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***Bordetella bronchiseptica* ASSOCIATED WITH PULMONARY DISEASE IN MOUNTAIN VOLES (*Microtus montanus*)**

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Abstract: *Bordetella bronchiseptica* was isolated from the lungs of all of six mountain voles (*Microtus montanus*) found dead or dying of pulmonary infection near the Bear River Research Station in northern Utah in January, 1973. The possibility of concomitant viral or mycoplasmal infection was not ruled out.

INTRODUCTION

Bordetella bronchiseptica is a common cause of infections of domestic and laboratory animals, sometimes as the primary disease agent and other times as a secondary invader. The small gram-negative bacillus causes bronchopneumonia and other respiratory infections in laboratory rabbits, guinea pigs, and rats, as well as in horses, swine, dogs, cats, and monkeys.¹ It is reported to complicate chronic pneumonia of laboratory mice when *Mycoplasma pulmonis* is the primary etiological agent.² It commonly complicates canine distemper, but is capable of inducing respiratory infection in dogs in the absence of the distemper virus.¹ That it plays a role in the pathogenesis of atrophic rhinitis in swine has been established, but the nature of that role is not clearly understood.^{1,3,4} Of the same genus as the organism responsible for whooping cough (*B. pertussis*), it occasionally causes a similar syndrome in man.^{1,5,6}

In spite of the frequency of its involvement in respiratory diseases of captive animals, there appear to be no reports of *B. bronchiseptica* having been identified as the cause of disease in any wildlife species. Switzer *et al.*,⁷ in a survey conducted in central Iowa, isolated the bacterium from the tracheas of 6 of 21

rats (*Rattus norvegicus*) trapped at a small town dump, as well as from 1 of 78 striped skunks (*Mephitis mephitis*), 2 of 105 opossums (*Didelphis marsupialis*), 4 of 108 foxes (*Vulpes fulva*), and 3 of 85 raccoons (*Procyon lotor*), all trapped by fur buyers. Since live trapping favored the capture of active, healthy animals, examinations for pathologic changes were not made (letter dated 15 April 1976 from William P. Switzer, Veterinary Medical Research Institute, Iowa State University, Ames, Iowa). In the course of a study comparing the antigenic structure of porcine strains of *B. bronchiseptica* with that of strains from other animal species, Pedersen⁷ isolated one from a mouse caught on the premises housing a swine herd, although it differed serologically from the serotype isolated from the pigs. Farrington *et al.*⁸ isolated *B. bronchiseptica* from the nasopharyngeal areas or tracheas of 6 of 13 short-tailed shrews (*Blarina brevicauda*), 1 of 36 raccoons, 2 of 34 opossums, 4 of 46 red foxes, and 1 of 46 house sparrows (*Passer domesticus*). Examinations for evidence of respiratory disease were not made.

An outbreak of pneumonia in mountain voles is the subject of this report. Whether *B. bronchiseptica* was the sole etiological agent was not unequivocally demonstrated.

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CASE HISTORY

An unusually high level of mountain vole activity was evident in a 12 × 16.5 m plot of lawn adjacent to the Bear River Research Station (BRRS) throughout January, 1973. When an 8-10 cm layer of snow began to thaw, numerous surface runways burrowed through the grass were revealed, and three or four apparently healthy, active voles could be seen at almost any time during daylight hours.

One dead vole was found on a concrete walk about 20 m from the lawn on February 1. Its nose was blood-caked, and a spot of still-wet bloody fluid about 2 cm in diameter evidently had been discharged through the nares.

Shortly after 1 February — the precise date was not recorded — activity on the lawn decreased markedly; only occasionally was a vole seen. Because of the likelihood that dead animals would be taken quickly by scavengers, baited live traps were set on the lawn in an effort to capture sick ones before they were captured by predators.

Between 1 February and 24 February, five additional specimens were collected, two found dead and three captured in traps. One of the latter three had died during the night and the other two died within an hour after their transfer to laboratory cages.

MATERIALS AND METHODS

Routine necropsies included gross external and internal examinations. Also, lung, liver, spleen, and heart blood were streaked on Difco² brain heart infusion agar (BHIA) plates, which were incubated at 37 C for 48 h. A portion of each tissue was preserved in 10% formalin for sectioning and staining with hematoxylin and eosin (H and E). Experimental infections were induced in lightly ether-anesthetized laboratory mice (Rocky

Mountain Laboratory strain) by intranasal instillation of one drop of 18-h Difco brain heart infusion culture from a 25 gauge hypodermic needle (about 4.0×10^6 bacterial cells, measured by plate counts on BHIA). Mice not surviving the infection were examined post mortem in the same manner as were the voles.

Facilities for the isolation of viruses and mycoplasmas were not available at the BRRS at that time.

RESULTS

At necropsy, no evidence of mechanical injury was found in the voles. Gross pathologic changes were confined to the lungs, which were consistently congested and edematous. About 0.2 ml of blood-tinged fluid was present in the pleural cavity of the first one found dead, and one lobe of the lung was hepatized in the second.

Histopathologic examination of the lungs confirmed the existence of congestion and edema and disclosed a considerable degree of atelectasis. The alveoli contained fluid, fibrin, inflammatory cells, and, in some cases, erythrocytes.

Cultures of lung (but no other tissues) yielded heavy, confluent growth of a small, gram-negative coccobacillus with these morphological and biological characteristics:

Colonies on BHIA pinpoint at 24 h reaching 6-7 mm after 4 days, if well isolated; circular, smooth, translucent.

Cells arranged singly, in pairs, and occasionally in short chains; motile.

Glucose, levulose, galactose, lactose, sucrose, xylose, mannose, maltose, dulcitol, and arabinose not fermented.

Gelatin not liquified; H₂S not produced; indol negative; nitrate

² Reference to trade names does not imply endorsement of commercial products by the Federal Government.

reduced to nitrite; urease positive; citrate utilized; catalase positive; oxidase positive; litmus milk alkaline.

On the basis of these findings, we identified the bacterium as *B. bronchiseptica*.

When the bacterium was isolated from the voles in 1973, one drop of broth culture given by intranasal instillation killed seven of eight adult laboratory mice. More recently (1979), the same number of cells of the same isolate killed six of eight young mice (4-6 weeks) but none of eight adult mice.

The experimental infection was usually acute, causing death in five of seven fatal cases in 1973 and in all of six cases in 1979. Pathologic changes in the lungs ranged from congestion only to bronchopneumonia, the alveoli containing fibrin or a mixture of fibrin, granulocytes, and

macrophages. There was evidence of necrosis in some areas.

DISCUSSION

The fact that *B. bronchiseptica* was present in great numbers in the lungs of infected voles and that the bacterium isolated from them induced an acute fatal infection in a high percentage of experimental mice suggests that it played a role in the etiology of the epizootic. However, the diagnosis of frank bronchopneumonia in some of the experimental animals, but in none of the voles leaves unresolved the question of whether one or more other etiological agents may have been at least partly responsible for the outbreak, particularly since the possibility of *B. bronchiseptica* being a concomitant of either a viral or a mycoplasmal infection was not ruled out.

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