An outbreak of listeriosis in a breeding colony of chinchillas

Melinda J. Wilkerson, Amy Melendy, Eric Stauber

*Listeria monocytogenes* is a facultatively anaerobic short gram-positive rod-shaped bacterium that infects a wide range of animals, including ruminants, monogastric animals, birds, fish, rodents, and humans. Infection with this obligate intracellular pathogen causes 3 distinct clinical entities, septicaemia, encephalitis, and abortion, which rarely occur simultaneously in the same animal. The septicemic form affects the viscera with or without meningoencephalitis and is common in monogastric animals, whereas encephalitis and abortion occur principally in adult ruminants. Chinchillas are considered one of the species more susceptible to visceral listeriosis, especially when reared in confinement. Listeriosis in chinchillas has been described in many areas of the United States during a period (1949-1955) when chinchilla pelts were prized commodities in the fur industry. More recently, chinchillas have become a widely used animal model for studies of noise-induced hearing loss.

An outbreak of disseminated visceral listeriosis occurred in a breeding colony of 130 chinchillas located in northern Idaho. The chinchillas in this operation were raised for pelts. Polygamous breeding was practiced; 1 male had access to 8 females. The feed, open sacks of commercially formulated chinchilla pellets and a bale of hay, was stored on a wood pallet. To promote maintenance of their hair coats, a dust pallet was provided for the animals. During December 1995, there was an unusually large influx of mice into the chinchilla building, and on several occasions the owners noted mouse droppings in the feed. By January 1996, the colony experienced a 23% mortality (30 of 130) in breeding animals of various ages. Approximately 4 days prior to death, the animals were anorectic and hunched, and some had tachycardia. Many animals were found dead without premonitory clinical signs. Four chinchillas (2 dead males, 1 dead female, 1 moribund female) were submitted to the Washington Animal Disease Diagnostic Laboratory for necropsy.

All 4 chinchillas were emaciated and lacked subcutaneous and retroperitoneal fat. Consistent gross lesions in the 3 dead chinchillas included multifocal 0.1-0.3-cm-diameter white-tan foci affecting the capsular surfaces and parenchyma of the liver, mesenteric lymph nodes, and the serosa of the small and large intestines, particularly the cecum and colon. Individual differences in postmortem lesions are listed in Table 1. Animal A, a dead female, had a 2-cm rectal prolapse and colonic intussusception in addition to the visceral foci. The intussusception consisted of a 3-cm segment of dark red proxi-

From the Washington Animal Disease Diagnostic Laboratory (Wilkerson, Melendy) and The Department of Veterinary Clinical Sciences (Stauber), College of Veterinary Medicine, Washington State University, Pullman, WA 991652037. Current addresses: Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University Manhattan, KS 66506 (Wilkerson), and 127 Elongrove Ave.#9, Providence, RI 02906 (Melendy). Received for publication July 5, 1996.
tissue sections were deparaffinized in Pro-par, dehydrated in 100% ethanol, blocked for endogenous peroxidases with 3% hydrogen peroxide in methanol, rehydrated in graded alcohols and water, blocked for nonspecific binding of primary antibody with 3% goat serum, and digested with 0.1% Pronase for 10 minutes at 37°C. The positive control tissue consisted of formalin-fixed fetal bovine liver with hepatic necrosis from which L. monocytogenes had been isolated in pure culture prior to fixation. Negative control for the test was normal (nonimmune) rabbit serum applied to the Listeria-infected bovine fetal liver section and to the chinchilla tissues. Using this technique, listerial antigen-positive debris was identified as principally intracellular (in neutrophils and macrophages) deep-red staining of short rods and clusters of organisms within microabscesses and necrotic foci in the brain stem of animal D, in pericardial abscess in animal B, and in liver and intestine of animals A, B, and C (Figs. 1B, 2B). Rare extracellular listerial antigens also were highlighted by this method.

Within 3 days of submission, numerous bacteria were isolated from cultures of lung and thoracic fluid (animal B), spleen (animal C), and brain samples (animals A, D) and identified as L. monocytogenes based on the following criteria: gram-positive stain, beta hemolysis on sheep blood agar, a positive catalase reaction, positive CAMP test with Staphylococcus aureus, negative D-xylose fermentation, and motility at 20°C in a mannitol agar stab. Confirmation was performed with 1 isolate using the API 20 STREP identification system. Listeria were recovered from 2 of 4 brains, including animal D, with brain stem microabscesses, and animal A, which had no macroscopic or microscopic lesions (Table 1). The Listeria isolates in all animals were susceptible to various antibiotics, including penicillin, tetracycline, trimethaphrine-sulfadiazine, and chloramphenicol. Mixed bacterial growth was cultured from the brains of animal A and C and from thoracic fluid of animal C, indicating possible contamination or postmortem overgrowth. Many S. aureus were recovered from the uterine swab of chinchilla D. No Salmonella or Campylobacter were isolated from the intestinal samples cultured as described previously. No Campylobacter was detected with Victoria blue staining. Although the intestinal samples were not cultured specifically for Listeria, infection of the intestinal tract was demonstrated immunohistochemically in animals A, B, and C (Table 1).

The most common manifestation of listeriosis in chinchillas is microabscessation of the liver, mesenteric lymph nodes, and small and large intestines. Intestinal intussusception and rectal prolapse has been reported as complications of enteritis. These lesions may be confused with those produced by Y. pseudotuberculosis and Salmonella in chinchillas. The bacteriologic isolation and immunohistochemical techniques confirmed the presence of Listeria in this breeding colony of chinchillas. Although Listeria is most frequently isolated from the liver, intestine, colon, and spleen in chinchillas, the organism also has been isolated from the lung, heart, and brain. Torticollis is a commonly described symptom in chinchillas with Listeria-induced meningoencephalitis. Chinchilla D had brain stem encephalitis without evidence of septicemia or digestive tract infection. Although the pathogen in chinchilla D could have accessed the brain via the oral cavity, as is speculated of Listeria invasion into the ruminant brain in which entry via axons and trigeminal nerves follows penetration of oral mucosa and the dental pulp when animals cut or lose teeth, no oral lesions were observed in this animal. However, macroscopic oral wounds are not essential to the development of meningoencephalitis; it can be reproduced in mice and rabbits by microscopic inoculation of L. monocytogenes in the lip. Because microscopic examination of trigeminal nerve and its branches to the oral cavity was not performed in these chinchillas, such a route of infection could not be confirmed. A hematogenous route of infection was not considered likely because of the lack of a septicemic process.

The predominance of digestive tract infection in chin-
Figure 1. Immunoperoxidase stain of a focus of hepatic necrosis in chinchilla liver with (A) negative control serum and (B) antiserum against *Listeria monocytogenes*, revealing few antigen-positive debris and short rods. AEC chromogen, counterstained with hematoxylin. Bar = 25 µm.

Figure 2. Immunoperoxidase stain of a transmural microabscess with necrosis in chinchilla cecum with (A) negative control serum and (B) antiserum against *Listeria monocytogenes*, depicting a myriad of intracellular and extracellular bacteria. AEC chromogen, counterstained with hematoxylin. Bar = 70 µm.
chillas A, B, and C is suggestive of oral entry via contaminated feed. Hay contaminated with rodent, chicken, or ruminant feces has been implicated in outbreaks of chinchilla listeriosis, and the removal of such contaminated feed often stops the development of new infections. Moreover, the visceral syndrome has been reproduced experimentally in adult nonpregnant chinchillas and white mice by oral inoculation with *L. monocytogenes*. Although the pelleted feed and hay was not cultured during this outbreak, they were strongly suspected to be contaminated with mouse droppings or ruminant feces. This pathogen also could have been transmitted easily among animals by coprophagia because animals defecated in the dust pan during dust baths and this dust pan was transferred from cage to cages. Recommendations to break the cycle of oral transmission included removal of contaminated feed, euthanasia and disposal of all moribund animals, and disinfection of cages, water bottles, and dust baths. The owners discontinued feeding hay, stored pelleted feed in a covered metal container, provided individual dust baths, euthanatized 30 sick animals, and removed mice from the premises. No problems were noted in the subsequent 6 months.

*Listeria monocytogenes* was isolated from tissues and identified readily within 72 hours by bacterial culture without cold enrichment. Listerial antigens could be detected in chinchilla tissues immunohistochemically utilizing commercially available rabbit polyclonal antiserum, which verified the presence of intracellular bacteria in microabscesses and necrotic foci even though a tissue Gram stain did not. Failure of Gram stains to detect Listeria has been reported for bovine brain sections with compatible histologic lesions and cerebrospinal fluid cells from human patients. The rapid immunohistochemical test used for these chinchillas could be utilized to verify Listeria organisms if tissues are unavailable for culture. Lack of Gram staining is likely due to the obligate intracellular life cycle of Listeria. This particular organism is capable of utilizing the host cell’s actin machinery to propel itself through the cytoplasm of 1 cell into another cell without exposure to the extracellular environment.

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### Sources and manufacturers

**a.** Becton Dickinson, Cockeysville, MD.  
**b.** *Listeria* O antiserum poly, serotypes 1 and 4, Difco, Detroit, MI.  
**c.** Vector Laboratories Burlingame, CA.  
**d.** BioGenex, San Ramon, CA.  
**e.** Anatech, Battlecreek, MN.  
**f.** Protease Type XIV, Sigma Chemical Co., St. Louis, MO.  
**g.** Biomerieux, Hazelwood, MO.

### References