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Authors: Brogden, Kim A., Cutlip, Randall C., and Lehmkuhl, Howard D.

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## Cilia-associated Respiratory Bacillus in Wild Rats in Central Iowa

Kim A. Brogden, Randall C. Cutlip, and Howard D. Lehmkuhl, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50010, USA

**ABSTRACT:** Twenty-eight wild rats were live-trapped in central Iowa (USA) to estimate the prevalence of the cilia-associated respiratory (CAR) bacillus. Both light and electron microscopy were used to look for the Gram-negative, filamentous bacterium among cilia in tracheal and lung tissue sections. The organism was observed in the trachea of 20 rats with chronic respiratory disease and in the trachea of three of eight normal rats. Therefore, the organism appears to be common among wild rats in central Iowa.

**Key words:** Cilia-associated respiratory bacilli, CAR bacillus, respiratory disease, wild rats, *Rattus norvegicus*.

A naturally occurring, Gram-negative, filamentous bacterium has been described in the respiratory tract of laboratory and wild rats with chronic respiratory disease (CRD) (MacKenzie et al., 1981; van Zwieten et al., 1980). This organism, called the cilia-associated respiratory (CAR) bacillus, cannot be readily isolated in vitro (Ganaway et al., 1985), but can be seen by both light and electron microscopy. It is 0.12 to 0.2  $\mu\text{m}$  by 6.0 to 8.0  $\mu\text{m}$  and has a typical Gram-negative bacterial morphology. The CAR bacillus is similar in size and shape to bronchial epithelial cilia and often is found lying parallel to and among the cilia of the nasal mucosa, eustachian tubes, trachea, bronchi, and bronchioles (van Zwieten et al., 1980; Matsushita and Joshima, 1989; Matsushita, 1991).

The host range of the CAR bacillus has not been extensively examined. Van Zwieten et al. (1980) observed the organism in an epizootic of CRD in laboratory rats in the Netherlands and MacKenzie et al. (1981) observed the organism from a wild rat (*Rattus norvegicus*) colony at a Houston, Texas (USA) grain elevator. In addition to rats, the organism has also been seen in laboratory mice (MacKenzie et al., 1981;

Matsushita et al., 1989), rabbits (MacKenzie et al., 1981; Kurisu et al., 1990), and guinea pigs (Matsushita et al., 1989). In this study, we determined if the organism occurred naturally in wild rats (*Rattus norvegicus*) in central Iowa.

Twenty-eight wild rats were trapped in central Iowa (41°44' to 42°01'N; 93°26' to 93°39'W) with no more than two rats coming from a single location. Adult rats were captured in Havahart live traps (Woodstream Corporation, Lititz, Pennsylvania, USA). In the laboratory, the rats were euthanized by inhalation of chloroform, and exsanguinated. At necropsy, the lungs were evaluated grossly and pieces of trachea and lung were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with Giemsa and hematoxylin and eosin stains. A piece of trachea also was fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), for 1 hr at 4 C. Fixed tissue was washed twice in cacodylate buffer and stained in 1.0% osmium tetroxide for 1 hr. The tissue was washed again in cacodylate buffer and dehydrated in a series of graded ethanol solutions. The dehydrated tissues were cleared in propylene oxide, and infiltrated and embedded in Epon 812 (Fisher Scientific Company, Fair Lawn, New Jersey, USA) as described by Luft (1961). Thin sections were stained with lead citrate and uranyl acetate (Venable and Coggeshall, 1965), and examined with an EM-410 electron microscope (Philips Electronic Instruments, Mahwah, New Jersey).

Tracheal scrapings were cultured on 0.5% defibrinated sheep blood in trypticase soy agar, on MacConkey agar, and in Hayflick's medium containing 10% yeast autolysate and 20% horse serum (both with and without 0.7% agarose) as previously

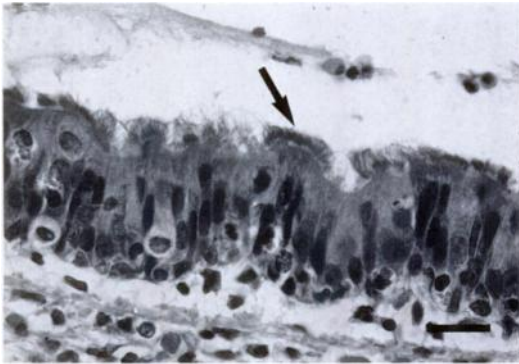


FIGURE 1. Tracheal mucosa from a wild rat (*Rattus norvegicus*) colonized with the filamentous CAR bacillus (arrow). Note the continuous mucous layer, interspersed with neutrophils, which has separated from the epithelial cell surface. H&E. Bar = 25  $\mu$ m.

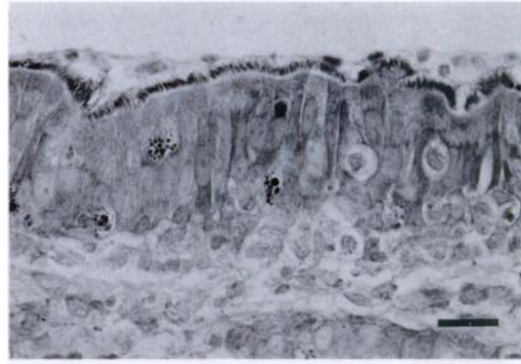


FIGURE 2. Tracheal mucosa from a wild rat (*Rattus norvegicus*) colonized with the filamentous CAR bacillus. Note the thick mats on cells on the epithelial cell surface. Giemsa. Bar = 25  $\mu$ m.

described (Brogden et al., 1988). Isolated bacterial species were identified by the methods of Kloos and Schleifer (1975), Hollis and Weaver (1981), Clark et al. (1984) and Hussain et al. (1984).

No gross lesions were seen in the rats at necropsy that were associated with the presence of the organism.

Histologically, 20 of 28 rats had CRD consisting of chronic focal interstitial pneumonia with interstitial thickening, perivasculitis, and peribronchitis. Cellular accumulations in submucosa consisted of lymphocytes, macrophages, plasma cells, and neutrophils. Bronchiectasis and catarrhal tracheitis and bronchitis were common features. In the 20 rats with CRD, CAR bacilli were observed by light microscopy in the lung of one rat and in the tracheas of seven rats (Figs. 1, 2; Table 1). The CAR bacilli were observed by electron microscopy in the trachea of 14 rats (Fig. 3). No CAR bacilli were seen by either light or electron microscopy in the tracheas of four rats.

Eight rats were normal and did not have any gross or histopathologic changes in their tracheas or lungs. However, CAR bacilli were seen by electron microscopy in three of these rats.

Small numbers of bacteria were isolated from trachea of the rats and included

diphtheroids, non-hemolytic *Streptococcus* species, *Staphylococcus epidermidis*, *Escherichia coli*, *Acinetobacter calcoaceticus*, *Proteus mirabilis*, *Serratia marcescens*, *Flavobacterium* species, and *Citrobacter freundii*. Mycoplasma was not isolated from the trachea of these rats by either broth or agar culture.

The importance of the CAR bacillus in the wild rat population is not known. The organism is associated with CRD resembling respiratory mycoplasmosis. The organism is transmissible among laboratory rats and mice and causes a respiratory disease characterized by focal atelectasis, bronchiectasis, and emphysema (Matsushita and Joshima, 1989). Rabbits and guinea pigs exposed to CAR bacilli seroconvert to the organism but no organisms or histological changes relating to the in-

TABLE 1. Prevalence of cilia-associated respiratory (CAR) bacilli in the tracheas of 28 wild rats (*Rattus norvegicus*), live trapped in central Iowa.

Lesions	Number of rats	CAR bacillus observed by:	
		Light microscopy	Electron microscopy
None	8	0 (0) <sup>a</sup>	3 (38)
CRD <sup>b</sup>	20	7 (35)	14 (50)

<sup>a</sup> Number (percent) of rats with CAR bacilli.

<sup>b</sup> Chronic respiratory disease characterized by submucosal accumulations of lymphocytes, plasma cells, and few neutrophils in the trachea and subacute interstitial pneumonia.

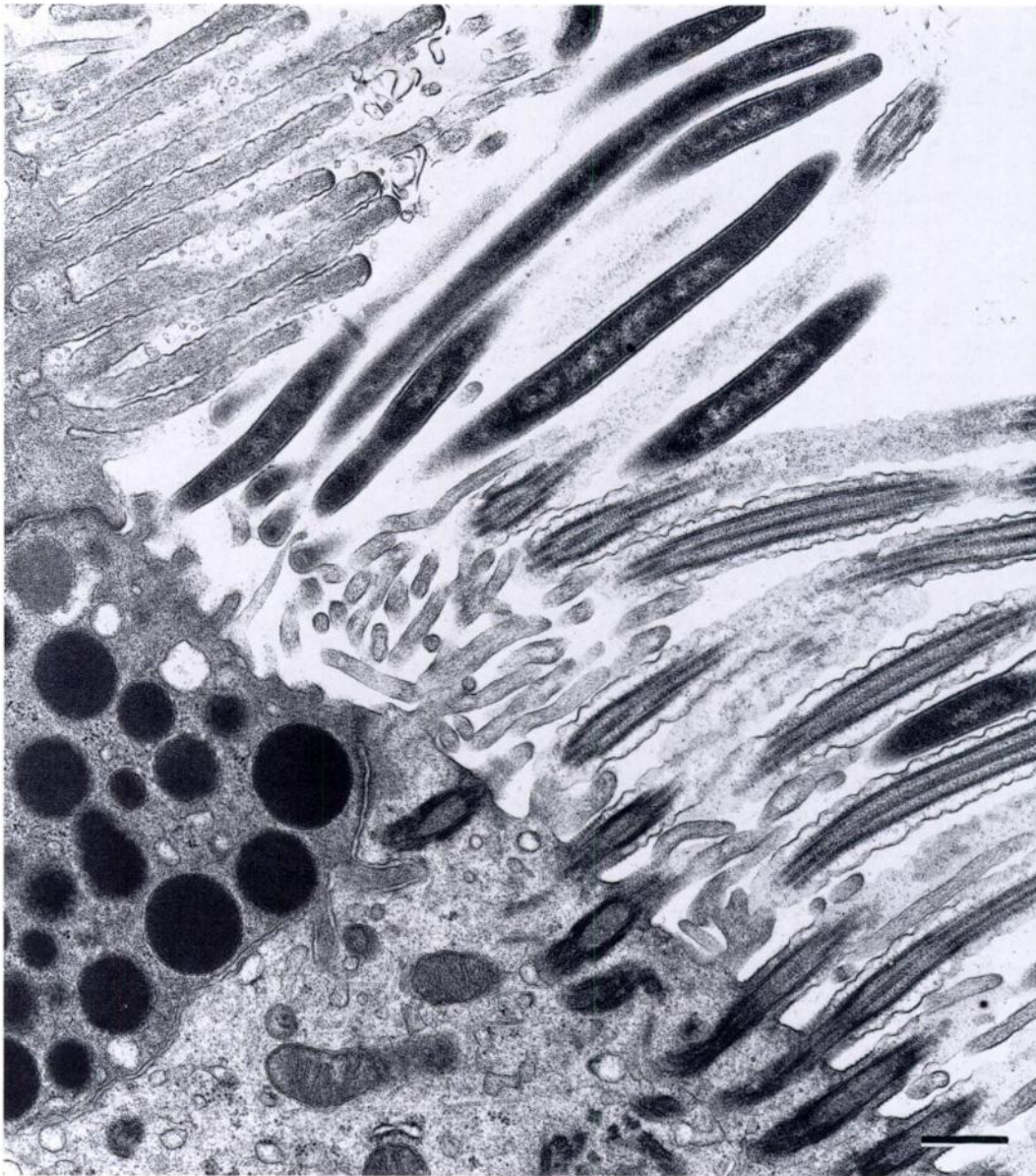


FIGURE 3. Tracheal Clara cell from a wild rat (*Rattus norvegicus*) colonized with CAR bacilli. Note the long, slender bacterial cells, with electron dense cytoplasm, lying parallel to and among the cilia of adjacent epithelial cells. Uranyl acetate and lead citrate stain. Bar = 0.5  $\mu$ m.

fection have been seen in the airways of either species (Matsushita et al., 1989). These animals may serve as carriers and spread the organism to other species of rodents and rabbits (Matsushita et al., 1989). Whether the organism is transmissible to agricultural or domesticated animals is not yet known.

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