Prevalence of Antibodies to *Legionella pneumophila* in Animal Populations

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We examined more than 2,800 human and animal sera for antibodies to four serogroups of *Legionella pneumophila* by using the microagglutination test. Antibody titers of $\geq 1:64$ were considered positive. The occurrence of positive equine sera (31.4%) was significantly higher than the occurrence of positive sera in cattle (5.1%), swine (2.9%), sheep (1.9%), dogs (1.9%), goats (0.5%), wildlife (0%), and humans (0.4%). The highest titer measured in horses was 1:512. The occurrence of positive sera in horses was related directly to age. In horses $\leq 1$, 2 to 3, 4 to 7, 8 to 12, and $\geq 13$ years old, the percentages of positive sera were 0, 10.1, 30.3, 44.9, and 58.1%, respectively. When we compared age-specific serogroup-specific rates in horses from Colorado and Pennsylvania, we found differences. With horses 8 to 12 and $\geq 13$ years old, there was a significantly higher ($P < 0.05$) occurrence of sera that reacted to serogroups II and III in horses from Pennsylvania. Of 242 positive sera, 43.8% reacted to a single serogroup (serogroup III or I most commonly), and 56.2% reacted to multiple serogroups (serogroups II and III or serogroups I, II, and III most commonly). A high percentage of seropositive horses suggested that horses are commonly infected with *L. pneumophila* or related organisms, and the age-specific rates of occurrence indicated that infection was related directly to duration of exposure. A definitive demonstration of equine infection will depend on isolation of the agent and repetition of this serological study with antigens obtained from organisms isolated from horses.

*Legionella pneumophila* is widely distributed geographically; it has been isolated in virtually all parts of the United States and in many other countries. Since the original description of Legionnaires disease in Philadelphia in 1977 (14) and the subsequent isolation of *L. pneumophila* from the lungs of individuals with fatal infections (19), much has been learned about the natural history of this organism. *L. pneumophila* has been isolated frequently from the environment. Isolates have been obtained from soil (21), and an epidemiological association with excavation was demonstrated during outbreaks in Pontiac, Mich. (15), and at St. Elizabeth's Hospital, Washington, D.C. (28). However, the evidence associating soil exposure with infection, obtained from serosurveys of construction workers, is equivocal (1, 26).

Water may serve as a source of *L. pneumophila* infections, especially when contaminated water becomes aerosolized. This organism was isolated from stream water and mud during an outbreak in Bloomington, Ind. (21); in other instances it was isolated from lake water (10, 11) and from the thermal effluent of a power plant (29). This bacterium has been shown to have the ability to survive in tap water for at least 1 year (24). Optimal conditions for aerosolization and dissemination of *L. pneumophila* occur when the organism becomes established in the contained water of air-conditioning cooling towers and evaporative condensers. Outbreaks of Legionnaires disease in Bloomington, Ind. (21), Memphis, Tenn. (4), Atlanta, Ga. (3), and Pontiac, Mich. (15), were associated with such cooling systems. An outbreak of nonpneumonic legionellosis in workers who were cleaning a steam turbine condenser suggests a similar mode of transmission (13). The recent description of Legionnaires disease in two patients in a renal transplant unit exposed to contaminated shower water may aid in understanding the sporadic cases which occur in addition to common source outbreaks (30).

The apparent ubiquity of *L. pneumophila* in the environment (10, 11), the prevalence of an antibody to this organism in normal human populations (8, 12, 18, 23, 27), and the associa-
tion of *L. pneumophila* with disturbed soil and water raises questions regarding the natural host range of this bacterium and role of animal populations in the natural history of human Legionnaires disease.

Naturally occurring infections in domestic or wild animal species have not been reported. Sentinel guinea pigs placed in the air-conditioning system of the Pontiac, Mich., Health Department in 1968 became ill (15), and their lungs eventually yielded *L. pneumophila*. Recent experiments designed to develop animal models of Legionnaires disease in laboratory animals have extended the host range. After experimental intraperitoneal challenge, Hartley strain guinea pigs, Sherman rats, and Monoglian gerbils were highly susceptible. Moderate to low susceptibility was demonstrated with ICR mice, Syrian hamsters, and New Zealand rabbits. White leghorn chickens, coturnix quails, and corneal pigeons were not susceptible (C. M. Patton, S. E. Johnson, A. F. Kaufman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B79, p.28).

The ubiquity of *L. pneumophila* in the environment and the susceptibility of laboratory animals to either natural or artificial infections raise the question as to whether domestic and wild animal species become infected naturally with *L. pneumophila*. There have been only limited attempts to determine the prevalence of *L. pneumophila* infections in animal populations, and these have been inconclusive. Page and Lattimer (20) found that 2 of 34 Iowa horses tested had low levels of complement-fixing antibody (1:4 and 1:16) to *L. pneumophila*. Pritchard et al. (22) found that none of 164 cattle and 112 pigs had antibody, as determined by the indirect fluorescent-antibody test. Because of the paucity of serological data and the lack of information concerning the role of domestic animals in the natural history of Legionnaires disease, we examined several animal populations for serological evidence of *L. pneumophila* infection.

### MATERIALS AND METHODS

**Serum specimens.** We tested a total of 2,586 animal sera. The animal species examined and their geographic origins are listed in Table 1. These sera were obtained from diagnostic laboratories to which they had been submitted for routine serological testing for infectious diseases under various control programs (equine infectious anemia, pseudorabies, bluetongue) or as part of surveillance programs (leptospiriosis, bovine leukemia). We assumed that the serum pools represented cross sections of the predominantly healthy animal populations in the areas sampled. Information concerning the health status of individual animals within the samples was unavailable.

A collection of 286 normal human sera was obtained from the Vector-Borne Disease Division, Centers for Disease Control, to assess the sensitivity of the antibody test system used and to compare the titers in the human and animal populations. These sera had been collected in Larimer County, Colo. in 1968 and 1970 and represented a cross section of age groups.

**Serological test.** Isolates of the four serogroups of *L. pneumophila* were obtained from the Centers for Disease Control. We used the microagglutination (MA) test of Farshy et al. (9), with minor modifications. The initial serum dilution used in this study was 1:2 instead of 1:4, 1:8, or 1:10 as described in previous reports (8, 18, 25). Antigens for each strain of *L. pneumophila* were grown on charcoal-yeast extract agar instead of enriched Mueller-Hinton agar. Carbol fuchsin (final concentration, 0.002%) was used instead of safranin to increase the visibility of the agglutination reaction. All sera were tested against the four serogroups of *L. pneumophila* as follows: serogroup I, Philadelphia 1 strain; serogroup II, Togus 1 strain; serogroup III, Bloomington 2 strain; and serogroup IV, Los Angeles 1 strain. Positive control goat sera, supplied by the Centers for Disease Control, and negative control rabbit sera were tested against each new lot of antigen produced.

### RESULTS

Table 2 shows the distribution of antibodies to the four serogroups of *L. pneumophila* in several animal species and humans.

MA titers of ≥1:64 were considered positive, which is consistent with the criterion set by other workers with the MA test (9, 25). The percentage of positive titers to *L. pneumophila* was significantly higher in horses than in the other species examined (*P < 0.001; chi-square test*) (Table 3). Of 636 horses tested, 31.4% were positive to at least one serogroup; reactions to serogroup II (17.3% positive) and serogroup III...
The following strains were used: serogroup I, Philadelphia 1; serogroup II, Togus 1; serogroup III, Bloomington 2; serogroup IV, Los Angeles 1.

(22.8% positive) were most common. Cattle had the second highest rate of occurrence of antibody; 5.1% of 533 sera had titers of ≥1:64 to at least one serogroup. Without exception wild animals were negative. Of 286 human sera, only 1 reacted to the serogroup II and III antigens at a titer of 1:64, giving an overall rate of occurrence of 0.4%; this was similar to the findings of other serological studies on normal human populations (2, 6, 8, 18, 27). The occurrence of antibody in horses clearly increased with increasing age of the animal (Table 4). In horses ≤1, 2 to 3, 4 to 7, 8 to 12, and ≥13 years old, the percentages of positive sera were 0, 10.1, 30.3, 44.9, and 58.1%, respectively.

The age-specific rates of occurrence in horses from Pennsylvania were compared with the rates in horses from Colorado (Fig. 1). There was no significant difference in the age-specific rates of occurrence between the horses from the two states for L. pneumophila serogroups I and IV. Older Pennsylvania horses had a significantly higher prevalence of antibodies (P < 0.05; chi-square test) to serogroup II and III antigens than Colorado horses.

We also analyzed the rates of occurrence of antibodies to single and multiple serogroups of L. pneumophila. Of the 242 positive sera, 43.8% reacted with only one serogroup. Of these, reactions to the serogroup III antigen were most common (17.8% of all positive sera), followed by reactions to the serogroup I antigen (16.5% of
TABLE 3. Prevalence of antibody to *L. pneumophila* by species<sup>a</sup>

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. tested</th>
<th>No. with antibodies to the following serogroups:</th>
<th>Total&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Horses</td>
<td>636</td>
<td>103 (16.2)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>110 (17.3)</td>
</tr>
<tr>
<td>Cattle</td>
<td>533</td>
<td>12 (2.3)</td>
<td>7 (1.3)</td>
</tr>
<tr>
<td>Sheep</td>
<td>464</td>
<td>5 (1.1)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Pigs</td>
<td>447</td>
<td>7 (1.6)</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Goats</td>
<td>219</td>
<td>1 (0.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dogs</td>
<td>104</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Wild animals</td>
<td>183</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Humans</td>
<td>286</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Titer of $\geq 1:64$ to each serogroup antigen.<br>
<sup>b</sup> Titer of $\geq 1:64$ to at least one serogroup antigen.<br>
<sup>c</sup> The numbers in parentheses are percentages.

all positive sera). The majority of the positive sera reacted with more than one serogroup. The most common combinations of positive titers were titers to serogroups II and III (17.8% of all positive sera) and titers to serogroups I, II, and III (11.6% of all positive sera). Of the 242 positive sera, 24 (9.9%) reacted with titers of $\geq 1:64$ to all four serogroups.

**DISCUSSION**

The MA test can be applied to sera of multiple animal species easily since no species-specific reagents are used, as with the indirect fluorescent-antibody test. The MA test has comparable accuracy and is also the least costly of the serological tests for *L. pneumophila*. Farshy et al. (8) demonstrated that the MA test detected elevated titers in 97.2% of the sera from patients with confirmed Legionnaires disease. With the MA test, the upper limit of normal titers in human sera was found to be 1:8. However, when this test was applied to a single serum specimen, a titer of 1:32 was considered indicative of *L. pneumophila* infection (not necessarily

FIG. 1. Comparison of prevalence of antibody to *L. pneumophila* in equine sera from Colorado (open bars) and Pennsylvania (cross-hatched bars).
disease) (18). Human sera with MA titers of 1:32 or more to *L. pneumophila* had bactericidal activity against this organism (25). A titer of 1:64 was considered suggestive of active disease (9). Thus, we chose to set the lower limit of positive titers indicative of a past or present *L. pneumophila* infection at 1:64. We recognize the possibility that some animal species may respond to *Legionella* infections with the production of non-agglutinating antibody; thus, the results of this study must be interpreted within the constraints of what is measurable by the MA test.

The high percentage of seropositive horses suggests that horses are infected with this agent commonly. However, in this study we did not examine the question of pathogenicity of *L. pneumophila* in horses. Other domestic animals are apparently infected with *L. pneumophila* as well, but much less frequently. Wild animals show no indication of infection. In comparison, sera from normal humans show a very low prevalence of antibody; this is consistent with the findings of other workers (2, 6, 16, 18, 27). This suggests that the MA test is appropriately sensitive and specific for measuring antibodies to *L. pneumophila*. The higher prevalence of antibody in domestic versus wild animals may indicate that an association of animals with humans or human habitats or animal husbandry practices may be related to the *L. pneumophila* infection rate or that there is a common environmental source of infection. The higher prevalence of titers in horses than in other domestic animals is probably not strictly a function of age, since we tested equivalent proportions of aged cattle and dogs.

It is also possible that these titers resulted from cross-reactions with other antigens. Cross-reactions have been demonstrated between *L. pneumophila* serogroup I antigen and antibodies to *Pseudomonas pseudomallei* (17) and some strains of *Bacteroides fragilis* (5). However, hundreds of other bacteria have been examined without evidence of cross-reactions, and the cross-reaction titers seldom if ever reach the levels found in this study (maximum, 1:512).

Serological reactions to both single and multiple serogroups occurred. Of the 242 animal sera with titers of ≥1:64 to *L. pneumophila*, 56.2% were positive to more than one serogroup. The two most common patterns of multiple serogroup reactions were reactions to serogroups II and III and reactions to serogroups I, II, and III. This finding is consistent with the findings of other workers, who have demonstrated both group-specific and common antigenic determinants on *L. pneumophila* (31–33).

Domestic animal sera that reacted to only one serogroup reacted most frequently with serogroup III. Our data indicated that serogroup III Bloomington 2 strain or related *L. pneumophila* strains may be more prevalent in the environment of domestic animals than other *L. pneumophila* serogroups. The low frequency of individual serogroup reaction emphasizes the importance of using multiple serogroups in serological tests.

The increasing occurrence of *L. pneumophila* titers with increasing age indicates that (i) antibody is long lived, (ii) infection is persistent, or (iii) horses are repeatedly exposed to the agent and acquisition of the infection is related directly to the duration of exposure. The latter hypothesis appears to be most plausible, considering that the MA test for *Legionella* infection measures predominantly immunoglobulin M antibodies, as shown by 2-mercaptoethanol treatment of sera (7), and that immunoglobulin M titers are generally considered indicative of recent infection. Since immunoglobulin M is the predominant antibody measured, a question may be raised concerning its stability when it is frozen. Positive equine sera were tested both fresh and after freezing at −20°C. No effect on the levels of antibody were observed. Furthermore, equine sera frozen for approximately 5 years had the same level of seropositivity as fresh sera.

A definitive demonstration of equine infection with *L. pneumophila* depends on isolation of the agent. Currently, we are attempting to isolate *L. pneumophila* from horses with respiratory tract

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**TABLE 4. Prevalence of antibody to *L. pneumophila* in equine sera**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. tested</th>
<th>No. with antibodies to the following serogroups:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>≤1</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>2–3</td>
<td>79</td>
<td>6</td>
</tr>
<tr>
<td>4–7</td>
<td>132</td>
<td>25</td>
</tr>
<tr>
<td>8–12</td>
<td>127</td>
<td>35</td>
</tr>
<tr>
<td>≥13</td>
<td>74</td>
<td>21</td>
</tr>
</tbody>
</table>

a Titer of ≥1:64 to each serogroup antigen.

b Titer of ≥1:64 to at least one serogroup antigen.

c The numbers in parentheses are percentages.
infections. Experimental infections of horse with \textit{L. pneumophila} are also in progress.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


ERRATA

Use of Arginine Aminopeptidase Activity in Characterization of Arginine-Utilizing Mycoplasmas

H. J. BALL, S. D. NEILL, AND L. R. REID
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Volume 15, no. 1, p. 28, column 1, line 15: "naphthylamide derivatives" should read "naphthylamide derivatives (8a)."
Page 28, column 2, line 14: "of Neill and Ball" should read "of Neill and Ball (8a)."
Page 29, column 1, line 30: "of Neill and Ball" should read "of Neill and Ball (8a)."
Page 33, column 2, line 8: "method of Neill and Ball" should read "method of Neill and Ball (8a)."

Hemadsorption Immunosorbent Technique for Determination of Mumps Immunoglobulin M Antibody

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Volume 15, no. 1, p. 82, abstract, line 2: "into" should read "onto."
Page 83, column 1, line 24: "400 × g" should read "40 × g."
Page 83, column 1, line 33: "1:20" should read "1:40."
Page 85, column 2, Literature Cited, reference 7, line 3: "hepatitis virus" should read "hepatitis A virus."

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Volume 15, no. 1, p. 130, column 1: The present address should be deleted.
Page 130, column 2, lines 24 and 25: "the prevalence of an antibody" should read "the prevalence of antibody."
Page 131, column 1, line 16: "Monoglian" should read "Mongolian."