An Agglutination Test for the Detection of 
Bordetella bronchiseptica Infection in Swine

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ABSTRACT

An agglutination test with the use of formalin-killed antigen of the cell carrying the capsule was developed and used for the detection of antibody in swine naturally infected with Bordetella bronchiseptica. Under optimum antigen concentration and reaction temperature 210 or 60% of 342 serum samples tested from 42 conventional swine herds were positive for Bordetella infection. In contrast, only 34 or 10% of 342 nasal swabs from the same animals were positive for Bordetella by culture technique.

The test was relatively free of cross-reactivity to related organism. However, 2.7 and 13.0% of sera from growing pigs and mature hogs, respectively, reacted with antigen of Pasteurella multocida. Because of this, only agglutinin reactions in 1:20 dilutions or higher to Bordetella were considered positive. The bulk of the antibody activity of selected sera tested from various age ranges of swine was mercaptoethanol sensitive, suggesting that serum antibody in Bordetella infection may be associated with immunoglobulin IgM. Because of the high agglutinability and stability of formalin-killed antigen the test may be useful as an auxiliary aid for the diagnosis of Bordetella infection where the organism cannot be identified by culture means.

INTRODUCTION

Respiratory tract infection of swine due to Bordetella bronchiseptica is enzootic in many herds. The bacterium is widely distributed in the swine population and some forms of the organisms are capable of causing turbinate atrophy while others may create a carrier state in some swine with no apparent signs of the disease. It
has been estimated that 50% of all United States market weight swine have some degree of turbinate atrophy (3).

Herd diagnosis of Bordetella infection is currently based on the isolation of the organism from live pigs. However, with the acceptance of Bordetella bronchiseptica as one of the primary causes of atrophic rhinitis of swine (AR), several studies to determine the level of antibody response to Bordetella rhinitis have been conducted with the persistent hope that serological tests may be useful in the diagnosis of the disease (1,5,6,7,8,9,13). Broth cultures of living organisms were used as antigen in an agglutination test that was thought to be useful for the diagnosis of field cases of AR (6). In a series of studies, killed antigen was used in an agglutination test to determine agglutinins in experimental AR (8,9). Japanese workers used formalin-killed organisms carrying the capsule (phase 1 cells) prepared from cultures grown on charcoal or blood agar for the diagnosis of B. bronchiseptica infection in pigs (13). Other workers developed a particular antigen settling test using plastic trays with U-shaped wells to detect antibody to pigs immunized against B. bronchiseptica (5).

More recently, a microtiter agglutination system was used to determine nasal and serum antibody in pigs vaccinated with B. bronchiseptica (1). This paper reports the results of an agglutination test developed and used to determine antibody levels in swine naturally infected with B. bronchiseptica.

MATERIALS AND METHODS

SOURCE OF SERA AND ANTIGEN

The sera used in the study were obtained from conventionally raised swine of south-eastern Alabama. At the time clinical samples were taken from these herds some had an active infection of Bordetella while others had previous histories of the disease on the farm or many herds had no record of encounter with the disease.

Blood for sera was collected from the cranial vena cava of growing swine (four to 12 wks) with an 18 gauge, one and one-half inch hypodermic needle and from the ear vein of mature hogs (one year and above) with partial vacuum container tubes. The sera were processed and stored at -20° until tested. Antiserum to phase I organisms was prepared in swine by intravenous injections of formalin-killed cells and was used as a reference serum.

PREPARATION OF ANTIGEN

Isolation and biochemical characterization of B. bronchiseptica was accomplished by using a method described by Kemeny and Amtower (9). Fresh isolates of the organism carrying the capsule were used as antigen and capsular staining of the organism was done according to a method described elsewhere (2). The antigen for the agglutination test was prepared by growing the organisms on trypticase soy agar (TSA)1. After 24 hours at 37°C, the cells were harvested with phosphate-buffered saline (PBS), 0.15, pH 7.2 and filtered through a layer of gauze and absorbent cotton. The organisms were killed with 1% formalin while being held at 4°C to 8°C for 48 hours with occasional shaking. The preparation constituted the stock antigen for the test that was maintained under refrigeration conditions for more than one year without any alteration in antigenic properties.

For preparation of antigen used in the test assay, stock antigen was routinely added to PBS to a concentration of 60% transmission (%T) at 625 nm on a Spectrophotometer2 or at various other %T's when used for the titration of the antigen. All antigens were preserved with 0.5% formalin and kept under refrigeration until used in the test. Antigens of Pasteurella multocida3, serotype D and Brucella suis, biotype 1 were prepared in a manner similar to B. bronchiseptica. A similar method was used to prepare antigens from Brucella canis and Klebsiella pneumoniae obtained from a clinically infected dog and pigs respectively.

Isolation of P. multocida was carried out by culturing nasal swab samples on TSA with 5% sheep blood added. Other identification criteria were based on acid pro-

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1Baltimore Biologic Laboratories, Cockeysville, Maryland.
2Spectronic 20, Bausch & Lomb, Rochester, New York.
3American Type Culture Collection, Rockville, Maryland.
duction in dextrose, lack of acid production in lactose and production of indole.

TEST OF Bordetella Suspect Sera

Prior to testing, the undiluted sera were inactivated at 56°C for 30 minutes in a water bath. The sera were serially diluted in 0.5 ml volumes with PBS starting with a 1:5 dilution in 13 x 100 serological tubes. The antigen (0.5 ml) was added, the tubes were shaken and were routinely incubated in a water bath at 55°C. For determining the effect of heat on the agglutination reaction, tests were incubated under various other temperature conditions (37°C, 42°C, 47°C, 52°C and 60°C). The incubation time for all tests was four hours at the desired temperature followed by 20 hours at 4°C to 8°C.

To determine the agglutination endpoint, tests were read under indirect fluorescent light from a desk lamp. The agglutinin pattern was judged as positive when definite granules of antigen were seen in a layer covering the bottom of the tube. The reciprocal of the highest dilution of serum giving a positive reaction was taken as the titer. Since cross-reactivity was previously observed in some of the sera obtained from mature swine against P. multocida, only agglutinin titers in 1:20 dilution and above were considered positive for Bordetella infection.

2-Mercaptoethanol Treatment of Sera

In the treatment of sera with 2-mercaptoethanol, a simplified version of a method described by Nowotny (10) was used. Briefly, 0.2 ml of serum and 0.2 ml of 0.2 M, 2-mercaptoethanol were added to 0.6 ml of physiological saline. After incubation at room temperature for one hour, the mixture was diluted twofold in PBS and 0.5 ml of antigen was added. The tubes were incubated, read and scored as above.

Statistical Evaluation

The mean and standard error of the mean (SEM) of agglutinin titers were calculated on an electronic calculator*. Mean titers were then subjected to analysis of variance and evaluated on the basis of the least significant difference test. Differences were considered significant at the 0.05 probability level.

RESULTS

Bordetella bronchiseptica containing the capsule was readily demonstrated in fresh isolate (Fig. 1) by capsular staining. Comparison of the agglutinating capacity of serum from different age ranges of swine with antigen of phase I (capsular organism) and phase III (noncapsular organism) indicated that organisms carrying the capsule had higher agglutinin titers compared to those lacking the capsule (Fig. 2). There was a significant increase in the agglutinating capacity of sera with phase I antigen compared to phase III in swine of the age ranges of eight to 12 weeks, gilts and sows (P < 0.5) in all cases. However, there was no significant increase of phase I over phase III antigen to serum of pigs in the ranges of four to seven weeks (P > 0.05). When various sera of different age ranges of swine were used to determine the optimal concentration of antigen, the highest agglutinin titers (P < 0.05) were obtained at 60% T (Fig. 3).

There was a significant increase in the agglutinin titers (P < 0.05) when the test was carried out at a reaction temperature of 55°C compared to 52°C (Fig. 4). In contrast, the agglutinin titers decreased sharply from 1:1920 to 1:640 (P < 0.05) when the reaction temperature was raised from 55°C to 60°C.

Sera of positive for agglutinin titers were then subjected to analysis of variance and evaluated on the basis of the least significant difference test. Differences were considered significant at the 0.05 probability level.


*Electronic Calculator, Model PC-1002, Sharp Electronic Corporation, Paramus, New Jersey.
Fig. 1. Bordetella bronchiseptica carrying the capsule.

Fig. 2. Comparison of agglutinin titers of swine sera with B. bronchiseptica Phase I and Phase III antigen (mean value ± SEM).

CROSS-REACTIVITY STUDIES OF RELATED ORGANISMS

Sera of growing pigs and mature hogs obtained from various sources did not react with antigens of Brucella suis, Brucella canis or Klebsiella pneumonia in dilution of 1:10 or higher (Table I). However, the mean agglutinin titers of sera obtained from growing pigs and mature hogs were 12.3 ± 1.4 and 17.27 ± 1.4 respectively, against Pasteurella antigen. The percent of cross-reactivity to Pasteurella antigen was 2.7% in growing pigs and 13% in mature hogs.

Fig. 3. Effect of antigen concentration on the agglutination reaction of sera produced in swine to B. bronchiseptica (mean value ± SEM).

Fig. 4. Effect of temperature on the agglutination reaction of sera produced in swine to B. bronchiseptica (mean value ± SEM).

AGGLUTININ TITERS OF CONVENTIONAL SWINE HERDS

Swine of various ages were tested for Bordetella by both the culture and aggluti-
nation procedure at different time intervals during 1976 (Table II). Of the 342 samples tested from 42 herds, 34 or 10% were positive by the culture procedure compared to 210 or 60% by the serological test.

2-MERCAPTOETHANOL TREATMENT OF SERA

Pooled serum samples of swine from convention herds of all age ranges fail to agglutinate *Bordetella* antigen following treatment with 2-mercaptoethanol (Table III).

DISCUSSION

An agglutination test with the use of formalin-killed cells was developed to detect agglutinins in the sera of swine naturally infected with *B. bronchiseptica*. Antigen made from organisms carrying the capsular (Phase I) gave consistently higher titers than those devoid of the capsular material (Phase III). It was noticed that fresh isolates of *B. bronchiseptica* isolated from the nasal cavity of swine frequently underwent changes to the immediate phase (having a thin capsular and partial capsular antigen) or phase III organism after subculturing. This observation supported similar findings previously reported (13). In our work the capsular form could be restored by growing the organism in media containing 5% blood serum. In view of this it may be concluded that the immunologic antigen is probably located on the surface of the cell and is associated with the capsular antigen. Serological analysis has not established the nature of the change as to whether it is only a variation of the proportion of the antigens present in phase I or the appearance of new antigens (16). Nevertheless, care must be taken in the selection of stable strains of *B. bronchiseptica* for the test. The concentration of antigen was also considered crucial, particularly in testing low titer serums in order to avoid false negative or positive sera.

Agglutinins in sera of swine naturally infected with *B. bronchiseptica* were detected over a wide range of reaction temperatures. However, maximum activity was obtained at 55°C. This is in agreement with previous findings (13).

Of the organisms tested belonging to the *Brucellaceae* family, only *P. multocida* cross-reacted with sera of swine naturally infected with *B. bronchiseptica*. This could be due to infection with *Pasteurella* spp. since the organisms are known to be associated with atrophic rhinitis (3, 4, 11). Since many swine may have antibody to *P. multocida* it is recommended that only titers of 1:20 and above be considered positive for *Bordetella* infection.

In this study, the prevalence of *B. bronchiseptica*, as measured by the serological test, was much greater in the swine population of conventionally reared swine compared to the number of bacterial isolations. This agreed with other workers who have also reported difficulty in recovering the organisms from swine (9, 12). Certain factors, such as contamination of nasal swabs at the time of collection, greater resistance of older animals to the infection and the use of medicated feed may contribute to low isolation percentages. Relocation of the organisms in the nasal cavity may also be a factor since *B. bronchiseptica* was isolated from the ethmoidal area on necropsy with greater frequency than pre-
TABLE I. Agglutinin Titters of Swine Serums to Antigens Related to Bordetella bronchiseptica

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Mean Agglutinin Titer of Serums&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number and Percent Cross-Reaction</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pigs&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Mature Hogs&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>12.3 ± 1.4</td>
<td>17.3 ± 1.4</td>
</tr>
<tr>
<td>Brucella suis</td>
<td>&lt; 1:10</td>
<td>&lt; 1:10</td>
</tr>
<tr>
<td>Brucella canis</td>
<td>&lt; 1:10</td>
<td>&lt; 1:10</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>&lt; 1:10</td>
<td>&lt; 1:10</td>
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</tbody>
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<sup>a</sup>Total of 108 serums were tested against each antigen together with a positive and negative Bordetella control serum
<sup>b</sup>Serum of pigs eight to 12 weeks old
<sup>c</sup>Serum of swine six months and older

TABLE II. Prevalence of Bordetella bronchiseptica Infection in Conventional Swine as Determined by Culture and Serological Methods

<table>
<thead>
<tr>
<th>No. of Herds Tested</th>
<th>No. of Animals Tested</th>
<th>No. of Organisms Isolated</th>
<th>No. of Serological&lt;sup&gt;a&lt;/sup&gt; Positives</th>
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<tr>
<td>42</td>
<td>342</td>
<td>34 of 10%</td>
<td>210 of 60%</td>
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<sup>a</sup>Positive in 1:20 dilution or greater

TABLE III. Agglutinin Titer of Swine Sera to Bordetella Antigen before and after Treatment with 2-mercaptoethanol

<table>
<thead>
<tr>
<th>Host</th>
<th>Geometric Mean Agglutinin Titer&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Pigs (4-7 weeks)</td>
<td>44 ± 12.3</td>
</tr>
<tr>
<td>Pigs (8-12 weeks)</td>
<td>148 ± 23.4</td>
</tr>
<tr>
<td>Mature hogs</td>
<td>82.23 ± 7.3</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reciprocal of endpoint dilution

The lack of correlation between isolation and serum titer of gilts could probably be attributed to certain management factors. Under most management regimes, gilts are usually set apart from the herd in preparation for breeding and thus are less subjected to herd associated stress factors.

The relatively high agglutinability of sera obtained from conventionally raised swine could have been the reflection of constant antigenic stimulation. In a similar study, it was thought that persistence of agglutinin levels in pigs and mature hogs was induced by prolonged antigenic stimulus (8). The contention was supported in this study by demonstrating that the bulk of serum antibody of growing pigs and mature hogs to Bordetella was mercaptoethanol sensitive. The results further suggested that new antibody, presumably macroglobulin (IgM), is probably being constantly synthesized in pigs infected with Bordetella. This may be the case of a certain kind of antigen (polysaccharide) selectively stimulating the synthesis of one kind of antibody (IgM). Studies investigating the synthesis of agglutinins to gram-negative somatic antigen in rabbits indicated the predominancy of IgM antibody with killed Salmonella typhosa or hyperimmunization with either somatic polysaccharide or whole enteric organisms (14, 15).

The diagnosis of Bordetella infection in swine herds is currently based on isolation of B. bronchiseptica from live pigs. Since the data presented in this study as well as others (6,7,8,9,13) have demonstrated antibody in the sera of swine naturally infected with B. bronchiseptica, it appears that the agglutination test may be useful for the identification of the carrier state where the disease cannot be detected bacteriologically.

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REFERENCES


