Mycobacterium paratuberculosis is the etiologic agent of ruminant paratuberculosis, commonly referred to as Johne’s disease. The disease is characterized by a chronic granulomatous ileocolitis that ultimately terminates in diarrhea, weight loss, debilitation, and death. The disease is transmitted among ruminants predominately through feces when animals are at a young age. In the United States, the national weight loss, debilitation, and death. The disease is transmitted among ruminants predominately through feces when animals are at a young age. In the United States, the national disease prevalence has been suggested to be 2.6% of the dairy and 0.8% of the beef cattle, although regional surveys suggest local prevalences of 11-17%. Cattle infected with M. paratuberculosis shed organisms in feaces often at concentrations approaching 10^8 colony-forming units (cfu) per gram. The environment of an infected herd is therefore considered heavily contaminated. In addition to feces, other body fluids may also shed M. paratuberculosis, including milk. Some studies have suggested that as many as 35% of clinically infected cattle and 11.6% of asymptomatic carriers have detectable quantities of M. paratuberculosis in their milk.

In recent years, there has been an interest in the possible association of paratuberculosis and human Crohn’s disease, a chronic granulomatous ileocolitis of unknown etiology. The possible etiologic association between these 2 diseases has largely been prompted by the isolation of M. paratuberculosis from human patients with Crohn’s disease in the United States, Australia, The Netherlands, and France. Of the primary groups currently engaged in the culture of human tissues, M. paratuberculosis has been isolated from 20%, 33%, and 38% of patients with Crohn’s disease, but from only 0.8% (1 in 121) of controls. These human isolates have been shown to be of bovine origin and are indistinguishable from strains isolated from cattle.

Recently, a species-specific insertion sequence in M. paratuberculosis, IS900, was used in the polymerase chain reaction to examine tissues from human patients for the presence of this organism. IS900 was detected in 65% of tissues from patients with Crohn’s disease but in only 12.5% of controls and 4.3% of cases of ulcerative colitis. The finding of IS900 in 12.5% of controls indicates a previously unrecognized environmental distribution of this organism, suggesting that human contact with M. paratuberculosis may be common. The low incidence of M. paratuberculosis in ulcerative colitis patients suggests that colonization of the disrupted or abnormal intestinal mucosa by M. paratuberculosis does not occur. Similar findings were obtained in pediatric cases; IS900 was detected in 78% of Crohn’s disease patients and in <20% of controls. The high prevalence (65-78%) in Crohn’s disease patients suggests some etiologic role for this organism in humans as well as other animals species.

Because the patient populations examined in these studies had no known direct contact with cattle or M. paratuberculosis, we examined indirect avenues of human contact. As part of that examination, the thermal resistance of M. paratuberculosis under laboratory conditions simulating pasteurization was evaluated.

Two strains of M. paratuberculosis of bovine origin (strains 3737 and ATCC 19698) and 2 strains isolated from diseased human tissues (strains Linda and Ben) were grown in 7H9 broth containing Dubos’ oleic albumin complex and 0.05% Tween-80 as previously described. All strains (except 19698) were grown from limited-passage frozen seed lot cultures having <5 passages and maintaining all original characteristics. Cultures were lightly sonicated to disrupt clumps, and initial number of colony-forming units per milliliter was estimated spectrophotometrically as previously described. A wild-type strain of Mycobacterium bovis (Hawaiian strain) and M. bovis BCG (TMC 1011) were also used.

Fresh milk, obtained aseptically from a Holstein cow known to be free from paratuberculosis, was chilled on ice and maintained at 4 C until use. To insure accurate colony-forming unit determinations, M. paratuberculosis and M. bovis were suspended in whole milk at approximately 1 x 10^3, 1 x 10^4, and 1 x 10^5 organisms/ml, thereby insuring a minimum of 100 colonies per undiluted inoculated tube at a 0% kill rate. Organisms were suspended in a total volume of 10 ml raw milk. Prior to and after heat treatment, milk samples containing M. paratuberculosis were vigorously mixed and subjected to serial lo-fold dilutions in phosphate-buffered saline containing 0.05% Tween-80, and 100-µl samples were inoculated onto 5 slants of Herr-ald’s egg yolk medium containing 2 µg/ml mycobactin. Colony-forming units were determined by colony counts before and after heat treatment. Only dilution tubes containing 5-50 colonies were counted to determine colony-forming units per milliliter of milk because >50 colonies could not be reliably counted and there was too great a variability in dilution tubes containing less than 5 colonies. All strains were exposed to 3 separate heat treatments on the same day employing the same stocks of spiked milk. Results are reported as the mean ± SD of the 3 heat treatments, thereby representing the average colony count of 15 tubes, or 1.5 ml of the total milk sample.

Thermal resistance was performed according to guidelines described for commercial pasteurization as defined by the Public Health Service, US Food and Drug Administration. The standard holder method was simulated by heating milk in a water bath to 63 C (145 F) and maintaining that temperature for 30 minutes with continuous mixing. To simulate
Table 1. Effect of heat treatment (63 C for 30 min) on strains of *Mycobacterium paratuberculosis*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pre-heat treatment (cfu)*</th>
<th>Room temperature</th>
<th>Post-heat treatment</th>
<th>Ice</th>
<th>% sur. vival</th>
<th>% sur. vival</th>
<th>% sur. vival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cfu</td>
<td>% kill</td>
<td>cfu</td>
<td>% kill</td>
<td>cfu</td>
<td>% kill</td>
</tr>
<tr>
<td>3737</td>
<td>$2.0 \times 10^4 \pm 5.5 \times 10^4$</td>
<td>$1.0 \times 10^4 \pm 1.8 \times 10^4$</td>
<td>99.8</td>
<td>5.2</td>
<td>$1.5 \times 10^4 \pm 2.9 \times 10^4$</td>
<td>92.7</td>
<td>7.3</td>
</tr>
<tr>
<td>19698</td>
<td>$1.8 \times 10^4 \pm 1.7 \times 10^4$</td>
<td>$1.5 \times 10^4 \pm 1.1 \times 10^4$</td>
<td>91.9</td>
<td>8.1</td>
<td>$1.5 \times 10^4 \pm 1.2 \times 10^4$</td>
<td>91.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Linda</td>
<td>$1.5 \times 10^4 \pm 6.7 \times 10^4$</td>
<td>$4.7 \times 10^4 \pm 9.6 \times 10^4$</td>
<td>68.4</td>
<td>31.6</td>
<td>$5.9 \times 10^4 \pm 7.4 \times 10^4$</td>
<td>60.7</td>
<td>39.3</td>
</tr>
<tr>
<td>Ben</td>
<td>$1.1 \times 10^4 \pm 2.4 \times 10^4$</td>
<td>$2.4 \times 10^4 \pm 6.7 \times 10^4$</td>
<td>78.6</td>
<td>21.4</td>
<td>$3.0 \times 10^4 \pm 4.7 \times 10^4$</td>
<td>73.6</td>
<td>26.4</td>
</tr>
</tbody>
</table>

* Mean ± SD number of colony-forming units (cfu) of 3 separate heat treatment cycles.

Table 2. Effects of heat treatment (72 C for 15 sec) on strains of *Mycobacterium paratuberculosis*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pre-heat treatment (cfu)*</th>
<th>Room temperature</th>
<th>Post-heat treatment</th>
<th>Ice</th>
<th>% sur. vival</th>
<th>% sur. vival</th>
<th>% sur. vival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cfu</td>
<td>% kill</td>
<td>cfu</td>
<td>% kill</td>
<td>cfu</td>
<td>% kill</td>
</tr>
<tr>
<td>3737</td>
<td>$2.0 \times 10^4 \pm 1.7 \times 10^3$</td>
<td>$6.7 \times 10^3 \pm 97.6$</td>
<td>96.7</td>
<td>3.3</td>
<td>$8.1 \times 10^3 \pm 88.4$</td>
<td>96.0</td>
<td>4.0</td>
</tr>
<tr>
<td>19698</td>
<td>$1.9 \times 10^4 \pm 1.5 \times 10^3$</td>
<td>$3.4 \times 10^4 \pm 23.2$</td>
<td>97.2</td>
<td>2.8</td>
<td>$6.6 \times 10^4 \pm 1.1 \times 10^4$</td>
<td>96.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Linda</td>
<td>$1.4 \times 10^4 \pm 1.5 \times 10^3$</td>
<td>$3.6 \times 10^4 \pm 7.4 \times 10^4$</td>
<td>75.2</td>
<td>24.8</td>
<td>$4.5 \times 10^4 \pm 1.1 \times 10^4$</td>
<td>68.7</td>
<td>31.3</td>
</tr>
<tr>
<td>Ben</td>
<td>$1.2 \times 10^4 \pm 1.3 \times 10^3$</td>
<td>$2.9 \times 10^4 \pm 2.3 \times 10^4$</td>
<td>75.1</td>
<td>24.9</td>
<td>$3.7 \times 10^4 \pm 2.9 \times 10^4$</td>
<td>68.5</td>
<td>31.5</td>
</tr>
</tbody>
</table>

* Mean ± SD number of colony-forming units (cfu) of 3 separate heat treatment cycles.
propiate. In our continued efforts to identify sources of human contact with this organism, studies are currently in progress to determine if *M. paratuberculosis* exists within pasteurized retail dairy products.

**References**


**Nocardiosis in a llama**

**Ching-Dong Chang, Timothy R. Boosinger, Patricia M. Dowling, Elizabeth E. McRae, Jeffrey W. Tyler, David G. Pugh**

An 8-year-old intact male llama weighing 136 kg was referred to the Auburn University Large Animal Clinic with a history of recumbency following an episode of heatstroke 2 weeks earlier. The llama had previously been treated with intramuscular injections of broad spectrum antibiotics, corticosteroids, and vitamin E/selenium. The llama was in a state of recumbency and could not be stimulated to rise. Rectal temperature was 103 F, heart rate was 60 beats per minute, and respiratory rate was 40 breaths per minute. Crackling, wheezing, and expiratory grunting were heard on auscultation of the dorsal lung fields. Percussion revealed consolidation of the ventral portions of the lung lobes.

The hemogram indicated anemia (packed cell volume = 26%), low red blood cell count (7.57 million/µl leukocytosis (undifferentiated white cell count = 18,700/µl), hyperfibrinogenemia (900 mg/dl), and hypoproteinemia (4.2 g/dl). Serum chemistries revealed increased total bilirubin (0.44 mg/dl), gamma glutamyltranspeptidase (52 IU/liter), and aspartate transaminase (48 IU/liter). Liver enzymes were also elevated, with alanine transaminase (ALT) at 118 IU/liter and lactic dehydrogenase (LDH) at 787 IU/liter.

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