Bacteriological study of caseous lymphadenitis in pre-scapular lymph nodes of sheep slaughtered in Urmia, northwestern Iran

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Summary

To identify the causative agent of caseous lymphadenitis in pre-scapular lymph nodes of sheep, the contents of 98 pre-scapular lymph nodes which showed the gross changes compatible with lymphadenitis were cultured. Bacteria isolated included Corynebacterium pseudotuberculosis (79.3%), Arcanobacterium pyogenes (9.4%), Staphylococcus aureus (7.5%) and Streptococcus alphahemolytica (3.8%). It was shown that Corynebacterium pseudotuberculosis is the main causative agent for lymphadenitis in northwestern Iran.

Key words: Caseous lymphadenitis, Pre-scapular lymph nodes, Sheep, Iran

Introduction

Caseous lymphadenitis (CLA) is a chronic infectious disease in sheep, which is presented as enlargement of superficial lymph nodes. The most important lymph nodes are pre-scapular, pre-femoral, inguinal, mediastinal and mesentric lymph nodes. The infection could spread to lung, liver, kidneys, spleen, brain and spinal cord. CLA usually will not be diagnosed clinically. Therefore, it has a high prevalence rate and the majority of cases are diagnosed during inspection in slaughterhouse. The disease was reported from Australia, North and South America, New Zealand, Kenya, Brazil, Iran and most European countries (Kuriaj and Ngatia, 1990; Ghanbparpou et al., 2002). Due to its importance in contamination of meat and meat products, CLA has a great economical importance (Howard and Smith, 1999). CLA also was reported in goats, deer, cattle, horses, camels and human beings (Goldberg et al., 1981).

The major causative agent of CLA is Corynebacterium pseudotuberculosis. Other bacterial agents which also have been isolated from CLA include Staphylococcus epidermidis, Corynebacterium equi, Streptococcus alphahemolytica, Moraxella spp., Arcanobacterium pyogenes, Staphylococcus aureus, Pasteurella spp., Pseudomonas aeruginosa, Escherichia coli, Micrococcus spp., Aeromonas and different species of Bacillus. It is to explain that the causative organisms have been isolated cases of CLA (Corynebacterium pseudotuberculosis) in sheep, which were affected by weak ewe syndrome.

Corynebacterium pseudotuberculosis is a Gram positive mycolic acid-containing facultative intracellular pathogen, which is phylogenetically related to Mycobacterium tuberculosis (Pascual et al., 1995).

The causative organism will enter through the damaged skin and mucous membrane and finally reach the regional lymph nodes and cause an inflammatory and necrotic changes (Simmons et al., 1998). CLA must be differentiated from actinobacillosis, tuberculosis, melioidosis and Morell disease.

CLA is a chronic disease characterized by the
formation of necrotic lesions that in sheep are typically located in superficial lymph nodes and the lungs (Batey, 1986). Transmission of the disease is thought to occur via contamination of shearing wounds with viable bacteria originating from the discharging lung abscesses of infected sheep (Ellis et al., 1987; Paton et al., 1995). In Australia, CLA is one of the most prevalent diseases of sheep and as a consequence, has an economic impact due to reduced wool production by infected animals and condemnation of carcasses and skins in abattoirs (Paton et al., 1994).

While the pathogenic process employed by *C. pseudotuberculosis* in causing CLA in sheep and goats is not well understood, at least two major virulence determinants have been identified. The toxic lipid in the cell wall mediates the bacterial resistance to phagocytic cells. The other identified virulence determinant is a sphingomyelin-degraded phospholipase D (PLD) exotoxin. PLD is thought to mediate dissemination of the pathogen within the host by increasing local vascular permeability (Batey, 1986).

This study was conducted to identify the major causative agent of CLA in our region. It could help us in controlling the disease (Radostitis et al., 2000).

### Materials and Methods

Ninety-eight pre-scapular lymph nodes of sheep with CLA collected from a slaughterhouse in Urmia, northwestern Iran. Swabs were taken from the contents of the nodes and transferred to the microbiology laboratory in tryptone soy broth culture. They were subjected to culture on blood agar (Merck) by streaking method. The cultures were then incubated at 37°C for 24–48 hrs. The colonies were differentiated based on their shape, size, color and the presence of hemolysis. Pure cultures were then made from these colonies. Using Gram stain and biochemical tests such as sugar fermentation and catalase, the isolated bacteria were finally identified (Carter and Cole, 1990; Quinn et al., 1994).

### Results

The isolated organisms include *Corynebacterium pseudotuberculosis* in 79.3% of samples, *Arcanobacterium pyogenes* in 9.4%, *Staphylococcus aureus* in 7.5% and *Streptococcus alphanemolytica* in 3.8% of samples (Table 1).

### Table 1: Relative frequency of bacterial isolates from infected lymph nodes.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of pure isolates</th>
<th>Percentage of pure isolates</th>
<th>Number of mixed isolates</th>
<th>Percentage of mixed isolates</th>
<th>Total Number of isolates</th>
<th>Total percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pseudotuberculosis</em> (%)</td>
<td>79</td>
<td>74.5</td>
<td>5</td>
<td>4.7</td>
<td>84</td>
<td>79.3</td>
</tr>
<tr>
<td><em>A. pyogenes</em> (%)</td>
<td>4</td>
<td>3.8</td>
<td>6</td>
<td>5.6</td>
<td>10</td>
<td>9.4</td>
</tr>
<tr>
<td><em>S. aureus</em> (%)</td>
<td>2</td>
<td>1.9</td>
<td>6</td>
<td>5.6</td>
<td>8</td>
<td>7.5</td>
</tr>
<tr>
<td><em>S. alphahemolytica</em> (%)</td>
<td>3</td>
<td>2.8</td>
<td>1</td>
<td>0.9</td>
<td>4</td>
<td>3.8</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>83.1</td>
<td>18</td>
<td>17.0</td>
<td>106</td>
<td>100</td>
</tr>
</tbody>
</table>
Discussion

In the present investigation the main isolated bacteria was *C. pseudotuberculosis* Which is the main causative organism for CLA In this study 84 cases of *C. pseudotuberculosis* were isolated. Unanian et al., (1985) reported that in 79 cases the isolated bacteria were in pure form and in 5 cases it was mixed by *A. pyogenes*, *S. aureus* and *S. alphahemolytica*. *A. pyogenes*, *S. epidermidis* and some *Streptococcus spp.* were isolated from the culture of infected lymph nodes. This organism may cause the infection either primarily or secondarily. *C. pseudotuberculosis* sometimes together with other bacteria such as *S. areus* or *A. pyogenes* is able to infect the lymph nodes experimentally.

*A. pyogenes* was isolated from 10 samples. Four out of these samples were pure and six were mixed with *S. aureus* and *C. pseudotuberculosis*. *S. aureus* was isolated from eight samples, two were pure and six were mixed with *A. pyogenes* or *C. pseudotuberculosis* (Kurij and Ngatia, 1990). Unanian et al., (1985) isolated *S. aureus* from infected lymph nodes. It is important to mention that *S. aureus* as another agent of the disease, is present on skin, pharynx, nasal mucosa, and oral cavity in healthy animals. Lesions like incision, wounds, immune suppression and primary viral infections were the most important causes for bacterial attack (Timony et al., 1992). Another isolated organism from four cases was *S. alphahemolytica*. Of these four cases, three were pure and one was mixed with *C. pseudotuberculosis*. In another study, *S. alphahemolytica* were isolated with *E. coli* from pre-ascalural lymph nodes (Hein and cargill, 1981). *Streptococcus spp.* was present on mucous membrane, animal digestive system and causes different lesions in hosts (Timony et al., 1992). Infected lymph nodes from the slaughterhouse could be a source of environmental contamination (Batey, 1986).

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References


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