Prevalence Of *Legionella* In Pharyngeal Secretions Of Patients With Pharyngitis

Dennis J. Baumgardner, M.D., Diana K. Johnson, M.T., and Joseph E. Ross, M.D.

Abstract: **A serological investigation has suggested that *Legionella pneumophila* may be associated with sore throat in adults. In a study of 177 adults and children with acute pharyngitis, *Legionella* species were not isolated from pharyngeal cultures, which utilized selective and nonselective buffered charcoal-yeast extract media. Group A beta-hemolytic streptococci were isolated from 14 percent of the 177 symptomatic patients. Throat cultures from 88 asymptomatic control subjects were negative for *Legionella* and beta-hemolytic streptococci. Further studies are needed to determine if *Legionella* species are associated with acute pharyngitis. (JABFP 1988; 1:24-28.)**

Pharyngitis is one of the most common illnesses seen by primary care physicians. It accounts for up to five percent of pediatric visits and 40 million annual adult visits.[1] Some 300 million dollars are spent each year in diagnosis and treatment of pharyngitis, and an estimated 100 million workdays are lost each year due to sore throat.[2,3] The approach to clinical diagnosis and appropriate use of antibiotics remains controversial.

While most cases of pharyngitis are attributed to streptococcal, viral, or mycoplasmal[1,4] infections, studies involving culture and serological data have failed to identify an etiologic agent in about one-half of the subjects.[5-7] In the case of *Chlamydia trachomatis*, for example, serological data suggesting an etiologic relationship with pharyngitis[4] were not confirmed by studies utilizing cultures.[2,3] Because one such serological study[6] suggested that *Legionella pneumophila* may be associated with acute pharyngitis, a controlled pilot study was undertaken to determine the prevalence of *Legionella* organisms in pharyngeal secretions of patients with acute pharyngitis.

Subjects and Methods

From July 8, 1985, until December 3, 1985, 177 experimental subjects were studied. This group represented all willing patients, or children with parental consent, who came to the Rockford, Illinois, Family Practice Residency Clinic during laboratory business hours. Included were patients seen at initial presentation for acute subjective sore throat and children over one year of age with febrile illness who had clinical signs of pharyngitis as defined by pharyngeal erythema. Excluded were patients who had received antibiotics in the previous 48 hours. A control population of 88 afebrile volunteers, without clinical signs of pharyngitis, who had been free of subjective sore throat for the preceding eight-week period, was selected from the same patient panel. After informed consent, a questionnaire was administered, and all patients received an examination and a throat culture by a resident or staff physician.

Throat cultures were obtained by swabbing the posterior pharynx with rayon-tipped double swabs in a mixed salts-phosphate-sodium thioglycolate transport medium (Precision Pledget Vial Double Swab). Specimens were processed within four hours by the second author at Biomedical Services Laboratory, University of Illinois College of Medicine at Rockford. One of the swabs was used for identification of group A beta-hemolytic streptococci using standard bacitracin disk/blood agar plates.[7]

The second swab was streaked on nonselective, L-cysteine and 0.1 percent alpha-keto-glutarate-supplemented, buffered, charcoal yeast-extract (GIBCO™) agar plates. Specimens were also plated on identical media containing cefamandole and polymixin B for selective culture.[8] *Legionella* cultures were incubated in humidified air at 95°F (35°C) for at least five to seven days. Acid wash pretreatment, which reduces the amount of back-

From the Department of Family Medicine and the Department of Biomedical Sciences, University of Illinois College of Medicine at Rockford. Address reprint requests to Dennis J. Baumgardner, M.D., Department of Family Medicine, St. Luke's Family Practice Center, Medical College of Wisconsin, 1110 S. 24th Street, Milwaukee, WI 53204

Supported by MSP Grant #2-6-51817 from the University of Illinois College of Medicine at Rockford.

ground normal flora in sputum cultures for *L. pneumophila*,[8] did not significantly reduce the amount of normal flora colonies surviving on the nonselective media when pharyngeal secretions were streaked for isolation (data not shown), and it was omitted from our protocol. Colonies with morphology typical of *Legionella* species were tested for gram stain characteristics and ability to be subcultured on buffered charcoal yeast-extract agar and on blood agar. Where necessary, biochemical tests[9] were performed. Media and culture conditions were routinely checked using a control *L. pneumophila* serogroup 1 strain.

The mean age of the 177 experimental group patients was 21.5 years (range, 1–67 years); the mean age of the control group was 22.1 years (range 1–62 years). There was no significant difference between the two groups regarding age and sex by decade. Both groups were 38 percent men. Smokers accounted for 12 percent of the experimental group and 23 percent of the control group ($P < 0.05$). Three of the 177 experimental patients had diabetes mellitus and four had asthma. Of the 88 control patients, one patient was diabetic; two had asthma; one was a child with bronchopulmonary dysplasia; and one child was receiving prednisone for nephrotic syndrome. No other patients from either group were receiving corticosteroids or immunosuppressive drugs. Exclusive of the previous 48 hours, eight percent of the experimental patients and 10 percent of the control patients had received a course of antibiotics in the prior eight weeks ($P > 0.50$).

Experimental patients had been ill an average of 6.5 days, and 17 percent of them had oral temperatures of 101°F (38.2°C) or greater at the time of presentation. Chills were reported by 36 percent of the experimental patients, 46 percent reported headaches, 55 percent had coughs, 61 percent had nasal congestion or rhinorrhea, seven percent had diarrhea, and 22 percent reported nausea or vomiting. Pharyngeal or tonsillar exudate was found in 31 percent of the experimental patients. Of the 177 pharyngitis cases, 16 had evidence of acute otitis media, four had signs of serous otitis media, and three patients had clinical evidence of pneumonia.

Results

Twenty-five experimental patients (14 percent) and none of the control patients had throat cultures positive for group A beta-hemolytic streptococci.

Pharyngeal cultures were negative for *Legionella* or *Legionella*-like organisms in all 177 experimental patients and in all control group subjects. Growth of normal flora organisms on the nonselective, buffered, charcoal yeast-extract agar was generally heavy but in most instances did not preclude observation of individual colony morphologies. We found, as did Bridge and Edelstein,[10] that media supplemented with cefamandole and polymyxin B appear to inhibit most normal oropharyngeal microorganisms. Two of the most common survivors included *Acinetobacter* and *Bacillus* species.

Discussion

Infections caused by the more than 20 recognized *Legionella* species and the properties of these unique organisms have been well described in a recent monograph[11] and reviews.[12,13] Classically, legionellosis has taken two basic forms. Legionnaires' disease, which may be epidemic or sporadic, has a low attack rate but is often a progressive, sometimes fatal, pneumonia. Pontiac fever has a very high attack rate but is a brief, self-limited disease characterized by fever, chills, cough, chest pain, and nausea. The clinical spectrum of *Legionella* disease is expanding and apparently includes upper respiratory tract disease, at least in compromised hosts.[14] Pharyngitis is not considered a symptom of legionellosis, though all five patients in a series of cases of Legionnaires' disease in Seattle had sore throat.[15]

Serological data suggest that minor *Legionella* infections may be common. Up to 20–40 percent of normal community subjects develop antibodies to *L. pneumophila* without having typical Legionnaires' disease.[15,16] While some of these incidences may represent Pontiac fever, nonspecific monoclonal antibody stimulation, or serologic conversion to other cross-reacting microorganisms, others could conceivably represent another type of upper respiratory tract infection. It is not known precisely how people acquire Legionnaires' disease, and person-to-person spread has not been described. Legionellae are ubiquitous in our environment,[11] and proposed routes of infection include those that are directly via aerosols or perhaps through the oropharynx.

Bridge and Edelstein[10] concluded that oropharyngeal colonization with *L. pneumophila* oc-

curs infrequently. Their study was based on 186 afebrile, pneumonia-free volunteers. Throat swabs were cultured for *Legionella* and used for direct fluorescent antibody test; however, no one in the study, which utilized a selective medium, was culture positive. No cultures were obtained from patients with pharyngitis. Komaroff, et al. in a study of 763 adult pharyngitis patients,[6] demonstrated a rise in serum *L. pneumophila* antibody titers in two percent of their cases. This study, however, did not include attempts to culture *Legionella* from the throat.

In our pilot study of 177 patients with acute pharyngitis, no *Legionella* organisms were isolated from pharyngeal secretions. Serology was not performed in this pilot study. Culture has been shown to be more sensitive than direct fluorescent antibody staining, with one organism per smear representing approximately 10^4 bacteria per mL of respiratory secretions.[10] If the serological responses reported by Komaroff, et al. were indeed indicative of *Legionella* pharyngitis rather than nonspecific or cross-reactive antibody stimulation or other *Legionella* infection, several explanations may be offered for our failure to demonstrate the organism by throat culture.

The number of patients may have been too small to observe a low prevalence of *Legionella* in pharyngitis. This study was conducted during the months of highest incidence of sporadic legionellosis;[17] however, no significant epidemic of Legionnaires' disease has ever been reported in Northwestern Illinois.

While the standard medium used in this study is often satisfactory for isolation of *Legionella* from sputum, it may not be optimum for isolation from pharyngeal secretions. Sodium-containing transport media, such as used here, may diminish recovery of *Legionella* from clinical specimens. Feeley, et al.[18] described lower recovery rates of *L. pneumophila* from tissue inoculum when supplemental sodium chloride was added to solid charcoal-yeast extract media. Bridge and Edelstein[10] found that calcium alginate or rayon swabs yielded higher recovery percentages from *L. pneumophila* suspensions when swabs were dissolved in distilled water rather than in Ringer solution with sodium hexametaphosphate. This difference, however, was less than an order of magnitude. Finally, one study has shown that a dialyzable substance, produced by several bacteria considered to be normal human pharyngeal inhabitants, specifically inhibited the growth of *L. pneumophila* in vitro.[19] The importance of this phenomenon in vivo, and implications regarding the ability of legionellae to cause pharyngitis or to be recovered in throat cultures, is uncertain. Further investigations are needed, perhaps in more endemic areas, to determine the role of *Legionella* species in acute pharyngitis. Definitive studies may require a large panel of patients, refinements in culture and transport media, and serological data. Moreover, many studies are still needed to define the role of other potential pathogens in unexplained pharyngitis. The scope of this problem in primary care should justify the increasingly sophisticated methodology that may be required.

We acknowledge Thomas Root for helpful discussions and the assistance of Jackie Ring, Dorice Murphy, and Rita Huwe.

References

1. Mandel JH. Pharyngeal infections. Causes, findings, and management. Postgrad Med 1985; 77:187-93, 196-9.
2. Gerber MA, Ryan RW, Tilton RC, Watson JE. Role of Chlamydia trachomatis in acute pharyngitis in young adults. J Clin Microbiol 1984; 20:993-4.
3. Huss H., Jungkind D, Amadio P, Rubenfeld I. Frequency of Chlamydia trachomatis as the cause of pharyngitis. J Clin Microbiol 1985; 22:858-60.
4. Komaroff AL, Aronson MD, Pass TM, Ervin CT, Branch WT Jr, Schachter J. Serologic evidence of chlamydial and mycoplasmal pharyngitis in adults. Science 1983; 222:927-9.
5. Baker LH, Hodges GR. Examination of pharyngeal secretions to determine the etiology of pharyngitis. Am J Med Sci 1976; 272:89-93.
6. Komaroff AL, Aronson MD, Pass TM, Ervin CT. The etiology of sore throat in adults. Clin Res 1980; 28:227A.
7. Anderson LJ, Patriarca PA, Hierholzer JC, Noble GR. Viral respiratory illnesses. Med Clin North Am 1983; 67:1009-30.
8. Edelstein PH. Improved semiselective medium for isolation of Legionella pneumophila from contaminated clinical and environmental specimens. J Clin Microbiol 1981; 14:298-303.
9. Feeley JC, Gorman GW. Legionella. In: Lennette EH, Balows A, Hausler WJ Jr, Truant JP, eds. *Manual of clinical microbiology*. 3rd ed. Washington, D.C.: American Society for Microbiology, 1980:318-24.
10. Bridge JA, Edelstein PH. Oropharyngeal colonization with Legionella pneumophila. J Clin Microbiol 1983; 18:1108-12.
11. Thornsberry C, Balows A, Feeley JC, Jakubowski W, eds. Legionella: proceedings of the 2nd international symposium. Washington, D.C.: American Society for Microbiology, 1984.
12. Woodhead MA, Macfarlane JT. The protean manifestations of Legionnaires' disease. J R Coll Phys Lond 1985; 19:224-30.
13. Helms CM, Viner JP, Weisenburger DD, Chiu LC,

Renner ED, Johnson W. Sporadic Legionnaires' disease: clinical observations on 87 nosocomial and community-acquired cases. Am J Med Sci 1984; 288:2-12.
14. Schlanger G, Lutwick LI, Kurzman M, Hoch B, Chandler FW. Sinusitis caused by Legionella pneumophila in a patient with the acquired immune deficiency syndrome. Am J Med 1984; 77:957-60.
15. Foy HF, Broome CV, Hayes PS, Allan I, Cooney MK, Tobe R. Legionnaires' disease in a prepaid medical-care group in Seattle, 1963-75. Lancet 1979; 1:767-70.
16. Plouffe JF, Baird IM. Age related immunity to Legionella pneumophila in a community population. In: Thornsberry C, Balows A, Feeley JC, Jakubowski W, eds. Legionella: proceedings of the 2nd international symposium. Washington, D.C.: American Society for Microbiology, 1984:186-7.
17. England AC 3d, Fraser DW, Plikaytis BD, Tsai TF, Storch G, Broome CV. Sporadic legionellosis in the United States: the first thousand cases. Ann Intern Med 1981; 94:164-70.
18. Feeley JC, Gibson RJ, Gorman GW, et al. Charcoal-yeast extract agar: primary isolation medium for Legionella pneumophila. J Clin Microbiol 1979; 10:437-41.
19. Flesher AR, Kasper DL, Modern PA, Mason EO Jr. Legionella pneumophila: growth inhibition by human pharyngeal flora. J Infect Dis 1980; 142: 313-7.

Editors' Comment

We, the Editors, feel this article has merit, and we are interested in publishing important pilot studies. For that reason, we offer the original manuscript, the reviewers' criticisms, and the authors' response. We recognize this is not a commonly used technique, but we believe it is an interesting innovation in medical journalism to offer the reader the opportunity to determine for himself the value of the study. In addition, it would seem to us that this technique would potentially stimulate others to investigate the questions.

"Prevalence of *Legionella* in Pharyngeal Secretions of Patients with Pharyngitis" was reviewed by five reviewers who had conflicting opinions about the manuscript. Their concerns were that this investigation of asymptomatic controls and patients with pharyngitis failed to grow *Legionella* from anyone. This was different from the expectation that approximately two percent of the patients with pharyngitis would have *Legionella* infection. If that prevalence is correct, the finding of no infection in 177 patients is right at the five percent level of significance by Fisher's exact test.

Other observations were that there were probably too few subjects. This, of course, would have assisted in the above problem. The common objection by all reviewers was that there was no serologic data presented that would have confirmed the two percent serologic conversion rate reported by other investigators. They also noted that there were no immunofluorescent staining data and that the investigator did not use accepted methods for processing specimens for plating on nonselected media; that is, no acid wash pretreatment. There was some concern that only 48 hours without antibiotics might be a little short in duration and that the average duration of illness at presentation might have been a little bit too long.

Authors' Comment

The *Journal* is to be commended for the obvious painstaking steps that were taken to solicit a thorough review of our paper. We do, however, take exception to several of the reviewers' criticisms.

First, let us reemphasize, as the title and introduction of our article state, that this work represented a *pilot* study utilizing culture to determine the *prevalence* of *Legionella* species in the pharyngeal secretions of patients with pharyngitis. This was not intended to represent a definitive study of *Legionella* as an etiologic agent in acute pharyngitis.

Its purpose, rather, was to guide further research in this area. Mindful of this, individual criticisms are now addressed.

Regarding subject number, we felt that while the results of the serological study cited in our article (Komaroff, Aronson, Pass, Ervin, Ref. 6) suggested a possible association of *L. pneumophila* with acute pharyngitis, the two percent seroconversion rate was not a reasonable indicator of positive outcomes in our study. The reasons are as follows:

Komaroff's report indicated that acute and convalescent sera for antibodies to Legionnaires' *Bacillus* were obtained from stratified patient samples. As this report was published in abstract form only, we could not fully evaluate the protocol utilized in the study or its direct applicability to our study.

In a letter dated September 20, 1984, the second author of this abstract, Mark D. Aronson, indicated that the serologic data in question were originally submitted as part of a publication in *Science* (Science 1983; 222:927-9). He indicated that the reviewers challenged the data and the authors unfortunately had to withdraw them.

Based on the time when the study was reported, we would presume that these antibodies represented four of the six now known serotypes of *L. pneumophila* and would not necessarily reflect serologic responses to the twenty-odd other known *Legionella* species. Our study utilizing culture would have potentially isolated nonpneumophila species of *Legionella* and other *Legionella*-like organisms.

It is also possible that the seroconversions reported by Komaroff were indicative of *Legionella* infection elsewhere or were, in fact, the result of nonspecific or cross-reactive antibody stimulation. This fact was pointed out in our article in the discussion.

Lastly, the study of Komaroff dealt with an adult population, and these numbers would not necessarily be applicable to our population, which was approximately one-half pediatric patients.

Serologic data would certainly be part of a definitive study on the subject. While our protocol called for performance of serological tests on culture-positive patients, serological tests on every patient were not obtained, because the aim of this pilot study was to ascertain whether or not *Legionella* organisms could commonly be isolated from pharyngeal secretions of symptomatic patients.

As mentioned, the other drawback to serology is that it tests only for *L. pneumophila* and not for the other species. In the same manner, direct immunofluorescent staining would certainly be considered for a more definitive study, but it was not chosen as part of our protocol. Aside from the facts that DFA staining is specific only for the pneumophila species and that there have been reports of other non-*Legionella* cross-reacting organisms, culture has been shown to be more sensitive than DFA staining for demonstration of *Legionella*. As we mentioned, legionellae need to be present in numbers of approximately 10^4 bacteria per mL of respiratory secretions in order to observe one organism per high-powered field with DFA stain.

It was clearly stated why an acid wash pretreatment was not used in our nonselective media protocol. In our hands, this acid wash pretreatment did not significantly reduce the amount of normal flora colonies surviving on nonselective media when pharyngeal secretions were streaked for isolation, with and without an acid prewash treatment. These are unpublished data, which are cited in our article.

Regarding our stipulation of only 48 hours without antibiotics prior to admission to the study, we agree that this is a point of controversy. Our local infectious disease consultant, who reviewed our protocol, felt that this was a reasonable cutoff. In actual fact, our data revealed that only eight percent of experimental patients and only 10 percent of control patients had received any antibiotics in the prior eight weeks.

The reviewers were also surprised that our patients had been ill for an average of 6.5 days at the time of presentation. In planning our protocol, we did not exclude patients on the basis of "days since onset of illness." This was done so as not to select out a group of patients with pharyngitis of a particular natural history. An informal investigation into these data suggested that the longer-than-expected duration of illness reflected a trend in our patient panel only to seek medical care for a more persistent sore throat. More brief illnesses were, perhaps, treated at home. It should be noted, however, that 14 percent of our pharyngitis patients were culture-positive for group A beta-hemolytic streptococci, and this compares favorably with the *Streptococcus* positive results of numerous other studies of acute pharyngitis.

In summary, we feel that many of the objections raised by the reviewers have a rational and scientific explanation in the context of a pilot study such as this one.

The authors acknowledge the assistance of Steven Lawrence and Marie Kania in preparing this response.