A Sandwich Enzyme-linked Immunosorbent Assay for the Detection of *Streptococcus suis*

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ABSTRACT

A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was developed for the detection and the identification of *Streptococcus suis* capsular types 1, 2, 1/2, 3 and 22. The specificity of this test was first evaluated using reference strains of *S. suis* capsular types 1 to 28 and 1/2 as well as 15 different bacterial species susceptible to be isolated from swine. The ELISA developed was very specific for capsular types 1, 3 and 22 but it could not discriminate between capsular types 2 and 1/2.

In a second study, *S. suis* isolates from 328, 493, 368 and 76 diseased pigs were used to detect capsular types 1, 2 or 1/2, 3 and 22 respectively. The relative specificity and sensitivity varied between 98% and 100%. The ELISA results were in excellent agreement with the standard techniques (biochemical tests, coagglutination and capsular reaction tests) in detecting both positive and negative strains. Kappa values were 0.80, 0.99, 0.97 and 1.00 for detecting *S. suis* capsular types 1, 2 or 1/2, 3, and 22 respectively. To evaluate the relative-sensitivity of the test, primary cultures from 73 diseased pigs and tissue samples from 67 diseased pigs were used directly for detecting these capsular types. With primary cultures, the relative specificity and sensitivity (95.9% and 91.6% respectively) remained high and the test was very suitable (Kappa = 0.87). The ELISA using tissue samples gave a good specificity (97.6%), a moderate sensitivity (62.5%) and a low agreement with standard tests (Kappa = 0.64). This test appeared to be suitable for detecting or identifying *S. suis* from pure and primary cultures, but it was less sensitive for detecting *S. suis* directly from tissues.

RÉSUMÉ

Un test ELISA-sandwich a été développé pour la détection et la sérotypie de souches appartenant aux sérotypes 1, 2, 1/2, 3 et 22 de *Streptococcus suis*. La spécificité de ce test a été déterminée à partir des souches de référence de *S. suis* et 15 espèces bactériennes diverses susceptibles d'être isolées chez les porcs. Ce test s'est avéré très spécifique pour la détection des sérotypes 1, 3 et 22, mais n'a pu discriminer entre les sérotypes 2 et 1/2.

Dans une seconde étude, des isolats de *S. suis* provenant de 328, 493, 368 et 76 porcs ont été utilisés pour évaluer la capacité du test à détecter respectivement les sérotypes 1, 2 ou 1/2, 3 et 22. La spécificité et la sensibilité relatives du test se situaient entre 98% et 100%. Les résultats ont montré que les échantillons positifs autant que les échantillons négatifs pouvaient être détectés et la valeur Kappa était respectivement de 0,80, 0,99, 0,97 et 1,00 pour les sérotypes 1, 2 ou 1/2, 3 et 22. Pour évaluer la sensibilité relative du test ainsi développé, des cultures bactériennes primaires provenant de 73 porcs malades et des échantillons d'organes provenant de 67 porcs malades ont été utilisés directement pour la détection des mêmes sérotypes. Avec les cultures primaires, la spécificité et la sensibilité relatives (95,9% et 91,6% respectivement) sont restées élevées et le test s'est avéré très performant (Kappa = 0,87). Cependant, l'ELISA utilisant des échantillons d'organes a montré une bonne spécificité (97,6%), une sensibilité modérée (62,5%), et un faible taux de concordance (Kappa = 0,64) avec les tests standards (tests biochimiques, coagglutination et réaction capsulaire). Le test ELISA, ainsi developpé, est apparu efficace pour détecter *S. suis* à partir des cultures pures ou primaires, mais pas suffisamment sensible pour en détecter la présence directement à partir d'organes.

INTRODUCTION

Streptococcus suis infections in pigs are of major importance in countries where the swine industry is developed (1–5). This microorganism is also an important zoonotic agent for people in contact with swine or pig products (6,7). Infections due to *S. suis* have also been reported in ruminants (8,9) and in a horse (10). Until now, 29 *S. suis* capsular types have been described (11–13) and capsular type 2 is the most prevalent capsular type found in diseased animals, followed by capsular types 3, 1/2, 8 and 4 (14).

Preliminary diagnosis may be made based on clinical signs, age of affected pigs, necropsy findings, and

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Supported in part by the Fonds pour la formation des chercheurs et l'aide à la recherche #91-AS-2865.

Submitted March 9, 1992.

demonstration of gram-positive cocci in the lesions. To confirm the diagnosis, *S. suis* must be isolated and its identification carried out using biochemical tests (15). When capsular typing is available, a minimal number of tests such as 6.5% NaCl, acetoin, amylase, trehalose and salicin is suggested (16–17). Capsular typing is currently performed using a coagglutination technique and confirmation is accomplished by the capsular reaction test (16). Because of the large number of capsular types and the need for a confirmatory test, capsular typing is not accessible to routine diagnostic laboratories.

Several authors have suggested immunological methods for the direct detection of *S. suis* capsular type 2 from tonsils or nasal cavities of pigs. The use of a selective medium containing antiserum against capsular type 2 has given good results and has allowed the rapid testing of a large number of specimens (18–20). Others have used an indirect fluorescent antibody test carried out directly from cultures on plates (19,21,22). The main disadvantage of these techniques is their ability to detect only a very limited number of different capsular types.

There is a need to develop an approach allowing diagnostic laboratories to rapidly detect *S. suis* and identify, at the same time, the capsular type. Serotyping is a prerequisite for the understanding of the epidemiology of *S. suis* infections, their pathogenesis, and to the elaboration of control methods such as vaccination. This paper presents a method based on a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA), to detect and simultaneously identify the capsular type of *S. suis* isolates directly from plates containing mixed cultures after primary isolation as well as from pure cultures. Because of the large number of capsular types, the development and the validation of the technique were carried out with five capsular types: 1, 2, 1/2, 3 and 22. These capsular types were chosen because capsular types 2, 1/2 and 3 are the most prevalent (14), and also because capsular types 1, 2, 1/2 and 22 present cross-reactions (11).

MATERIAL AND METHODS

BACTERIAL STRAINS

Reference strains of *S. suis* capsular types 1 through 8 and 1/2 were kindly supplied by Dr. Jorgen Henrichsen, Statens Seruminstitut, Copenhagen, Denmark. Reference strains of *S. suis* capsular types 9 through 28 were obtained from our laboratory. A total of 15 other bacterial strains, representing different bacterial species susceptible to be found in swine [*Streptococcus bovis* (ATCC 9809), *Enterococcus faecalis* (ATCC 19433), *Enterococcus faecium* (2440), *Streptococcus equisimilis* (field strain), *Streptococcus pneumoniae* (ATCC 6319), *Actinobacillus suis* (3500), *Pasteurella multocida* (A:1062), *Haemophilus parasuis* (field strain), *Actinomyces pyogenes* (field strain), *Actinobacillus pleuropneumoniae* capsular type 1 (4074), *Erysipelothrix rhusiopathiae* (field strain), *Bordetella bronchiseptica* (strain 276), *Proteus vulgaris* (field strain), *Bacillus subtilis* (field strain) and *Escherichia coli* (field strain)] have been used to evaluate the microbiological specificity of the ELISA test. *Streptococcus suis* isolates originating from 493 diseased pigs, primary cultures of different tissues from 73 diseased pigs, and tissue samples from 67 diseased pigs were included in the study. Each pig provided only one *S. suis* isolate. All *S. suis* isolates had previously been identified with biochemical tests and capsular type determined using the coagglutination and the capsular reaction test.

PRODUCTION OF ANTIGENS FOR IMMUNIZATION

Reference strains of capsular types 1, 2, 3 and 22 were grown overnight, aerobically, on blood agar plates (Tryptic Soy agar containing 5% bovine blood), then in Todd-Hewitt broth (Difco Laboratories, Detroit, Michigan) for about 18 h at 37°C. Bacteria were killed by adding formalin to the Todd-Hewitt cultures to a final concentration of 0.5% (V/V). After overnight incubation at 25°C, the formalin-treated cultures were centrifuged at 7,000 × *g* for 15 min; cell pellets were suspended in phosphate buffer solution (PBS, pH 7.2) containing 0.5% formalin. The formalinized suspensions were adjusted to a concentration of 10^9 CFU/mL and were used to immunize rabbits and cows.

PRODUCTION OF *STREPTOCOCCUS SUIS* ANTISERA

New Zealand White rabbits and adult cows were immunized against *S. suis* capsular types 1, 2, 3 and 22. Animals were housed and fed according to the guidelines issued by the Canadian Council on Animal Care. Prior to immunization, sera from all rabbits and cows were tested for capsular types 1, 2, 1/2, 3 and 22 by slide agglutination test (23) to ascertain the absence of anti-*S. suis* antibodies.

Rabbits were injected, intravenously, twice a week for four weeks with increasing doses of the formalinized suspensions (0.5, 1.0, 1.5, 2.0, 2.5 mL).

Cows were injected, intramuscularly twice in the first week, with 5 mL of the bacterial solution emulsified with an equal volume of Freund's incomplete adjuvant (Gibco Canada Inc., Burlington, Ontario). Then, series of injections were performed, intravenously, twice a week by injecting 8 mL of the bacterial suspension without adjuvant for the next three weeks.

One week after the last inoculation, the antibody response of rabbits and cows was monitored by the slide agglutination test. Blood samples were collected, sera were centrifuged at 4,000 × *g* for 20 min, heated at 56°C for 30 min and stored at −20°C.

IMMUNOGLOBULIN G PURIFICATION

Bovine immunoglobulin G class antibodies were purified by affinity chromatography using a HR 16/5 protein A-superose column coupled to a FPLC System (Fine Chemical, Uppsala, Sweden). Sera were diluted with an equal volume of starting buffer consisting of 1.5 M glycine (pH 8.9) and 3.0 M NaCl and eluted with 0.1 M citric acid solution adjusted to pH 3.0. The column was eluted at a rate of 1.0 mL/min and the peaks monitored at 254 nm with a UV-M detector. Purified lgG fractions were dialysed against a phosphate buffered saline (pH 7.4), concentrated

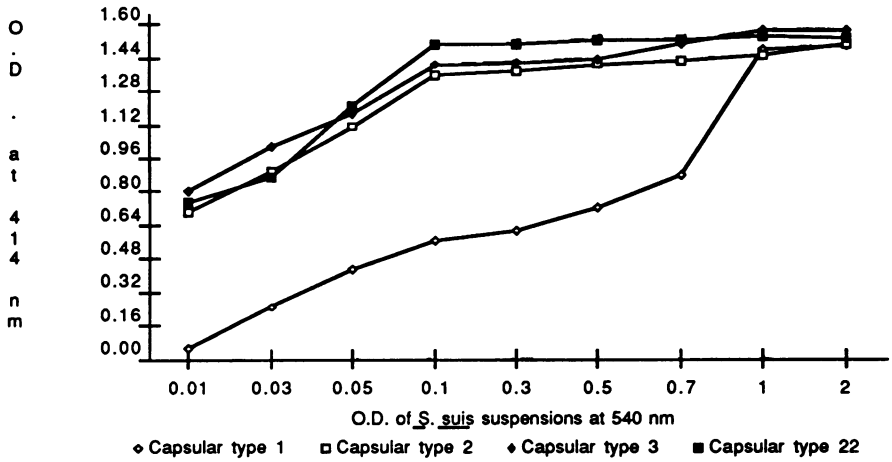

Fig. 1. Effect of different dilutions of known concentrations of *S. suis* capsular types 1, 2, 3 and 22 on ELISA results using the homologous antisera.

(3:1) using a Speed-Vac, and stored at −20°C.

PREPARATION OF ANTIGENS FOR ELISA

In order to determine the analytical sensitivity, different dilutions of known concentrations of reference *S. suis* capsular types 1, 2, 3 and 22 (10^7 through 2 10^9 cells/mL) were tested by a double-antibody sandwich ELISA to detect capsular types 1, 2, 3 and 22. This preliminary study showed that the optimal antigen working concentrations were 10^8 cells/mL (optical density (OD) of 0.1 at 540 nm) for *S. suis* capsular types 2, 3 and 22, and 10^9 cells/mL (OD of 1.0 at 540 nm) for capsular type 1 (Fig. 1).

Colonies from pure and primary bacterial cultures were mixed with 1 mL of a solution of 0.05% polyoxyethylene Sorbitan monolaurate (Tween 20, Sigma Chemical, St. Louis, Missouri) in 0.02 M phosphate buffered saline (PBS; pH 7.4). Tissue samples were individually sampled with a sterile cotton-tipped applicator which was shaken in 1 mL of PBS-Tween 20. These suspensions were used fresh, at their optimal concentration, for the ELISA test.

In another experiment, reference strains of *S. suis* capsular types 1, 2 and 1/2 were either heated at 60°C for 30 min, boiled for 15 min, or treated with 0.5% formalin.

DOUBLE-ANTIBODY SANDWICH ELISA PROCEDURE

A volume of 50 µL of bovine lgG diluted in 0.1 M carbonate buffer (pH 9.6) was added to the wells of polystyrene round-bottomed 96-well microtiter plates (Nunc-Immuno Module; Maxisorp U16; Gibco Canada Inc., Burlington, Ontario). Plates were incubated overnight at 4°C and washed for three periods of 3 min with PBS-Tween 20. Fifty µL of each antigenic solution were used, in duplicate, in lgG-coated wells, incubated for 15 min at 20–24°C, and plates were washed three times with PBS-Tween 20. Fifty µL of rabbit antiserum, raised against the same capsular type as the bovine lgG used to coat the wells, diluted in PBS-Tween 20 were added. Negative control included wells without antigen, rabbit antisera or both, positive control consisting of capsular type 2 bovine lgG optimally diluted, a reference *S. suis* capsular type 2 adjusted at an OD of 0.1 at 540 nm, and a rabbit antiserum raised against capsular type 2 optimally diluted were included. After an incubation of 15 min at 20–24°C, plates were washed, and 50 µL of a commercial goat antirabbit immunoglobulin G conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, Mississauga, Ontario), made up to its optimal dilution, were added to each well. After further incubation for 15 min at 20–24°C and subsequent washing, 100 µL of 0.4 mM, 2,2' azino-bis (3-ethyl-benzthiazoline-6-sulfonic acid) (Sigma Chemical, St. Louis, Missouri) and 0.5 M H_2O_2 in 0.05 M citrate solution adjusted at pH 4.0 were added to each well. The reaction was then allowed to develop for 30 min at 20–24°C and the OD recorded at 414 nm on a ELISA reader (Titertek Multiskan photometer, Flow ICN Biomedicals, Mississauga, Ontario). Four ELISA systems differing only by the antigen concentrations and the specificities of the sera, were named to detect capsular types 1 and 1/2 (syst 1), 2 and 1/2 (syst 2), 3 (syst 3) and 22 (syst 22). Because the ELISA test is biologically similar to standard tests, the ELISA results could be compared with the results of standard tests. For this purpose, animals positive to all standard tests are assumed to be diseased, and animals negative are considered disease-free. Relative-sensitivity and relative-specificity could also be estimated (24). To determine the optimal cut-off value which would give a good specificity without affecting the sensitivity, ELISA results were calculated with a gradual number of cut-offs (0.40, 0.42, 0.45, 0.47, 0.50, 0.52, 0.55, 0.57, 0.60, 0.62, 0.65, 0.67 and 0.70). For each cut-off, the relative-sensitivity and the relative-specificity were estimated according to Martin (24):

— Sensitivity (SENS) is the ability of a test to detect an animal with a specified disease. In our case, it corresponds to the proportion of the homologous *S. suis* capsular type that cause disease and also give a positive result on ELISA test.

— Specificity (SPEC) is the ability of a test to detect healthy animals or those not having the specified disease. In our case, it represents the proportion of the heterologous *S. suis* capsular types and all bacteria other than *S. suis* that give a negative result on ELISA test.

Optimal cut-off was the OD which corresponds to the meeting point of relative-specificity and relative-sensitivity curves. The different optimal cut-off used to detect *S. suis* capsular types 1, 2, 3 and 22 were 0.50 (SPEC and SENS, 99%), 0.53 (SPEC and SENS, 98%), 0.53 (SPEC and SENS, 98%) and 0.46 (SPEC and SENS, 100%) respectively. The performance of the technique (double-antibody sandwich ELISA) was determined by measuring agreement between ELISA and standard tests (biochemical, coagglutination and capsular reaction tests) using kappa statistics (25,26).

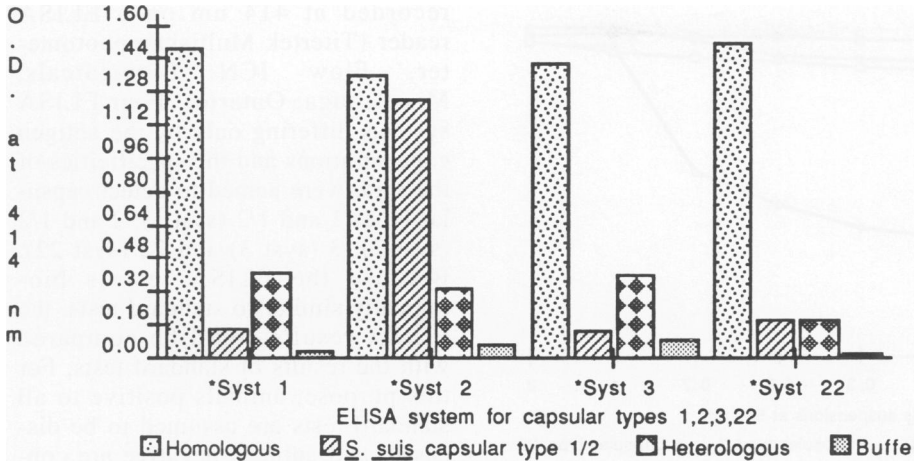

Fig. 2. Optical density reading of *S. suis* type-specific antisera and homologous capsular type, *S. suis* capsular type 1/2, other *S. suis* capsular types and other bacteria (heterologous). *Syst 1, 2, 3 or 22: ELISA test carried out for the detection of *S. suis* capsular types 1, 2, 3 or 22 respectively.

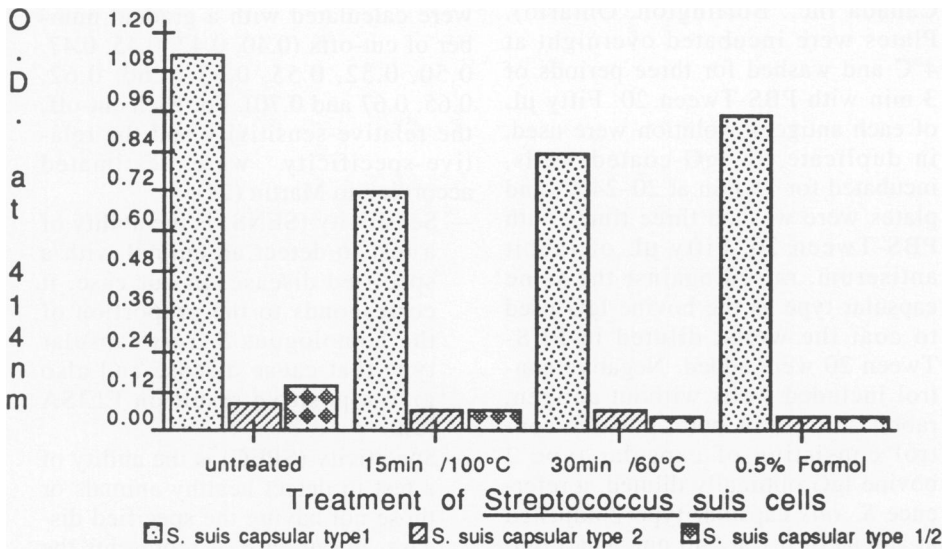

Fig. 3. Effect of the different treatments of *S. suis* capsular types 1, 2 and 1/2 on ELISA results using antisera to *S. suis* capsular types 1.

RESULTS

IMMUNOSPECIFICITY OF ELISA

Preliminary tests were carried out in order to standardize the technique in terms of optimum antigen concentration (Fig. 1), serum and conjugate dilutions.

Figure 2 represents an assay of *S. suis* type-specific sera with homologous reference capsular types 1, 2, 3 and 22, reference capsular type 1/2 and heterologous antigens represented by all other *S. suis* reference strains, and other bacterial strains susceptible to be isolated from swine. In this study, all homologous reactions were positive. Bovine IgG and rabbit sera raised against *S. suis* capsular types 1, 2, 3 and 22 reacted with the homologous antigen and gave an average OD of 1.50, 1.35, 1.40 and 1.48 respectively. Reactions in ELISA with the heterologous antigens (other than *S. suis* capsular type 1/2) gave OD which were below the respective determined cut-off in all cases. Bovine IgG and rabbit serum raised against *S. suis* capsular types 1, 3 and 22 did not present a positive reaction with capsular type 1/2. On the other hand, capsular type 2 antisera gave high OD (1.25) with this capsular type. It should be noted that there was no cross-reaction between capsular types 2 and 22 (data not shown). In order to see if disrupted or capsule fixed cells could produce a positive reaction between capsular type 1 antisera and *S. suis* capsular type 1/2, different treatments (cells boiled for 15 min, heated at 60°C for 30 min or formalinized with 0.5%) were performed on reference capsular types 1, 2 and 1/2 antigens. The treated antigens, as well as the untreated, were then tested by ELISA with the antisera to *S. suis* capsular type 1. Figure 3 shows that, in the four cases, the homologous reaction was positive, no cross-reaction between capsular types 1 and 2 was observed and the antisera raised against *S. suis* capsular type 1 did not recognize capsular type 1/2. This suggests that the results obtained previously were independent of the type of treatment.

VALIDATION OF THE DOUBLE-ANTIBODY SANDWICH ELISA

Streptococcus suis field isolates from 493 diseased pigs were used to evaluate the performance of ELISA in detecting *S. suis* capsular types 2 or 1/2. Because of the earlier results (Fig. 2), no attempt was made to discriminate between these two capsular types. *Streptococcus suis* field isolates from 328, 368 and 76 diseased pigs were used to evaluate the performance of ELISA in detecting *S. suis* capsular types 1, 3 and 22 respectively. Tables I and II show the kappa statistics used to determine the agreement between the double-antibody sandwich ELISA and standard tests (biochemical, coagglutination and capsular reaction tests) to detect and identify the capsular type of pure culture isolates of *S. suis* capsular types 1, 2 or 1/2, 3 and 22, respectively. The ELISA results gave a good agreement with standard tests in detecting both positive and negative isolates. The kappa values were 0.796, 0.995, 0.972 and 1.000 for detecting *S. suis* capsular types 1, 2 or 1/2, 3 and 22 respectively. These kappa values were obtained with 95% confidence limits of 0.42–1.00, 0.993–0.996, 0.94–0.99 and 1.00–1.00 for the detection of *S. suis* capsular types 1, 2 or 1/2, 3 and 22 respectively.

TABLE I. Comparison of double-antibody sandwich ELISA and standard tests for the detection of *Streptococcus suis* capsular type 1 and 2 or 1/2

Detection of capsular type 1				Detection of capsular type 2 or 1/2			
	Standard tests[a]				Standard tests[a]		
Test results	+	−	Total	Test results	+	−	Total
ELISA[b] +	2	1	3	ELISA[b] +	155	1	156
ELISA[b] −	0	325	325	ELISA[b] −	0	337	337
Total	2	326	328	Total	155	338	493
Agreement observed	= 0.997			Agreement observed	= 0.997		
Agreement due to chance	= 0.985			Agreement due to chance	= 0.568		
Kappa	= 0.796			Kappa	= 0.995		
SE (K)	= 0.182			SE (K)	= 0.001		
95% Confidence limits (K)	= 0.42 −1.00			95% Confidence limits (K)	= 0.993 − 0.996		

[a]Biochemical, coagglutination and the capsular reaction tests
[b]Double-antibody sandwich ELISA

TABLE II. Comparison of double-antibody sandwich ELISA and standard tests for the detection of *Streptococcus suis* capsular type 3 and 22

Detection of capsular type 3				Detection of capsular type 22			
	Standard tests[a]				Standard tests[a]		
Test results	+	−	Total	Test results	+	−	Total
ELISA[b] +	40	2	42	ELISA[b] +	2	0	2
ELISA[b] −	0	326	326	ELISA[b] −	0	74	74
Total	40	328	368	Total	2	74	76
Agreement observed	= 0.994			Agreement observed	= 1.000		
Agreement due to chance	= 0.801			Agreement due to chance	= 0.948		
Kappa	= 0.972			Kappa	= 1.000		
SE (K)	= 0.013			SE (K)	= 0.000		
95% Confidence limits (K)	= 0.94 −0.99			95% Confidence limits (K)	= 1.00 − 1.00		

[a]Biochemical, coagglutination and the capsular reaction tests
[b]Double-antibody sandwich ELISA

TABLE III. Comparison of double-antibody sandwich ELISA, using primary cultures or tissue samples as antigens, and standard tests for the detection of *Streptococcus suis* capsular types 1, 2 or 1/2, 3 and 22

Test with primary isolates				Test with infected tissues			
	Standard tests[a]				Standard tests[a]		
Test results	+	−	Total	Test results	+	−	Total
ELISA[b] +	22	2	24	ELISA[b] +	15	1	16
ELISA[b] −	2	47	49	ELISA[b] −	9	42	51
Total	24	49	73	Total	24	43	67
SPEC and SENS %	= 95.9 and 91.6%			SPEC and SENS %	= 97.6 and 62.5%		
Agreement observed	= 0.945			Agreement observed	= 0.850		
Agreement due to chance	= 0.558			Agreement due to chance	= 0.574		
Kappa	= 0.875			Kappa	= 0.649		
SE (K)	= 0.049			SE (K)	= 0.106		
95% Confidence limits (K)	= 0.77 −0.97			95% Confidence limits (K)	= 0.44 − 0.85		

[a]Biochemical, coagglutination and the capsular reaction tests
[b]Double-antibody sandwich ELISA

RELATIVE SENSITIVITY OF THE DOUBLE-ANTIBODY SANDWICH ELISA

Primary isolates of tissue samples from 73 diseased pigs and tissue samples from 67 diseased pigs were used to evaluate the relative-specificity and relative-sensitivity of the ELISA developed above. Table III summarizes the results. With primary isolates, relative-specificity and relative-sensitivity (95.9%, 91.6% respectively) were not affected by the heterogeneity of antigenic suspensions. The kappa value was 0.875 with a 95% confidence limit of 0.77–0.97, and shows that the ELISA, compared to standard tests, was suitable. The ELISA using tissue samples as antigen, had a good relative-specificity (97.6%) and a moderate relative-sensitivity (62.5%) compared with standard tests because nine false negatives were observed in 24 samples identified as positive by the standard tests. Also, the kappa value of 0.649 with a 95% confidence limit of 0.44–0.85, indicated a low level of agreement between ELISA and standard tests.

DISCUSSION

The major objective of this study was to develop a rapid and specific double-antibody sandwich ELISA for the detection and the capsular typing of *S. suis*. In this study, the technique was validated for capsular types 1, 2, 3 and 22. The ELISA test was very specific because it did not present any cross-reaction between *S. suis* and the 15 bacterial isolates used in this test. It was also sensitive for detection of specific epitopes of the homologous capsular type; nonspecific background reactions were very low, and cross-reaction between capsular type 2 and capsular type 22 was not detected. Capsular type 1/2, which possesses both capsular types 1 and 2 antigenic determinants (13) reacted only in the test developed for capsular type 2. Cross-absorption experiments showed that capsular polysaccharide-type 1/2 antigen possesses an antigenic component identical to that of capsular type 2; this is in accordance with Perch *et al* (27) who also mentioned that the capsular type 1 fraction of capsular type 1/2 was similar but not identical to the antigen of capsular type 1. The ELISA test carried out with disrupted cells eliminated the hypothesis that common antigenic epitopes between capsular types 1 and 1/2 could be masked and not detected by the capsular type 1 antiserum in the ELISA test. Experiments performed by Jacques *et al* (28) showed that the capsular material of capsular type 1/2 reference strain was best stabilized with antiserum against capsular type 2 rather than against capsular type 1. Thus, it is possible that the component 1 of capsular type 1/2 is rapidly degraded. The ELISA test developed to detect capsular type 1 was less sensitive than that for capsular types 2, 3 and 22; this could explain the fact that there was no

reaction betweeen capsular type 1 antiserum and capsular type 1/2 antigens.

Based on the results obtained with pure culture isolates, the ELISA method described here for detecting capsular types 1, 2 or 1/2, 3 and 22, appears to be an efficient technique in terms of specificity and sensitivity. It agrees closely with standard tests in detecting both negative and positive cultures. The test is also more practical and faster than other tests previously used (18–20) because a large number of samples can be tested at the same time, and it does not require a primary identification using biochemical tests. With the ELISA test, using primary cultures and tissue samples, the microbiological sensitivity and specificity calculations were relative and based on biologically related tests. It was not possible to assemble a sufficiently large representative group of samples in order to determine the specificity of the test, but our relative estimates indicate a low variability in microbiological specificity and sensitivity. The ELISA, using primary cultures, was more rapid and advantageous than that using pure isolate cultures because it does not require sub-culturing of the isolate and allows the detection of mixed *S. suis* infections. At present, no report mentioned the detection of a mixed infection which involved two or more *S. suis* capsular types. With the ELISA test, two capsular types (2 and 3) were detected in a primary culture. The two capsular types were isolated and their identification was confirmed by coagglutination and capsular reaction tests (data not shown).

Detection and identification, directly from tissue samples, appeared rapid and advantageous, since detection of live and dead bacteria is possible. The ELISA test evaluated for detecting *S. suis* directly from tissue samples of diseased pigs showed a low sensitivity and a low agreement with standard tests. The ELISA developed in this study, for detecting *S. suis* from pure or primary cultures, has a significant advantage over standard tests, and it may be considered as a valuable means for the detection of the different capsular types of *S. suis*.

ACKNOWLEDGMENTS

We acknowledge the invaluable technical assistance of Francine Bernard. We also are indebted to Sophie Radacovici for her help in the purification of the antibodies.

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