Pathogenesis of *Streptococcus zooepidemicus* infection after intratracheal inoculation in llamas

Christopher K. Cebra, VMD, MS; Jerry R. Heidel, DVM, PhD; Margaret L. Cebra, VMD, MS; Susan J. Tornquist, DVM, PhD; Bradford B. Smith, DVM, PhD

**Objectives**—To test whether generalized *Streptococcus zooepidemicus* infection could be induced by intratracheal inoculation in llamas and to characterize this infection.

**Animals**—6 test and 3 control llamas.

**Procedure**—Test llamas received 1 of 3 dosages of *S. zooepidemicus* by intratracheal injection, whereas control llamas received sterile culture medium. Physical examination variables and results of clinico-pathologic analyses of blood, peritoneal fluid, and tracheal wash fluid were compared in test llamas between, before, and during the development of bacteremia and with control llamas. Bacteriologic culture was performed on all collected body fluids and tissue specimens that were collected at necropsy. Tissue specimens that were collected at necropsy were examined histologically.

**Results**—Infection induced fever, anorexia, and signs of depression. Five of 6 infected llamas developed specific signs of inflammation in the thorax or abdomen, bacteremia, neutrophilic leukocytosis with toxic changes and high band neutrophil cell counts, hyperfibrinogenemia, and high peritoneal fluid WBC counts and protein concentrations. On development of bacteremia, llamas had significant decreases in serum iron (from 118 ± 25 to 6 ± 4 µg/ml) and increases in serum glucose (from 131 ± 5 to 253 ± 48 mg/dl) concentrations.

**Conclusions and Clinical Relevance**—*Streptococcus zooepidemicus* spreads rapidly to other body compartments after intratracheal inoculation in llamas. Fever, anorexia, and signs of depression are the most consistent clinical signs, although other signs are possible. Clinico-pathologic analysis of body fluids yields evidence of inflammation. Infection by *S. zooepidemicus* can be proven by bacteriologic culture of body fluids before death or of tissue specimens after death. (Am J Vet Res 2000;61:1525–1529)

Little is known about bacterial infections in camelids, although they are the most common cause of death and referral of llamas to secondary care centers in North America. There is a growing body of evidence, supported by increasing experience with llamas and alpacas, that *Streptococcus zooepidemicus* is an important primary pathogen of camelids in North America. This and other Lancefield group C streptococci have been isolated from blood, milk, uterine discharges, peritoneal exudates, and intrabdominal abscesses from camelids with evidence of sepsis, pneumonia, mastitis, metritis, or colic. There is additional evidence from abroad that these microorganisms are important pathogens of camelids worldwide, specifically as the cause of alpaca fever in South America and of pneumonia, mastitis, and peritonitis in camels. The prevalence of these infections has not been studied, but it is known that diseases of the types caused by streptococci account for more than 35% of New World camelid deaths in North America, and it is reasonable to presume that a portion of those deaths are the result of streptococcal infection.

We know little about the pathogenesis of streptococcal infections in camelids. Focal and generalized infections have been observed, with focal infections often found in internal sites such as the abdomen. In South America, where *S. zooepidemicus* is thought to be a commensal organism of the oral cavity, superficial disease is often attributed to wound infection, and systemic infection is presumed to develop secondary to wound infection or ingestion of the organism. In other species, streptococcal bacteremia commonly is thought to develop secondary to respiratory tract infection. In our experience, *S. zooepidemicus* is a rare contributor to oral infections and is not isolated from the distal portion of the respiratory tract of healthy camelids. This suggests that camelids in North America do not commonly harbor this organism and that infection through the respiratory tract could develop. To our knowledge, the possibility of bacteremia secondary to respiratory tract infection has not been investigated.

Our study was undertaken to test whether bacteremia and the generalized form of *S. zooepidemicus* infection could be induced following intratracheal inoculation and, in so doing, create a model of the disease. Because the model is based on a disease seen in clinical practice, we hoped to characterize the clinical signs, clinico-pathologic abnormalities, and lesions seen with streptococcal infection. Knowledge of the appearance of this disease would enable better recognition and thereby, timely treatment. Furthermore, a model that consistently caused easily measurable physical and clinical pathologic abnormalities would provide a valuable tool for further study on the pathogenesis of infection, treatment efficacy, and possible immunoprophylaxis.

**Materials and Methods**

**Animals**—Nine llamas (2 females and 7 sexually intact males) between 10 and 19 months of age weighing between
of the third gastric compartment to be used for sample collection. Tracheal wash fluid and peritoneal fluid were collected in clot tubes for bacteriologic culture, and blood was collected, for biochemical analysis. In addition, 20 ml of blood was collected using sterile techniques, and divided between 2 vials, each containing 100 ml of Columbia broth for bacteriologic culture. All 3 fluids were collected in tubes coated with potassium EDTA for cytologic analysis.

Statistical analysis—To test for effects of collecting samples, data from control llamas were tested for significant changes over time, using a 1-way ANOVA. To test for effects of infection, preinfection data from test llamas were compared with data from the first day of detectable bacteremia, using paired t-tests, and data from test llamas on the first day of detectable bacteremia were compared with data from control llamas (on the fourth day after sham infection, chosen because all samples were collected that day), using unpaired t-tests. Choosing the first detectable day of bacteremia rather than a specific day after inoculation was done to compensate for the different rates of development of disease with the different bacterial inocula. Data from the test llama that did not develop bacteremia were excluded from all calculations. For all calculations, significance was determined when values were P < 0.05.

Results

Clinical signs—Control llamas did not have any signs of disease throughout the trial. Four of the 6 test llamas developed progressive disease characterized by all or some of the following clinical signs: fever (39.2 to 40.7 °C), anorexia, depression, weakness, recency, tachycardia, tachypnea, dyspnea, cough, nasal discharge, abnormal respiratory sounds, including crackles and pleural friction rubs, hunched posture, gastric atony, tenesmus, colic, and diarrhea. Respiratory signs were subtle in most llamas and detectable only briefly. Weakness, recency, dyspnea, pleural friction rubs, hunched posture, and colic were grounds for immediate euthanasia. Fever and signs of depression began 8 hours after inoculation in 2 group-1 llamas and progressed until euthanasia was performed 48 hours after infection. Two llamas in group 2 developed fever 24 hours after infection that persisted until euthanasia on day 5. Whereas 1 of these llamas also developed progressive disease, the other llama had a lower fever (< 39.4 °C) throughout and appeared to be recovering despite a single episode of high fever (40.7 °C) and diarrhea prior to euthanasia. Rectal temperatures of llamas with bacteremia were significantly higher than preinfection temperatures (P = 0.001) or rectal temperatures of control llamas (P = 0.002). Clinical signs of disease were first observed 24 hours after infection in 1 llama in group 3 and progressed slowly until euthanasia on day 7. The other test llama in group 3 had mild abnormal pulmonary sounds on day 2 and fever on day 7 (coincident with the only positive culture result from that llama) but, otherwise, appeared completely unaffected. Because of this llama's apparent rapid clearance of the infection, data from the llama were not included in calculations for statistical comparisons.

Clinical pathologic findings—There were no significant changes over time in the clinical pathologic data from the control llamas. Nonsignificant hematologic changes that were observed in test llamas at the
Table 1—Mean ± SD for selected clinicopathologic data from llamas before and after intratracheal inoculation with Streptococcus zooepidemicus and from control llamas

<table>
<thead>
<tr>
<th>Variables</th>
<th>Test llamas (n = 5)</th>
<th>Control llamas (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preinfection</td>
<td>Bacteremia</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (× 10^3 cells/µL)</td>
<td>18.6 ± 3.5</td>
<td>24.7 ± 11.7</td>
</tr>
<tr>
<td>Band neutrophils (× 10^3 cells/µL)</td>
<td>0 ± 0</td>
<td>6.6 ± 6.7</td>
</tr>
<tr>
<td>Lymphocytes (× 10^3 cells/µL)</td>
<td>5.3 ± 1.0</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>Total plasma protein (g/dL)</td>
<td>5.8 ± 0.2</td>
<td>6.2 ± 0.3*</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>200 ± 140</td>
<td>560 ± 320*</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>131 ± 5</td>
<td>253 ± 481†</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.2 ± 0.2</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>151 ± 5</td>
<td>147 ± 5</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>5.0 ± 0.7</td>
<td>4.0 ± 0.6*</td>
</tr>
<tr>
<td>Calcium (g/dL)</td>
<td>9.5 ± 0.2</td>
<td>8.7 ± 0.31</td>
</tr>
<tr>
<td>Total CO₂ (mEq/L)</td>
<td>26.6 ± 3.0</td>
<td>23.5 ± 1.73</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>114 ± 26</td>
<td>6 ± 51</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td></td>
<td></td>
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<tr>
<td>Protein (g/dL)</td>
<td>3.1 ± 0.7</td>
<td>5.4 ± 0.8‡</td>
</tr>
<tr>
<td>WBC (× 10^3 cells/µL)</td>
<td>3,125 ± 2,229</td>
<td>39,631 ± 60,454</td>
</tr>
</tbody>
</table>

*Significantly (P < 0.05) different from before infection value. †Significantly (P < 0.01) different from before infection value. ‡Significantly (P < 0.05) different from control value. §Significantly (P < 0.01) different from control value. n/N = 4 for the test llamas.

serum total carbon dioxide concentrations were lower than control llamas (P = 0.035).

Tracheal wash usually yielded little fluid and did not reveal evidence of pulmonary inflammation. Peritoneal fluid analysis yielded more evidence of inflammation. All 5 test llamas that developed bacteremia had increases in peritoneal fluid protein concentration from the first sample to the last. Final protein concentration was > 5 g/dl in 3 test llamas (and no control llamas), all of which had final values more than double their initial values. Llamas with bacteremia had a significant increase in peritoneal fluid protein concentration (P = 0.027). Peritoneal fluid cell count also increased but not significantly (Table 1). Test llamas with detectable bacteremia also had significantly higher peritoneal fluid protein concentrations than control llamas (P = 0.008). The 2 llamas in the first group had counts > 5000 cells/µl, and the other 3 llamas had counts > 10,000 cells/µl. However, 3 llamas (2 test llamas and 1 control) had initial peritoneal fluid cell counts between 3,300 and 5,900 cells/µl. Cell counts were higher in llamas with clinical evidence of abdominal pain and gross evidence of peritonitis at necropsy. Intracellular and extracellular bacterial cocci were visible in all 4 peritoneal fluid samples with positive bacteriologic culture results.

Bacterial Isolation—Streptococcus zooepidemicus was not isolated from any of the control llamas or from any of the preinfection samples from the test llamas. Tracheal wash samples had positive bacteriologic culture results for S. zooepidemicus at all time points for 5 of the 6 llamas and at 1 time point for the sixth. Four llamas had evidence of diffuse infection on or near the day of euthanasia, as the organism was isolated from all tissue and fluid samples that were tested. Llamas with positive tissue specimen bacteriologic culture results included 2 in the group with the largest inoculum and 1 each from the other 2 groups. The other 2 llamas appeared to have cleared the infection, except from the inoculation site. Escherichia coli also was isolated from...
several of the body fluids and tissue specimens collected at necropsy from 2 llamas given the largest inoculum. *Streptococcus zooepidemicus* bacteremia was first detected on day 1 in the first group, day 4 in the second group, and day 7 in the third group for llamas with positive tissue specimen bacteriologic culture results; those llamas were euthanatized within 24 hours of detection of bacteremia. Of the 2 llamas with negative tissue specimen bacteriologic culture results, 1 had transient bacteremia (low numbers of *S. zooepidemicus* on day 1 only), and the other never had evidence of disseminated infection.

**Necropsy lesions**—Important gross or histologic abnormalities were not identified in any of the control llamas. The 2 test llamas from which *S. zooepidemicus* could not be isolated after death also had no lesions. Other test llamas had peritonitis, pleuritis, or both, with fibrinous exudates and multiple adhesions between serosal surfaces of viscera and body wall surfaces. Histologic lesions consisted predominately of fibrin and neutrophil accumulations on the serosal surfaces of thoracic and abdominal viscera. One llama had pulmonary parenchymal lesions characterized by thickening of interlobular septa caused by fibrin, neutrophil infiltration, and edema. Gastric ulcers were not found in any of the llamas.

**Discussion**

The ease with which streptococcal bacteremia was induced in these llamas after experimental inoculation suggests that naturally occurring infections in camelids may only require small inocula. The largest inoculum in this trial was a fifth that used to induce mild chronic disease in foals,\(^7\) yet caused severe peracute disease in llamas. The lower doses, with as few as 40,000 colony-forming units of bacteria (1 µl of media), also were capable of causing bacteremia and severe disease, although more slowly and less reliably. Given this evidence of susceptibility, limiting exposure of camelids to sources of *Streptococcus zooepidemicus* would appear prudent.

Why these llamas were so sensitive to *Streptococcus* spp. in comparison to other species is not fully understood. A possible explanation is that llama and alpaca mucosal surfaces are more permeable to bacteria than in more vascular organs.\(^1\) Camelids are more likely to develop fibrinous exudates than cattle,\(^1\) and it is possible that the stress and debilitation caused by streptococcal bacteremia may be exacerbated by the immune response, which is weaker in these large animals. Therefore, the visceral pleura receives its arterial blood from the bronchial artery.\(^\text{16}\) Once in the pleura or peritoneal space, bacteria tend to replicate rapidly, because the immune response is weaker there than in more vascular organs.\(^\text{16}\)

With a less extensive omentum than cattle, camels tend to develop fibrinous exudates in response to abdominal infections. The organism is able to survive within the exudate as a facultative anaerobe and may even have greater cytotoxic activity in the low oxygen tension environment.\(^\text{20}\) It is likely that all these factors are important in the pathogenesis of this infection. Because of the lack of parenchymal disease in these llamas, most clinical signs and clinical pathologic abnormalities were nonspecific and referable to the inflammatory response (CBC changes, fever, anorexia, depression, tachycardia, tachypnea, hunched posture, colic, tenesmus), not dysfunction of specific organs. Clinical signs of infection also varied among llamas, with a broad spectrum of possible appearances. Several other conditions, including heat stress, gastric ulceration, intestinal impaction, and other infectious diseases, can cause similar clinical signs and are more widely known than *S. zooepidemicus* infection.\(^\text{21}\) Without bacteriologic culture of blood, tissue, or body cavity fluids, streptococcal infection easily could be misdiagnosed as 1 of these other diseases. It is also possible that the stress and debilitation caused by streptococcal infection contributes to the development of these other diseases (as witnessed by the development of hyperthermia, evidence of stress, and gram-negative sepsis in several of the test llamas). In any case, veterinarians should consider streptococcal disease as a differential diagnosis in any camelid with fever, signs of depression, or other clinical signs referable to sepsis or infection of the pleural or peritoneal cavities.

This model provided information about llamas’ acute response to bacterial infection. Knowledge of this response may aid in differentiating this disease from other causes of fever, signs of depression, and colic. Hematologic changes included neutrophilic leukocytosis, high band cell counts, toxic changes in neu-
isms among individuals is known to occur in other species. Peritoneal fluid incubated with bacteria and protein concentrations rose consistently in llamas that developed clinical disease. Healthy llamas also had counts > 5,000 cells/µl, but protein concentrations > 5 gm/dl, cell counts > 10,000 cells/µl, and presence of bacteria all were specific to peritoneal fluid from infected llamas. Iron sequestration as the result of acute infection has not been described previously in llamas, but this and the other hematologic abnormalities were similar to those seen in other species with gram-positive bacterial infection. Hematologic and peritoneal fluid abnormalities also were similar to those seen in the few reports of this disease, except that llamas given the larger inocula developed severe disease before they developed hyperfibrinogenemia and high peritoneal fluid cell counts. Because similar inflammatory changes are likely to develop in camels exposed to other bacterial pathogens, this model may be useful for further research on camels’ inflammatory and immune responses.

We believe this model provides a plausible explanation for some animals with natural infection with *Streptococcus zooepidemicus*. The subtle and transient nature of respiratory signs may explain why this route of infection has not been considered in the past; it is remarkable that gastrointestinal signs were more prominent than respiratory signs, despite the route of infection. It was important to establish the respiratory route of infection for 2 reasons. First, it indicates that prophylactic measures should be directed toward decreasing damage and increasing immunity in the airway. Dust control, elimination of other respiratory pathogens, and vaccines that promote mucosal immunity are likely to be important. Second, it highlights that infected llamas may shed the organism in airborne particles or respiratory exudates. Aerosol and fomite transmission of streptococcal organisms among individuals is known to occur in other species, and the sudden occurrence of fever and a positive tracheal wash bacteriologic culture result in 1 llama housed with a sick llama may indicate that such transmission also occurs in infected llamas. Further study is necessary to determine the source of infection, likelihood of animal-to-animal or animal-to-man transmission, and prevalence of infection.

References


