Distribution of *Legionella pneumophila* Antibody Among Primate Species

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Sera representing 16 different primate species were surveyed by indirect immunofluorescence for evidence of antibody to *Legionella pneumophila*. The presence of antibody in Old and New World monkeys and in apes supports previous observations of the ubiquity of *Legionella pneumophila*.

Impressive progress has been made in the recognition and delineation of the agent *Legionella pneumophila* (1) since the epidemic of Legionnaires disease (LD) in Philadelphia during 1976. Current as well as retrospective diagnoses of LD have indicated that outbreaks in many parts of the United States, as well as in Spain, the United Kingdom, Denmark, The Netherlands, Australia, and Israel (9, 10) have been due to *L. pneumophila*. Several important epidemiological parameters of LD remain to be determined. These include information on vectors, reservoirs, and natural modes of transmission. Preliminary reports on experimental infections of animals and their tissues have recently been made (6; R. J. Arko, K. H. Wong, W. O. Schalla, and W. L. Peacock, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B78, p. 28; C. M. Patton, S. E. Johnson, and A. F. Kaufmann, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B79, p. 28).

To understand the possible role of animals, particularly nonhuman primates, as reservoirs, a survey for antibodies to *L. pneumophila* was undertaken. In a seroepidemiological survey of 1,200 normal human sera, a 22.5% incidence of *L. pneumophila* antibody was reported (2). These investigators screened sera at 1:16 by using a microhemagglutination test and an indirect fluorescent-antibody (IFA) technique. We have employed a comparably sensitive IFA assay and demonstrated reactivity to *L. pneumophila* in sera from a variety of nonhuman primates in the wild and in captivity.

MATERIALS AND METHODS

IFA reagents, Philadelphia type 1 antigen, LD-positive human control serum, yolk sac diluent, and fluorescein-conjugated rabbit anti-human globulin were provided by the Biological Products Division, Centers for Disease Control, Atlanta, Ga. (catalog no. KK0003, 78-0290K and 79-0103K). Fluorescein-labeled anti-human immunoglobulin G produced in goats was obtained through commercial sources (Melyo Laboratories, Inc., Springfield, Va.).

Procedures used were those of Wilkinson (13). Eight-well (6-mm), epoxy-coated slides (Bellco Glass Inc., Vineland, N.J.) were observed with a Leitz Dialux 20 microscope equipped with a Ploemopak epi-illumination source (Ploemopak, Wetzlar, Germany). All sera exhibiting fluorescence at a screening dilution of 1:20 were subsequently titrated at twofold serial dilutions through 1:1,280. Positive human control serum was titrated with each battery of tests. The titer of a serum was the last dilution that exhibited fluorescence.

As the National Institutes of Health and World Health Organization Collaborating Center for Reference and Research in Simian Viruses, laboratories at the Southwest Foundation for Research and Education (SFRE) function as a repository for simian viruses and antisera, as well as for sera from various species of primates (Table 1), and serve as diagnostic facilities for primate centers worldwide (4). Sera for fluorescent-antibody testing were obtained from this collection, and unless indicated specifically otherwise, were selected to represent random surveys of normal humans and animals of the various species.

RESULTS

Levels of *L. pneumophila* antibody in human and ape sera are shown in Fig. 1. The dotted line at ca. 1:256 designates the level which the Centers for Disease Control suggest as indicative of infection with the *L. pneumophila* at some undetermined time in humans. All groups tested had prior exposure to *L. pneumophila* or organisms with cross-reacting antigens and responded by the production of various levels of antibody. Antibody levels and frequency were lowest in orangutans and highest in captive chimpanzees and gibbons. Of 14 chimpanzees newly arrived from Africa, 8 displayed titers $\geq 1:20$; these levels were generally lower than those for captive chimpanzees.

Twenty-seven human sera were randomly selected from samples of SFRE staff members, including administrative, technical, animal support, and new personnel. Only one of these sera had a titer greater than 1:256 (1:320), one had a

TABLE 1. Source of primate sera used in *L. pneumophila* antibody survey

Common name (Species)	Source	Yr collected
Human (<i>Homo sapiens</i>)	SFRE	1978
Gorilla (<i>Gorilla gorilla</i>)	Yerkes Regional Primate Research Center, Atlanta, Ga., and Gladys Porter Zoo, Brownsville, Tex.	1978, 1979
Gibbon (<i>Hylobates</i> sp.)		
Orangutan (<i>Pongo</i> sp.)		
Chimpanzee-African (<i>Pan troglodytes</i>)	SFRE, Africa	1967
Chimpanzee-captive (<i>Pan troglodytes</i>)	SFRE	1978, 1979
Baboon-African (<i>Papio cynocephalus</i>)	SFRE, Africa	1968
Baboon-captive (<i>Papio cynocephalus</i>)	SFRE, San Antonio, Tex.	1964-1979
Cynomolgus (<i>Macaca fascicularis</i>)	Lederle Laboratories, Pearl River, N.Y.	1973
Rhesus (<i>Macaca mulatta</i>)	SFRE	1973
Squirrel monkey (<i>Saimiri sciureus</i>)	SFRE	1973
Capuchin (<i>Cebus apella, albifrons</i>)	SFRE	1972, 1973
Spider monkey (<i>Ateles</i> sp.)	Gulf South Research, New Iberia, La.	1978
Owl monkey (<i>Aotus trivirgatus</i>)	Baylor University School of Medicine, Houston, Tex.	1974-1976
Howler monkey (<i>Alouatta palliata</i>)	National Institute of Hygiene, Caracas, Venezuela	1971
Marmoset (<i>Saguinus oedipus</i>)	SFRE	1967-1975
Japanese macaque (<i>Macaca fuscata</i>)	Dryden Colony - Arashiyama West, Primate Research Ranch, Laredo, Tex.	1978
African green (<i>Cercopithecus aethiops</i>)	SFRE	1973

FIG. 1. *L. pneumophila* antibody levels in human and ape sera. * Sera with titers of 1:20 or greater divided by total number tested (percent $\geq$ 1:20).

titer of 1:160, and the remaining 25 sera had titers of 1:80 or less. Few sera (1 from a human, 5 from chimpanzees, and 4 from gibbons) had titers greater than 1:256.

Figure 2 shows antibody in sequential bleedings of captive chimpanzees that had revealed elevated ($\geq$ 1:80) *L. pneumophila* titers in the survey. Two juvenile animals, 4X50 and 4-38 (born in 1976 and 1975, respectively), exhibited a fourfold or greater antibody rise between 1976 and 1978. Whereas clinical records of older animals (born in the period from 1957 to 1962) revealed no remarkable sequelae, the juveniles

were noted to have experienced runny noses during the fall of 1978 and spring of 1979.

This ill-defined syndrome, with its common-cold like manifestations, is frequently observed among young captive chimpanzees in spring or fall. Although of no apparent serious clinical consequence, these incidences in our colony traditionally inspire throat, rectal, and hematological samplings of the animals involved. To date no microbial etiology has been ascribed.

In Table 2 are listed the serological results for an experimental group of young chimpanzees from which acute and convalescent sera (12/

FIG. 2. Persistence of *L. pneumophila* antibody in captive chimpanzees.TABLE 2. Persistence of *L. pneumophila* antibody in infant and juvenile chimpanzees

Animal no.	Birth date (mo/yr)	Serological results ^a on following sample date (mo/yr)				
		12/75	1/76	4/77	4/78	2/79
4X8	4/74	40	20	40	40	40
4X9	4/74	40	80	<20	NT ^b	NT
4X10	7/74	20	40	<20	<20	40
4X16	10/74	<20	<20	<20	<20	<20
4X23	11/74	<20	80	20	NT	NT
4X41	10/75	40	<20	20	<20	40
4X43	11/71	640	640	NT	40	<20
4X54	11/72	640	320	320	160	160

^a Given as reciprocal of IFA titer.^b NT, Not tested.

1975 through 1/1976) were obtained relative to an outbreak of the above-mentioned runny nose syndrome. There were no remarkable *L. pneumophila* seroconversions within the group, a possible exception being animal 4X23. To determine whether younger animals (<2 years) might have subsequently converted to levels seen in animals 4X43 and 4X54, sera from later samplings were tested. Again, no seroconversions were evident. One (4X54) of two animals with elevated titers in January 1976 maintained substantial antibody over a 3-year period; the titer of the other animal (4X43) subsided to <1:20.

L. pneumophila antibody profiles of several groups of Old World primates are shown in Fig. 3. Japanese macaques were unique among all species tested in total absence of antibody; i.e., none was positive at a 1:20 dilution. Generally, baboons exhibited low incidences of antibody, with only 5 of 30 captive and 1 of 15 African animals having titers $\geq$ 1:20. A group of infant ($\leq$ 1 year) baboons had marginal antibody levels,

with a single interesting exception. The animal which displayed a titer of 1:80 was, in fact, a fetus, cesarean-derived in the third trimester; maternal serum titer was 1:40.

An additional group of sera from sick baboons which were hospitalized with various clinical manifestations was assayed for *L. pneumophila* antibody. Of 18 samples from this group, 3 had minimal titers of 1:20; 15 were <1:20 (data not shown). Clinical profiles indicated that the animals had been removed from experimental or breeding situations to be treated for such symptoms as weakness, anorexia, diarrhea, and trauma. Two of these were described as having respiratory distress (see below) that was suggestive of possible tuberculosis infection. These two, which died shortly after admission, displayed extensive pulmonary consolidation at necropsy. No viable organisms were recovered, but one lung smear revealed positive acid-fast staining. *L. pneumophila* antibody titers in both were <1:20.

Distribution of *L. pneumophila* antibody in sera of New World primate species is shown in Fig. 4. A high incidence of antibody in spider and capuchin monkeys is in marked contrast to a single positive incidence in the owl monkey and marmoset groups.

DISCUSSION

Clinical evaluations based on testing single serum specimens are particularly tenuous; moreover it remains to be determined what levels of antibody, as detected by various assay systems, were definitive in the diagnosis of LD in experimental animal systems in which acute and convalescent sera had not been examined. More obscure still is the significance of antibody levels

FIG. 3. *L. pneumophila* antibody in Old World monkeys. See legend to Fig. 1 for explanation of labeling.

FIG. 4. *L. pneumophila* antibody in New World monkeys. See legend to Fig. 1 for explanation of labeling.

observed in normal animal (simian) populations. Is the arbitrary titer of 1:256 designated as the titer for humans the same as that for simians? Until data are available providing a specific answer, this question remains an enigma. In a recent survey of normal human sera (2), microhemagglutination and IFA assays compared favorably (94.9% agreement). A screening of 1,200 sera at a 1:16 dilution with the microhemagglutination technique showed a 22.5% incidence of antibody $\geq 1:16$.

Although sera were screened against only one (Philadelphia type 1) of several serotypes, survey data described herein substantiate previous reports of the ubiquity of *L. pneumophila* (2, 8). Although our own data clearly indicate a wide distribution of *L. pneumophila* antibodies in

primates, often at substantial levels, the relatively small numbers of samples among the species surveyed preclude definitive statistical evaluation. Accordingly, attempts to reconcile our 55% (15 of 27) incidence of *L. pneumophila*-positive human sera with the above-mentioned 22.5% incidence are unwarranted.

Previous studies in this laboratory (5) as well as in those of others (11) have demonstrated the suitability of using the human immunofluorescence reagents as employed herein, although some sensitivity may be lost in testing New World monkey sera. However, if sensitivity decreases among the more divergent species tested, one would anticipate a concomitant reduction in incidence and levels of antibody. In view of this, *L. pneumophila* antibody levels such as those

observed in spider monkeys are particularly striking.

There is a twofold purpose in defining the disposition of the nonhuman primate relative to Legionnaires disease. The availability of a primate model which reflects the pathogenesis and immune response to *L. pneumophila* akin to the human disease would be of value for evaluating a vaccine if so desired. Limited studies have described varied susceptibility in laboratory rodents; a lack of susceptibility was seen in fowl species (Arko et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B78, p. 28; Patton et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B79, p. 28). To date, a single report (6) describes *L. pneumophila* infection in primate tissue: in vitro replication and destruction of cynomolgus monkey alveolar macrophages. In addition, mention is made of unpublished observations of successful aerosol-induced LD infection in the same species.

A second aspect is the veterinary implications of LD. Long an enigma to the primate veterinarian, respiratory illnesses are most often clinically presented when the subject has finally reached moribund conditions or at necropsy. Furthermore, this typical scenario most often precludes the possibility of obtaining suitable paired serum samples from such animals. Preliminary studies were unable to relate *L. pneumophila* to incidents of mild respiratory distress in chimpanzees or in groups of hospitalized baboons. However, the preponderance of epidemiological reports indicate an attack rate of 23% in humans (3) with the clinical picture of LD seldom limited to pulmonary findings (12). When one considers current evidence of widespread geographic outbreaks of LD and the persistence of the organism in natural environs (8), a report describing a 32-year-old isolate of *L. pneumophila* is not really surprising (7). Newly recognized, but hardly new, this fastidious but tenacious microbe has apparently long been in our midst.

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