JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1982, p. 249–252
0095-1137/82/020249-04$02.00/0

Vol. 15, No. 2

Enzyme-Linked Immunosorbent Assay for Detection of Antibody to *Treponema hyodysenteriae* Antigens

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Received 21 July 1981/Accepted 21 September 1981

The enzyme-linked immunosorbent assay (ELISA) was evaluated and compared with the microtitration agglutination test for the detection of swine antibody to *Treponema hyodysenteriae* lipopolysaccharide antigens. Cells of *T. hyodysenteriae* serotypes 1 and 2 were extracted with hot phenol-water (68°C). The lipopolysaccharide fraction from the aqueous phase was coated on plastic wells at concentrations of 1 μg (serotype 1) and 10 μg (serotype 2) of carbohydrate per ml. The ELISA was serotype specific when lipopolysaccharide antigens were reacted against sera from convalescent swine. Seroconversion of infected pigs was detectable with the ELISA within 1 to 2 weeks postinoculation and with the microtitration agglutination test 2 to 3 weeks postinoculation. Antibody titers could be detected in convalescent pigs as long as 19 weeks postinoculation by the ELISA and 12 to 13 weeks postinoculation by the microtitration agglutination test. Therefore, the ELISA may be useful for the detection of asymptomatic carriers.

The clinical diagnosis of swine dysentery is often difficult to establish because of the presence of asymptomatic carriers within infected herds (1, 8, 18). This problem increases in severity when low levels of drugs are used to control the disease, preventing the detection of *Treponema hyodysenteriae* by culture (unpublished observation).

Serological tests which have shown promise in the detection of *T. hyodysenteriae* infections in pigs are a plate agglutination test and a microtitration agglutination test (MAT) (9, 11). However, these tests lack the sensitivity needed for the detection of infections in individual pigs. Also, both tests require an extensive incubation period before completion. In this report, we describe the use of an enzyme-linked immunosorbent assay (ELISA) to measure antibody to lipopolysaccharide (LPS) extracted from cells of *T. hyodysenteriae*. We also report here a comparison of the ELISA with the MAT.

MATERIALS AND METHODS

Bacterial antigens. *T. hyodysenteriae* isolates B234 and B204 and *Treponema innocens* isolates B1555a and B256 were grown in Trypticase soy broth with dextrose as described by Joens et al. (12). To obtain biologically active antigen for testing in the ELISA, isolate B204 (serotype 2) was cultured in Trypticase soy broth medium without dextrose. Cells from a 60-h, 1-liter culture were harvested by centrifugation at 4°C and washed once in 0.01 M phosphate-buffered saline (PBS), pH 7.2. The cells were pelleted and extracted with hot phenol-water as previously described (2), with the following modification. The aqueous phase was dialyzed against 100 volumes of distilled water overnight and precipitated 2× with 6 volumes of 90% ethanol and 10 mg of sodium acetate at −20°C overnight. The precipitate was dissolved in 2 ml of distilled water, filter sterilized, and stored at 4°C.

Antisera. Antisera against *T. hyodysenteriae* isolates B234 and B204 were prepared in pigs (weighing approximately 16 kg each) as previously described (R. D. Glock, D. L. Harris, R. A. Goodnow, and J. M. Kinyon, Proc. Int. Pig Vet. Soc. Cong. 5th, abstr. no. 245, 1980). Sera were also collected from pigs orally exposed to isolates B234 or B204. Sera from these two treatment groups were used in the assay as positive reference sera. Sera from unexposed swine of different ages were used as negative reference sera.

ELISA. The ELISA was performed essentially as described by Engvall and Perlmann (5, 6). Ela-cuvettes (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) were coated with 0.3 ml of solution containing LPS antigen in 0.05 M carbonate-bicarbonate buffer (pH 9.6) for 24 h at 4°C. The cuvettes were covered during incubation to prevent evaporation. After coating, the antigen solution was removed by aspiration, and the Ela-cuvettes were washed three times with PBS (pH 7.2; 0.01 M) containing 1% Tween 80 and 0.5% bovine serum albumin (PBT). Sera from experimental swine were diluted 1:100 in PBT (unless otherwise stated), and 0.3 ml of the diluted sera was added to each well and incubated for 2 h at 37°C. After incubation, the wells were washed three times with PBT, and 0.3 ml of a dilution of conjugate was added to each well. After 1.5 h of incubation at 37°C, the conjugate was aspirated from the wells, and the wells were washed four times with PBT. The substrate

TABLE 1. Titers obtained by the ELISA on sera of hyperimmunized, convalescent, or unexposed pigs

Serum	ELISA titer[a] with following isolate:	
	B234 Ag (serotype 1)	B204 Ag (serotype 2)
SD 1908 (hyperimmune)	>32,768	
SD 1913 (hyperimmune)		16,384
S 570 (convalescent)	1,024	
S 941 (convalescent)	2,048	
S 939 (convalescent)		1,024
S 940 (convalescent)		1,024
S 957 (negative)	<64	<64
GES (negative)	64	128

[a] Numbers represent the highest doubling dilution which provided an OD greater than that of the background absorbance.

solution containing hydrogen peroxide and 2,2'-azino-di-(3-ethyl-benzy-thiazoline sulfonic acid) in citric acid (pH 4.0) was then added to each well (0.3-ml volume). The reaction was read at 405 nm (Gilford PR 50 reader) after incubation with gentle shaking for 4 min at room temperature.

Determination of optimal antigen concentration for coating cuvettes. Polystyrene cuvettes were incubated with 0.3 ml of antigen (0.01, 0.1, 1.0, 10, and 100 μg of carbohydrate per ml [2]) in a carbonate-bicarbonate buffer (pH 9.6) and were tested against a 1:100 dilution of positive and negative reference sera and a constant dilution of conjugate. The concentration of LPS which demonstrated optimal sensitivity was 1 μg of carbohydrate per ml for serotype 1 and 10 μg of carbohydrate per ml for serotype 2. These antigen concentrations were used in all subsequent testing.

Conjugate dilution. An optimal dilution of conjugate was determined for the ELISA by using constant amounts of antigen and positive reference sera and by varying the concentration of conjugate in the test. Five dilutions were run (1:100, 1:200, 1:300, 1:400, and 1:500), and the highest dilution yielding an absorbance of 1.0 to 1.5 at 0.405 nm after 1.5 h of reaction time was chosen as the acceptable working dilution. The conjugate dilution of 1:400 was chosen for all subsequent testing of swine sera in the ELISA.

A reaction 2× greater than that of the negative reference sera was considered positive for *T. hyodysenteriae* antibody. Serum samples (1:100 dilution) which reacted at a level of ≥1.0 optical density (OD) units in ELISA were positive, and those samples which were lower than 1.0 OD unit were negative. The background level in the ELISA was designated as the control range.

The serum samples used in the comparison of the ELISA with the MAT were collected from pigs intragastrically inoculated with isolated B234, B204, or Trypticase soy broth medium. The serum samples were diluted 1:100 in PBT for testing in the ELISA or serially diluted (twofold) in a 1% formolized PBS buffer for the MAT.

Conjugated antisera. Rabbit anti-porcine immunoglobulin conjugated to horseradish peroxidase was prepared according to the method of Saunders et al. (16) and supplied by G. M. Brown, National Veterinary Services Laboratory, Animal Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa. The conjugate was filtered, sterilized, and stored at 4°C.

Agglutination test. The MAT was performed as described by Joens et al. (11) with the following modification: cells from *T. hyodysenteriae* isolate B234 were harvested with a concentrator (Millipore Corp., Bedford, Mass.), washed with PBS, and suspended in 0.2% formolized saline (I. T. Egan and D. L. Harris, personal communication).

RESULTS

Sensitivity and reproducibility of the ELISA. Reference sera from pigs hyperimmunized with either isolate B234 (serotype 1) or B204 (serotype 2) *T. hyodysenteriae* had titers of ≥1:16,384 (Table 1). Reference sera from swine orally infected with either isolate B234 or B204 had titers of ≥1:1024. Reference sera from unexposed swine had little or no activity in the ELISA, with background reactions occurring at serum dilutions of ≤1:128.

Antigen extracted from both serotypes was reacted in replicate with a 1:100 dilution of homologous reference sera from convalescent pigs. Four replicates of serotype 1 LPS reaction against a reference serum sample from a convalescent pig gave a mean absorbance value of 1.280 ± 0.130 (standard deviation). Five replicates of serotype 2 LPS reaction against a reference serum sample from a convalescent pig gave a mean absorbance value of 1.456 ± 0.178 (standard deviation).

Antigenic specificity of LPS in ELISA. Cross-reactivity occurred between serotypes when LPS from cells of *T. hyodysenteriae* was tested against antisera from hyperimmunized swine. Cross-reactivity between serotypes was not detected when LPS antigen was reacted against sera from pigs orally infected with *T. hyodysenteriae*. Antibody activity was not detected in sera from pigs orally infected with *T. hyodysenteriae* when tested against *T. innocens* LPS (Table 2).

Comparison of the ELISA with the MAT. Serum titers of pigs infected orally with *T. hyodysenteriae* isolate B234 (serotype 1) are shown in

TABLE 2. Antigenic specificity of *T. hyodysenteriae* LPS in the ELISA

Treatment[a]	Serum no.	Antisera against *T. hyodysenteriae* serotype[b]	ELISA values (OD_{450}/4 min)[c]	
			Serotype 1 Ag	Serotype 2 Ag
Hyperimmune antisera	SD1908	1	2.765	1.739
	SD1913	2	2.765	2.764
Convalescent antisera	S570	1	1.356	0.225
	S939	2	0.208	1.310
	S940	2	0.358	1.675
Negative sera	GES		0.367	0.238
	S957		0.294	0.062
	1692		0.414	0.368
	1306		0.510	0.367

[a] Swine serum samples (reference sera) were diluted 1:100 in PBT buffer before testing in the ELISA.
[b] Serotype of the isolate used in the production of antisera.
[c] OD values as read on a PR 50 Gilford reader. OD_{450}, OD at 450 nm.

Fig. 1. Antibody activity was detected in serum from pigs in the ELISA at the time pigs were showing clinical signs of swine dysentery, 7 to 10 days postinfection. Positive MAT titers were present in the sera from pigs 2 weeks postinfection. Serum titers reached their highest level at 3 weeks postinfection in both tests. ELISA titers remained at a relatively high level throughout the 19-week observation period, whereas agglutinating titers decreased after 3 weeks postinfection and subsided into the control range at 12 to 13 weeks postinfection.

Pigs inoculated orally with isolate B204 (serotype 2) or Trypticase soy broth (controls) were examined for the presence of antibody as detected by the ELISA and the MAT (Fig. 2). The results indicate that antibody was detected by ELISA at 2 weeks postinoculation, whereas agglutinating antibody (MAT) was detected at approximately 3 weeks postinoculation. Titers of control pigs remained below 0.65 (OD) (ELISA) or 4 (reciprocal of dilution) (MAT) units throughout the 7-week period.

DISCUSSION

These results demonstrate that the ELISA is a highly sensitive and specific test for the detection of antibodies in swine sera to *T. hyodysenteriae* antigen. The high sensitivity and specificity of the ELISA has been shown in other studies (3, 4, 7, 14, 15, 17).

The reaction of sera from convalescent pigs to the LPS antigen appears to be serotype specific, and only in sera from hyperimmunized pigs did we see cross-reactivity. Serotypes 1 and 2 are the only two serotypes known to be present in the United States (2). Therefore, until more serotypes are detected, it is likely that only LPS from serotypes 1 and 2 will be needed to screen sera for *T. hyodysenteriae* antibodies. The speci-

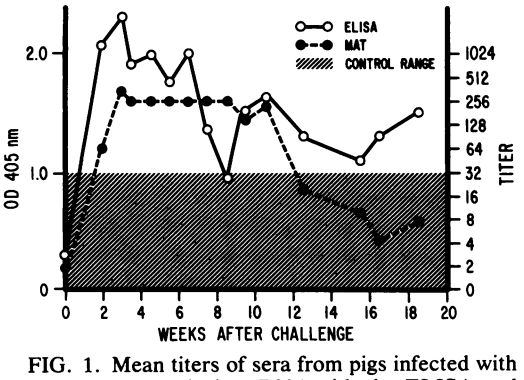

FIG. 1. Mean titers of sera from pigs infected with *T. hyodysenteriae* isolate B234 with the ELISA and MAT (each value is the mean value for four pigs). The control range refers to a serological response which is considered negative in both the ELISA and MAT. Serum was diluted 1:100 for testing in the ELISA.

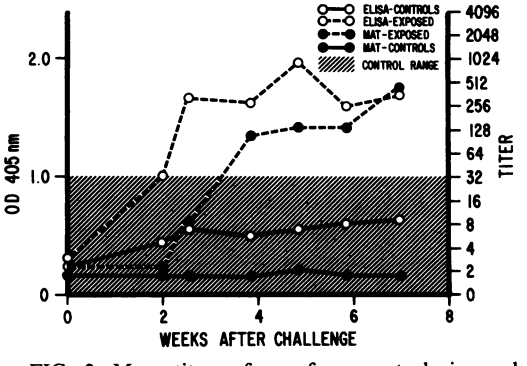

FIG. 2. Mean titers of sera from control pigs and pigs infected with *T. hyodysenteriae* isolate B204 with the ELISA and the MAT (each value is the mean value for four pigs). The control range refers to a serological response which is considered negative in both the ELISA and MAT. Serum was diluted 1:100 for testing in the ELISA.

ficity shown by the LPS in the ELISA may aid in determining the distribution of the two serotypes when testing sera from the field.

Presently, the most widely accepted test for the detection of *T. hyodysenteriae* antibody in swine sera is the MAT as described by Joens et al. (11) and modified by I. T. Egan and D. L. Harris (personal communication). A comparison of the MAT with cultural isolation of the spirochete as methods of diagnosis showed that *T. hyodysenteriae* infections in herds could be identified with the MAT (13). However, this test was not effective in the diagnosis of infection in individual pigs. The MAT will detect agglutinating antibody to *T. hyodysenteriae* in serum of swine 2 to 4 weeks after inoculation. The agglutinating titer will usually peak at 4 to 7 weeks postinfection and drop to a level similar to that observed in uninfected controls after 10 to 14 weeks (10). In the present study, an ELISA titer was detected in pigs at 1 to 2 weeks postinfection, peaked at 3 weeks postinoculation, and remained at a level 2 to 3 times higher than that of the background absorbance up to 19 weeks postinoculation. The reason for the decline in antibody levels in the sera of pigs at 8 to 9 weeks and 14 to 15 weeks postinoculation was unknown. This indicates that the ELISA is more sensitive than the MAT and that it may be useful in the individual diagnosis of *T. hyodysenteriae* infection.

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