

## Lack of Airborne Spread of Infection by *Legionella pneumophila* Among Guinea Pigs

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Many investigators find no spread of Legionnaires disease from person to person. The present study examined the question of airborne transmission of infection by *Legionella pneumophila* serogroup 1 from guinea pigs inoculated nasally with the agent to healthy guinea pigs. The nasal inoculation produced confluent peribronchiolar pneumonia similar to the pulmonary lesions observed in humans, but by techniques of clinical observation, serology, culture, and pathology, there was no evidence of airborne spread of infection from 26 inoculated guinea pigs to 64 uninoculated guinea pigs. The results, compatible with epidemiological studies of Legionnaires disease that fail to demonstrate airborne person-to-person transmission of the illness in humans, are useful for scientists who work with animal models of Legionnaires disease.

*Legionella pneumophila*, the causative agent of Legionnaires disease, is cultured from a variety of environmental samples, including fresh water, soil, cooling towers, evaporative condensers, and shower heads (8, 16, 18, 19). Legionnaires disease is presumably transmitted from these environmental sources to man by inhalation of aerosols containing the agent (4, 6, 8-10, 19). Many investigators find no evidence of spread of Legionnaires disease from person to person (4, 9, 14, 17, 22), but infrequent reports suggest possible secondary spread (2, 5, 11, 21, 23), raising the question of airborne and respiratory transmission droplet of infection by *L. pneumophila* from ill to healthy persons. The aim of the present study was to test that question in an animal model that was developed previously (12, 13), guinea pigs inoculated intranasally with *L. pneumophila*.

### MATERIALS AND METHODS

**Inoculation.** Ninety pathogen-free male Hartley guinea pigs (400 to 500 g) were used. Twenty-six (group 1) were inoculated with 0.5 ml of a sterile suspension of Bovarnicks SPG (1) containing  $5 \times 10^6$  virulent organisms of *L. pneumophila* serogroup 1 (courtesy of Bureau of Laboratories, Commonwealth of Pennsylvania). Quantitation was by the dilution and spread plate method (20). Prior work (12) demonstrated that this dose, when inoculated nasally, produced pneumonia in most guinea pigs and that roughly 50% of the guinea pigs survived. Each animal was inoculated individually under a class II biological safety cabinet, using a nose dropper and alternating one drop every 5 s, deep into each nostril. Forty-five uninoculated guinea pigs (group 2) were housed in quarters

contiguous with those inoculated. Nineteen uninoculated guinea pigs (group 3) were placed in a separate room that was adjacent to animals in group 1 and 2. In three separate experiments, inoculated animals remained quarantined in the biological safety cabinet for three intervals according to Table 1. Quarantine attempted to test the effects of: (i) spread from sneezing the initial inoculum from the upper respiratory tract and (ii) possible airborne transmission from the lower respiratory tract. Guinea pigs given *L. pneumophila* intranasally may sneeze for 3 h after inoculation. Thus, animals quarantined for 1 h continued to sneeze after removal from the biological safety cabinet, and by contrast, sneezing after removal from the cabinet was not seen in animals quarantined for 4 to 24 h.

**Housing and handling.** Each animal was housed in a separate cage and kept in the same cage throughout the study. Each cage measured 8 (width) by 13 $\frac{3}{4}$  (length) by 7 inches (height) (ca. 20 by 58 by 18 cm) and had closed metal sides, a closed metal floor, and a wire mesh lid that was open to the air. Cages were arranged so that inoculated animals of group 1 were placed on racks surrounded by uninoculated animals of group 2. The horizontal distance between each cage was less than 1 inch (2.54 cm). The verticle distance between cages was 6 inches (15.24 cm). There was no bodily contact among the animals. Moreover, when an animal was removed from a cage for daily temperature measurement or for other manipulations, a pair of sterile gloves was used by the handler, and all surfaces in contact with animals were scrubbed with alcohol. The two rooms used for housing animals each measured 9.3 (width) by 24 (length) by 8.6 feet (height) (ca. 2.8 by 7.3 by 2.6 m). The area of containment was under negative pressure, and the air in each room was compartmentalized. The air system in the animal quarters was separate from that of the rest of the building.

**Examination for infection by *L. pneumophila*.** Rectal temperatures were taken daily on all animals from 1

TABLE 1. Duration of quarantine of guinea pigs inoculated with *L. pneumophila*

Experiment	Duration of quarantine (h)	No. of animals <sup>a</sup>		
		Group 1	Group 2	Group 3
1	1	16	25	10
2	5	5	10	5
3	24	5	10	4

<sup>a</sup> Group 1, infected nasally; group 2, not infected, but housed in the same room, near infected guinea pigs; group 3, not infected but housed in a separate room.

week before inoculation to 2 months after inoculation. During the same time interval, all animals were observed daily for clinical signs of illness, including runny nose, watery eyes, ruffled fur, and cachexia. Serum samples obtained from all animals 1 week before, 2 weeks after, and 2 months after inoculation were tested for antibodies to *L. pneumophila* by the conventional indirect immunofluorescent antibody procedure (25) and by card agglutination test (15). To document the presence or absence of *L. pneumophila* in tissue, necropsies were performed within 10 days after inoculation on the five animals from group 1 that died spontaneously and on four animals from group 2 and two animals from group 3. The animals from group 3 were randomly selected for sacrifice. Postmortem examination consisted of gross and microscopic examination of all internal organs. Microscopic study included hematoxylin and eosin stains, Dieterle stains (24), and direct immunofluorescence (3, 11) to visualize *L. pneumophila*. Culture of lung and spleen on the following media were also performed: blood agar, MacConkey agar, thioglycollate broth, Feeley-Gorman agar (7), and charcoal yeast extract agar (7a).

## RESULTS

Within 1 week after inoculation, 19 of 26 guinea pigs (73%) from group 1 developed a fever of equal to or greater than 103°F (39.4°C), and 24 of 26 guinea pigs exhibited clinical signs of illness, as described above. Of the guinea pigs from group 1, 5 (19.6%) died spontaneously within 10 days, and the 21 other guinea pigs in group 1 survived for the remainder of the study. Three were from experiment 1, one was from experiment 2, and one was from experiment 3. By contrast, all animals in groups 2 and 3 remained clinically healthy and afebrile during the period of observation. The pneumonia, seen at necropsy in all five guinea pigs from group 1 who died spontaneously, was characterized by bronchiolar exudate and peribronchiolar consolidation of histiocytosis and macrophages. Acute splenitis, detected in four of five fatalities from group 1, featured sinus histiocytes and microabscesses. Neither pulmonary nor other systemic abnormalities were noticed in the tissues of the four control animals of group 2 and

the two animals of group 3. Direct fluorescent antibody studies and cultures of lung and spleen documented *L. pneumophila* in 5 of 5 lung specimens from group 1 and in 3 of 5 spleen specimens from group 1. These tests also showed an absence of the bacterium in splenic and pulmonary tissue of the six animals from groups 2 and 3.

Paired sera of 19 of 21 survivors in group 1 demonstrated a fourfold rise in antibody titer to *L. pneumophila*. Paired sera of all animals in group 2 and group 3 displayed no detectable antibodies to *L. pneumophila*, even at 2 months after exposure to ill animals.

## DISCUSSION

The present study found no evidence of airborne transmission of infection by *L. pneumophila*—either clinical or subclinical—from ill to healthy guinea pigs. We did not evaluate transmission by bodily contact or transmission by direct exposure to infected mucus or infected internal organs; rather, the experimental design was specifically tailored to assess airborne transmission in guinea pigs inoculated nasally. Altering variables of the experimental design, including the dose of *L. pneumophila*, preparation of inoculum, route of inoculation, ratio of infected animals, physical properties of the quarters, and host immune competence, might conceivably yield different results.

The findings of our study will be helpful to scientists who work with animal models of Legionnaires disease. The data are also compatible with the epidemiological data that failed to demonstrate airborne person-to-person transmission of Legionnaires disease in humans.

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