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Detection of Type-Specific Antigens in the Lungs of *Haemophilus pleuropneumoniae*-Infected Pigs by Coagglutination Test

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Specific diagnosis of *Haemophilus pleuropneumoniae* infection by the conventional culture and identification method usually requires 3 to 4 days. Since *H. pleuropneumoniae* may be disseminated from infected individuals during this period, this amount of time required for diagnosis may be too slow to aid in epidemic control. To obtain an earlier diagnosis, a coagglutination test was successfully used to detect serotype-specific antigens in lung extracts of infected pigs. A total of 19 lung tissues from experimentally infected pigs, 240 lung tissues from naturally infected pigs that died of pleuropneumonia, and 571 lung specimens from an apparently healthy pig population were examined for culture isolation as well as for antigen detection. The results showed that detection of antigens in lung tissues by the coagglutination test is an extremely rapid, simple, quite specific, and highly sensitive procedure for the diagnosis of *H. pleuropneumoniae* infection. Further, detection of antigens in lung tissues was found to be a much simpler and much more rapid method than culture isolation. The coagglutination test seems to be especially useful for detecting *H. pleuropneumoniae* in pigs exposed to chronic infection as well as those in which multiple serotypes are involved.

Haemophilus pleuropneumoniae is an important causative agent of pleuropneumonia in swine. A definite diagnosis of *H. pleuropneumoniae* infections requires the isolation of the organism from affected pigs. Culture examinations are relatively insensitive, especially in chronic cases, and also are time consuming.

The rapid detection and identification of etiological agents in bacterial diseases can be of great value in prompt diagnosis and treatment. In recent years, techniques based on immunological principles have found favor, since they are usually sensitive, specific, and rapid. The presence of pneumococcal antigens in the sputum, urine, and sera of patients with lobar pneumonia has been reported to correlate very closely with the presence of pneumococcal disease (2).

The previously described rapid methods of antigen detection directly from the tissues or secretions in diseases are the fluorescent antibody technique, the coagglutination test, and counter-immunoelectrophoresis (1, 2, 5). A simple technique that would permit identification of *H. pleuropneumoniae* directly from the lungs would allow immediate application of the appropriate antimicrobial therapy in the management of *H. pleuropneumoniae* infections. It would also be valuable as an aid in the detection of *H. pleuropneumoniae* antigens in health surveys. Such a method appears to be the coagglutination test.

This study presents an evaluation of the coagglutination test by comparing it with the culture isolation procedure for detection of *H. pleuropneumoniae* antigen(s) in lungs of pigs with and without *H. pleuropneumoniae* infection.

MATERIALS AND METHODS

Cultures of known serotypes. Five *H. pleuropneumoniae* strains representing serotypes 1 to 5 were received from A. Gunnarsson of the National Veterinary Institute, Uppsala, Sweden. Strains representing serotypes 6 and 7 were received from S. Rosendal of the University of Guelph, Ontario, Canada.

Specimens of lung tissues. A total of 240 lung tissue specimens submitted for bacteriological diagnosis were obtained from pigs which died of acute pleuropneumonia. Another batch of 571 lung specimens were obtained from apparently healthy pigs at the slaughter house. All lung tissues were examined by culture methods for the presence of *H. pleuropneumoniae* (4). After the culture examination, the tissues were frozen in a plastic bag or kept in 10% Formalin at room temperature and examined for the detection of antigens within a few weeks of storage.

Preparation of type-specific antisera. For the preparation of antisera against capsular antigens, reference strains representing all seven serotypes were used. Antisera were prepared in rabbits by intravenous inoculation of formalinized whole-cell antigens of each serotype as described by Mittal et al. (3).

Preparation of lung extract for detection of antigen. A small piece of lung tissue (ca. 2 g) was homogenized in about 3 ml of saline in a mortar with the help of a 60-mesh Norton Alundum RR (Fisher Scientific Co., Pittsburgh, Pa.). The lung tissue saline suspension was kept in a small glass tube, boiled in a water bath for 10 min, and centrifuged at 8,000 × *g* for 30 min to remove the particulate material. The clear supernatant was examined for the presence of *H. pleuropneunomiae* antigens by the coagglutination test.

Experimental infection of pigs. Forty piglets, 4 to 8 weeks of age, were obtained from a closed, *H. pleuropneumoniae* infection-free herd. The piglets were either infected by intranasal administration of 2.0 ml of an overnight culture or by aerosol inhalation as described earlier (K. R. Mittal, R. Higgins, S. Larivière, and D. LeBlanc, Am. J. Vet. Res., in press). Of 40 piglets, 36 were infected with a culture of serotype 1, and the remaining 4 were infected with that of serotype 5. Lung tissues from pigs which died of infection or those which were sacrificed 7 weeks postinfection were examined for bacterial isolation and antigen detection.

RP test. The Ring precipitation (RP) test was performed in a Pasteur pipette with lung tissue saline extracts as the antigen and with the reference type sera prepared in rabbits. The details of this technique have been described earlier (3).

Coagglutination test. The details of the preparation of coagglutination reagents and the procedure of the coagglutination test have been described in the accompanying paper (4).

Comparison between the coagglutination and RP tests. Saline extracts of several lung pieces obtained from pigs known to be positive by both culture isolation and antigen detection tests were used. Tenfold or twofold dilutions of the lung extracts were prepared in 0.5-ml volumes of saline. Different dilutions of the extract were tested against type-specific antisera or coagglutination reagents in the RP and coagglutination tests, respectively. The reciprocals of the highest dilution of the lung extract giving a positive reaction was considered the titer.

RESULTS

Results of comparison between the RP and coagglutination tests for detection of antigens in lung tissues showed that the coagglutination test was more sensitive and easier to read than was the RP test. Thus, the coagglutination test was the only test which was consistently used throughout this study.

TABLE 1. Comparison of the culture isolation technique with detection of antigen(s) by the coagglutination test in lung tissues of 240 pigs which died of acute pleuropneumonia

Isolation test result	No. of lungs tested for antigen by coagglutination test	
	Positive	Negative
Positive	131	5
Negative	28	76

TABLE 2. Results of culture examination and detection of type-specific antigens by the coagglutination test in the lung tissues of 240 piglets which died of acute pleuropneumonia

Serotype	No. (%) of pigs positive by	
	Culture examination	Antigen detection
1	102 (75)	109 (69)
2	2 (1.5)	5 (3)
5	26 (19)	31 (19.5)
7	2 (1.5)	2 (1)
1 and 2	1 (1)	1 (0.5)
1 and 3	0	1 (0.5)
1 and 4	0	1 (0.5)
1 and 5	3 (2)	7 (5)
4 and 5	0	1 (0.5)
1, 2, and 5	0	1 (0.5)

Lung tissues from experimentally infected piglets. *H. pleuropneumoniae* serotype 1 was isolated from the lungs of all 15 pigs that died shortly after experimental infection with serotype 1. Saline extract from these lungs elicited a positive reaction for serotype 1 antigen alone in both coagglutination and RP tests. All four piglets which survived experimental infection with serotype 5 were sacrificed 7 weeks postinfection. Although attempts to isolate *H. pleuropneumoniae* were unsuccessful, the lung extract contained only serotype 5 antigens, as shown by coagglutination and RP tests.

Lung tissues from naturally infected piglets with acute pleuropneumonia. Lung tissues obtained from 240 pigs with acute pleuropneumonia were collected in the autopsy room of our diagnostic service. The tissues were cultured and tested for the presence of antigens. Antigen detection by the coagglutination test was successful in 131 pig lungs from which isolation of *H. pleuropneumoniae* was made. Antigen was demonstrated by the coagglutination test in 28 pig lungs from which *H. pleuropneumoniae* was not isolated. When bacteriological isolation was made, the corresponding type-specific antigen(s) was always demonstrated in the lungs by the coagglutination test, except in five cases in which we failed to detect antigens in the lungs (Table 1). The isolates from these five lungs belonged to serotypes 1, 2, and 5. Of the pigs from which type-specific antigens were demonstrated, ca. 7% yielded a positive reaction for more than one serotype-specific antigen; the most frequent combination was due to serotypes 1 and 5 (Table 2).

Lung tissues from apparently healthy pigs from healthy herds. Although *H. pleuropneumoniae*

isolation was not made from any of the lung tissues of 571 pigs from the slaughter house, antigens were demonstrated in 35 (6%) of these pigs. The antigens demonstrated were serotype 1 specific, serotype 5 specific, or both.

DISCUSSION

Results of antigen detection in lung tissues from experimentally infected piglets as well as the results shown in Tables 1 and 2 clearly indicate that the coagglutination test is quite specific in detecting the serotype-specific antigens and that it is more sensitive than the culture isolation procedure. Failure to isolate *H. pleuropneumoniae* in some cases, especially chronic infections, may be due to the limitation of the isolation procedure. Hence, serological detection of antigens in the lung tissues is a much more sensitive method to verify the incidence of *H. pleuropneumoniae* infection in the field, especially when dealing with chronic cases as well as infections with multiple serotypes. It may also be possible that the detection of antigens in the lungs may not indicate the presence of live organisms, but it may indicate that the animals had been exposed to infection sometime in the past. Culture isolation is not necessarily always successful, and it is also time consuming. Rapid and accurate diagnosis of the causative agent in bacterial diseases is often of clinical importance. The choice of therapy may depend on this information, and effective treatment may be delayed when diagnosis is based on conventional bacteriological culture techniques.

Of the 571 apparently healthy pigs which came from seropositive herds and which experienced an episode of acute pleuropneumonia several weeks earlier, 35 were positive for antigen detection. It is noteworthy that none of the pigs from the slaughter house were found positive by culture. This situation may be similar to that observed with four pigs which were infected experimentally with serotype 5. This would suggest that demonstration of antigens by coagglutination is a much more superior method to detect *H. pleuropneumoniae* in animals exposed to chronic infection than is culture examination. It would appear that ca. 5 to 10% of the pigs which survived exposure to *H. pleuropneumoniae* may remain carriers for several weeks.

These findings suggest that a simple, specific, and a sensitive tool is available to investigate the epidemiology of *H. pleuropneumoniae* infection. A suitable control program based on antigen detection in the lungs of slaughtered pigs could be adopted in certain herds to certify them as free from *H. pleuropneumoniae* infection.

Detection of antigen in the lung tissues by coagglutination test can also be useful even if the specimens received in the laboratory are in a state of deterioration in which all *H. pleuropneumoniae* cells are dead and disintegrated. Lung tissues preserved in 10% Formalin solution were also found to be suitable for antigen detection.

Failure to detect antigen in 5 culture-positive lungs and inability to isolate the culture from 28 antigen-positive lungs indicate that there is no perfect correlation between infection and antigen detection by the coagglutination test. This discrepancy may perhaps be due to the selection of different sites on the lung piece for culture examination and antigen detection. Results of experimental aerosol infection of pigs showed that *H. pleuropneumoniae* multiply preferentially in certain areas of the lung and that the presence of hemorrhagic lesions correlated with higher numbers of organisms (6).

Although antigen detection by the coagglutination test provided a wider safety margin than did cultural examination, neither was absolute. It is therefore suggested that in diagnostic laboratories the combination of antigen detection by the coagglutination test and culture examination would greatly improve the accuracy of diagnosis.

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