

## CHAPTER 229

*Legionella*

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**HISTORY**

Legionnaires' disease is an acute pneumonic illness caused by gram-negative bacilli of the genus *Legionella*, the most common of which is *Legionella pneumophila*. Pontiac fever is a febrile, nonpneumonic, systemic illness closely associated with, if not caused by, *Legionella* species. *Legionellosis* is the term that encompasses all diseases caused by, or presumed to be caused by, the *Legionella* bacteria, including legionnaires' disease, focal nonpulmonary infections, and Pontiac fever.

Legionnaires' disease was first recognized when it caused an epidemic of pneumonia at a Pennsylvania State American Legion convention in Philadelphia in 1976; 221 people were affected, and 34 died. Despite intensive laboratory investigation, the cause of the outbreak went undetected for many months. An epidemiologic investigation determined that the disease was most likely airborne and focused primarily at one convention hotel, which closed because of adverse publicity.<sup>1</sup> About 6 months later, two investigators at the United States Centers for Disease Control and Prevention, Joseph McDade and Charles Shepard, announced that they had discovered the etiologic agent, a fastidious gram-negative bacillus.<sup>2</sup> Because of the historical association with the American Legion convention, this disease is now called legionnaires' disease, and the etiologic agents belong to the family *Legionellaceae*, with *L. pneumophila* being the agent responsible for the 1976 Philadelphia epidemic. Use of an antibody test for the disease showed that several prior unsolved outbreaks of pneumonia had been legionnaires' disease, including epidemics investigated in the 1950s and 1960s.<sup>3,4</sup> The availability of diagnostic tests uncovered a 6-year-long epidemic of legionnaires' disease amongst British tourists staying at one hotel in Spain.<sup>1</sup> An unsolved epidemic of a nonpneumonic febrile illness was also found to have resulted from exposure to *Legionella* bacteria; this illness was termed Pontiac fever, after Pontiac, Michigan, where this had occurred.<sup>6,7</sup> As with legionnaires' disease, prior epidemics of Pontiac fever had occurred as early as 1949 without determination of an etiology.<sup>9</sup> Bacterial culture isolates from the 1940s through the 1960s were found to be *Legionella* bacteria, although at the time they had been thought to be rickettsial agents.<sup>9,14</sup> Thus, both the organism and the disease had been studied decades before, but major advances in technology and epidemiology were required to properly classify the disease and determine its cause.

Even with identification of *L. pneumophila* in 1977 as the cause of legionnaires' disease, the source of the bacterium, factors promoting its multiplication and spread, and ways to abort epidemics of legionnaires' disease remained uncertain for several years. Epidemics of the disease, especially nosocomial ones, commonly lasted for years even though the cause of the disease was recognized.<sup>15-21</sup> Eventually it was discovered that *L. pneumophila* and other *Legionella* species were naturally occurring aquatic bacteria that had a propensity for growing in warm water, most particularly in cooling towers, water heaters, and potable-water plumbing. These discoveries led to the end of several multiyear outbreaks of the disease, and to methods for preventing the disease.-- Now, because of improved diagnostic, environmental detection, and remediation methods, it is unusual for outbreaks of legionnaires' disease to last more than a week or two.

Legionnaires' disease still occurs, both in sporadic and in epidemic form, sometimes involving many hundreds of victims.<sup>23,24</sup> The disease, although a relatively rare cause of community-acquired pneumonia,

can cause high morbidity and mortality if treated improperly. Our current knowledge about the disease has resulted in the ability to abort epidemics in days, to effectively treat affected patients, and to reduce the frequency of the disease by making modifications in building design and maintenance. From a scientific basis, discovery of the disease led to major advances in the study of intracellular pathogens and their interactions with host cells.

**THE ETIOLOGIC AGENT**

The *Legionella* species are small gram-negative bacilli with fastidious growth requirements. Proteins rather than carbohydrates are used as an energy source. Obligate aerobes, the bacteria grow at temperatures ranging from 20° to 42° C. The *Legionella* bacteria are in the taxonomic order *Legionellales*, which includes the families *Coxiellaceae* and *Legionellaceae*. *Coxiella burnetii*, an obligate intracellular parasite and the etiologic agent of Q fever, is a member of the *Coxiellaceae*, and the closest relative of the *Legionellaceae*. Three different genera have been proposed for the *Legionellaceae*: *Legionella*, *Fluoribacter*, and *Tatlockia*; however, the latter two generic names have never been widely used or accepted, and the single genus *Legionella* is almost universally used to describe all species. L-Cysteine is required for the growth of all but one of the clinically important *Legionella* species, and this amino acid is needed for the initial growth of all described *Legionella*, species from environmental or clinical sources. Soluble iron is required for optimal growth and for the initial isolation of the bacterium from both clinical and environmental sources. Iron, L-cysteine, a-ketoglutarate, and charcoal-containing yeast extract agar buffered with an organic buffer (BCYEa agar) is the preferred growth medium for clinical isolation. Clinically important *Legionella* species grow best at 35° C in humidified air on BCYEa medium, usually in 2 to 5 days after inoculation of plates. An incubation of up to 14 days may very rarely be required for the isolation of unusual *Legionella* species.

More than 49 different *Legionella* species have been described, 20 of which have been reported to infect humans.<sup>25,26</sup> *L. pneumophila* contains at least 16 different serogroups; seven other species contain two different serogroups, with the remaining species containing only one serogroup each. *L. pneumophila* serogroup 1 caused the 1976 Philadelphia outbreak and is the cause of 70% to 90% of all cases of legionnaires' disease for which there has been a bacterial isolate.<sup>27,28</sup> *L. pneumophila* serogroup 1 can be further divided into multiple subtypes using a variety of serologic, other phenotypic, and genetic methods. One particular subtype of *L. pneumophila* serogroup 1 causes 67% to 90% of cases of legionnaires' disease due to *L. pneumophila*, and 85% of cases due to *L. pneumophila* serogroup 1; this subtype is distinguished by its reactivity with a particular monoclonal antibody, and it is variously termed the Pontiac, the Joly monoclonal type 2 (MAb2), or the Dresden monoclonal type 3/1 (MAb 3/1) monoclonal subtype.<sup>29</sup> The predominance of the Pontiac subtype has implications for diagnosis (see later).

Most clinical microbiology laboratories should be able to identify *Legionella* bacteria to the genus level by detection of their typical colony morphology and Gram stain appearance, by determination of L-cysteine growth dependence, and by excluding possible mimics by using standard microbiology identification techniques (Fig. 229-1). Identification of *L. pneumophila* serogroup 1, the most common clinical isolate, can be accomplished by sophisticated clinical microbiology laboratories using relatively simple serologic testing. Identification of other *L. pneumophila* serogroups, and other *Legionella* species, is often much more difficult and is best left to specialized reference laboratories.<sup>26</sup> This is because these bacteria are relatively inert in the use of commonly tested biochemical substrates, and they require sophisticated phenotypic, serologic, and molecular testing. Reference laboratory-based phenotypic testing of bacteria, including determination of cellular fatty acids and ubiquinones and protein electrophoresis, can often be used to identify the bacteria. Definitive identification is based on both immunologic detection of surface antigens and bacterial DNA sequencing.