Rapid Detection of Group C Streptococci from Animals by Latex Agglutination

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A group C latex agglutination reagent, included as the negative control in the PathoDx Strep A latex agglutination test (Diagnostic Products Corp., Los Angeles, Calif.), was compared with culture for rapid detection of group C streptococci (Streptococcus equi, S. equisimilis, S. zooepidemicus, and S. dysgalactiae) from swabs of veterinary specimens. The overall sensitivity of the latex test was 78%, and specificity was 97.6%. Only 25% of S. dysgalactiae isolates were detected, thereby accounting for the relatively low sensitivity. Ninety-three percent of the group C streptococci other than S. dysgalactiae were isolated from horses. When the latex test was evaluated for detection of group C streptococci other than S. dysgalactiae from horses, the sensitivity and specificity were 95.3 and 100%, respectively. We found the group C latex agglutination test to be a rapid and accurate method for the detection of the major pathogenic group C streptococci from swabs of equine specimens.

Group C streptococci are etiologic agents of wound, eye, reproductive tract, and respiratory tract infections, arthritis, and mastitis in animals as well as occasional pathogens of humans. The group C streptococcal species include Streptococcus equi (the primary agent of streples), genital infections, and mastitis in horses), S. zooepidemicus (a cause of wound, respiratory, and genital infections in a wide variety of animals and occasionally pharyngitis and glomerulonephritis in humans), S. equisimilis (a cause of streples, wound and genital infections, and mastitis in horses and other animals and the most common group C streptococci causing pyogenic infections in humans), and S. dysgalactiae (an important cause of bovine mastitis and polyarthritis of lambs) (2, 5). Infections due to streptococci, particularly S. equi, may be highly contagious, and rapid detection of the agent is important when considering isolation measures, treatment efficacy, and returning the animals to their normal activities (6).

Latex agglutination tests and other rapid antigen detection tests have become popular in human medicine because they can be used in the physician's office. They are simple to perform, and results can be obtained in a few minutes, enabling appropriate therapy to be started before the patient leaves the office. The most widely used bacterial detection test used in pediatricians' offices today is for detection of group A streptococcal pharyngitis. One such test is the PathoDx Strep A latex agglutination test, marketed by Diagnostic Products Corp., Los Angeles, Calif. The PathoDx kit contains a group A streptococcal latex agglutination reagent and a negative control latex reagent conjugated to antibody to group C streptococcal antigen. The purpose of this study was to determine the sensitivity and specificity of the group C PathoDx latex agglutination reagent for detection of group C streptococci from swabs of veterinary specimens.

MATERIALS AND METHODS

Latex agglutination test. The PathoDx latex agglutination test contains purified antibody to the streptococcal group A or group C carbohydrate that is covalently bound to latex beads, antigen extraction and neutralization reagents, and control swabs containing the group A or group C carbohydrate. For our purposes the group C reagents were used for the test and the positive control, and the group A reagents were used for the negative control. The PathoDx test was designed for antigen detection from swabs, and therefore only swabs from various veterinary specimens were used. The Marion Culturette swabs (Marion Laboratories, Inc., Kansas City, Mo.) were used for all referral specimens submitted for routine culture. Dacron swabs were used to collect specimens from within the Veterinary Teaching Hospital. If fluid specimens were assayed, a Dacron swab was immersed in the fluid and used for the test. Fluids were not centrifuged because in field situations and in most clinicians' offices (for which the test was designed) centrifuges would not be available.

Two drops each of reagents 1 and 2 were added to a 12- by 75-mm disposable glass tube. The swab was incubated in the mixture for 1 min at room temperature, and 4 drops of reagent 3 were added. The reagents were mixed with the swab, and the swab was pressed against the side of the wall and removed. The positive and negative control swabs were processed in the same manner each day tests were done. Each lot of latex reagent was also tested once with extracts of quality control stock cultures of S. zooepidemicus and Streptococcus (Enterococcus) faecalis. The group A latex reagent was negative with both quality control organisms, and the group C latex reagent was positive only with S. zooepidemicus. Samples (50 μl) of each extract were placed in two wells of the slide provided, and 1 drop of Strep C latex was added to one row of wells and 1 drop of Strep A latex was added to another row of wells. The slide was rotated for 4 to 5 min on a rotary shaker and examined immediately for agglutination. A 2+ to 4+ reaction was considered positive (50 to 100% agglutination), whereas a 0 to 1+ reaction (0 to 25% agglutination) was considered negative.

Specimens and culture procedures. All swabs were submit-
ted by mail to the Washington Animal Disease Diagnostic Laboratory and cultured on Columbia agar containing 5% bovine blood and on MacConkey agar. Swabs were not in transit more than 1 or 2 days and were cultured the same day they were received. Swabs (but not fluid specimens) were then inoculated into thioglycolate broth, removed, and then frozen at −70°C. Culture plates were incubated at 35°C in air and examined after approximately 18 and 42 h. If no growth occurred on culture plates, broth cultures were examined daily and subcultured on blood and MacConkey agar after turbidity developed or after 7 days. Swabs that were culture positive for streptococci were collected, thawed, and tested with the latex agglutination reagent. Ninety percent of the specimens were referral cases from outside the hospital of the College of Veterinary Medicine, and therefore duplicate specimens for culture and for latex agglutination could not be obtained. Specimens from within the hospital were collected in duplicate so that one specimen was cultured and the other was tested directly by latex agglutination.

All streptococcal isolates were identified to species by conventional biochemical tests (2, 3, 5) and with the API 20S kit (Analytab Products, Plainview, N.Y.). Isolated streptococci were grouped by using the Streptex latex agglutination serogrouping kit (Burroughs Wellcome Co., Research Triangle park, N.C.). Nonstreptococcal bacteria mixed with streptococci in specimens were identified to genus or species level, depending on significance, by standard procedures (2, 3, 5).

Statistics. The percent sensitivity of the PathoDx test compared with culture was calculated by the equation [true positives/(true positives + false-negatives)] × 100, and the percent specificity was calculated by the equation [true negatives/(true negatives + false-positives)] × 100.

RESULTS

One hundred-nine streptococcal isolates representing 10 species were isolated from clinical swabs. Clinical specimens from nine animal species included swabs of the upper and lower respiratory tract, reproductive tract, eye, ear, wound or lymph node abscesses, milk, and joint fluids (Table 1). Sixty-four isolates were group C streptococci, and 45 isolates were non-group C streptococci. All specimens containing S. equi (n = 8) and S. equisimilis (n = 2) were strongly positive by latex agglutination, and cultures of these specimens yielded high numbers of each streptococcal species (greater than 500 colonies per plate). All isolates of S. equi and S. equisimilis were obtained from horses. Ninety-one percent of the S. zooepidemicus isolates were from horses, and 31 of 34 specimens containing S. zooepidemicus were positive by latex agglutination. Two of the three specimens that were culture positive but latex agglutination test negative were swabs of transtracheal aspirates, and the third was a vaginal swab. The transtracheal aspirate specimens may have been negative due to the dilute nature of these specimens, and culture of the vaginal swab yielded less than 25 colonies of S. zooepidemicus. Twenty specimens were culture positive for S. dysgalactiae, but only five were positive by latex agglutination; the positive reactions were only 2+. Thirteen of these specimens yielded more than 500 colonies per plate on culture, and therefore failure to detect S. dysgalactiae was not apparently due to insufficient antigen. Since similar specimens positive by latex agglutination assay with the other group C streptococci were negative for S. dysgalactiae, we concluded that this species was not reliably identified by the group C latex agglutination assay. S. dysgalactiae was isolated from the widest variety of animal species; 35% of the isolates were obtained from horses.

The sensitivity of the latex agglutination test for the detection of all group C streptococci from all animal specimens tested was 78.0%. When the test was examined for all group C streptococci from horses, the sensitivity increased to 87.3%. When S. dysgalactiae was excluded from the test, the sensitivity was 93.6% from all animals and 95.3% from horses. The specificity of the latex agglutination test was 97.8% for specimens from all animals and 100% for specimens from horses (Table 2).

<table>
<thead>
<tr>
<th>Species</th>
<th>Lancefield group</th>
<th>No. tested</th>
<th>Respiratory</th>
<th>Urogenital</th>
<th>Wound</th>
<th>Eye and ear</th>
<th>Joint fluid</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. zooepidemicus</td>
<td>C</td>
<td>34</td>
<td>10 e (1)</td>
<td>6 e (1)</td>
<td>12 e</td>
<td>3 e</td>
<td>2 c</td>
<td>1 g (1)</td>
</tr>
<tr>
<td>S. equi</td>
<td>C</td>
<td>8</td>
<td>2 e</td>
<td>6 e</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. equisimilis</td>
<td>C</td>
<td>2</td>
<td>1 e</td>
<td>1 e</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. dysgalactiae</td>
<td>C</td>
<td>20</td>
<td>1 h</td>
<td>3 c (3)</td>
<td>1 e</td>
<td>1 c (1)</td>
<td></td>
<td>1 b (1)</td>
</tr>
<tr>
<td>S. faecalis</td>
<td>D</td>
<td>24</td>
<td>5 e (4)</td>
<td>1 e (1)</td>
<td>1 b (1)</td>
<td>2 f</td>
<td>1 p (1)</td>
<td>3 c (3)</td>
</tr>
<tr>
<td>S. canis</td>
<td>G</td>
<td>10</td>
<td>1 a</td>
<td>1 a</td>
<td>1 a</td>
<td>2 b</td>
<td>1 g</td>
<td>1 f</td>
</tr>
<tr>
<td>S. bovis</td>
<td>D</td>
<td>3</td>
<td>1 f</td>
<td></td>
<td></td>
<td></td>
<td>2 b</td>
<td></td>
</tr>
<tr>
<td>S. uberis</td>
<td>E</td>
<td>4</td>
<td>1 a</td>
<td>3 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. acidominimus</td>
<td>–</td>
<td>3</td>
<td>1 l</td>
<td>1 l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. suis</td>
<td>D</td>
<td>1</td>
<td>1 p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Animal species of specimens: e, equine; c, canine; g, guinea pig; h, human; b, bovine; f, feline; p, porcine; a, avian; l, llama. The numbers within parenthesis indicate the numbers of false-negatives for group C streptococci and the numbers of false-positives for non-group C streptococci.

*– Some or all isolates could not be classified by the Lancefield grouping system.
TABLE 2. Sensitivity and specificity of the group C streptococci latex agglutination test

<table>
<thead>
<tr>
<th>Streptococcal isolates</th>
<th>No. of isolates</th>
<th>Group C culture positive</th>
<th>Group C latex positive</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>From animals</td>
<td>109</td>
<td>64</td>
<td>46</td>
<td>78.0</td>
<td>97.8</td>
</tr>
<tr>
<td>From horses</td>
<td>49</td>
<td>48</td>
<td>41</td>
<td>87.3</td>
<td>100</td>
</tr>
<tr>
<td>Other than S. dysgalactiae, isolated from animals</td>
<td>49</td>
<td>44</td>
<td>41</td>
<td>93.6</td>
<td>97.8</td>
</tr>
<tr>
<td>Other than S. dysgalactiae, isolated from horses</td>
<td>42</td>
<td>41</td>
<td>39</td>
<td>95.3</td>
<td>100</td>
</tr>
</tbody>
</table>

a Of 18 false-negative latex agglutination tests, 15 were with specimens containing S. dysgalactiae.

Of the 45 specimens containing non-group C streptococci, S. faecalis was the most common isolate, 1 specimen (Streptococcus canis) was positive by latex agglutination, and 1 specimen (Streptococcus bovis) was obtained from a horse. Most specimens (66%) contained one or more bacterial species in addition to the streptococci. The most common bacterial isolates mixed with streptococci were Staphylococcus species, members of the family Enterobacteriaceae, Corynebacterium species, Actinobacillus species, Actinobacter species, and Pasteurella species. Fifty percent of group C streptococci other than S. dysgalactiae were isolated in pure culture, including one isolate of S. zooepidemicus that was negative by latex agglutination. All isolates of S. dysgalactiae were obtained from specimens containing one or more nonstreptococcal bacterial species. The presence of heterologous bacteria in a specimen did not interfere with the latex agglutination assay.

DISCUSSION

Conventional biochemical tests for identification of group C streptococci may require up to 7 days to complete (3). Rapid detection of group C streptococci would aid in preventing spread of infections and initiating appropriate therapy. Bannister et al. (1) evaluated the API 20S and Rapid Strep (API System S. A., Montalieu-Vercieu, France) tests for rapid speciation of group C streptococci from horses. Although the Rapid Strep test correctly identified the species of all group C isolates tested (S. dysgalactiae was not included), the assay required isolation of the bacteria and an additional 24 h for complete acidification of the carbohydrate. Identification to species of a group C streptococcus may not be necessary, because the treatment of choice for all equine streptococcal infections is penicillin (12). The development of "bastard strangles" after penicillin treatment of animals with immature abscesses due to S. equi has not been clinically proven (12). Therefore, rapid detection of a group C streptococcus infection would facilitate management and initiation of treatment. Direct antigen detection provides group identification within 10 min, without the need for extensive laboratory facilities. Therefore, the test can be done in the field or in the office.

Latex agglutination and other antigen detection tests have been widely used for rapid detection of group A streptococci from throat swabs. The Culturette Brand 10-Minute Group A Strep kit (Marion Laboratories, Kansas City, Mo.) and the Directigen Rapid Group A Strep Test (Hyson, Wescot and Dunning, Baltimore, Md.) have been studied the most extensively. A summary of the sensitivities and specificities of direct detection systems for group A streptococci from 27 published studies has recently been reported (4). Of the latex agglutination tests studied, sensitivity of the tests has ranged from 62 to 100%, and specificity has ranged from 88 to 100%.

Commercial kits for direct detection of group B streptococci from urogenital swabs have also become available but have not been evaluated as extensively as group A antigen detection tests (7, 13). We used the PathoDx group C latex agglutination reagent because this reagent was developed for the detection of antigen from swabs, rather than for serotyping cultured bacteria, and because the sensitivity and specificity of the PathoDx group A reagent have been reported to be 96.7 and 97.9%, respectively (false-negative and -positive tests, however, were thought to be due to sampling errors) (9), and 97.3 and 98.6%, respectively (Diagnostic Products Corp.; data on file).

The group C latex agglutination test was highly sensitive and specific for identifying S. zooepidemicus, S. equi, and S. equisimilis. All S. equi and S. equisimilis isolates and 31 of 34 S. zooepidemicus isolates were correctly detected. The capability of the test to detect group C streptococci other than S. dysgalactiae was particularly impressive, considering that most of our specimens were referral cases from outside the hospital and that the same swab was therefore necessarily used for culture and the latex agglutination test. The standard culture procedure used in the laboratory included brief immersion of the swab into thioglycolate broth, thereby introducing a dilution effect that may have reduced the sensitivity of the test. The limit of antigen detection has not been determined for the group C latex reagent, but a similar reagent designed to detect group A antigen from throat swabs has been shown capable of detecting the equivalent of 10 or more colonies by plate culture (11). Swabs obtained from within the hospital that contained group C streptococci (and which were not diluted) all gave + reactions with the latex agglutination assay but also had more than 50 colonies of group C streptococci per plate on culture. However, many referral swabs that were diluted also gave + reactions in the latex agglutination assay.

Since most of the specimens spent some time in transit, there was some concern that a high false-positive rate might occur if the bacteria were present but had lost viability. However, only one specimen (canine) was positive by latex agglutination but negative by culture. Whether this false-positive reaction was a true false-positive or was due to the presence of nonviable group C streptococci in the specimen was not determined. Some specimens contained a large number and variety of other bacterial species in addition to group C streptococci. However, we did not find any correlation between the presence of heterologous bacterial species in a specimen and an increase in false-negative or false-positive reactions.

In contrast to the favorable detection of group C antigen from S. equi, S. zooepidemicus, and S. equisimilis, the PathoDx reagent detected group C antigen in only 25% of specimens containing S. dysgalactiae. Recent taxonomic studies have shown that isolates of S. dysgalactiae are biochemically and genetically very similar to streptococci of serogroups G (large colony type) and L as well as C (other than S. equi and S. equisimilis) (10). Therefore, if some of our isolates biochemically identified as S. dysgalactiae belonged to serogroup G or L, they would not have reacted with the group C latex reagent.

Since 93% of group C streptococci other than S. dysgalactiae were from horses and only one non-group C streptococcus was isolated from a horse, the group C latex reagent
seemed most useful and reliable with specimens from horses. The sensitivity and specificity of the test for group C streptococci other than *S. dysgalactiae* from horses were 95.3 and 100%, respectively. Furthermore, if the test were used only on specimens from horses the failure to detect *S. dysgalactiae* would be of little significance, since this species is not normally considered an equine pathogen (2, 8). We conclude that the PathoDx group C latex agglutination test is a rapid, accurate, and simple method for use in the field or office to detect the major pathogenic streptococci from horses.

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LITERATURE CITED