Streptococcus zooepidemicus Infection in Sheep

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ABSTRACT

Fibrinous pericarditis, fibrinous pleuritis and pneumonia associated with Streptococcus zooepidemicus were observed in two lambs in a small flock of sheep. These lesions were reproduced in lambs inoculated intratracheally with Streptococcus zooepidemicus. Clinical signs included pyrexia, serous to mucopurulent nasal discharge, dyspnea and depression followed by death in six to seven days. Histologically the tissue changes were characterized by an acute inflammatory response involving bronchioles and alveoli, fibrinous pleuritis and fibrinous pericarditis.

INTRODUCTION

Streptococci have been isolated from the lungs of sheep (6, 8) but few reports have included a description of the biochemical or serological characteristics of the species isolated. Rafyi and Mir Chamsy (12) reported an outbreak of a septicemic condition in sheep in Iran caused by Streptococcus zooepidemicus and were able to reproduce disease in sheep. Cultural, biochemical and serological characteristics of the organism along with macroscopic lesions were given but histopathological observations were not reported. This is the first report in North America of lambs naturally and experimentally infected with Streptococcus zooepidemicus.

MATERIALS AND METHODS

NATURAL DISEASE

This laboratory maintains a flock of 42 sheep of mixed breeds for experimental purposes. During the summer the sheep are maintained on pasture and have access to shelter. In August 1971, two lambs (1 and 2) were depressed and dyspneic and were examined as described below.

BACTERIOLOGY

For routine isolation attempts, 10% sheep blood agar and MacConkey's agar were used. Other media used are given in the results and were selected according to criteria cited in Bergey's Manual of Determinative Bacteriology (1).
Streptococcus zooepidemicus isolated from the pleural cavity of lamb 1 was tested for its pathogenicity. Six 10.0 ml volumes of nutrient broth were inoculated with single colonies of the organism and incubated for 18 hrs at 37°C. Five tubes were pooled and thoroughly mixed. Plate counts of the pooled broth cultures were carried out according to the method of Miles and Misra (10). The number of organisms/ml was calculated to be 3.4 x 10⁶. This culture was used for experimental inoculation of lambs.

Tissues for bacteriological examination of experimental animals included nasal mucosa, pharynx, trachea, lung, bronchial and mediastinal lymph nodes, pleural cavity and spleen.

**Experimental Disease**

**Experiment A** — A lamb (3AT) and a ewe (4AP) were used in a preliminary trial to test the pathogenicity of the organism isolated from lamb 1. The lamb was inoculated intratracheally (T) with 5.0 ml of a nutrient broth suspension of *Streptococcus zooepidemicus* and 2.0 ml of the suspension was injected into the left pleural cavity (P) of the ewe (Table I).

**Experiment B** — Ten lambs, approximately ten months of age were removed from the flock and housed in a separate building. Nasal swabs were taken from both nostrils of each lamb and examined for the presence of *Streptococcus zooepidemicus*. Daily clinical examination, rectal temperature and blood were taken starting six days prior to inoculation and for the duration of the experiment. Nasal swabs were obtained prior to inoculation and daily until death or slaughter.

One control (c) lamb was inoculated intranasally (N) with 5.0 ml of nutrient broth and was killed on postinoculation day (pid) 4. A second control lamb was inoculated intratracheally with 5.0 ml of nutrient broth and killed on pid 8 (Table I). Both lambs were housed in a building removed from the subject lambs.

Four lambs were inoculated intranasally (N) with 5.0 ml of an overnight broth culture of *Streptococcus zooepidemicus* and one lamb was killed on pid 2, 4, 6 and 8. The remaining four received a similar inoculum intratracheally (T) and were necropsied on the same days unless death intervened (Table I).

All lambs were stunned with a captive bolt pistol and exsanguinated.

**Histopathology**

Tissues for histopathological examination were fixed in 10% buffered neutral formalin and/or formol sublimate. Paraffin sections were cut at five microns and routinely stained with hematoxylin and eosin. Other stains used were Pollak's trichrome (11), van Giesen and Mallory (5).

**TABLE I. Route of Inoculation of Streptococcus zooepidemicus in Sheep**

<table>
<thead>
<tr>
<th>Sheep Number</th>
<th>Inoculum</th>
<th>Route of Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Experiment A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 AT</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intratracheal</td>
</tr>
<tr>
<td>4 AP</td>
<td>2 ml <em>Streptococcus zooepidemicus</em></td>
<td>Left Pleural Cavity</td>
</tr>
<tr>
<td>5 CN</td>
<td>5 ml Nutrient Broth</td>
<td>Intrasanal</td>
</tr>
<tr>
<td>6 CT</td>
<td>5 ml Nutrient Broth</td>
<td>Intratracheal</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 BN</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intrasanal</td>
</tr>
<tr>
<td>8 BN</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intrasanal</td>
</tr>
<tr>
<td>9 BN</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intrasanal</td>
</tr>
<tr>
<td>10 BN</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intrasanal</td>
</tr>
<tr>
<td>11 BT</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intratracheal</td>
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<tr>
<td>12 BT</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intratracheal</td>
</tr>
<tr>
<td>13 BT</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intratracheal</td>
</tr>
<tr>
<td>14 BT</td>
<td>5 ml <em>Streptococcus zooepidemicus</em></td>
<td>Intratracheal</td>
</tr>
</tbody>
</table>

A — Experiment A
T — Intratracheal
P — Intrapleural
N — Intrasanal
B — Experiment B
RESULTS

CLINICAL EXAMINATION

Depression and dyspnea were the only clinical signs observed in the two naturally infected lambs. In experiment A pyrexia, depression and anorexia were observed in both animals. The ewe was found dead on the morning of pid 4 and the lamb was killed on pid 5.

No abnormalities were observed in the control lambs and the maximum rectal temperature recorded was 103.5°F. Total and differential white blood cell (WBC) counts were within the normal range (4). Clinical signs were not observed in lambs inoculated intranasally with Streptococcus zooepidemicus. A maximum rectal temperature of 105°F was recorded in lamb 8BN on pid 2 and lamb 10BN on pid 3. There was a coincident increase in the total WBC count and a “shift to the left” (4). The mean temperatures are shown in Fig. 1. Pyrexia was not observed in lambs 7BN and 9BN.

Immediately following intratracheal inoculation there was considerable coughing which was followed in two days by serious nasal exudation. By pid 3 the nose and muzzle area was covered by a crusty mucopurulent exudate which remained until the animals died. Lambs 13BT and 14BT were depressed on pid 5 and lamb 13BT was found dead on the morning of pid 6. Lamb 14BT was recumbent and exhibited grinding of the teeth, salivation and dyspnea the day before it died. Rectal temperatures of 106.6°F or greater were recorded at least once in each of the lambs and temperatures above 104.5°F were recorded throughout the observation period. The mean temperature response was always higher for lambs inoculated intratracheally than intranasally. In lambs 13BT and 12BT there was a marked increase in the total WBC count and in all four there was a marked “shift to the left”. The results of the total WBC counts were similar when expressed as the absolute differential leukocyte count per mm³ of blood (4).

BACTERIOLOGY

An organism of the genus Streptococcus was isolated from the pleural cavity of lambs 1 and 2 on 10% sheep blood agar. All other isolation attempts from other sites including nasal cavities failed. The colonies were small, circular, convex, entire, translucent and exhibited a wide zone of beta hemolysis. The organism was gram positive and long chains were observed on dark field examination of serum broth cultures. A fine granular growth was formed in the bottom of tubes of nutrient and serum broths and the supernatant liquid was clear.

The addition of two percent equine serum was necessary to promote growth in differential media. Acid was produced from salicin, lactose and sorbital. Mannitol, inulin, raffinose, trehalose and glycerol were not fermented. Gelatine was not liquefied, indol was not formed and nitrate was not reduced. Litmus milk was acidified. Serologically, the organism belonged to Group C of Lancefield’s classification. It was concluded that the organism under study was Streptococcus zooepidemicus.

Tests with selected antibiotic sensitivity discs revealed the organism to be sensitive to chloramphenicol, erythromycin, penicillin and ampicillin.

A nasal swab of animal 4AP at the time of death revealed Streptococcus zooepidemicus and a similar swab from lamb 3AT was negative.

The remainder of Streptococcus zooepidemicus isolates were made only from experimental animals inoculated intra-

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tracheally. Culture attempts were negative for pharynx and trachea of lambs 11BT and 14BT, negative for spleen of 12BT and negative for nasal swabs from lamb 11BT. Nasal swabs were positive for lambs 12BT, 13BT and 14BT for days 3 and 4, days 2 to 6 and days 6 and 7 respectively. All other isolation attempts for intratracheally inoculated lambs were positive (Table II).

**GROSS PATHOLOGY**

Macroscopic lesions in lambs 1 and 2 were confined to the thoracic cavity and consisted in both lambs of bilateral fibrinous pleuritis, fibrinous pericarditis, focal areas of consolidation in all lobes of the lung and enlargement of the bronchial and mediastinal lymph nodes. Similar lesions were observed in the ewe (4AP) and the lamb (3AT) (Fig. 2). No significant macroscopic lesions were observed in the control or intranasally inoculated animals. In all of the intratracheally inoculated lambs the trachea was inflamed around the inoculation site. In the lamb killed on pid 2 (11BT) there were focal linear areas of collapse in both apical and cardiac lobes of the lung. The bronchial and mediastinal lymph nodes appeared normal, as did the remaining organs. In the lamb killed on pid 4 (12BT) there were acute fibrinous pleuritis and acute fibrinous pericarditis with adhesions between the pleura, pericardium and diaphragm and of the lobes of the lung. Areas of consolidation were present mainly in the right apical and cardiac lobes and in the middle of the left diaphragmatic lobe. The abdominal cavity was normal.

More advanced lesions of fibrinous pleuritis and pericarditis with adhesions were observed in the lambs found dead on pid 6 (13BT) and pid 7 (14BT) (Fig. 3). The anterior ventral half of the lung was consolidated and strands of fibrin were present on the diaphragmatic surface of the liver.

**HISTOPATHOLOGY**

The salient features in the two naturally infected lambs were fibrinous pleuritis and acute bronchiolitis. Most alveoli contained a serous exudate with neutrophils and a few macrophages (Fig. 4) whereas other alveoli, particularly beneath the inflamed pleura, contained mostly macrophages and there was hypertrophy and hyperplasia of the alveolar lining cells. The peribronchial, perivascular and subpleural lymphatics were distended. Except for the pre-

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**TABLE II. Recovery of Bacteria from Tissues of Lambs Inoculated with *Streptococcus zooepidemicus* in Experiment II**

<table>
<thead>
<tr>
<th>Sheep Number</th>
<th>Days Post Inoculation</th>
<th>Nasal Swab Cultures (PID)</th>
<th>Pharynx</th>
<th>Trachea</th>
<th>Pleural Cavity</th>
<th>Lung</th>
<th>LBLN</th>
<th>MLN</th>
<th>Spleen</th>
</tr>
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<tbody>
<tr>
<td>5 CN</td>
<td>K 4</td>
<td>-</td>
<td>NE</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6 CT</td>
<td>K 8</td>
<td>-</td>
<td>NE</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>7 BN</td>
<td>K 2</td>
<td>-</td>
<td>NE</td>
<td>-</td>
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<tr>
<td>8 BN</td>
<td>K 4</td>
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<td>NE</td>
<td>-</td>
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</tr>
<tr>
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</tr>
<tr>
<td>10 BN</td>
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</tr>
<tr>
<td>13 BT</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14 BT</td>
<td>D 7</td>
<td>+ 6-7</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

C = Control  
N = Intranasal  
T = Intratracheal  
NE = Not Examined  
K = Killed  
D = Died  
LBLN = Left Bronchial Lymph Node  
MLN = Mediastinal Lymph Node  
PID = Postinoculation Day  
B = Experiment B
contained increased numbers of neutrophils and histiocytes. The bronchiolar mucosa was intact and had not been infiltrated by neutrophils. By pid 8 (10BN) the number of affected alveoli had increased and bronchioles within these areas were inflamed and were usually filled with a purulent exudate (Fig. 5). The bronchi and trachea were normal in all intranasally inoculated lambs. Increased numbers of neutrophils were observed in the cortex and sinuses of the bronchial and mediastinal lymph nodes.

In the intratracheally inoculated lambs lesions were present by pid 2 (11BT) and were confined to the respiratory tract and associated lymph nodes. The mucosa and submucosa of the trachea of all intratracheally inoculated lambs were inflamed and there was an acute cellulitis surrounding the injection site. Neutrophils were fewer in the mucosa distal to the injection site. The tracheal lumen contained purulent exudate. The bronchi and bronchioles were inflamed and usually filled with a purulent exudate. Many alveoli were collapsed and within these areas there was an increased number of neutrophils. Other alveoli contained a serous exudate mixed with neutrophils and macrophages. The subpleural,

Fig. 2. Fibrinous pleuritis and pericarditis and focal consolidation of the lung in lamb 3AT killed five days after intratracheal inoculation of Streptococcus zooepidemicus.

Fig. 3. Fibrinous pericarditis and pleuritis in a lamb six days after being inoculated intratracheally with Streptococcus zooepidemicus.

Fig. 4. Serous exudation and neutrophil infiltration of alveoli, purulent exudate in bronchioles and distention of the subpleural and perivascular lymphatics in the lung of lamb 2. H & E. X30.
perivascular and peribronchial lymphatics were very prominent due to the presence of large numbers of neutrophils. The bronchial and mediastinal lymph nodes were acutely inflamed.

The lesions in the trachea and lymph nodes in lamb 12BT examined on pid 4 were similar to those of lamb 11BT but the pulmonary changes were more widespread and more acute. The pleura was inflamed and covered with a thick layer of fibrin. Lymphatic vessels, particularly the pleural lymphatics, were distended with neutrophils, debris and cocci. The interlobular septa were thickened due to the presence of a serofibrinous exudate and numerous neutrophils. Surrounding inflamed bronchioles, the alveoli contained a serous or serofibrinous exudate and varying numbers of neutrophils whereas more peripherally placed alveoli contained a serous exudate and increased numbers of attached and free alveolar macrophages. Spread of the infection appeared to be via the lymphatics and bronchioles.

The distribution of lung lesions in lambs 13BT and 14BT examined on pid 6 and 7 respectively was similar to that seen on pid 4. Larger areas of lung were consolidated and bacterial microcolonies were more numerous in the lymphatics and in alveoli (Fig. 6). The bronchial and mediastinal lymph nodes were inflamed and numerous bacteria were observed. There was sloughing of the tracheal mucosa and numerous bacteria were present in the hemorrhagic submucosa.

Lesions in the heart of lamb 13BT were confined to the pericardial sac, the epicardium and to the adjacent myocardium. The lesions consisted of neutrophil infiltration of the myocardium, a zone of bacteria and neutrophils and, peripheral to this, a zone of fibrin of varying thickness involving the epicardium and pericardium.

**DISCUSSION**

The organism recovered from the initial two naturally infected lambs was identified on its physical and biochemical characteristics as *Streptococcus zooepidemicus* and serologically classified as a member of Lancefield's Group C. Our results were essentially similar to those reported by Rafyi...
and Mir Chamsy (12) who isolated *Streptococcus zooepidemicus* from the heart blood of sheep in Iran. In one of their outbreaks the mortality rate was as high as 90% in lambs and 60% in adult sheep. In the present investigation the organism was demonstrated in only two of 23 lambs and only these two were clinically ill. None of 17 ewes and two rams were affected. Since no additions had been made to the flock it was suspected that the infection was either inherent to the flock or that infection was acquired from contact with other animals.

The flock was in contact with seven ponies which were exercised in the same pasture. Since *Streptococcus zooepidemicus* is a common inhabitant of the genital tract of mares (9) and it was not uncommon to find the lambs pawing through the horse manure it is conceivable that this was the source of the organism. Genital and rectal swabs from the ponies were not examined at that time but subsequent examinations revealed the presence of *Streptococcus zooepidemicus* in one of three genital swabs and all seven rectal swabs from the ponies.

In the experimental disease clinical signs were minimal or absent in lambs inoculated intranasally, whereas in lambs inoculated intratracheally there was pyrexia, serous to mucopurulent nasal discharge, anorexia and depression followed by death in four to six days. Rafyi and Mir Chamsy (12) reported clinical signs in experimentally inoculated sheep which included a local inflammatory response, pyrexia, anorexia and death in from five to ten days. In the present investigation clinical signs were not specific since they could be confused with those described for sheep experimentally infected with *Pasteurella hemolytica* (3, 13).

The macroscopic lesions could be mistaken for those associated with *P. hemolytica* infection but histologically the lesions differed. In the present investigation the predominant changes consisted of serous or serofibrinous exudation, increased numbers of neutrophils and cocci. In pneumonia associated with *P. hemolytica* i.e. so-called enzootic pneumonia (14) the salient features are characteristic elongated cells within alveoli, extensive necrotic areas and increased numbers of alveolar macrophages. Gram-negative coccobacilli are usually numerous but neutrophils are not (14).

Working with *P. hemolytica* Smith (13) found that it was difficult to infect sheep using the intratracheal route but was successful using the intrabronchial route. The intrabronchial route was also used by Berstein *et al.* (2) who commented on the ability of the ovine lung to tolerate enormous numbers of bacteria. Although studies on the pulmonary clearance of bacteria have not been carried out in sheep it has been shown in calves that 92% of inhaled *P. hemolytica* and 95% of inhaled *Staphylococcus aureus* were cleared from the lungs in eight hours (7). The mild response seen in intranasally inoculated lambs suggests that perhaps some of the inoculum entered the lungs and was soon cleared by the pulmonary defence mechanism but probably the majority was swallowed. The results achieved with intratracheal inoculation were unexpected. Reasons for this success may be related to the virulence of the organism, the dose given or the resistance of the lambs. Studies using different doses were not done but it is conceivable that the dose given was sufficient to overwhelm the defence mechanism of the lung thus allowing the establishment of an active infection.

Reports of *Streptococcus zooepidemicus* in sheep are rare. Reasons for this are not clear. It may be that sheep are frequently infected with the organism but under most conditions recover with or without antibiotic treatment. Another possibility is that in most cases of fibrinous pleuritis, diagnosed on the basis of gross pathology, infection is assumed to be due to *P. hemolytica*. Even in those cases from which a beta hemolytic streptococcus is isolated it is possible that the organism is either not identified further or is assumed to be *Streptococcus pyogenes*. Perhaps this report will stimulate further investigation of the role *Streptococcus zooepidemicus* in infections in sheep.

**ACKNOWLEDGMENTS**

I wish to thank Dr. J. R. Long, Ontario Veterinary College, University of Guelph, Ontario for the serological identification of the organism. The excellent technical assistance of Miss Olive Wood is gratefully acknowledged.
REFERENCES


Book Review


Veterinary Anesthesia by Lumb and Jones covers a wide range of topics of interest to veterinary practitioners. What veterinarian has not on occasion had difficulty in maintaining a large cow in dorsal recumbency, or perhaps in anesthetizing a monkey or your client's pet snake. The authors have done an excellent job of covering the wide ranging anesthetic problems encountered in veterinary practice.

The pharmacology of the intravenous anesthetics, the gaseous anesthetics, the local anesthetics and the agents used for chemical restraint receives reasonably good coverage in this book. The clinical nature of the material and its orientation to the practical situation makes it most appropriate for the practitioner. All of us have had problems obtaining unbiased information on new drugs that are introduced into the veterinary market, i.e. ketamine and xylazine. The fact that this book is new makes it a handy reference in this situation although it too will become obsolete in time.

The techniques of nerve blocking, spinal anesthesia and intravenous anesthesia receive good coverage in this text. These procedures tend to be widely understood and well established in veterinary medicine and there is not a lot that the authors can add that is new. The coverage of inhalation anesthetics is excellent ad the many flow charts, graphs and pictures will help the reader in solving problems encountered in his practice. The information supplied in this section of the book may well be worth the price of the whole book.

Any study of anesthesia relies heavily on the normal functioning of the respiratory system. The coverage of the physiology of respiration is therefore a review of basic information that will be useful to even the most up-to-date veterinary. Euthanasia and anesthetic emergencies both receive creditable coverage in the book.

In summary, the information in the book is basically accurate and the recent publication makes the information current and useful to students and practitioners alike. The reviewer cannot judge whether or not this book is superior to other veterinary anesthesia books available but it certainly is useful and deserves careful inspection and consideration by all persons involved in veterinary practice. — W. D. Black.