 1 min interval for cooling on ice. Samples of the disrupted cells were examined by dark-field microscopy to confirm maximum cell disintegration. Antigen was diluted in PBS to an optical density of 0·025 at an absorbance of 415 nm using a Titertek Multiscan ELISA reader (Flow Laboratories, Sydney, Australia), with 100 μl of antigen in a Nunclon flat-bottom microtitre tray.

Experimental animals

All swine for experimentation were obtained at 4 weeks of age from a herd free of SD and were culture negative for *T. hyodysenteriae*. Swine were housed in separate isolated sheds and fed grower rations without antibiotics. Faecal swabs were negative for *Salmonella* spp. during the trials.

Vaccination trial

This trial consisted of post-weaning swine ($n = 30$) divided into three groups. Group 1 ($n = 10$) was experimentally vaccinated at days 0 and 11 by intramuscular injection of 2 ml of Negatrep Vaccine® (E. R. Squibb). Group 2 ($n = 10$) was injected with 2 ml of 2×10^8 *T, hyodysenteriae* strain 5380 cells/ml in saline at days 0 and 11. Group 3 ($n = 10$) served as a control non-vaccinated group. All groups were observed for 68 days.

Experimental challenge trial

Twelve post-weaning swine were used in this trial, divided into two groups. Group 4 ($n = 6$) consisted of non-vaccinated control swine held together with group 5 swine for the same period. Group 5 consisted of swine ($n = 6$) experimentally vaccinated at days 1 and 11 by intra-muscular injection of 2 ml of Negatrep Vaccine® (E. R. Squibb). Both groups 4 and 5 were fasted for 24 h prior to being challenged orally on day 22 with 2×10^{10} cells of *T. hyodysenteriae* strain 5380, cultured on blood agar and suspended in skim milk to a volume of 100 ml.

Natural infection trial

This trial consisted of 40 16-week-old swine from two units in a piggery with a clinical outbreak of SD. At the commencement of the trial several swine, in both units, had clinical signs of SD with moderate to severe diarrhoea and swine dysentery was confirmed at post-mortem.

Field trial

Thirty-one herds were involved in this trial. A complete history of medication used in each herd was provided. Eight herds were not using any medication. Twenty herds were using medication for diseases other than swine dysentery and three herds were using medication to control *T. hyodysenteriae*.

Microbial examination of swine

After clinical signs of swine dysentery were observed, rectal swabs from swine prior to acute clinical disease and swabs of colonic mucosa from swine that died after challenge were taken to recover *T. hyodysenteriae*.

ELISA

Nunclon flat-bottom microtitre plates (Nunc, Denmark) were coated with 0·1 ml of the antigen preparation, sealed and incubated at 4 °C overnight. Following coating, the antigen solution was removed by washing the plate three times with 0·3 ml of PBS (pH 7·4, 0·01 M) containing 0·5% (w/v) Tween 20 (PBS-T) and 5% (w/v) skim milk. Remaining absorbent sites were 'blocked' by adding 0·1 ml of PBS-T-5% skim milk for 2 h at room temperature. The blocking reagent

was removed and the plate washed three times with PBS-T. Sera from experimentally infected or naturally infected swine were diluted 1:400 in PBS-T and 0·1 ml of the diluted serum added to the wells in columns 4 to 12 in rows A to H. Wells in column 1 served as PBS-T control and were used for blanking the ELISA reader. Wells in column 2 served as positive reference control sera serially-diluted twofold from 1:400 to 1:51200 in rows A through G, respectively. Wells in column 3 served as negative reference sera diluted 1:400 and were obtained from swine with no previous history of SD. All of these swine had mean ELISA optical density (OD) readings of less than 0·4 at 415 nm. After incubation for 1 h at room temperature, the plate was washed with PBS-T as described above. Goat anti-porcine IgG horseradish peroxidase – labelled conjugate (KPL Laboratories, Maryland, USA) at an optimal 1:400 dilution in PBS-T (0·1 ml) was added for 1 h at room temperature. The plate was washed in PBS-T twice then once with distilled water and incubated with 0·1 ml of substrate solution (1 mM 2,2′ azino-di-3-ethyl benzthiazolinsulphate, 2·5 mM-H_2O_2 in 0·1 M citrate/phosphate buffer, pH 4·2) for 30 min at room temperature. The plate was read on a Titertek Multiscan ELISA reader (Flow Laboratories, Sydney, Australia) using a 415 nm filter. A positive serum antibody titre to *T. hyodysenteriae* was considered if a reaction of 3–4 times that of the mean OD reading for the negative control sera was obtained.

SDS–PAGE and immunoblotting

SDS–PAGE was performed as described by Laemmli [13] by using a 0·75 mm, 12·5% (w/v) polyacrylamide resolving gel with a 4% (w/v) stacking gel. Samples of 10 μg whole cell extracts in SDS buffer of pronase-digested cell extracts (10 μg cell protein: 1 μg pronase, 37 °C for 16 h) were solubilized in SDS sample buffer at 100 °C for 3 min before SDS–PAGE. Gels were electrophoresed at 50 V per gel for 1·5 h. After SDS–PAGE, gels were electrophoretically transferred onto nitrocellulose membranes (pore size 0·1 μm, Schleicher and Schull) using the procedure of Towbin and co-workers [14]. After transfer, the immunoblots were washed in 10 mM Tris-100 mM saline (TN buffer, pH 7·4) and blocked with 5% skim milk in TN buffer containing 0·05% (w/v) Tween 80 (TNT buffer, pH 7·4) for 2 h at room temperature and then washed three times with TNT buffer. The immunoblots were probed with ELISA-positive serum from naturally infected (1:100) or vaccinated swine (1:200) diluted in TNT buffer for 2 h at room temperature. The blotted membranes were then washed three times with TNT buffer and incubated for 1 h with horseradish peroxidase-conjugated sheep anti-swine immunoglobulin G (IgG) or goat anti-swine immunoglobulin M (IgM) diluted 1:200 in TNT buffer. The immunoblots were then washed twice in TNT buffer, twice in 10 mM Tris-HCl, pH 7·4 and then antigens were visualized by incubation with substrate (3 mg of 4 chloro-1-naphthol per ml in 0·01% hydrogen peroxide-10 mM Tris-HCl, pH 7·4) for 10 to 20 min, washed in distilled H_2O and photographed.

RESULTS

Vaccination trial

The mean circulating antibody levels in swine in groups 1, 2 and 3 prior to vaccination were OD = 0·08 (standard deviation, SD ± 0·03), OD = 0·13 (SD

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Table 1. *ELISA for swine IgG titres to* T. hyodysenteriae *from vaccination and experimental challenge trials*

Trial		Group	No. swine	Mean IgG titre* (SD)† Pre-vaccinated	11 days post-vaccinated	20 days post-challenge
Vaccination trial	(1)	Vaccinated	10	0·13 (0·06)	1·93 (0·29)	NA
	(2)	Vaccinated (saline)	10	0·10 (0·06)	0·49 (0·19)	NA
	(3)	Control	10	0·08 (0·03)	0·315 (0·14)	NA
Challenge trial	(4)	Control/ experimentally infected	6	0·18 (0·03)	0·49 (0·21)	0·86 (0·36)
	(5)	Vaccinated/ experimentally infected	6	0·26 (0·21)	2·23 (0·26)	2·53 (0·07)

* OD reading at a dilution of 1 in 400 porcine serum.
† Standard deviation (StD) in brackets.
NA, Not applicable.

$\pm$0·06) and OD = 0·10 (SD$\pm$0·06), respectively (Table 1 and Fig. 1). When group 1 swine were vaccinated with Negatrep Vaccine, the mean circulating antibody (Immunoglobulin IgG) level after vaccination, as measured by ELISA, increased to OD = 1·93 (SD$\pm$0·29) in comparison with group 3 non-vaccinated swine with OD = 0·315 (SD$\pm$0·14). Group 2 swine displayed a low level of circulating antibody, 2 weeks post-vaccination, with OD = 0·49 (SD$\pm$0·19) after immunization with *T. hyodysenteriae* cells in saline.

The group 1 antibody levels remained consistently high for a further 6–8 weeks after vaccination while those of group 2 vaccinates and group 3 non-vaccinates remained low (Fig. 1).

Productive antibody response during the experimental challenge trial

The mean circulating antibody levels in swine in the experimental challenge trial prior to vaccination were OD = 0·18 (SD$\pm$0·03) and OD = 0·26 (SD$\pm$0·21) for the group 4 and group 5 swine, respectively (Table 1 and Fig. 2). After vaccination the group 5 mean antibody response to *T. hyodysenteriae* was evaluated to OD = 2·23 (SD$\pm$0·26) while the control group 4 antibody level remained low at OD = 0·49 (SD$\pm$0·21).

All swine were challenged on day 22 (11 days post-vaccination) with virulent *T. hyodysenteriae* strain 5380 isolated from an SD outbreak. All group 4 non-vaccinated swine developed clinical signs of dysentery and diarrhoea within 10 days of challenge and two swine in group 4 died within 17 days. The two swine which died were examined post-mortem and found positive for *T. hyodysenteriae* by microscope and histological examination of the intestinal tract and culture of the spirochaete. At 20 days post-challenge the remaining group 4 swine were bled and then placed on medication to recover. The *T. hyodysenteriae* isolated from colonic lesions was identical to the *T. hyodysenteriae* inoculum based upon biochemical testing, SDS–PAGE analysis of cellular protein and Western blot analysis to confirm serotype specificity.

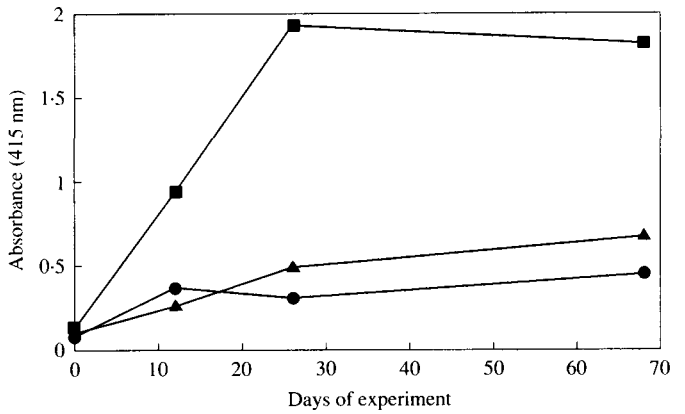

Fig. 1. Optical density versus bleed of vaccinated and non-vaccinated swine in the development of antibody (IgG) response against whole cell antigen of *T. hyodysenteriae*. ■——■, Group 1, vaccinated swine; ▲——▲, Group 2, vaccinated (saline) swine; ●——●, Group 3, non-vaccinated control swine. Serum samples were collected from vaccinated and non-vaccinated swine on day 0 (first vaccination), day 11 (second vaccination), day 26 and day 68, and the IgG titres measured by ELISA as described in the text.

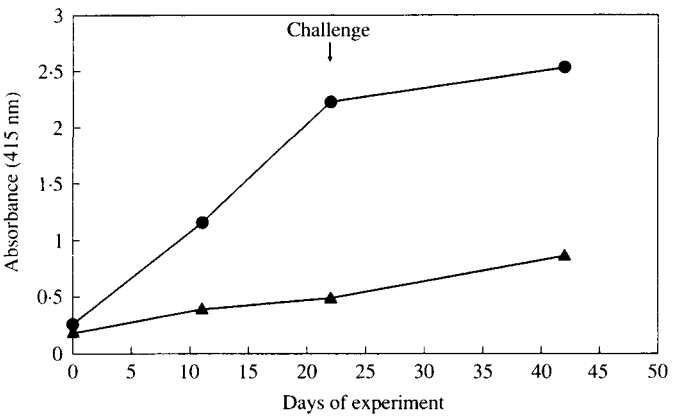

Fig. 2. Optical density of antibody responses of sera from vaccinated swine and swine experimentally infected with *T. hyodysenteriae* isolate 5380. ▲——▲, Group 4, experimentally challenged swine; ●——●, Group 5, vaccinated swine. Serum samples were collected from Group 4 and 5 swine on the following days: day 0 (experimental vaccination, Group 5), day 11 (experimental vaccination, Group 5), day 22 (challenge) and day 42 (20 days post-challenge) and the IgG titres measured by ELISA as described in the text.

All group 5 vaccinated swine were resistant to challenge and showed no histological or microscopic evidence of changes caused by *T. hyodysenteriae* infection. At 20 days after challenge the mean antibody level of group 2 swine remained relatively high at OD = 2·53 (SD ± 0·07) whilst the antibody response in the remaining group 4 swine was OD = 0·86 (SD ± 0·36) illustrating an increase in IgG level after clinical signs of SD (Fig. 2). It was noticeable that the antibody level to *T. hyodysenteriae* did not develop to the same extent in the control challenged swine as occurred in the vaccinated swine.

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Table 2. *Swine antibody response in natural field infection trial*

Unit	No. swine	Category*	No swine	Mean IgG titre†	Mean IgM titre†
1	20	1	4	0·21	NT
		2	5	0·3	NT
		3	6	0·47	NT
		4	5	0·72	1·51
2	20	1	9	0·22	NT
		2	9	0·33	NT
		3	1	0·47	NT
		4	1	0·7	1·66

* Categories based upon IgG titres.
† OD reading at a dilution of 1 in 400 porcine serum.
NT. Not tested.

Natural infection trial

Serum from 40 16-week-old swine, 20 from each of two separate grower units (1 and 2), were examined for IgG response to *T. hyodysenteriae* by ELISA (Table 2). Optical density readings of greater than 3–4 times that of the mean O.D. reading for the negative control sera were considered positive for antibody to *T. hyodysenteriae* and were classified as category 3 and 4, respectively. OD readings below these values were considered to be either negative (category 1) or indeterminable (category 2), for low levels of antibody to *T. hyodysenteriae* (twice that of the mean O.D. reading for the negative control sera).

Eleven swine in unit 1 (55%) and two swine in unit 2 (10%) demonstrated positive titres (category 3 and 4) to *T. hyodysenteriae*. Five of the swine of unit 1 (25%) and one in unit 2 (5%) demonstrated category 4 (high positive) titres (OD = 0·6–0·9) indicative of *T. hyodysenteriae* infection. These swine also demonstrated strong IgM titres (Table 2) to *T. hyodysenteriae*.

Reaction pattern of sera from pigs with T. hyodysenteriae *infection*

Figure 3A shows the immunoblotting reactions observed when whole cell extracts of *T. hyodysenteriae* and *T. innocens* were probed with pooled sera taken from category 4 positive-titre swine (unit 1) in the natural infection trial. The IgG antibodies reacted with at least 20 immunoreactive polypeptides with apparent molecular masses in the 16 kDa to 90 kDa range (Fig. 3A). These results are similar to the immunoreactive polypeptides observed using serum from vaccinated swine (data not shown). The 16 kDa LPS antigen was identified in two *T. hyodysenteriae* isolates of known serotype strain B204 (serotype 2) and to a lesser extent B78 (serotype 1). The IgG response to the LPS antigen after pronase-treatment of cell extracts demonstrated specific reactivity with only serotype 2 (B204) (Fig. 3B). There was no immunoreactive response to the LPS from different serotypes of *T. hyodysenteriae* or *T. innocens* (Fig. 3B Lane 10).

When IgM antibodies were measured using the ELISA they showed a strong ELISA positive response. When used in the Western blot the IgM antibodies reacted strongly with the polypeptides with differences in the immunoreactivity of the high M_r polypeptides (Fig. 4A). IgM also reacted with the 16 kDa LPS but

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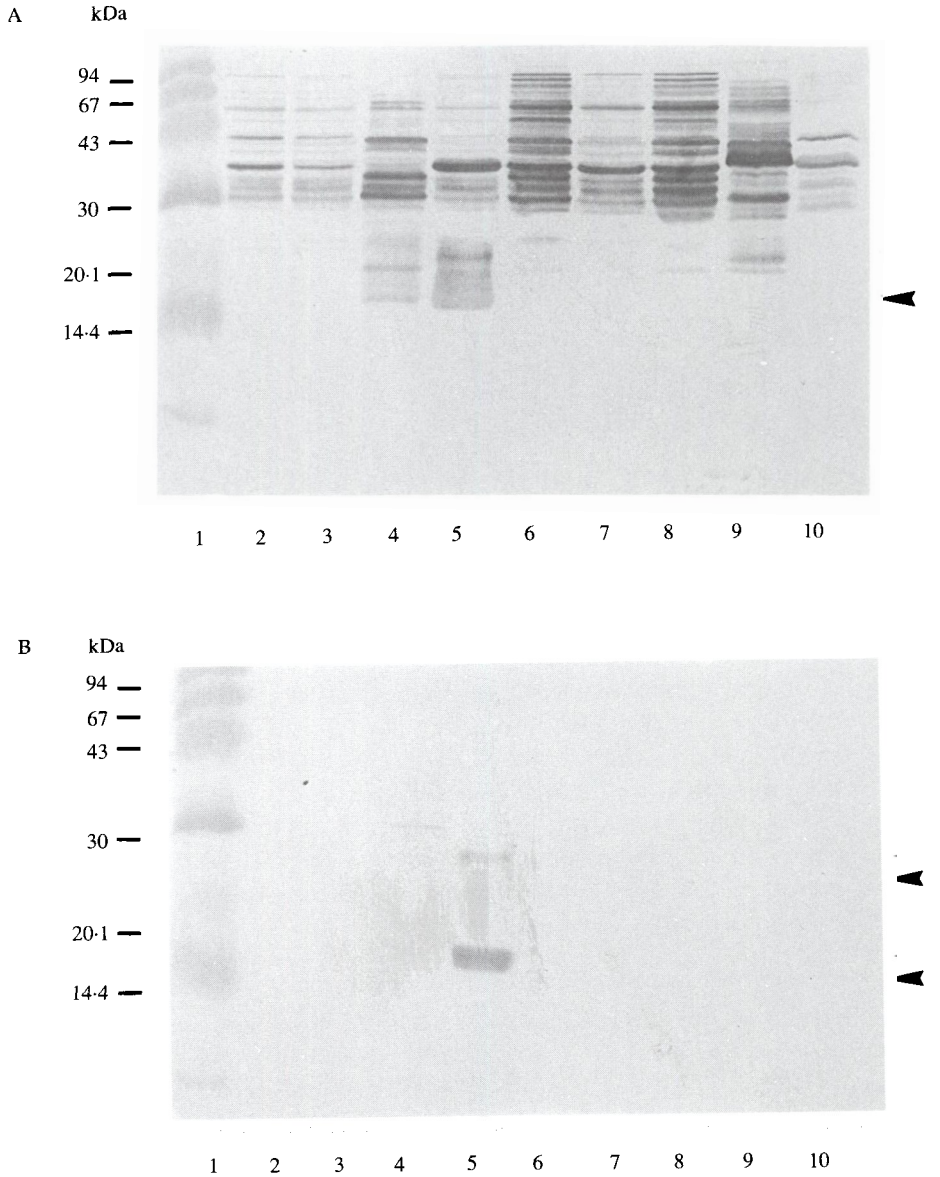

Fig. 3. Swine immunoglobulin G responses to *T. hyodysenteriae* and *T. innocens* whole cell solubilized proteins and pronase-treated whole cell lysates using sheep anti-swine IgG conjugate. Cell lysates and pronase-treated cell lysates were prepared as described in the text, separated by SDS–PAGE and transferred to nitrocellulose for Western blot analyses. Antigens were detected using category 4 positive-titre porcine serum Panel A, Cell lysates; Panel B, Pronase-treated cell lysates. Lane 1, prestained low molecular mass standards (Bio-Rad); Lane 2, 5380; Lane 3, 70A; Lane 4, B78; Lane 5, B204; Lane 6, B169; Lane 7, A1; Lane 8, 8929; Lane 9, non-pathogenic spirochaete 594; Lane 10, *T. innocens* B256. Arrows indicate the position of the LPS.

was cross-reactive with all serotypes observed. The IgM response was strongest to serotypes 1 and 2 (B78, B204) and gave the least response to the LPS antigen from the Australian *T. hyodysenteriae* isolates 5380 and 70A.

IgM also reacted to the LPS after pronase treatment of cell extracts (Fig. 4B).

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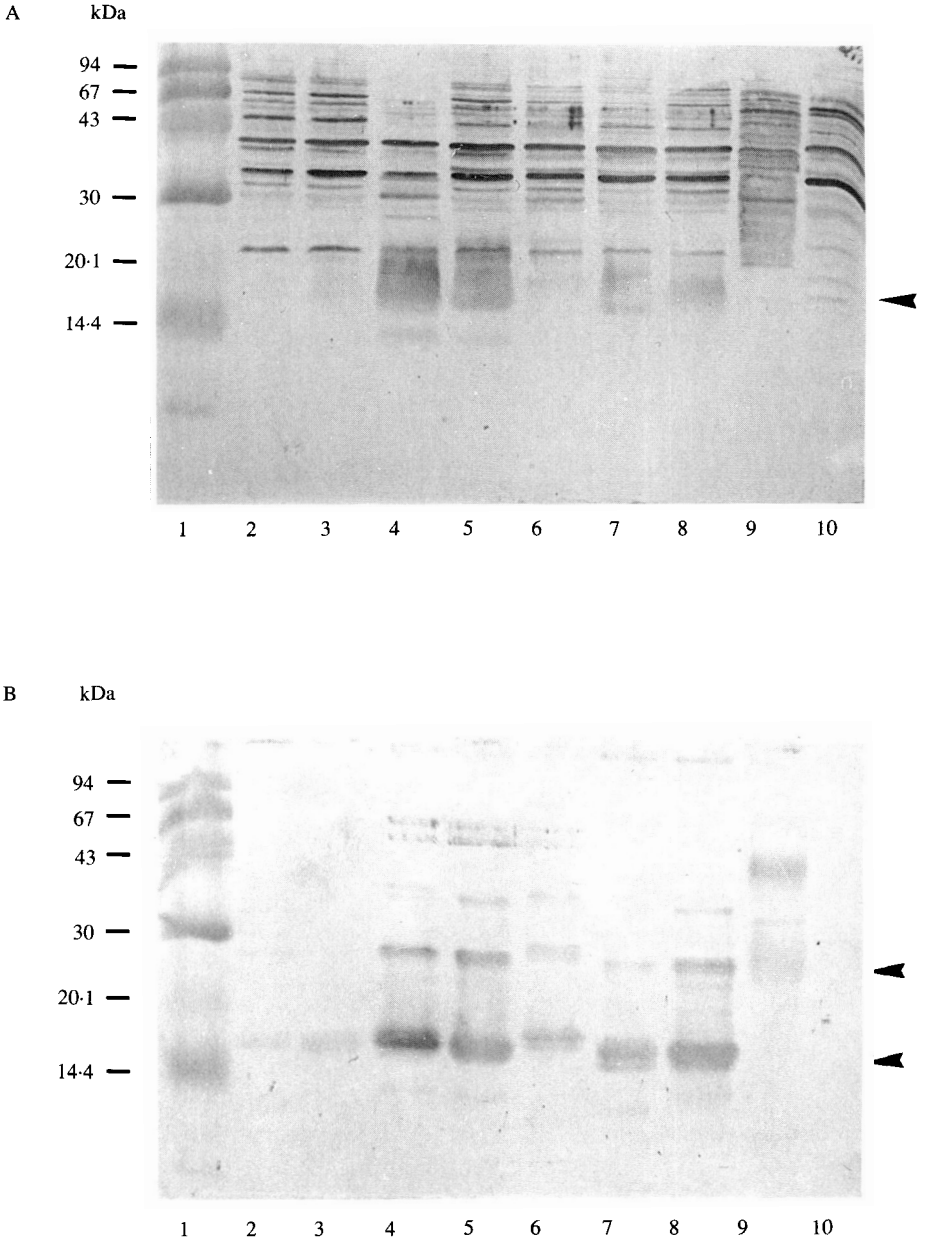

Fig. 4. Swine immunoglobulin M responses to whole cell lysates and pronase-treated whole cell lysates of *T. hyodysenteriae* and *T. innocens* isolates using goat anti-swine IgM conjugate. Whole cell lysates and pronase-treated cell lysates were prepared as described in the text, separated by SDS–PAGE and transferred to nitrocellulose for immunoblotting. Antigens were detected using category 4 positive-titre porcine serum. Panel A, Whole cell lysates; Panel B, Pronase treated cell lysates. Lane 1, prestained standards; Lane 2, *T. hyodysenteriae* 5380; Lane 3, 70A; Lane 4, B78; Lane 5, B204; Lane 6, B169; Lane 7, A1; Lane 8, 8929; Lane 9, non-pathogenic spirochaete 594; Lane 10, *T. innocens* B256. Arrows indicate the positions of the LPS.

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The IgM response was less definitive than the IgG response, reacting with the LPS from all *T. hyodysenteriae* serotypes. In addition, IgM antibodies recognized higher molecular weight LPS in non-pathogenic spirochaete 594, but not the higher molecular weight LPS from *T. innocens* B256 (Fig. 4B).

Application of ELISA/Western blot detection of antibodies in naturally-infected herds

A field examination of swine serum antibodies to serotypes of *T. hyodysenteriae* using ELISA and Western blotting of pronase-treated cell extracts of *T. hyodysenteriae* was undertaken. Serum was collected from swine from 6 herds with current outbreaks of moderate to severe diarrhoea (and diagnosed as clinical SD), 2 herds diagnosed as non-specific scours and 23 herds with no recent clinical history of SD. Thirteen herds (9 herds using medication unlikely to be effective against *T. hyodysenteriae*, 2 herds with current SD and using medication to control *T. hyodysenteriae* and 2 herds with current SD and not on medication), contained swine with ELISA-positive (category 3 and 4) antibody responses to *T. hyodysenteriae*. These were confirmed as *T. hyodysenteriae* specific responses by Western blotting of pronase-treated cell extracts. The reactivity of the swine sera in these blots was used to identify the *T. hyodysenteriae* serotype(s) involved. The distribution of serotypes involved was: serotype 1–6 herds, serotype 2–2 herds and serotype 4–9 herds. No herd contained antibody specifically against LPS of serotype 3.

DISCUSSION

The present study has demonstrated that an ELISA based upon sonicated whole cell antigens of *T. hyodysenteriae* can be used to detect circulating antibody (IgG) response in individual swine naturally or experimentally infected with *T. hyodysenteriae* and in swine vaccinated with Negatrep® vaccine and, when used in conjunction with a Western blotting procedure, is highly specific for *T. hyodysenteriae*.

Joens and colleagues [8], reported that an ELISA, used to measure antibody to *T. hyodysenteriae* LPS, could identify porcine IgG antibodies to a particular serotype of *T. hyodysenteriae* in experimentally infected swine up to 19 weeks post-infection. However, their ELISA assays were dependent on knowing the serotype of *T. hyodysenteriae* present in the herd or of developing ELISA's with LPS antigens that represent every known serotype. Wright and co-workers [15] found that the ELISA was most reliable at 6–8 weeks post-infection of experimentally infected swine and Burrows and colleagues [16] found maximum ELISA titres 3–4 weeks post-onset of clinical disease. In this study, using sonicated whole cell antigens of *T. hyodysenteriae*, we have detected an IgG response in experimentally infected and naturally infected swine up to 8 weeks post-infection, which is in agreement with Wright and colleagues [15] and Joens and colleagues [8]. Importantly, since the protein antigens of *T. hyodysenteriae* are common to the whole species, the sonicated cell antigen ELISA system is not serotype specific and so is more valuable for screening herds. We can use this ELISA system to detect all serotypes involved in swine dysentery outbreaks, whereas the LPS ELISA of Joens and co-workers [8] can only screen for antibodies against *T. hyodysenteriae*

serotypes 1 and 2, and antibodies specifically against the LPS of serotypes 3 and 4 (and others), would go undetected. Moreover, using our ELISA system we have not detected the non-specific cross-reactivity which resulted in elevated IgG titres in several non-infected control swine, comparable to IgG titres in infected swine which Wright and co-workers [15] found. In addition, we have also used the ELISA to detect significant swine IgM antibodies in the early weeks of post-infection (2 weeks), which may be used along with the IgG antibodies in immunoblotting. The strength of this assay lies in the broad screening of the ELISA using whole cell antigen coupled with the addition of the Western blot procedure which is necessary to eliminate non-specific false positives that may occur with the ELISA system. The ELISA is an excellent primary screening assay. However, antibody to other spirochaetes will cross-react with *T. hyodysenteriae* antigens. When the Western blotting method is used in conjunction with the ELISA, the LPS of the various *T. hyodysenteriae* serotypes can be clearly identified and readily differentiated from the LPS of *T. innocens* and other spirochaetes.

The means ELISA titres of vaccinated swine prior to and after experimental challenge were significantly different ($P < 0{\cdot}001$) from the mean ELISA titres of experimentally infected swine showing clinical signs of SD (Table 1, Trial 2). The mean IgG responses of clinically ill, experimentally infected swine in the Challenge Trial (OD = 0·86, SD $\pm$ 0·36) were similar to that of naturally infected swine with clinical signs of SD (OD 0·7–0·9). These results indicate that the antibody response to *T. hyodysenteriae* infection is significantly delayed and is only produced after clinical or subclinical symptoms of the disease are manifested.

Swine that recover from infection and so develop a higher antibody titre appear more resistant to further infection. Rees and co-workers [17] have proposed that IgG levels are correlated with the duration of clinical signs of disease and not directly related to protection against SD. However, Wannemuehler and colleagues [18] have shown that after intramuscular vaccination with outer membrane antigens, experimentally challenged swine developed IgG levels which resulted in less severe clinical signs of SD than in non-vaccinates. Mean IgG response of swine vaccinated against *T. hyodysenteriae* using Negatrep Vaccine® was OD = 1·93 and OD = 2·28 in Trials 1 and 2, respectively. Trial 2 vaccinated swine were resistant to challenge with virulent *T. hyodysenteriae* and these results have been observed in other vaccine trials (data not shown). These results indicate a strong protective immune response to *T. hyodysenteriae* infection after Negatrep vaccination and, in agreement with Wannemuehler and colleagues [18], suggest that high serum antibody titres lead to enhanced protection against re-infection. The results, however, do not exclude the potential role of other mechanisms in protection against *T. hyodysenteriae* infection (for example, cell-mediated immune mechanisms) and suggest that the mechanism and formulation for antigen delivery is critical in stimulating the appropriate protective immune responses.

We have used both the techniques of ELISA and immunoblotting to monitor the sera from 31 herds for antibody directed to *T. hyodysenteriae* during infection. The detection of anti-*T. hyodysenteriae* antibodies in individual swine from a given herd has provided information on the incidence of SD, the presence of carrier swine in the herd, and allows the specific serotype of the *T. hyodysenteriae* causing

clinical SD to be identified. In four of the SD-positive herds, more than one *T. hyodysenteriae* serotype was identified and this may suggest the possibility of more than one serotype involved in infection. In addition, these techniques can be used to assess the level of protection provided by vaccination against *T. hyodysenteriae* infection in swine dysentery vaccination programmes.

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