Characterization of Pasteurella spp isolated from healthy domestic pack goats and evaluation of the effects of a commercial Pasteurella vaccine

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Objective—To characterize Pasteurella spp isolated from healthy pack goats and evaluate the effects of administration of a commercial Pasteurella vaccine.

Animals—45 goats.

Procedure—Pharyngeal swab specimens and blood samples were collected on day 0 before vaccination with a Pasteurella (Mannheimia) haemolytica serotype A1 bacterin. Samples were also collected from 17 goats on days 21 and 35. Isolated Pasteurella spp were assigned to biovariant groups on the basis of results of biochemical utilization tests and serotyped. Serum antibody titers were determined.

Results—Multiple strains of Pasteurella spp were isolated from swab specimens and assigned to 30 non-hemolytic and 14 β-hemolytic biovariant groups. The most common biovariant isolated was nonhemolytic P. haemolytica belonging to group 2. This strain was isolated from 41 goats. Nonhemolytic P. haemolytica strains were isolated from 31 goats, whereas β-hemolytic strains of P. trelasi and P. haemolytica were isolated from 8 and 35 goats, respectively. Vaccination with the A1 serotype did not affect the proportion of goats from which we isolated each biovariant group or the number of biovariant groups isolated.

Conclusions and Clinical Relevance—Multiple strains of P. haemolytica and P. trelasi belonging to nonhemolytic and β-hemolytic biovariant groups were isolated from the pharynx of healthy domestic pack goats. Because hemolytic activity correlates with leukotoxin production, β-hemolytic strains may have a greater potential to cause disease in naive populations of wild ruminants. However, vaccination with an A1 serotype bacterin did not decrease the proportion of culture-positive goats.

Goats can be easily managed on minimal trails and taken to areas that are not used by many hikers. In addition, goats can be sustained by browsing on available forage in most areas. Because of their increased use in wilderness habitats, there is concern regarding potential transmission of disease agents from pack goats to wild ruminants, particularly bighorn sheep.

Respiratory disease attributable to infection with Pasteurella spp appears to develop only sporadically in most wild ruminant species. However, multiple epidemiologic studies in bighorn sheep populations have been reported. A number of Pasteurella strains can be found as commensals in the upper respiratory tract of healthy free-ranging bighorn sheep. The majority of isolates from bighorn sheep are nonhemolytic P. trelasi. Although β-hemolytic Pasteurella strains have been isolated from healthy bighorn sheep, other β-hemolytic strains, including some from domestic sheep, have a high potential to cause respiratory disease in bighorn sheep. The risk of transmission of strains with high disease potential from pack goats to wild animals in wilderness habitats is unknown. However, owners of pack goats have been advised to vaccinate their goats against Pasteurella spp prior to packing into certain wilderness habitats.

Because little is known about the diversity of Pasteurella organisms carried by domestic pack goats, the primary objective of the study reported here was to identify and classify Pasteurella spp isolated from the pharynx of pack goats. A secondary objective was to determine whether vaccination with a commercial Pasteurella vaccine would affect our ability to isolate Pasteurella organisms from these goats. We used results of biochemical utilization tests to classify Pasteurella isolates. Although it has been recommended that trehalose-negative organisms identified as P. haemolytica be placed in a new genus, Mannheimia, divided into 5 named and several unnamed species, we used the biovariant differentiation system of Jaworski et al in the study reported here to assign Pasteurella isolates to biovariant groups. This system is based on the Bisgaard and Mutters biogrouping system and allows for a greater ability to detect differences in isolates than could be achieved by use of the proposed Mannheimia nomenclature. Therefore, the genus name Pasteurella has been reserved in this report for both P. haemolytica and P. trelasi.

Materials and Methods

Goats—Forty-five goats ranging from 14 months to 7 years of age were used in this study. Goats were of several...
breeds, including Alpine, Nubian, Toggenburg, Saanen, La Mancha, Boer, and Pygmy, from 9 owner groups (herds) identified as herds A through I. On test days, owners transported their goats 12 to 30 miles to a central processing area.

Sample collection—Pharyngeal swab specimens and blood samples were collected on day 0 from all goats immediately prior to vaccination with a P haemolytica serotype A1 bacterin (2 mL/goat, SC) produced for use in cattle. Swab specimens and blood samples were also collected from 17 goats in herds A, B, C, and G on days 21 and 35. To collect pharyngeal swab specimens, a handler held each goat’s head, and a sheathed projectable swab system was inserted to the back of the throat. Swabs were placed on ice for transport to the laboratory, where they were submitted for bacteriologic culture within 2 hours. Blood samples that were collected by jugular venipuncture were allowed to set to room temperature (approx 22°C) for 4 hours prior to centrifugation at 1,500 X g for 10 minutes. Serum was collected, aliquoted into 4-mL vials, and stored at -70°C until serologic tests were conducted.

Bacteriologic culture procedures—Pharyngeal swab specimens were inoculated onto Columbia blood agar with 5% sheep blood (CBA) and Columbia blood agar with 5% bovine blood containing antibiotics selective for Pasteurellae.29 Inoculated media were incubated at 37°C in an atmosphere containing 10% added CO2 and examined after 24 and 48 hours for colonies characteristic of Pasteurella spp. Representative colonies were selected and tested by use of biochemical utilization tests for identification and classification into biovariant groups as described.19,25 Pasteurella haemolytica and P trehalosi isolates were serotyped by use of standard plate agglutination.26

Serologic assays—Serum agglutinating and leukotoxin-neutralizing antibody titers against P haemolytica serotype A1 were determined by use of a direct agglutination assay.19,26 Washed formalinized cells from an overnight broth culture of P haemolytica strain 280697 were diluted to a working concentration (optical density of 1.8 at 525 nm). Two-fold serial dilutions of each serum sample were placed in U-bottomed 96-well microtiter plates with equal volumes of antigen suspension (ie, washed formalinized bacteria). Plates were sealed and incubated overnight at room temperature. Antibody titers were expressed as the log2 of the reciprocal of the endpoint dilution.

Leukotoxin-neutralization antibody titers were measured, using an established colorimetric assay.19,26,27 Briefly, the assay was performed by preparing 2-fold serial dilutions of each serum sample in RPMI 1640 tissue culture medium in U-bottomed microtiter plates. Control wells received 200 µL of RPMI 1640 medium containing 7% fetal bovine serum. Two hundred microliters of a P haemolytica serotype A1 leukotoxin preparation was added to each test well, and an equal volume of RPMI 1640 was added to control wells. Plates were incubated at room temperature for 30 minutes. Twenty microliters of a bovine lymphoma cell (BL3 cells) suspension (1.2 X 105 viable cells/mL) was then added to each well, and plates were incubated at 37°C in an atmosphere containing 5% added CO2 to allow uptake of neutral red dye by living cells. Cells were washed twice with PBS solution and lysed by addition of 200 µL of a 0.05M acetic acid/0.5% SDS solution. The optical density of each well was determined at 540 nm, using an automated spectrophotometer. Titers were expressed as the reciprocal of the highest log2 of the dilution of serum that resulted in neutralization of ≥ 50% of the cytotoxicity, compared with that in control wells.

Statistical analyses—Serum agglutinating and leukotoxin-neutralizing antibody titers were compared within and among herds over the 3 test days by use of linear regression and calculation of 95% confidence intervals.30 Proportion of goats that were culture-positive for β-hemolytic and non-hemolytic Pasteurella strains, proportion of goats that were culture-positive for each of the biovariant groups, and number of different biovariant groups isolated were compared within and among herds over time by use of ANOVA followed by the Fisher protected least significant difference method.31 All analyses were performed, using commercially available software, and significance was set at P ≤ 0.05.

Results

Bacteriologic results—Pasteurella spp were isolated from all swab specimens collected. Of the 186 isolates, 99 were identified as P haemolytica, 86 as P trehalosi, and 1 as P multocida biotype U1 (Table 1). Pasteurella trehalosi was isolated from all 45 goats and

Table 1—Pasteurella biovariants isolated from pharyngeal swab specimens from pack goats in 9 herds (herds A through I)

<table>
<thead>
<tr>
<th>Species isolated</th>
<th>Biovariant group</th>
<th>Hemolytic activity</th>
<th>No. of culture-positive goats/all goats (herd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P haemolytica</td>
<td>β</td>
<td>1/7 (D)</td>
<td>9/51 (A), 4/41 (H)</td>
</tr>
<tr>
<td>P trehalosi</td>
<td>β</td>
<td>1/7 (G)</td>
<td>2/41 (D), 1/6 (I), 1/4 (H)</td>
</tr>
<tr>
<td>P multocida</td>
<td>U1</td>
<td>1/7 (F)</td>
<td>1/7 (H), 1/5 (A), 1/4 (I)</td>
</tr>
</tbody>
</table>

β = β-hemolytic. nh = Nonhemolytic.

*Biovariant group identified on the basis of results of biochemical utilization tests.** Hemolytic activity assessed by growth on Columbia blood agar with 5% sheep blood. †Significantly different from proportion in other herds.
was the only Pasteurella spp cultured from 7 goats, including 4 of 4 goats in herd F. Pasteurella haemolytica was cultured from 38 goats. Forty-four biovariant groups were identified on the basis of hemolytic activity on CBA and results of biochemical utilization tests.

The 86 P. trelahosi isolates were assigned to 14 biovariant groups, 11 and 3 of which were variants of biogroups 2 and 4, respectively, as identified by Bisgaard and Mutters.23 Organisms belonging to nonhemolytic P. trelahosi biovariant group 2 were isolated from 32 goats distributed among all herds. Beta-hemolytic P. trelahosi organisms were isolated from 8 goats, including animals in all herds except C and D. Fifty-six of the 86 P. trelahosi isolates were characterized with standard typing sera as serotype T3, 18 were serotype T4, I was serotype T10, 1 was serotype T15, 7 were untypeable, and 3 were not tested for serotype. Antisera produced against 1 of the untypeable isolates agglutinated 3 isolates that did not react with standard typing sera.

The 99 P. haemolytica isolates were assigned to 29 biovariant groups, 18 of which were nonhemolytic and 11 of which were β-hemolytic. Beta-hemolytic P. haemolytica strains were cultured from goats in all herds except D and F. Biovariant group 1 was the most common β-hemolytic biovariant isolated; organisms belonging to this biovariant group were isolated from all goats in herds A (n = 5) and H (4). Other β-hemolytic P. haemolytica biovariants included biovariant groups 3, Uα, and Uβ (Table 1). Hemolytic isolates were also identified in biovariant groups 1αβ, 7, 7α, 8, 16α, 16β, and U0.

Of the 99 isolates, only 12, including isolates in biovariant groups 7, 7α, 16α, 16β, and U0, reacted with the following standard typing sera: serotypes A8 (n = 3 isolates), A11 (6), and A163 (3). Two isolates agglutinated in normal rabbit serum, and the remainder were untypeable, because they did not react with any of the standard typing antisera. Fifteen isolates in the latter group agglutinated in antiserum produced against 1 of the untypeable isolates (G454A).

The number of goats from which each biovariant group was isolated was compared among herds. The proportion of biovariants isolated from which we isolated P. haemolytica organisms identified as β-hemolytic biovariant group 1 was significantly greater in herds A (5/5 goats) and H (4/4), compared with all other herds; biovariant group-1 organisms were not detected in any other herd. Similarly, some β-hemolytic P. haemolytica organisms were isolated from a significantly greater proportion of goats in herds B (biovariant group 3), E and G (biovariant group U3), and D and I (biovariant group Uβ) than in other herds (Table 1). The proportion of biovariants isolated from which we isolated β-hemolytic P. trelahosi biovariants did not differ significantly among herds.

Sixteen biovariant groups were isolated from the 7 goats in herd G, which was significantly greater than the number of biovariant groups isolated from goats in herds D, E, F, and H. Goats in herd F carried P. trelahosi exclusively. Neither the proportion of goats from which we isolated each biovariant group nor the number of different biovariant groups isolated varied significantly among specimens obtained on days 0, 21, and 35.

Organisms belonging to nonhemolytic biovariant group 2 were the only organisms isolated from at least 1 goat in each herd, but the proportion of goats from which this biovariant group was isolated did not significantly differ among herds. The proportion of goats from which we isolated organisms belonging to biovariant group 2 was significantly higher than that for all other biovariant groups. However, the next 6 most prevalent biovariant groups (Uα1, U03, U0β, and Uα2) comprised β-hemolytic strains. Overall, organisms belonging to 30 nonhemolytic and 14 β-hemolytic biovariant groups were isolated.

Serologic results—Serum agglutinating antibody titers varied from 0 to 4 on day 0; 2 goats in herd I had titers of 4 prior to vaccination. Although additional serum samples were not obtained from these goats or those in herds D, E, F, or H, titers in the majority of 17 goats in herds A, B, C, and G tested on days 0, 21, and 35 remained stable over time. A 4-fold increase in titer was detected from day 0 to day 35 in 1 goat from each of herds B and G. Agglutinating antibody titers did not significantly differ among herds on any test day.

Serum leukotoxin-neutralizing antibody titers ranged from 5 to 11 on day 0. Although no significant differences were detected in leukotoxin-neutralizing antibody titers among herds, titers did increase 4-fold on days 21 and 35 in 3 of 4, 3 of 4, and 2 of 4 goats in herds A, C, and G, respectively, compared with day-0 values. Mean leukotoxin-neutralizing titer was significantly higher in herd A, compared with herd G, on days 21 and 33.

Discussion

The diversity of the Pasteurellaceae family, which includes the Actinobacillus, Haemophilus, and Pasteurella genera, has been evaluated extensively and discussed in numerous reports.30-34 Characterization of isolates by use of biochemical utilization tests proved to be a useful means of differentiating and categorizing organisms isolated from goats in the present study. All but 1 isolate had basic characteristics typical of P. haemolytica or P. trelahosi.35 Pasteurella isolates that were catalase-negative and fermented trehalose were, therefore, identified as P. trelahosi and assigned to multiple biovariant groups. Similarly, trehalose-negative isolates were assigned to multiple biovariant groups of P. haemolytica.

Although β-hemolysis is 1 of the major characteristics by which organisms assigned to the P. haemolytica-complex were originally differentiated from other Pasteurella species,18 both β- and nonhemolytic strains have been recognized.12,13 Most reports regarding nonhemolytic isolates describe organisms isolated from wild ruminants. The majority (30/43) of P. haemolytica and trelahosi biovariant groups isolated from goats in this study were nonhemolytic and, in that regard, resembled those commonly isolated from wild ruminants. However, β-hemolytic strains of P. haemolytica or trelahosi were isolated from goats in all but 1 herd. In a previous study,36 β-hemolytic activity of Pasteurella spp evaluated on sheep blood agar was highly correlated with the presence of the structural lltA gene, which
is essential for production of leukotoxin. Because results of in vitro tests indicate that neutrophils from bighorn sheep are more sensitive to leukotoxin than those from domestic sheep, the β-hemolytic strains that we isolated from goats in the present study are of greatest concern as potential pathogens for bighorn sheep.

Most standard \( P\) \( haemolytica \) and \( P\) \( t\) \( r\) \( e\) \( h\) \( a\) \( l\) \( o\) \( s\) serotypes belong to β-hemolytic biovariant groups 1 and 2, many of which have been associated with disease. Therefore, the β-hemolytic isolates belonging to biovariant group 1 cultured from all goats in herds A and H and β-hemolytic biovariant group 2 from goats in 5 herds are of greatest concern as potential pathogens. Most Pasteurella isolates reported to be associated with disease in ruminants are serotypeable, with those from goats identified by others as serotypes A2, A5, A6, A7, A8, and A11.6-8,38 Although none of the serotypes belong to biovariant group 1 cultured from all goats in the present study agglutinated in standard typing sera, 12 isolates identified in serotype A8 (n = 3), A11 (6), or A16 (3). The majority (63/69) of biovariant group-2 isolates agglutinated in standard typing sera. Many of the additional Pasteurella organisms isolated from these goats were identified in biovariant groups shared with other domestic livestock, wild ruminants, or both.39

Although serum antibodies specific for \( P\) \( h\) \( a\) \( e\) \( m\) \( o\) \( l\) \( y\) \( t\) \( i\) \( c\) a A1 cells or leukotoxin were detected in the majority (15/17) of goats following vaccination with a commercial \( P\) \( h\) \( a\) \( m\) \( o\) \( l\) \( y\) \( t\) \( i\) \( c\) a bacterin, the greatest increase in titer over time was detected for leukotoxin-specific antibodies. In another study in which goats were vaccinated with either \( P\) \( h\) \( a\) \( e\) \( m\) \( o\) \( l\) \( y\) \( t\) \( i\) \( c\) a serotypes A1, A2, or A6, goats had low serum antibody titers against all serotypes prior to vaccination, and titers did not change significantly until after transthoracic injection of \( P\) \( h\) \( a\) \( e\) \( m\) \( o\) \( l\) \( y\) \( t\) \( i\) \( c\) a into the lungs. Goats immunized with A1 were protected against challenge with serotypes A1, A2, and A6. However, vaccination with serotype A2 or A6 did not protect goats from challenge with the A1 serotype.

Vaccination of calves with a \( P\) \( h\) \( a\) \( m\) \( o\) \( l\) \( y\) \( t\) \( i\) \( c\) a serotype A1 bacterin results in reduction of nasal and tonsillar colonization with the homologous serotype.6 The goats in the present study were also vaccinated with a \( P\) \( h\) \( a\) \( m\) \( o\) \( l\) \( y\) \( t\) \( i\) \( c\) a serotype A1 bacterin. However, because serotype A1 organisms were not isolated from any goat either before or after vaccination, it was impossible to determine whether vaccination had any effect in these goats. Vaccination did not appear to alter the isolation rate of any biovariant detected in this study, suggesting that the vaccine strain was not closely related antigenically to organisms carried by these goats.

Although \( P\) \( h\) \( a\) \( m\) \( o\) \( l\) \( y\) \( t\) \( i\) \( c\) a serotypes 1 and 2 belonging to biovariant group 1 appear to have the greatest potential for causing disease in bighorn sheep and other wild ruminants, these serotypes were not isolated from goats evaluated in this study. However, this does not eliminate concerns regarding potential transmission of strains that may cause disease in wildlife. In evaluation of data collected in our laboratory relative to animal host and disease associations for more than 4,000 Pasteurella isolates, no other \( P\) \( h\) \( a\) \( m\) \( o\) \( l\) \( y\) \( t\) \( i\) \( c\) a biovariant group isolated from goats in this study have been associated with disease. However, β-hemolytic \( P\) \( t\) \( r\) \( e\) \( h\) \( a\) \( l\) \( o\) \( s\) strains such as those isolated from goats in 5 of the 9 herds have been incriminated as the cause of disease in both domestic and bighorn sheep.3,24 Pasteurella spp may be transmitted when animals are in close proximity, particularly by nose-to-nose contact. Therefore, when domestic pack goats are in wildlife habitats, they should be managed to prevent contact with wild ruminants. Wildlife managers should also be concerned about the potential for transmitting pathogens when previously separated populations of wild ruminants are intermingled as a result of translocation to augment populations or control depredation.

References


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