BACTERIOLOGICAL SURVEY OF LEGIONELLA SPECIES IN THE NORTHERN STATES OF NIGERIA

BY

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DECLARATION

I declare that the work reported in this thesis was carried out solely by me at the Laboratory of the Department of Microbiology, Ahmadu Bello University, Zaria, Under the Supervision of Professor N. E. Gomwalk, Professor A. A. Ahmad and Dr. (Mrs) L. E. Odama all of the Department of Microbiology, Ahmadu Bello University, Zaria. The work of other researchers are duly acknowledged and referred to appropriately. I also declare that no part of this work has been submitted elsewhere for a degree.

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CERTIFICATION

This thesis, BACTERIOLOGICAL SURVEY OF *LEGIONELLA* SPECIES IN THE NORTHERN STATES OF NIGERIA, by SABO E. YAKUBU, meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to: My Late Mother, Jummai Hassan Yakubu who, by the Grace of God gave me the needed support and encouragement during my primary education and stood by me thereafter until her departure to eternal rest in Christ Jesus; My Wife, Ladi Yakubu; My children, Rebecca, Yasid, Elizatbeth, Sarah, Ruth and Joy; and all who seek to contribute to our knowledge of the "Killer diseases".

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ABSTRACT

The incidence and densities of *Legionella* species in 663 water samples from both potable and raw water sources in the Northern States of Nigeria were determined by the direct immunofluorescence antibody (DFA) method and, buffered charcoal yeast-extract (BCYE) culture confirmation. Polyvalent pool conjugates reactions detected *Legionella* - like cells in virtually all potable water samples. Using serospecific antisera the presence of each species and serogroup was highlighted. Of the first six serogroups of A. *pneumophila,* serogroup 1 was the most predominant; it was detected in every sample. Serogroup 3 was second in predominance, 87.5% of the water samples exhibited fluorescing cells of *Legionella* morphology. The least detected of the serogroups was serogroup 5; it was found in only 25% of the samples. Serogroup 6 also was detected in 42.5% of the samples. Among the remaining species of *Legionella. L. gormanii* predominated in 85% of the samples, followed by *L. micdadei* (82.5%). *L. bozemanii* was the least detected of all *Legionella* species; only 17.5% of the water samples showed cells that were consistent with *Legionella* morphology.

Detection rates was highest in public and private tap water samples (100%). Hotels water samples comprising mainly of water from air-conditioning cooling systems were 96% positive whereas hospitals were 94% positive for *Legionella* - like bacteria. This high percentage rate dropped for boreholes water samples. Isolation rates were much lower; public and private tap water samples had culturable legionellae in 20% of the samples. Hotels water samples had isolatable legionellae in 66.7% of the samples, while hospitals water samples were 38% positive for legionellae. Boreholes water samples had culturable

legionellae in 25% of the samples screened, while well water samples were 60% positive for *Legionella* spp.

Raw water samples (246) from all the states demonstrated *Legionella* - like cells at varying degrees, from 40% in Abuja to 58% in Bauchi State by polyvalent pool conjugate reaction. Using serospecific antisera, the detection rates increased across all the states sampling areas, the lowest detection rate was 61% in Katsina state to 86% in Gongola State. The interstate variation in relative abundance of *Legiomllaceae* was less than the intraspecies variation. Among the species in all the states. *L. bozemanii* serogroup 1 was the least detected of all, only 47% of the samples demonstrated fluorescing cells characteristic of *Legionella* morphology. Among the serogroups of *L. pneumophila*, serogroup 5 was the least detected (51%). The occurrence of other serogroups or species ranged from 65% to 98% of/,, *pneumophila,* whereas the other species were 47.1% to 86%. The population densities, for the most part ranged from 10^4 to 10^6 cells/ml.

L. pneumophila, the type species, demonstrated the wide spread nature of the first six serogroups in all the states. However, there was a remarkable intraserotypic as well as intrasampling area variation with one or more serotypes predominating in any one given state. Serogroup 5 and 6 were least detected in most states' sampling areas.

Of the 98% (49 out of 50) positive samples, by immunofluorescence, only 46% (23 samples) were positive by culture. These positive samples comprised of 5 rivers. 11 dams. 3 lakes. 2 creeks, one stream, and one pond. However, there were no culturable legionellae in water samples from Benue and Gongola States.

This study has demonstrated the wide distribution of legionellae in our environmental water samples and underscored the need for surveillance and continued monitoring of this group of pathogenic bacteria especially in public buildings and hospitals.

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CHAPTER ONE

INTRODUCTION

During the summer of 1976, an outbreak of a disease of an unknown etiology struck without warning members of the American Legions who were attending their annual convention at a hotel in Philadelphia. The occasion was the 58th annual convention held at the Bellevue-Strafford Hotel in Philadelphia, Pennsylvania, the United States of America (USA). The convention held from July 21 through 24, was attended by some 4, 400 delegates, members of their families and other conventioneers. Drs. Fraser and McDade (1979) reported that between July 22 and August 3, 149 of the conventioneers developed what appeared to be the same puzzling illness, characterised by fever, coughing and pneumonia. It was an unusual explosive outbreak of pneumonia with no apparent cause. Because most of the attendees had returned to their respective homes before becoming ill, it was not until August 2 that reports to the Pennsylvania Department of Health made it clear that an epidemic had occurred among those who had attended the convention. Little did any one realise that the convention was destined to be recorded in the (history of the) annals of medicine. Rightly stressed by Fraser and McDade, "that day marked the start of one of the largest and most complex investigations of an epidemic ever undertaken". Public and scientific attention was focused on the epidemic of pneumonia at the Pennsylvania American Legion convention. "Legionnaires' disease", as the illness was quickly named by the press, was to prove a formidable challenge to epidemiologists and laboratory investigators alike. Only after many months of tireless searching was the causative agent discovered, and more months were to pass before the etiologic agent of the mysterious disease was characterized as a previously unknown bacterium. Meanwhile the dreaded disease

had affected a total of 221 people, and 34 of them had died of pneumonia or its complications (McDade, *et al.*, 1977).

By early January 1977, the etiologic agent of Legionnaires' disease (LD) had been isolated by McDade of the Centers for Disease Control (CDC), Atlanta, Georgia (USA). This *Legionnaires'* disease bacillus (LDB), was unrelated by phenotypic and genetic analysis to any previously described families of bacteria. The bacterium was, therefore, placed in a new family, *Legionellaceae*, as a new genus, *Legionella* (named after the Philadelphia victims), and species, *pneumophila*, after the predilection of the bacterium for the lung (Brenner *et al.*, 1979). Since this initial epidemic of LD., many outbreaks and sporadic cases have been reported in many parts of the world. The CDC, by November 7, 1978, had confirmed 496 sporadic cases and 558 cases associated with outbreaks of LD in the United States. Cases have also occurred in almost all the States in the US, and in many countries, including Canada, Scotland, England, Wales, the Netherlands, Sweden, Denmark, Spain, Italy, Israel, France, Germany and Australia (Meyer *et al.*, 1980).

Since the 1976 outbreak in Philadelphia, health authorities in developed countries have been aware of Legionnaires' disease. In England and Wales in 1984, 151 cases were recorded, of which 15 proved fatal. Most of these were isolated cases or minor outbreaks. However, the worst outbreak was yet to come. A town of less than 60,000 inhabitants, Stafford in England was to achieve notoriety as the home of the world's worst recorded outbreak of LD, (Sherwood, 1985), while Strafford Hotel, Philadelphia in America remains of equal notoriety as the birth place of the world's first recorded major outbreak of Legionnaires' disease. The Stafford outbreak struck only the patients who had come to the hospital on a day visit basis. Consequently, the out patients took the disease home with them, and thus there was no evidence

of an outbreak in the hospital itself. After an intensive investigation, the Stafford District Hospital, a modern showplace among British hospitals was implicated as the source of the outbreak which claimed 39 lives, and possibly 46, with a total of 163 cases, (Sherwood, 1985).

In these two major outbreaks of Legionnaires' disease, Philadelphia 1976 and Stafford 1985, and in subsequent outbreaks and sporadic cases, in many parts of the world, air-conditioning cooling systems and potable water supply have been implicated and proven as sources of outbreaks and cause of the disease (Fraser *et al.*, 1977; Sherwood, 1985; Fox, 1985; Garbe *et al.*, 1985; Shands *et al.*, 1985; Fraser, 1985; Addis *et al.*, 1989; Watson and O'Mahony, 1990).

In the African continent however, the bacterium and the disease it causes have rarely been mentioned. In Southern Africa where a Legionellosis Research Laboratory was established in 1979, Mauff and Koornhof (1984) reported some cases of LD in Johannesburg area and in a Zambian copper mine. They were able also to isolate *L. pneumophila* from environmental samples as well as from patients with the disease or who had died of it - Legionnaires' disease. To my knowledge, however, this is the first study of *Legionellaceae* of this magnitude in the African continent.

I am not aware of any reported case of Legionnaires' disease in West African sub-region, or in Nigeria in particular. However, sporadic outbreaks of mysterious deaths, seasonal epidemics of diseases, "killer diseases" and/or "killer virus" and many unreported deaths occur almost regularly in many parts of Nigeria. The death-toll in our clinics and hospitals is very high, especially on the elderly and debilitated, and post-operative patients.

That epidemic diseases still occur in the twentieth century with heavy toll in human lives is a lesson developing countries can ill-afford to miss. If the modern day America and Britain

could suffer the worst epidemics of the 20th century, and still be threatened by yet another "monster killer" reappearing in the form of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS), the developing world should better learn to value and invest in medical and other scientific endeavors and/or training. This may be the only option, however expensive to minimize the devastating effects of epidemics and 'killer viruses', and to contain or cope with them should they strike in spite.

In view of public health significance of Legionnaires' disease the objectives of this work are to:-

1. Determine the incidence and prevalence of *Legionella* species in the Northern States of Nigeria by carrying out a bacteriological survey of this organism;
2. Document the presence of *Legionella* spp and identify habitats where the organism is found in our particular environment;
3. Isolate *Legionella* spp and compare the isolates with those already identified in America and Western Europe;
4. Develop knowledge of the bacterium and the diseases it causes within the national health service scheme;
5. Define the clinical and epidemiological features of the disease caused by *Legionellaceae;*
6. Suggest preventive measures for Legionnaires' disease;
7. Get the medical practitioners/clinicians and microbiology laboratories aware of the bacterium and the diseases it causes so as to involve them, and encourage continued surveillance and.
8. Generate data that will stimulate interest in *Legionella* species and form a baseline for investigation in the event of an outbreak of Legionellosis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Aspect of Legionnaires' Disease Bacterium

A dramatic and historic outbreak of serious pulmonary infection associated with the American Legionnaires' Convention in Philadelphia, July (1976), led to the recognition of a discrete disease entity, Legionnaires' disease (LD), (Fraser *et al.*, 1977). An exhaustive investigation by McDade *et al.* (1977) led to the isolation and identification of the etiologic agent, (Chandler *et al.*,1977). This organism was shown to be a bacterium that was phenotypically unique, whose deoxyribonucleic acid (DNA) was unrelated to the DNAs of all other species tested (Brenner *et al.*, 1978, 1979 and 1981; Moss *et. al.*, 1977).The bacterium was therefore classified by Brenner *et. al.* (1979) in a new genus and species, *Legionella pneumophila*, in the new family *Legionellaceae*.

Legionella pneumophila was subsequently shown to be a major cause of both pneumonia and an acute, self-limiting febrile disease called Pontiac fever (Kaufmann *et al.*,1981). The public health significance of LD stimulated many intense investigations. It was soon learned that *Legionella pneumophila* had first been isolated in 1947 by Jackson who recovered the organism (OLDA) from a sick guinea pig that had been inoculated with the blood of a patient with a febrile respiratory illness (Jackson *et al.*,1952). Subsequently, it became apparent that neither the bacterium nor the disease was new. The second organism, tagged WIGA was recovered by Bozeman in eggs, from guinea pigs inoculated with lung tissue taken from a patient who had died of what the US Navy called "skin diver's disease". Both organisms, OLDA and WIGA did not grow on bacteriological media and could not be related to the patient's illness with complement fixation tests of a premortem serum (Bozeman

et al,1968). Failure of the two organisms to grow on bacteriological media led to the designation of the organisms as "rickettsia-like agents". Neither had been classified. Both are gram-negative, bacillary-shaped organisms that are somewhat larger than rickettsiae and have a moderate degree of pleomorphism (McDade *et al.*, 1977).

The recognition of the morphologic and biologic similarities of the first isolates, OLDA and WIGA, and the etiologic agent of Legionnaires' disease (McDade *et al.* 1977) has stimulated a resurgent interest in rickettsia-like agents. More so because in both cases the isolation of these organisms was achieved by a procedure designed to isolate a rickettsia, a very small bacterium-like organism. Rickettsias are special in that they do not grow on synthetic culture media as most bacteria do but grow only in living host such as the arthropods, experimental animals such as guinea pigs, and embryonated eggs. Fraser and McDade (1979) reported that one rickettsia, *Coxiella burnetii* which causes a type of pneumonia called *Q. fever* was isolated by inoculation of guinea pigs with tissue specimens obtained from patients at autopsy. Worthy of note is the similarities of symptoms of rickettsial pneumonia and viral influenza to Legionnaires' disease. In the world's worst recorded epidemic outbreak of Legionnaires' disease at Stafford, England, (May 1985), influenza was the initial diagnostic consideration because of the clinical presentation and the presence of antibodies to influenza virus in the first sera collected (Rashed *et al*.,1986). This outbreak, originally misdiagnosed as influenza claimed at least 39 lives, and possibly 46, with a total of 163 cases. The 1976 outbreak in Philadelphia that gave the disease its name caused 36 deaths. (Sherwood, 1985; Fraser and McDade, 1979).

2.2 The Bacterium - In Historical Perspective

The Legionnaires' disease bacterium (LDB) was isolated early in 1977 by McDade of the CDC. It is the 1976 epidemic in Philadelphia that brought Legionnaires' disease (LD) worldwide attention and led to the isolation of the etiologic agent. The earlier isolation by Marilyn Bozeman, of two organisms that subsequently turned out to be the first recorded isolates of LDB, was the OLDA strain in 1949 (McDade *et. al.*, 1979) and the WIGA strain (Hebert *et. al*, 1980). She also isolated HEBA strain in 1959 (Hebert *et. al.*1980). The bacterium was not identified at the initial encounter because it did not grow on any bacteriologic media in use at the time. This special growth requirement probably prevented its being isolated as a human pathogen earlier (McDade *et. al.*, 1979). Another LDB isolated in Pittsburgh (Pasculle et.al.1980) was initially called the "Pittsburgh pneumonia agent" (PPA), and was later known to have been isolated much earlier on (Hebert *et al.*, 1980).

The bacterium is known to date, to survive and remain viable from water temperatures of 5.7 to 63°C, indicating that a great range of habitats is capable of harboring this bacterium (Fliermans *et. al.*, 1981). Some members of LD bacteria have since been known to be more of soil dwellers than water, and that association with gardening in soil rather than exposure to cantaminated water appeared to be the major environmental risk factor for the acquisition of LD (Steel *et. al.*, 1990a). Its relationship to thermal environments is suggested by its cellular fatty acid composition which is similar to that of known thermophilic bacteria (Moss *et. al.*,1977).

2.3 Classification of the Legionnaires' Disease Bacterium:

When Fraser and McDade (1977) finally isolated the Legionnares' disease bacterium barely six months after the Philadelphia explosive outbreak of pneumonia, he opened the door to the characterization and classification of the bacterium. The first four strains of the LD bacterium isolated were called Philadelphia 1,2,3 and 4. Strains subsequently isolated were also identified by city to distinguish them from the Philadelphia strains. This convention (was used and) has now been adopted at the Centers for Disease Control (CDC), Atlanta, and all strains are designated by city of origin and the number of isolates from that city.

The strains thus far examined were gram-negative, weakly oxidase - positive, catalase-positive, non-motile, fastidious organisms that had narrow optimal pH and temperature ranges and did not grow anaerobically. They did not reduce nitrates, use carbohydrates, degrade urea, or appear to possess decarboxylases for lysine and ornithine or an arginine dihydrolase (Weaver, 1979). They were rod shaped (0.3 to 0.4 μm x 2 to 3 μm) or filamentous (McDade *et. al.*, 1977). Although the *in-vitro* and *in-vivo* studies of four clinical isolates of Keel *et al.* (1978), showed the gram-negative rods to be 0.5 to 0.7 μm wide and varying from 2 to 20 μm in length. Electron micrographs confirmed the rod shape of the organism and its variability in length. Brenner and colleagues (1979) also showed that LD bacterium DNA had a guanine-plus-cytosine content of 39% and a genome size of approximately 2.5×10^9. This DNA relatedness studies indicated that the first isolates belonged to the same species and showed no significant relatedness between the LDB and many other species, genera, and families of known bacteria whose guanine-plus-cytosine content was above 45%. Despite these informative data, a scientific name for LD bacterium was not proposed for two reasons.

1. Many more genera with a compatible guanine-plus-cytosine content were yet to be tested for relatedness to the LD bacterium, and
2. Because the name "Legionnaires' disease bacterium" was universally used and understood, the lack of a formal name did not cause confusion or hinder communication (Brenner, 1978).

Brenner and co-investigators (1978) further tested "atypical" LD bacterium strains for DNA relatedness to LD bacterium Philadelphia 1. Included in this category were many environmental isolates. Labeled, fragmented, single-stranded DNA from LD bacterium Philadelphia 1 was allowed to re-associate with similarly prepared, unlabeled DNA from LD bacterium Philadelphia 1 (homologous control reaction) and that from other known or suspected LD bacterium strains. Strains previously shown to belong to the same species as Philadelphia 1 (Brenner *et al.*, 1978), served as known positive controls. These included Albuquerque 1, Flint 1,Bloomington 1 and 2,and Togus 1, which were 82-94% related to Philadelphia 1 in reaction done at optimal temperature, 60 °C. They were related 71-85% in reactions done at 75 °C, (stringent condition). Other bacteria tested were 0-7% related and 0-4% related to LDB in reactions at 60 °C and 75 °C respectively (Brenner *et al.*, 1978).

With the above experimental prove and other studies, including unusual fine structure characteristics and chemical composition of the peptidoglycan layer of LDB, Brenner *et al.* (1978) affirmed their believe that LD bacterium was a previously undescribed species. That its biochemical reactions, growth pattern, and DNA relatedness were sufficiently unique to warrant placing it in a new genus. Thus they proposed the name *Legionella pneumophila* sp. nov. The type strain of *Legionella pneumophila* is Philadelphia 1. *Legionella pneumophila* was proposed as the type species of the genus *Legionella*. *Legionella* was also proposed as the

type genus for a new family *Legionellaceae*. And since there was only one species in the genus, the description given for the species sufficed for the description of the new genus and the new family (Brenner *et al* (1978).

With ever growing accumulation of knowledge on *Legionella*, the organism is still considered as one genus with over 38 species and over 50 serogroups (Thacker *et al*., 1985 and 1886; Wilkinson *et al*., 1986; Benson *et al*., 1989; Thacker *et al*., 1990; Watson and O'Mahony, 1990; Benson *et al*., 1991; Pasculle.1993).

However, Brown and colleagues have proposed the formation of three genera from the first 5 species: *Legionella pneumophila, L. bozemanii, L. gormanii, L. micdadei* and *L. dumoffii* (Brown *et al*, 1981; Garrity *et al*, 1980). These three proposed genera are: *Legionella*, with only one species - *Legionella pneumophila*; *Tatlockia*, with only one species - *Tatlockia micdadei*; and *Fluoribacter*, with three species, *Fluoribacter bozemanii, Fluoribacter dumoffii*, and *Fluoribacter gormanii*. This proposal is yet to gain popularity in the scientific community. In fact, Brenner *et. al.* (1985) opposed the proposal on the ground that there is not a genetic definition for a genus. That it is doubtful that the relatedness values among the three species placed in *Fluoribacter* would fit the genetic definition of a genus, if there were one. Furthermore, the level of relatedness between *Fluoribacter* species is as low as 12%, with as much as 25% relatedness between these species and *L. pneumophila* (Brenner *et. al*., 1979, 1980; Feeley *et. al*., 1979; Morris *et. al*., 1980). Even in Brown *et. al.* proposal, *Fluoribacter* species showed as little as 4% interrelatedness, compared with values of up to 14% with *L. pneumophila* and up to 10% *with L. micdadei* (Brown *et. al*., 1981; Garrity *et. al*., 1980)

The problem in creating three genera out of the first 5 species will be compounded when one tries to create more genera out of the 22 *Legionella* species. Brenner *et al.* (1985) using DNA relatedness tried that and found that, at 40% relatedness level, there were 14 genera. Similarly at 30% relatedness level, 11 genera would emerge with species which do not auto-fluoresce grouped with autofluorescing members; while a few species are more than 30% related. However, at 25% relatedness level, as advocated by Brown and colleagues 9 genera would be formed with the first genus comprising of the same species as at the 30% level (Appendix A).

Brenner and colleagues (1985), opposing this creation further stated that even if these genetic genera could be defined and separated by phenotypic tests, none of these alternative approaches is appealing. However, the fact remains that legionellae can be identified to species only by serogrouping or DNA hybridization. And with a combination of biochemical tests and analyses of cellular fatty acids and ubiquinones, legionellae can be defined at the family level and form species groups, but cannot be identified to individual species (Brenner *et al.*,1985). Furthermore, grouping of species on the basis of DNA relatedness creates additional problems due to mixed phenotypes. Thus some species which do not autofluoresce could be grouped with the bluish white-autofluorescing species (Brenner *et al.*, 1985).

However, where genetic and phenotypic similarities are not in agreement, Brenner and colleagues suggest that a genus based on phenotype is preferable than one based on genetic relatedness (Brenner *et al.*, 1985). Thus *Legionella* is certainly a good phenotypic genus because:

- all species are isolated by the same methods and cultivated on the same media.,

- all species have similar biochemical profiles and are easily differentiated from other gram-negative bacteria by their unique fatty acid composition and isoprenoid quinone content.,
- When motile, they all have a common flagellar antigen;
- all species that have been isolated from humans cause the same disease, and the same treatment regimen is used against all species known to cause human diseases.

"For all of the above reasons", concludes Brenner *et al.* (1985),"It would be of little, if any, benefit to create additional genera in the family *Legionellaceae* at this time".

2.4 General Characteristics

Recognition of the diversity of the genus *Legionella* has increased steadily in the past one-and-a half decades. There is every reason to believe that the complexity will continue to increase. For in the first one decade of the recognition of *Legionella*, 22 species were included in the genus (Wilkinson, 1986a). Currently, however there are over 38 species (Appendix B) in the single genus *Legionella*. Alternative taxonomic proposals have been advanced for some species (Garrity *et al.*, 1980), but this is yet to receive universal acceptance.

Legionella species are non-spore-forming; narrow, gram-negative rods. They are aerobic, non-acid-fast (with the exception of *L. micdadei in vivo*), and unencapsulated bacilli that measure 0.3 to 0.9 μm in width and vary from short forms 1.5 to 2 μm in length to longer and filamentous forms (Brenner *et al.*, 1979). They are usually short and thin or coccobacillary when seen in direct smear of clinical specimens but more variable in length following growth in culture media. Forms greater than 20 μm long are not unusual (Brenner, 1986, and Koneman

et al., 1992). Pine *et al.*,(1979) reported that bacilli in tissue are only rarely filamentous, but elongated forms may predominate in some culture media.

Legionellae are nutritionally fastidious organisms. They require the amino acid L-cysteine and iron salt, ferric pyrophosphate for growth which are provided in buffered charcoal yeast - extract (BCYE) agar. Such specific nutritional constraints are unusual. Many bacteria require supplementary cysteine or iron for growth, but the double requirement is rare (Fraser and McDade 1979). Another unusual characteristic of the bacterium observed was that it could not consistently be stained by techniques that normally are effective for bacteria. Rather it could be stained dependably by a modification of the Dieterle silver-impregnation technique - an age long procedure for staining the spirochete of syphilis. The bacterium does not grow on the usual blood agar and differential agar media commonly used for respiratory specimens in clinical microbiology laboratories (Fraser and McDade,1979; Koneman *et al.*,1992). Carbohydrates are neither oxidized nor fermented. Amino acids provide the primary source of energy. L-cysteine is required for isolation of all species and is also necessary for continued subculture of all species except *L. oakridgensis*. All species except this latter organism and two other newer species. *L. nautarum* and *L. londinensis* are motile by means of polar or subpolar flagella (Wentworth *et al.*, 1987; Balows *et al.*,1991). Flagella, which occur singly or occasionally in pairs, are unsheathed and 14 to 20nm in diameter and possess a structure typical of other gram-negative bacteria. Pili are also present.

2.4.1 Biochemical Characteristics:

Winn, Jr. (1988) has rightly observed that *Legionella* was born in the molecular age, and the sophisticated resources of molecular biology have greatly expanded our knowledge of

the biochemical composition of the genus. All of the species of *Legionella* have the ultrastructural appearance of gram-negative bacilli, including inner and outer membranes. *L. micdadei* has a distinctive dense layer in the periplasmic space (Herbert *et al.*,1984). *Legionella* species produce characteristic branched - chain fatty acids and ubiquinones with 10 or more isoprene units in the side chain in their cell walls (Isenberg, 1979; Lambert and Moss.1989; Moss, 1981; Moss *et. al.*,1981; Rodgers and Pasculle, 1991). Most species are weakly catalase and peroxidase positive (Brenner *et al.*, 1984). With the exception of *L. micdadei*, *L. feelei* and *L. wadsworthii*, gelatin is liquefied (Orrison *et al.*,1981a; Brenner *et al.*, 1985; Koneman *et al.*, 1992).

The sodium hippurate hydrolysis test, which is positive for *L. pneumophila* and negative for the majority of *Legionella* species isolated from clinical materials, provides a useful presumptive procedure for differentiation between *L. pneumophila* and the other *Legionella* species. (Koneman *et al.*, 1992). Nitrates are not reduced to nitrites, carbohydrates are not utilized and urease is not produced. Nine species are known to produce blue-white or red autofluorescence when colonies are observed with a long wavelength ultraviolet light (Winn, 1987; Brenner *et al.*, 1985; Koneman *et al.*, 1992).

Biochemical characterization however, is not enough for differentiation among species of *Legionella*, but may be helpful as additional presumptive evidence that the isolate belongs to the genus. (Additional tests are required for definitive identification or for characterization of isolates that resemble *Legionella* since some species may not react with specific antisera.) All species, except *L. erythra*, contain prominent branched chain fatty acids by gas-liquid chromatography (Brenner *et. al.*, 1985). Isoprenoid quinones with more than 10 isoprene units in the side chain may be detected by reversephase, high performance liquid chromatography.

The ultimate identification is provided by the determination of genetic relatedness, using DNA hybridization (Brenner *et. al.*,1985).

2.5 LEGIONELLOSIS

Legionnaires' disease (LD), came to public attention after the first recognized outbreak of the disease in Philadelphia, Pennsylvania, in 1976. The disease occurs both sporadically in the form of community-acquired pneumonia (Broome and Fraser, 1979; Winn, 1988; Fang *et al.*, 1989, 1990), and in epidemics (Blackmon *et. al.*, 1981). In addition to LD a milder form of illness, called Pontiac fever (PF) also occurs, as well as illness involving anatomic regions of the body outside the chest cavity (Fraser and McDade, 1979; Koneman *et. al.*, 1992). Thus the term Legionellosis includes all forms of diseases or ailments caused by the species of bacteria in the genus, *Legionella*. The bacteria may produce either acute pneumonia, (Legionnaires' disease) (Fraser *et. al.*,1977), or a flu-like syndrome,Pontiac fever (Glick *et. al.*,1978) and asymptomatic form of *Legionella* infections (Dowling *et. al.*,1984).

The two syndromes, Legionnaires' disease and Pontiac fever though common in epidemics are differentiated on clinical and epidemiologic grounds (Broome and Fraser, 1979). Legionnaires' disease has a long incubation period of 1-10 days, a low attack rate but with 15-30% mortality rate, radiographic evidence of pneumonia and a prolonged convalescence. Approximately half of the recognized species of *Legionella* have been associated with this syndrome (Winn, 1987). On the contrary, PF has a short incubation period of only 5-6 hours, and up to 1-2 days, a high attack rate of up to 95-100%, absence of pneumonia on radiographs and a short course without residual defects (Winn, 1987; Koneman *et. al.*,1992). To date, only

L. pneumophila serogroups 1 and 6, and *L. feeleii* have been incriminated as causes of Pontiac fever (Glick *et. al.*, 1978, Herwaldt *et. al.*,1984; and Mangione *et. al*,1985).

But approximately 85% of the documented cases of Legionellosis have been caused by *L. pneumophila*. Serogroups 1 and 6 have accounted for up to 75% of the legionellae reported to cause human illness (Reingold *et. al.*, 1984). In addition to *L. pneumophila*, many other species have been isolated from clinical specimens collected from humans (Appendix C). Of these other species, *L. micdadei* has been the species most commonly implicated in pneumonia, followed less often by *L. bozemanii*. Others less frequently implicated have been *L. dumoffii*, *L. longbeachae*, *L. maceachernii*, *L, wadsworthii L. birminghamensis*, *L. cincinnatiensis*, *L. oakridgensis*, *L. anisa*, and *L. tucsonensis*. (Fang *et. al.*,1989; Thacker *et. al.*, 1989).

Legionella is thought to account for between 1 and 13% of all pneumonias seen in hospitals in the United States, Canada, the United Kingdom and Germany (Broome, 1984), where most research on LD has been conducted. Even in these countries and elsewhere, Legionnaires' disease poses a challenge to the medical community because its diagnosis is complicated by the fact that the clinical features of the disease are common to many other respiratory tract infections (Yu *et. al.*,1982; Meyer, 1983; Winn, 1985). Legionellosis has most commonly been recognised as a form of pneumonia (Yu,1990). Severity of the illness varies substantially, from the subclinical through a mild process described as "atypical pneumonia" to acute, rapidly progressing pulmonary disease.

2.5.1 **Occurrence of Legionellosis**

Legionnaires' disease are commonly found in middle-aged or older, from late fourties and above (mean age about 55 years). However, the disease can occur in persons of any age,

including children (Fisher-Hoch *et. al*,1981;Koneman *et al*., 1992).

Conditions known to predispose people to legionellosis include diabetes mellitus, ethanolism, cigarette smoking, construction work, residing near excavation or construction sites, travel, and immunosupressive medications (Cohen, et al., 1979), Band and Fraser, 1981; Heltberg *et al*., 1988). Also at risk are patients with chronic obstructive pulmonary disease, and cardiovascular disease, cancer, and those who have received renal homografts (Broome *et. al*., 1979; Haley *et. al*.; 1979; Meyer and Finegold 1980). Another predisposing factor is exposure to concentrated virulent *Legionella* organisms in the environment. Thus legionellosis has occurred with higher frequencies among travellers (Broome and Fraser,1979; England III, *et. al*., 1981; Winn, 1988; Yu, 1990), especially to an epidemic sites such as a hotel or hospital with an ongoing hyperendemic problem or in a highly endemic area and in hospitalized patients who have developed pneumonia during hospitalization.

2.5.2 **Signs and Symptoms of Legionellosis**:

The earliest symptoms typically include a rundown feeling, muscle aches, and a slight headache. There is a rapid onset of dry cough and elevated temperature of up to 39 °C to 40 °C or higher with shaking chills. The fever tends to be non remitting (Meyer *et. al*., 1980; Koneman *et. al*., 1992). Extrapulmonary symptoms and such complaints as fatigue, malaise, myalgia, and arthralgia may suggest the diagnosis of viral infection and mislead the clinician (Rashed *et. al*.,1986). Thus the often misdiagnosed cases in epidemics and sporadic cases. A summary of clinical manifestations is given in Appendix D.

In recent years, the clinical spectrum of legionellosis has expanded. The illness may involve essentially any organ system of the body, with or without pneumonia. Examples of such extrapulmonary involvement have been reported. Central nervous system manifestations such as headache, lethargy, confusion, stupor, and other less common manifestations including ataxia, coma, and seizures were reported in nearly half of the patients with Legionnaires' disease in the US (Johnson *et. al.*,1984; Meyer *et .al.*, 1980). Gastrointestinal symptoms such as abdominal pain, nausea, vomiting and diarrhea occur in many patients (Koneman *et. al.*, 1992). Bacteremia has also been reported by several workers (Edelstein, 1984 and Edelstein *et. al.*,1979; and Rihs *et al.*, 1985).

Many of the symptoms, including cough, are common to LD and PF. Sputum is not always produced and tends to be non-purulent. Similarly, legionellae are not usually demonstrable in respiratory secretions. But on occasion, secretions may be purulent and bacteria may be demonstrated, especially if material is obtained directly from the lung (Liu and Wright, 1984, and Winn *et al.* 1978). In spite of the similarity of the two main clinical syndromes experts' view (Beaty 1984), is that the typical patient with LD has the abrupt onset of high fever, nonproductive cough, chills, and headache or myalgia. That the peak symptomatic involvement occurs within 48 hours with the patient appearing rather toxic, flushed face and near prostration. Patients often are too breathless to talk comfortably and cough occurs in uncomfortable paroxysms. However, Beaty (1984) observed that though the presentation of "typical" signs may heighten a suspicion of LD, many patients may show markedly differnet symptioms. Some patients undergo slow deterioration while others who failed to develop fever finally died due to pneumonia (Fay et al., 1980; Beaty, 1984).

2.5.3 Diagnosis of Legionellosis

The first diagnosis of *Legionella* infection was accomplished retrospectively by inoculation of guinea-pigs with tissues of Legionnaires who had died of the disease (Fraser and McDade, 1979). This procedure was most tidious and expensive. However, the frustrating early days are over, "we have advanced to the point where it may be easier to identify with reasonable confidence infections caused by legionellae than those caused by more venerable or old-time pathegens,such as *Streptococcus pneumoniae* "(Winn, 1987). In summary, *Legionella* infection can be diagnosed by:

(1) demonstration of a serological response to the bacteria,

(2) recognition of bacterial antigen or nucleic acid in clinical specimens, and

(3) recovery of the pathogen in culture (Winn, 1988).

2.5.3.1 Serological Diagnosis

A variety of serological tests have been developed for detection of antibodies to *Legionella* spp. Of the several such tests, the indirect immunofluorescence antibody (IFA) test has been the most completely evaluated. The demonstration of a fourfold rise in serum indirect immunofluorescent titer to 128 or greater is diagnostic of LD, and it is the standard (Winn, 1988). Even then, the diagnosis of sporadic cases by serology is not as firm as that from culture. Sompolinsky and associates (1986) have encountered sera that contained both immunoglobulins M and G (IgM and IgG) antibodies relative with *Proteus hulgaris*, *Rickettsia typhi* and *L. pneumophila*. Serological diagnosis also has the additional disadvantage of being retrospective, although a seroconversion can be detected in many patients within the first week

of illness (Zuravleff *et. al.*,1983). It is important to use reagents that detect both immunoglobulins M and G (Naginton *et al.*,1979; Zimmerman *et al.*,1982).

2.5.3.2 **Nuclei Acid Probes - Gen - Probe**

Gen - Probe, Incorporated (1986) developed a DNA probe test designed to detect all 22 known *Legionella* species directly in clinical specimens within 2 hours (Bryan and Enns,1986). This DNA probe is highly specific for members of the genus *Legionella* (Edelstein, 1986; Bryan and Enns, 1986; Wilkinson *et.al.*,1986) and sufficiently rapid to achieve results in 1 to 4 hours (Kohne, 1986), a much needed time in clinical setting for a life threatening disease diagnosis. This potentially important molecular tool performs as well or better than direct immunofluorescence; it yields a numerical result and does not require judgements to be made concerning intensity of fluorescence, organisms shape, and staining patterns (Bryan and Enns, 1986). It can detect invading pathogens by specifically complexing with the nucleic acids of the infecting microbes. Thus detection and identification are performed simultaneously obviating the need for isolation of the organism. The DNA probe tests are therefore an advanced method for diagnosing infectious diseases, such as Legionellosis (Kohne, 1986). Kohne *et. al.*, (1984) emphasized that the specificity of this probe makes it possible to detect and quantitate the presence of *Legionella* species, even in the presence of large numbers of non related bacteria or mammalian cells. Wilkinson *et. al.*, (1986) also confirmed the specificity of this probe to be 100%, with a sensitivity greater than 98% in both clinical and environmental specimens. However, one disadvantage of the kit is that a radiolabeled DNA probe with a short half-life must be used (Winn,1988). The kit is also expensive and needs a gamma counter.

2.5.3.3 **Direct Detection of *Legionella* by DFA**

Direct detection of *Legionella* spp. in clinical specimens is by direct immunofluorescence antibody (DFA) assay. This has become the most rapid diagnosis of Legionnaires' disease (Edelstein, 1983, 1984; Edelstein *et .al*, 1980; Zuravleff *et al*., 1983). Although it has a low sensitivity (25% - 75%), its specificity is quite high (99% - 100%) (Edelsteins, 1987; Edelstein *et. al.*, 1980; Winn,1987). Fluorescein-conjugated polyclonal sera, in which immunoglobulins against multiple species and serogroups have been pooled is used. And provided the procedure is followed carefully, and meticulously by an experienced microbiologist, using a good fluorescence microscope, cross-reactions can be prevented. Koneman *et. al.* (1992) noted that most, cross-reactions result from (1) processing a positive control slide in the same container with specimens, (2) the microscopists' acceptance of fluorescing morphologic forms such as cocci or debris that are not typical of *Legionella* species, and (3) the contamination of rinse water, reagents, or slides by environmental *Legionella* species. The Genetic System (1985) has also developed a monoclonal antibody which has proven to be useful for DFA testing of both culture isolates and patient specimens. This test system has a sensitivity of 100% and a specificity of 98% that could be raised to 100% if a routine Gram stain was included in the test procedure.

2.5.3.4 **Culture Method**

Culture isolation of *Legionella* spp. from clinical specimens is another major test method employed for the clinical diagnosis of LD. It has become a sensitive means for detecting legionellosis by the majority of clinical microbiology laboratories (Edeslstein, 1983, and Winn, 1985). It is now the mainstay of diagnosis of *Legionella* infection; culture

specificity is 100%, although its sensitivity is 50 to 80%. Buffered charcoal yeast extract agar with alpha-ketoglutarate (BCYE-a) is the mainstay of microbiological diagnosis. Selective versions have been formulated to facilitate the recovery of legionellae from contaminated oral secretions (Phillips and Nash, 1985). If species other than *L. pneumophila* are sought, a medium with vancomycin rather than a cephalosporin should be used (Winn, 1987). Pre-treatment of specimen with acid or heat has also been added to assist and increase the yield of legionellae (Philips and Nash, 1985; Brenner *et al*., 1979).

Dye-containing media was also developed to assist in the recognition and preliminary identification to species of legionellae (Vickers *et al*., 1981), but has not been demonstrated to increase isolation (Winn, 1987). On the surface of charcoal agar (BCYE-a) the appearance of legionellae is characteristic. Colonies are smooth, convex and entire. When viewed with a stereomicroscope, they have a mottled appearance with shades of blue, pink, or green. The colonies are multifaceted and have been described as resembling "cut glass" or "ground glass" especially when observed with oblique light (Winn,1987). However, the colony surface is regular; but as the colony enlarges, the center and eventually the whole becomes opaque. It is useful, therefore, to observe plates daily, at least for the first 5-7 days. Colonies have a sticky consistency and cannot be removed clearly from the plate. If a bacteriologic loop is removed slowly from a colony, a tenacious strand of bacteria may be demonstrated (Winn, 1987). At least 2 days are usually required for recognition of colonies, but most cultures are positive within 5 days of incubation at 35 °C. *L. pneumophila* grows well in air, but other species may require low concentrations of CO_2, not exceeding 5% (Winn, 1987).

The broad clinical spectrum and severe morbidity and mortality of Legionnaires' disease emphasize the need for rapid and accurate laboratory diagnosis. When Legionellosis is

suspected clinically, lower respiratory tract specimens should be collected for both culture and direct fluorescent antibody (DFA) testing. Appropriate specimens for rapid examinations include expectorated sputum, materials collected using bronchoscopy (such as bronchial brush, biopsy, lavage, or washings), transtracheal aspirates, closed-and open-lung biopsy material, fine needle aspirates of lung, and pleural fluid (Edelstein *et al.*, 1980; Edelstein, 1987; and Winn, 1987). These specimens should be carefully collected to avoid aerosolization and transported to the laboratory at ambient temperature in sterile leak proof containers. They should be examined within 2 hours after collection. If less than 2 days delay is anticipated specimen should be refrigerated or packed on wet ice. For more days or weeks delay, -70°C or colder is required for storage (Edelstein 1984).

2.5.4 **Antimicrobial Therapy**

Patients with Legionnaires' disease usually require hospitalization, and for several days after admission their condition deteriorates. Pontiac fever patients however feel very sick for from two to five days, but they all recover, apparently completely (Fraser and McDade 1979).

Investigation of the clinical records of the Pennsylvania Legionnaires suggested that Erythromycin, in contrast to the antibiotics commonly prescribed for other pneumonias with similar signs and symptoms, was the most effective therapeutic agent (Fraser *et al.*, 1977). Broome and colleagues at the CDC observed during a 1977 epidemic in Vermont (USA) that only one person out of 22 who received erythromycin died, whereas 10 of 41 persons who did not receive the drug died. Since then Erythromycin has been effective in reducing the case-mortality rate and is still the drug of choice for legionellosis to date (Fraser and McDade, 1979; Winn, 1988; Yu, 1990).

The clinical response to Erythromycin therapy may occur immediately or be delayed for several days. However, the therapy is a prolonged high-dose, course of the drug, with two to four grams per day, administered for three weeks, occasionally supplemented with Rifampin (Baine, 1979; Beaty, 1984; Broome, 1984; Meyer, 1983). Rifampin is known to be very active *in vitro* (Thornsberry *et al.*, 1978), and could be given in addition to Erythromycin to some patients who are seriously ill or fail to respond to Erythromycin alone. But Rifampin should not be given alone because rapid one-step resistance may develop (Meyer *et al.*,1980). Cephalosporins, aminoglycosides, and penicillins are not effective for Legionnaires' disease (Baine, 1979; Winn, 1985). Some species of *Legionella* produce β-lactamase (Thornsberry and Kirvan, 1978).

If Erythromycin cannot be given, Deoxycycline in combination with Rifampin has been recommended for moderately or severely ill patients. Additional alternative agents include Trimethoprim-sulfamethoxazone, Imipenem, and Ciprofloxacin (Yu,1990). Appropriate treatment with Erythromycin has shown to reduce the mortality rate from 80% among immunosuppressed persons to 24%. In previously healthy patients, a 25% mortality rate was reduced to 7% with recommended therapy (Kirby *et. al.*,1980). The prognosis is said to be linked to the time required for a diagnosis to be reached and Erythromycin therapy initiated.

2.6 **Isolation of *Legionella* from Environmental Samples:**

L. pneumophila was first isolated from natural habitat in the mud of a stream by Morris *et al.* (1979). Subsequently *L. pneumophila* and other legionellae have been isolated from virtually every imaginable habitat. From fresh water (Fliermans *et al.*, 1981; Yakubu and Pass 1983) to brakish or estuarine water (Ortiz-Rogue and Hazen, 1987); from pond water

(Fliermans *et al.*, 1981; Tison *et al.*, 1983,and Bercovier *et. al.*, 1986) to water on leaves of epiphytes (Ortiz-Rogue and Hazen, 1987). From rain water and surface streams (Turner *et al.*, 1984; Morris *et al.*, 1979), to potable water (Tobin *et al.*,1981; Tison and Seilder, 1983; Stout *et al.*, 1982; Yakubu and Pass, 1983; Bartlett, 1984; Fraser,1985; Shands *et al.*, 1985; Bornstein, *et al.*, 1985 and Baree *et al.*, 1987); from the blast zone of Mount St. Helens (Tison *et al.*,1983; Campbell *et al.*, 1984), hot springs, and thermally polluted water (Fliermans *et al.*, 1981; Tyndall *et al.* 1984) to medically accepted spring spa waters in France (Bornstein *et al.*, 1989; Bornstein *et .al*,1989a). From air conditioning system of multistory buildings in the US and UK (Edelstein 1985; Fox, 1985; Gorman *et al.*, 1985; Orrison *et al.*, 1981b and 1983b; Wilkinson *et al.*, 1988; Sherwood, 1985) to an air conditioning system of a bus in Australia (Benson *et al.*,1989), and from saw dust to potting and gardening soils (Steele *et al.*,1990a; 1990b).

Environmental isolation of *Legionella* is accomplished by direct plating of environmental water samples on BCYEα agar, the best basal medium for culture of all *Legionella* species (Barbaree *et al.*, 1987; Edelstein, 1983, Feeley, 1984; Keathley and Winn, 1984). This method is now more sensitive and more practical than the age long inoculation of guinea pigs for recovery of *Legionella* (Feeley, 1984). Environmental specimens are generally collected from sites such as potable (tap) water systems, water heaters, air conditioning systems, respiratory therapy equipment associated with water, and whirlpools. All of these sites can serve as reservoirs, amplifiers, and disseminators of *Legionella* (Bartlett,1984; Jakubowski *et al.*,1984; Bolling *et al.*, 1985).

For bacteriological analysis, water specimens can be classified into two types, "low

count" and "high count" (Feeley, 1984). This is determined according to the concentration of non-*Legionella* microorganisms rather than the number of legionellae present.

"Low count" waters, such as potable water, should be collected in quantities of 1 to 10 liters in sterile jars and concentrated prior to plating, either by centrifugation (Edelstein, 1984) or filteration through 0.2µm polycarbonate filters (Gorman *et al.*,1983). Centrifugated sediment is resuspended by vortexing well (Edelstein, 1984). Sediment on filters is resuspended by placing the soiled filter in a small jar containing sterile water and sonicating the jar and contents in a sonicating bath of the type used to clean jewelry for 10 minutes. This sonicating procedure is recommended for dispersing bacterial aggregates in "high count" water samples as well (Gorman *et al*,. 1983).

"High count" waters, such as cooling tower may be collected in small volumes in sterile jars or tubes (Gorman *et al*.,1983). Samples from taps (e.g. sinks, and showers), may be collected by removing the outermost fitting and swabbing the inside of the pipe, showerhead, or aerator. The swab is then suspended in a small amount of water, vortexed, and 0.1ml of the sample is plated directly. An additional sample after concentration by centrifugation is also plated (Edelstein, 1984).

Both types of water samples either can be inoculated directly to media or can be exposed to low-pH (2.2) treatment for 10 minutes (Bopp *et al*., 1981; Gorman and Feeley, 1982) or heated at 60 °C for 1 to 2 minutes (Edelstein *et al* 1982), or at 50 °C for 30 min. (Harrison and Taylor, 1988) and then inoculated to media. Samples should be refrigerated for later acid wash treatment and replating if necessary. The CDC recommends that environmental specimens be incubated at 35 to 37 °C in a candle jar, or in a humidified incubator in air supplemented with 2.5 percent CO_2 (Gorman *et al*., 1983).

When collecting samples from hyperchlorinated sites (i.e. free residual chlorine greater than 0.5mg/l) sodium thiosulphate ($Na_2S_2O_3.5H_2O$) must be added to neutralize the chlorine that may otherwise inhibit some *Legionellae*. Approximately 0.8mM thiosulphate inactivates about 20mg/ml of free residual chlorine without toxicity to *Legionella* (Edelstein, 1984). If upon plating bacterial overgrowth occurs, samples should be pretreated with heat or acid (Edelstein, 1984; Gorman *et al.*, 1983).

2.7 MEDIA FOR LEGIONELLA CULTURE (Buffered Charcoal Yeast -Extract [BCYE] Agar)

The best medium for growing all species of *Legionella* is BCYE-a agar (Edelstein, 1981; Feeley, 1984; Keathley and Winn, 1984). Media for *Legionella* culture have undergone many improvement in the period since *L. pneumophila* was first isolated by McDade *et al.*, (1977), with the use of guinea-pigs and embryonoted chicken eggs (McDade *et. al.*, 1977). After Mueller-Hinton chocolate agar (M-H agar) was discovered to allow some growth of *Legionella* (Feeley *et al.*, 1978). Feeley and co-workers went on to develop an improved medium known as F-G (Feeley-Gorman) agar. This was based on the fastidious requirement of *Legionella* for ferric pyrophosphate and L-cysteine, and optimal growth at pH 6.9. F-G agar was later modified to provide better growth and was renamed charcoal yeast extract (CYE) agar (Feeley *et al.*, 1979). When later an ACES (N-2-acetamido-2-aminoethane-sulphonic acid) buffer was added by Pasculle *et al.* (1980), the medium became known as buffered charcoal yeast extract (BCYE) agar. Finally, Edelstein described another modification, the inclusion of 0.1 percent alpha ketoglutarate to arrive at the current BCYE-a

agar (Edelstein,1981). This semiselective medium is now the best basal medium for culture of all *Legionella* species (Edelstein,1983; Feeley,1984; and Keathley and Winn, 1984).

While BCYEα agar meets the fastidious growth requirements of *Legionella*, it also supports the growth of many other organisms. These other organisms, or contaminants usually outgrow *Legionella* (Wilkinson, 1982; Yakubu and Pass, 1983) and may in fact, be inhibitory to legionellae. (Zuravleff *et al.*, 1983). For this reason many specimens are much more successfully cultured on BCYEα -based media containing selective ingredients. Such preparations in common use include: BMPAa, BCYE with cefamondole, polymyxinB, anisomycin, and α-ketoglutarate (Edelstein, 1981), and GVPC, BCYE plus glycine, vancomycin, polymyxin and cycloheximide (Dennis *et. al.*, 1984a).

A number of other selective formulations are especially useful for isolating *Legionella* spp from environmental specimens. Those in common use are GVP, which is BCYE-a supplemented with glycine, vancomycin, and polymyxin B (Wadowski and Yee, 1981), and GPAV, containing anisomycin as well (Gorman *et al*, 1983). Another one known as MWY (modified Wadowsky and Yee medium) containing all of the above ingredients plus bromthymol blue and bromcresol purple is also in use (Edelstein, 1982). These media can be used to isolate the more sensitive *Legionella* species and may occasionally be helpful in the selective plating of contaminated clinical specimens, however, they do not inhibit enteric organisms (Edelstein, 1983; and 1984).

2.8 **Ecology of *Legionella* species**

Legionellaceae are ubiquitous in natural fresh-water habitats although they constitute only a small proportion of the total bacterial count in the aquatic environment. The first isolation of *L.*

pneumophila from a natural habitat was from the mud of a stream (Morris *et al*.,1979). Subsequently *L. pneumophila* and other members of *Legionella* have been isolated from lakes, rivers, streams, natural thermal lagoons and ponds, thermally polluted waters, mud samples, the shores of lakes and rivers, from ponds located in the blast zone of volcanic activity, and ground water (Fliermans *et al*.,1981; Bartlett *et al*., 1986; Tison and Seidler, 1983; Yakubu and Pass , 1983). Other water related habitats have also been reported to have among their inhabitants *Legionellae*: hot spring water used for hydrotherapy (Bornstein *et al*.,1989), rain water (Turner *et. al*., 1984; Ortiz-Roque, 1987). There appear to be higher concentration of *Legionella* species in warmer waters, such as 30 °C to 45 °C, than in water at cooler temperatures (Wadowsky *et al*., 1985). Ortiz-Rogue and Hazen (1987), in Puerto Rico reported recovery of legionellae from marine waters and from epiphytes in trees. Fliermans *et al*. (1979) demonstrated legionellae in 792 of 793 water samples examined in the South-eastern United States, thus demonstrating their ubiquitous nature among natural aquatic environments. Although isolation of *Legionella* from dry soil has not been reported, wind-blown dust from an excavation site was implicated for disseminating the organism in one of the earlier outbreaks of Legionnaires' disease (Broome and Fraser, 1979; and Fraser and McDade,1979). However, *Legionella* species including *L. pneumophila* and *L. bozemanii* have been isolated from samples of water of wet soil (Morris *et. al*, 1979). More recently, Steel and colleagues (1990a), isolated *L. pneumophila*, *L. longbeachae* serogroup 1, *and L. micdadei* from potting soils in Australia. In the same study, *L. longbeachae* serogroup 1 was also isolated from natural soil and from pine sawdust. Epidemiologic and microbiologic studies from South Australia in association with an outbreak of LD due to *L. longeachae* serogroup 1 suggested that soil, and not water, could be the natural habitat of *L. longeachea* serogroup 1 and that soil could be the

source of the organism in human disease. In this study (by Steele and colleagues), gardening in soil, rather than exposure to water contaminated with *L. longbeachae* appeared to be the major environmental risk factor.

However, most environmental studies on legionellae have concentrated on artificial or man-made aquatic habitats. These habitats are believed to function as amplifiers of *Legionellaceae*, e.g. hot-water tanks; or disseminators, e.g. aerosol-producing cooling towers, air conditioners, vaporizers and shower heads (Seidler, 1984). Outbreaks of *L. pneumphila* infection since the 1976 Philadelphia explosive pneumonic infection, have been traced to cooling towers, evaporative condensers, and steam turbine condensers (Glick *et al.*, 1978; Fraser and McDade, 1979; Politi *et. al.*, 1979; Cordes *et. al.*, 1980), all of which are artificial or man-made water related environments. In the Philadelphia (1976) epidemic, the investigators suggested that potable water might be involved in the transmission of Legionnaires' disease because of a significant association between disease and drinking of water from one hotel (Fraser *et al.*, 1977). This suggested association of LD with potable water has been strengthened by similar findings both in the United Kingdom (Tobin *et al.*, 1980) and in the United States (Cordes *et. al.*, 1981, and Stout *et al.*,1982).

It has become clear therefore that legionellae are ubiquitous in man-made water systems, both in the absence as well as in the presence of clinically demonstrated legionellosis. The organisms are frequently present in water cooling towers and have been found in tap water, shower water, hot water tanks, the insides of shower heads, rubber gaskets, and metal surfaces within plumbing systems. Potable water has been implicated as the most likely source of *Legionella* in many epidemics and sporadic cases of legionellosis that were not epidemiologically associated with cooling towers, or faulty air conditioning, or heat exchange

systems (Arnow *et al.*, 1982; Cordes *et al.*, 1981a; Fraser and McDade, 1979; Muder *et al.*,1986; Shands *et al.*, 1985).

Whirlpool spas have also been implicated in cases of legionellosis (Fallon *et al.*, 1990; Bornstein *et al.*, 1989). When Whirlpools are inadequately chlorinated, condition may favor the growth of legionellae and the organisms may be aerosolized into the atmosphere and inhaled by unsuspecting susceptible bathers. This has been supported by Breiman *et. al* (1990) report of an outbreak investigation of nosocomial Legionnaires' disease at a hospital in South Dakota (USA). The findings of their investigation have strengthened the hypothesis that aerosolized shower water can serve as the vehicle for spread of *Legionella pneumophila* (Breiman *et al.*, 1990).

Thus, numerous reports have associated the presence of *L. pneumophila* in institutional potable hot water with the occurrence of legionellosis. *Legionellae* appear to survive the usual chlorination procedures of municipal water treatment facilities and thus, not unexpectedly, may be present in potable water supplied to homes, apartment buildings, hotels, hospitals, and other buildings (Cordes, *et al.*, 1981a; Orrison, *et al.*, 1981; Best *et al.*, 1983; Helms *et al.*, 1983; Kusnetsov, *et al.*, 1993). Potable hot water, as Breimans' report (1990) has shown, especially if it does not exceed 55 °C, has contained heavy concentrations of *Legionella* in some instances. World Health Organisation (WHO) report concerning hot water related environments has stressed that the colonization of such environments has been related, among other factors, to the water temperature which appears to be the most important determinant in the isolation of legionellae in these environments (WHO, 1990). *Legionellae* are thus aquatic bacteria which survive at temperatures that are not tolerated by most other aquatic organisms. They are not thermophiles but their tolerance of higher temperatures may give them an ecological advantage.

uniquely fitting them for colonizing man-made water systems which are often at higher than ambient temperatures (Harrison and Taylor, 1988). In addition to water temperature, the construction of the plumbing system also seems to play an important role. It has been shown to correlate with the presence of certain kinds of resins in gaskets, the presence of dead ends or cul-de-sacs, where there is stasis, obstruction, or stagnation in water flow, and the presence of biofilms or slime layers on the surface of pipes containing other commensal bacteria, protozoa, and algae (Neideveld *et al*., 1986; Lee *et al*., 1988; States *et al*., 1989; WHO, 1990).

Factors that promote the growth of legionellae in plumbing systems are however incompletely known and are poorly understood in spite of a large accumulation of literature. Legionellae are fastidious organisms that require enriched media for growth in the laboratory. But that drinking water supplies all growth and energy requirements for legionellae is most unlikely. However, there are several bacterial characteristics that may enhance its ability not only to survive but to thrive in water systems.

2.8.1 Interaction with other Microbes

The first suggestion of symbiosis between *Legionella* species and other organisms was put forth by Tison when he isolated *L. pneumophila* from algal mat in a thermally polluted lake (Tison *et al*., 1980). Bohach and Snyder (1983) later found that when algae were removed from a culture that contained *Legionella* spp., the number of viable *Legionella* spp. in the mixture decreased. Thus suggesting some form of association. In the same study Tison found that of the algal species, *Chlorella* spp supported growth of colonies of the bacteria, while *Gloeocystis* did not. The relationship of *L. pneumophila* with green algae (chlorophyta) and blue-green algae (cyanobacteria) is thought to be due to the production of carbon compounds

which support the growth of *L. pneumophila*. However, *Chlorella* spp and *Gloeocystis* spp. are common contaminants in water systems which create aerosols, and so inhalation of a single algal cell carrying *L. pneumophila* may be an infective dose sufficient to cause the disease. LD (Hume and Hann, 1984).

Although *L. pneumophila* does not grow in sterile tap water, naturally occurring legionellae do multiply when they are associated with environmental bacteria (Wadowsky and Yee, 1985; Wadowsky *et al*., 1988). Wadowsky and Yee (1983) also demonstrated satellite growth of *L. pneumophila* with an environmental isolate of *Flavobacterium breve*.

Parasitic relationship with other environmental microorganisms have been proposed as an explanation, based upon observations. Rowbotham (1980a) was the first to document the phagocytosis of legionellae by environmental amoebae (aquatic and soil amoebae of the genera *Acanthamoeba* and *Naegleria*). Others have confirmed and extended these observations to include other amoebae such as *Hartmannella* and the ciliate *Tetrahymena* (Fields *et. al*., 1989; Newsome *et. al*., 1985; King *et. al*., 1988; Wadowsky *et. al*., 1988). States *et. al.* (1989), went further to explain that amoebae phagocytize legionellae, as they do other bacteria in nature, and the legionellae, instead of being digested as food, survive and multiply within nutritionally deficient habitats by living symbiotically within the amoebae or ciliates. Those amoebae which form cysts, might offer further protection to legionellae within the cysts against the effects of chlorine, thus perpetuating the continued presence of these pathogens in water.

In their study, using *Acanthamoeba palestinensis* Anand and colleagues (1983), cocultivated *L. pneumophila* and *A. palestinensis* and found that there was increased intracellular multiplication of the bacteria, especially at higher temperature (35 °C). Limited multiplication of legionellae was observed at lower temperature (20 °C). The study further

suggested that since *Legionella* and *Acanthamoeba* species were isolated from the same water and interacted on coculture, it implied legionellae infect amoebae in nature and multiply in them at a rate influenced by temperature. If this assumption holds, then sites colonized by amoebae could well act as a focus for multiplication of *Legionella* spp. Consequently cysts of *A. palestinensis* containing legionellae can provide protection to the later so to enable the bacteria to survive more adverse conditions than they could as free bacteria. This would also mean that cysts resistant to levels of chlorination used to treat domestic water supplies could protect legionellae harbored within them. The bacteria could then be released later into domestic piped water supplies, thus perpetuating the bacteria in potable water (Anand *et al*.,1984).

Barbaree *et al*., (1986) also demonstrated the ability of *L. pneumophila* cells to reside inside free living protozoa, thus offering a possible mechanism by which these bacteria can persist through adverse conditions (extreme cold in winter, in temperate climate and, extreme heat and drying in tropical climate) and propagate rapidly in favorable conditions. The ingestion of *L. pneumophila* by protozoa and the subsequent intracellular multiplication of the bacterium is in no small way a protective mechanism. This may explain, at least in part, the continued prevalence of legionellae in potable water, the biocidal water treatment notwithstanding. This condition is further enhanced by the fact that amoebae can revert from the trophozoite stage to the cyst stage and vice versa as environmental conditions change. The protozoa, *Tetrahymena* spp and amoebae were observed to be able to ingest, but not kill, virulent *L. pneumophila* fed to them in the laboratory, so they may be amplifiers of legionellae in nature as well as being natural hosts to these bacteria (Barbaree *et al*.,1986, Rowbotham, 1984). Because of the presence of protozoa (amoebae and ciliates) in air-conditioning system

and in hot water tanks, and the fact that these protozoans frequently contaminate pipe borne water, the increase of legionellae in domestic water will continue to be a health problem.

Recently, Wadowsky *et al.*, (1988) implicated Hartmannellid amoebae as growth factors for *L. pneumophila*. The predominant forms identified were *Hartmannella veriformes* and *Hartmannella cantabrigiensis*. Thus the relationship of legionellae and amoebae has 'improved' from amoebae feeding on legionellae to amoebae releasing growth factors for legionellae (Wadowsky *et al.*, 1988, Fields, *et al.*, 1989).

2.8.2 **Interaction with Plants Other than Algae**

A less-studied association reported is that observed between *L. pneumophila* and *Myriophillum spicatum*, a submerged aquatic macrophyte. In a study by Fliermans (1984) it was observed that when the plant died back each season in the Fall (August-December) of the year, large populations of *L. pneumophila* became associated with the degradation of the plant material. Such associations however, were not observed microscopically during the time of the year when the plant was actively growing. This association also was further confirmed by the use of direct fluorescent antibodies with subsequent culturing in which *L. pneumophila* serogroup 1 was isolated (Fliermans, 1984). Because of the global ubiquity of this macrophyte, *M. spicatum*, the relationship between it and legionellae is thought to be quite important for explaining the growth of legionellae in a variety of habitats and the subsequent role of those habitats as inoculum for amplifiers. Fliermans (1984) also noted that this association appeared to be rather specific in that other aquatic plants did not show any relationship with *L. pneumophila*.

2.8.3 Effects of other Factors on the Survival of Legionellae in Pipe-borne Water

It has remained a puzzle that a fastidious organism, like *Legionella* can survive and multiple in domestic water supply. The association of this organism with algae, water plants and other microorganisms is well documented (Tison *et al.*,1980; Pope *et al.*,1982; Fliermans, 1984; Rowbotham, 1980a; Wadowsky, *et al.*,1982).

However, explanations are yet to be complete as to how legionellae may survive and multiply in potable water, such as tap water. Temperature and water characteristics are known to play a significant role. *L. pneumophila* can multiply in tap water at temperatures as high as 42 °C. It has been isolated from hot-water tanks at 30 and 54 °C, but not from tanks held at 71 to 77 °C (Wadowsky *et al.*,1982). But legionellae survive less well in oil and gas water, heaters in which the heating element is at the bottom of the tank adjacent to the sediment (Jolly,1985).

Individual components of plumbing systems, such as rubber fittings and gaskets are known to support growth of legionellae (Colbourne *et al.*,1984; Niedeveld *et al.*, 1986). While a variety of metals are toxic to legionellae in drinking water, some, such as iron and zinc, can stimulate growth when present in low concentrations (States *et al.*,1985). Stout and co-workers (1985), have also demonstrated that a combination of factors are operative in plumbing systems. That the concentration of sediment was directly related to survival of legionellae, and the presence of environmental bacteria also improved survival, and that these two factors are additive.

In addition to biological factors known to encourage growth of *L. pneumophila* in pipe-borne water, there appear to be growth stimulating physical factors too, that are not well defined. These factors, in addition to elevated temperature (35-45 °C),include stagnation and obstruction, sediment formation, and the presence of biodegradable substances such as rubber

washers (Colbourne *et al.*, 1984; Plouffe *et al.*, 1983; Stout *et al.*,1985; Wadowsky *et al.*,1982; Ciesielki *et al.*,1984; Yee and Wadowsky, 1982).

For aquatic bacteria, legionellae are surprisingly fastidious, which perhaps implies a close relationship with other organisms. Pope *et al* (1982a) suggested symbiotic association of *L. pneumophila* with axenic algae and cyanobacteria, but *L. pneumophila* has been isolated from many dark habitats. Some cyanobacteria in particular, execrete amino acids and peptides into the surrounding environment and as *L. pneumophila* has been grown in defined amino acid media (Tesh and Miller,1981),such compounds might be utilized by legionellae for growth. Cyanobacterial filaments are also known to be colonized by bacteria, e.g. *Bacillus polymyxa*, source of the polymyxin used in selective media for legionellae. However, other environmental bacteria, e.g *Pseudomonas aeruginosa*, are very inhibitory to *L. pneumophila* in vitro (Rowbotham, 1980b). What these other organisms derive from their association with *L. pneumophila* is yet to be reported.

2.9 Pathogenicity of *Legionella*

Legionellae are facultative intracellular pathogens. The pathogenesis of these microorganisms is said to be multifactorial, and critical to the phenomenon are the occurrence of (bacterial) virulence factors, the host state of health, and the dose of infecting bacteria (Rodgers and Pasculle, 1991).

The mechanisms by which *Legionella pneumophila* exhibits its pathogenicity in producing cell and tissue damage in the lungs, and systemic illness are yet to be fully understood. However, a number of toxins have been identified. They include a hemolysin, cytotoxins, endotoxic activity (Baine *et al.*,1978; Friedman *et. al.*, 1980; Fumarola *et. al.*,

1984), and a number of extracellular enzymes. These enzymes, include lipases, proteases, phosphatases, DNases, and a susbstance with chymotrypsin-like activity (Baskerville 1986). It is also thought that extensive proteolytic degradation of important human proteins may be a virulence factor of legionellae. *In vivo* work will help to prove :-

a. whether the various toxins and enzymes are responsible for tissue damage;

b. whether damage to tissues in the lungs is caused solely by free, viable *L .pneumophila* organisms and their toxins after release from the phagocytes,

c. whether dead bacteria may provide another component such as endotoxin - in the response (Baskerville,1986).

Critical to pathogenesis also is the health status of the animal or patient. The compromised patients, those with chronic respiratory disease, and other conditions such as diabetes, cancer, renal disease and immunosuppressive disorders are highly susceptible

As to the infective dose, the available literature does not provide the number of *Legionella* bacteria to be inhaled to develop legionellosis. Strains virulence and individual susceptibility appear to play an important role. Baskerville (1986) reported the possibility that larger inhaled particles are involved in Pontiac fever and that the resulting different deposition pattern or inoculum size may cause different pathogenesis and clinical disease. This makes the conclusion that some host factor is involved inescapable. Hume and Hann (1984) also reported that inhalation of a single algal cell carrying *L. pneumophila* may be an infective dose sufficient to cause the disease (LD). Similarly, inhalation of a cyst of amoeba with legionellae when passing through areas where soil has been freshly turned, or of aerosolized cooling tower water may equally cause legionellosis (Rowbotham 1980b).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1. Sampling equipment

- 1-2 litre wide-mouth screw-cap plastic bottles (BEL-ART Products, Calvert Industrial Supplies, Calvert, Kentucky, USA).
- -Insulated container (Freeze Safe, Polyfoam Parker Corp, Wheeling, Illinois, USA).
- Ice packs (Stanbel, Springfield, Massachusetts)
- Thermometer
- Portable pH-meter (Griffin Portable pH-meter,UK).
- Portable conductivity-meter
- pH-indicator paper

3.1.2 Processing equipment

- Vacuum pump PCR3 (Vacuum Pump Products, Division, Radcliffe, Manchester, England).
- Sonicating bath (Senicor Instrument Corporation, Capaigue, New York)
- Vortex machine (WK Glasintrumente Apparate)
- Incubation chambers
- Filtering assemblies (Millipore Corporation)
- 150ml wide-mouth, screw-cap round jars

(Kerrs Glass Manufacturing Corporation, Lancaster, Pennsylvania, USA).

- Filter membrane (Nuclepore Corp., Pleasanton, California, and Millipore Corp., Redford Massachusetts)
- Coplin jars
- Stereomicroscope (Olympus, VT-II, 211892, Tokyo, Japan).
- Fluorescence microscope (AO Vertical Illuminator for Incident light fluorescence Microscopy, Model 2070, American Instrument Division Buffalo, NY).
- Fluocroslides and cover glass (Erie Scientific Sybron Corporation, Ohio).
- Screw-cap test tubes (13 x 100mm)
- Assorted pipettes (Cooks Laboratory Products, Dynatech Laboratories, Alexandria, Virginia, USA),
- Pasteur Capillary pipettes (cat. No 7095B, Corning Glass Works, Science Products Division, Corning, N.Y).
- Microlitre pipette dropper (Glasfirm, G1. 10, W. Germany)
- Measuring cylinders
- Wash bottles
- Round bottom flasks
- CO_2-incubator (NAPCO Controlled Environment Model 5100, Portland Oregon, USA).
- Biological Safety cabinet (LABCONCO Corporation, Glove Box Filter, Model 50014, Kansa City, Missouri, USA).

- Water bath (Grant Instruments [Cambridge] Ltd., Barrington, Cambridge, England).
- Assorted beakers
- Assorted measuring cylinders.

3.1.3 **Processing Reagents and Chemicals**

- Phosphate Buffered Saline (PBS), pH 7.6
- Glycerol Mounting Medium
- KCl-HCl Buffer, pH 2.2
- Alkaline neutralizer
- Sodium thiosulphate

3.1. 4 *Legionella* **culture media (Composition in Appendix E)**

3.1.4.1 Buffered charcoal Yeast - Extract (BCYE)

- Agar base (GIBCO Ltd., Paisley, Scotland)

3.1.4.2 Antibiotic supplements

- Polymyxin B sulphate (Sigma Chem. Co., Mo., USA)
- Anisomycin
- Vancomycin

3.1.4.3 Nutritional Additives

- L-cysteine HCl (Gibco Laboratories, Life Technologies Inco. Ohio. USA).

  Ferric Pyrophosphate

3.1.5 *Legionella* Polyvalent Pool conjugates A, B, and C.

3.1.6 *Legionella* Specific Antisera for DFA Products

- *L. bozemanii* Serogroup 1 (WIGA Isolate) Control Antigen for F.A. (BA 1727).
- *L. bozemanii* Serogroup 1 (WIGA Isolate) FITC-Labeled Rabbit Globulin for FA. Contains TMRITC - labeled Rabbit Serum (NRS) as Diluent. (BE 1859).
- *L. gormanii* Serogroup 1 (LS-13 Isolate) Control Antigen for FA, (BA 1781).
- *L. gormanii* Serogroup 1 (LS-13 Isolate) FITC-Labeled Rabbit Globulin for FA (with TMRITC-Labeled NRS Diluent), (BE 1967).
- *L. micdadei* Serogroup 1 (TATLOCK Isolate) Control Antigen for FA, (BA 1729).
- *L. micdadei* Serogroup 1 (TATLOCK Isolate) FITC-Labeled Rabbit Globin for FA (with TMRITC-Labeled NRS Diluent), (BE 1858).
- *L. pneumophila* Serogroup 1 (BELLINGHAM-1 Isolate) Control Antigen for FA, (BA 1893).
- *L. pneumophila* Serogroup 1 (PHILADELPHIA-1 Isolate) Control Antigen for FA, (BA 1629).
- *L. pneumophila* Serogroup 1 (KNOXVILLE-1 Isolate) FITC-Labeled Rabbit Globulin for FA (with TMRITC-Labeled NRS Diluent), (BE 1975).

- *L. pneumophila* Serogroup 2 (TOGUS-1 Isolate) Control Antigen for FA. (BA 1630).
- *L. pneumophila* Serogroup 2 (TOGUS-1 Isolate) FITC-Labeled Rabbit Globulin for FA (with TMRITC-Labeled NRS Diluent), (BE 1792).
- *L. pneumophila* Serogroup 3 (BLOOMINGTON-2 Isolate) control Antigen for FA. (BA 1631).
- *L. pneumophila* Serogroup 3 (BLOOMINGTON - 2 Isolate) FITC-Labeled Rabbit Globulin for FA (with TMRITC-Labelled NRS Diluent), (BE 1793).
- *L. pneumophila* Serogroup 4 (LOS ANGELES-1) Control Antigen for FA. (BA 1632).
- *L. pneumophila* Serogroup 4 (LOS ANGELES-1) FITC-Labeled Rabbit Globulin for FA (with TMRITC-Labeled NRS Diluent), (BE 1825).
- *L. pneumophila* Serogroup 5 (DALLAS-1E Isolate) Control Antigen for FA. (BA 1723).
- *L. pneumophila* Serogroup 5 (DALLAS-1E Isolate)FITC-Labeled Rabbit Globulin for FA (with TMRITC-Labeled NRS Diluent), (BE 1926).
- *L. pneumophila* Serogroup 6 (CHICAGO-2 Isolate) Control Antigen for FA. (BA 1725).
- *L. pneumophila* Serogroup 6 (CHICAGO-2 Isolate) FITC-Labeled Rabbit Globulin for FA (with TMRITC-Labeled NRS Diluent), (BE 1827).
- *L. pneumophila* Polyvalent Pool A (Serogroups 1,2,3 and 4) FITC-Labeled Rabbit Globulin for FA (with TMRITC-Labeled NRS Diluent), (BE 1894).

FITC-Labeled Normal Rabbit Control Globulin for *Legionella* FA. (BE 1874).

Legionella polyvalent Pool B FITC-Labeled Rabbit Globulin for Direct FA. *L. pneumophila* Serogroup 5, (DALLAS *E), Serogroup 6, (CHICAGO 2), *L. dumoffii* Serogroup 1, (TEX-KL), *L. longbeachae* Serogroup 1, (LONGBEACH 4). Contains TMRITC-Labeled NRS Diluent), (BE 1875).

Legionella Polyvalent Pool C. FITC-Labeled Rabbit Globulin for Direct FA. *L. micdadei* Serogroup 1, (TATLOCK), *L. bozemanii* Serogroup 1, (WIGA), *L. gormanii* Serogroup 1, (LS-13), *L. longbeachae* Serogroup 2, (TUCKER-1) (with TMRITC-Labelled NRS Diluent), (BE 1876).

These specific Antigens/Antisera were obtained from Centers for Disease Control, Atlanta, Georgia. 30333, USA.

3.2 METHODS

3.2.1 Introduction:

A total of 663 water samples were collected from various sources in all the Northern States, including Abuja, the Capital Territory. Figure 4-1 shows the areas covered by this study and the location of sample sites. Sampling sites were concentrated in and around the states capitals and routes leading to and through them. The choice of state capitals was based partly on the rate of developments, including construction works, industries, hospitals, population density and potable (drinkable) water systems; and partly because of the concentration of man-made water environments such as lakes, dams, ponds, swimming pools and water storage tanks.

3.2.2 **Water Sampling**

Water samples were categorized into two main groups, namely, potable water and raw water.

3.2.2.1 **Potable water** - this was subdivided into,

A. *Pipe-borne water associated with buildings*:

1. Public and private homes taps
2. hospitals taps
3. hotels taps
4. air-conditioning systems
5. swimming pools
6. decorative fountains

B. *Boreholes*

C. *Wells*

3.2.2.2 **Raw Water** - This was subdivided into:

(a) Rivers, Streams and Creeks

(b) Lakes

(c) Dams

(d) Ponds

3.2.3 **Potable Water Samples Collection**

(a) Potable water ('low count') samples were collected aseptically into pre-sterilized, wide-mouth screw-cap plastic bottles. A 0.1ml Sodium thiosulphate ($Na_2S_2O_3$. $5H_2O$) was added into each of these collection bottles to neutralize the residual chlorine that may inhibit the growth of legionellae. Approximately 0.8mM thiosulfate inactivates about 20mg/l of free residual chlorine, without toxicity to *Legionella* (Edelstein, 1984).

To loosen debris and scales within the pipes so that to facilitate the detection of *Legionella* that may be growing on the biofilm, pipes were vigorously 'tapped on' before samples were collected. Large buildings such as hospitals and hotels (because of epidemiological considerations) were sampled 2-3 times more than other buildings, especially if they were within easy reach. Samples (1-2 litres) were collected, and temperature and pH recorded simultaneously. They were transported to the laboratory in insulated containers with ice packs to prevent exposure to extreme heat. Upon arrival to the laboratory, samples were refrigerated at 4 °C. Under this condition, samples can be stored over an extended period of time (Bopp *et. al.*, 1981). However, most of these samples were processed within 4-6 weeks, while a few others were stored for much longer time.

(b) Water from air-conditioning systems ('high count' water) was collected directly from cooling towers, evaporative condensers; and one bleed point along the water distribution lines where access to cooling towers or condensers was not possible. From closed systems water was collected along the distribution line at release valves.

(c) Samples from swimming pools and decorative fountains were collected on the surface with swimming pools in use. Swimming activities in a way helped to agitate and mix the water to ensure that a representative sample was taken.

(d) Borehole samples were collected aseptically as was pipe-borne water samples. However, there was no need for sodium thiosulphate in sample bottles. In some boreholes, water was pumped manually directly into the sample bottles. In others, there were overhead tanks into which water was mechanically pumped, and stored in storage tanks, and later supplied through pipes. This latter category has additional variables such as storage tanks contamination and warming up, or heating by the sun, and pipe system (pipes, joints and gasket/rubber washers).

(e) For the well samples, a bucket was let down and allowed to sink for about a metre, then brought up to surface and sunk again. This procedure was repeated several times in a bid to mix the water before finally bringing the sample out of the well. For an index of human feacal contamination of well water, test for bacteria of coliform group was conducted by multiple test tubes method specified by the Standard Method for Water Analysis and Waste Water Treatment (APHA, 1985).

3.2.4 Raw Water Samples.

Water Samples of 2 litres or less were aseptically collected in sterile plastic bottles. Samples of greater than 2 litres were collected in new plastic gallons each flushed several times with excess samples. All samples were measured for pH and temperature simultaneously. Some were measured for conductivity. Samples not reaching the laboratory

the same day were refrigerated over night and transported the following day in Freeze Safe Polyfoam containers. Samples reaching the laboratory same day were transported at ambient temperature. Those not processed immediately were refrigerated at 4°C until they were processed.

Raw water samples were, for the most part, collected at the edge of the sample source, such as rivers, lakes, dams and ponds. However, at Lake Chad three samples were collected assisted by the lake attendant who took us in a boat through the canal, to the edge of and about a kilometre into the lake. Similarly at Lake Alao, also in Borno State, a fisherman helped us to collect a second sample some metres into the lake. At Kangimi Dam, in Kaduna State, we also collected a sample some distance about 366 meters into the lake in addition to one at the edge. Rivers, streams and creeks were running when samples were collected.

3.2.5 **Processing of the water samples**.

All samples from various sources were processed the same way as described:

3.2.5.1 *Procedure for Potable Water*.

Each water sample was filter-concentrated 100 - 200 folds using sterile filtration unit with a 0.2 μm porosity type polycarbonate filter membrane (Nuclepore Corporation, Pleasanton, Carlifornia U.S.A). Each sample of 1 - 2 litres was filtered 300 - 500ml aliquots under vacuum pressure. At the end of each sample, the funnel was rinsed with sterile distilled water. The filter membrane containing the deposit from the filtered water was aseptically removed, using sterile forceps, and transferred into a sterile 150ml, screw-cap

wide mouth bottle containing 10ml of the sample water for re -suspension. For the visibly turbid and all raw water samples, the above procedure was followed, but a 0.45μm porosity size type HA millipore filter membrane (Millipore Corporation, Bedford, Massachusetts. U.S.A) was used to facilitate filtration. And the deposit was resuspended in 20ml of the water sample because of the greater amount of inert materials accumulated on the filters. Flow-chart, Figure 3- 1 illustrates the step-wise process.

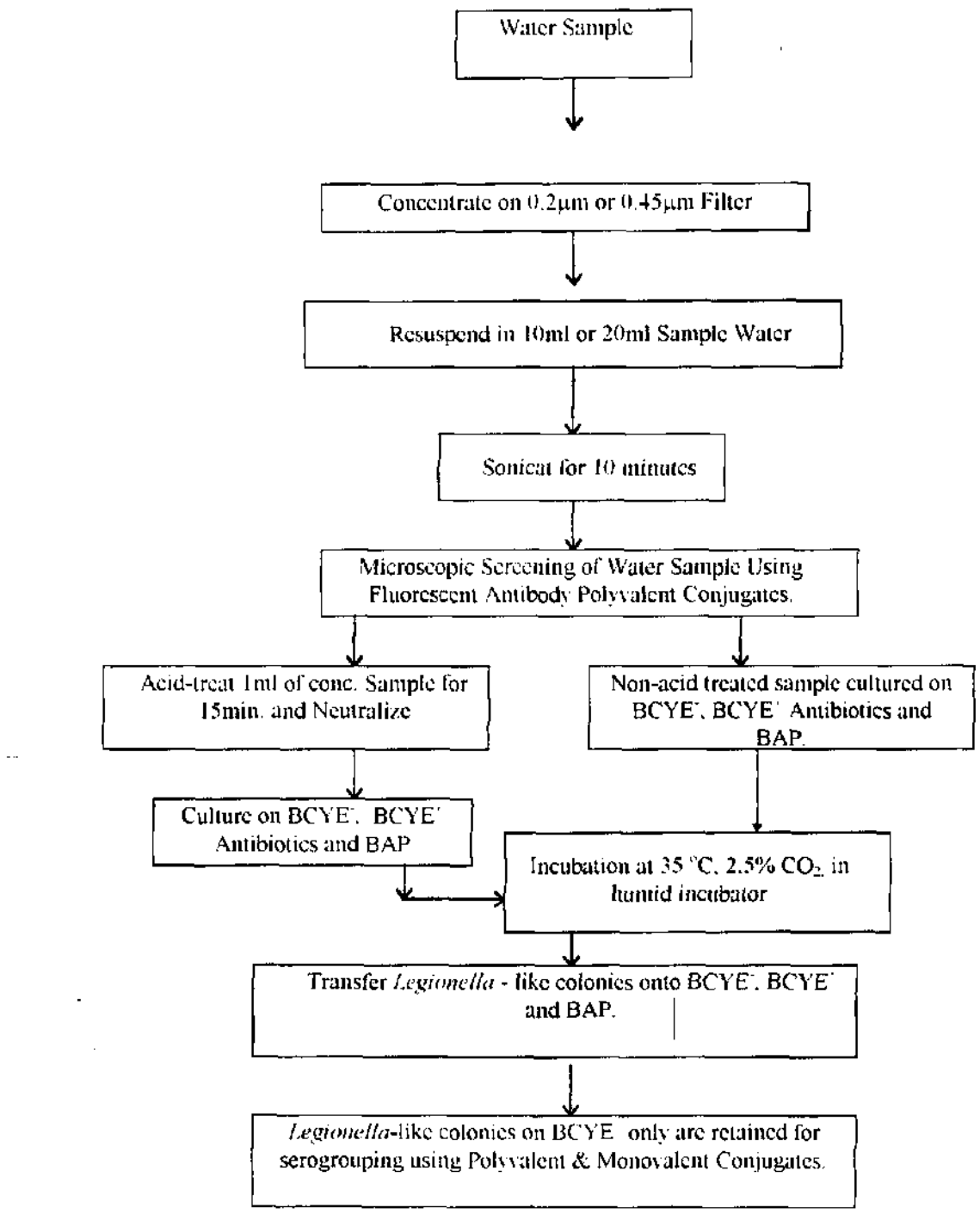


Figure 3 - 1: Procedure for screening water samples using DFA and BCYE.

BAP = Blood Agar Plate

BCYE = Buffered Charcoal Yeast-Extract

BCYE+ = BCYE plus antibiotics and L-cysteine

BCYE- = BCYE without L-cysteine

3.2.5.2 **Sonication**

The concentrated samples were then placed in a sonicating bath (Sonicor Instrument Corporation, Copaigue, New York) for 10 minutes to re-suspend and disperse cells trapped on the filter membrane. For effective sonication, the water in the bath was just enough to reach the level of the samples. When the sample bottles were placed into the bath, the caps were screwed on lightly to allow heat to dissipate. Samples may also be vortexed for 30 seconds.

3.2.5.3 **Preparation of the Antigen (Bacterial Cells)**

After sonication, each bottle was swirled several times to thoroughly mix the sample. Then, using a microlitre pipette dropper (Cooks Laboratory Products, Dynatech Laboratories, Alenxandria, Virgina, USA), a 0.05ml aliquots were delivered onto a two wells fluoroslide (Fluoro-Slides, Erie Scientific Sybron Corporation, Ohio). The aliquots were air-dried, and gently heat-fixed by passing the slide over the flame 2-3 times. (care was taken not to over heat the slides and distort the antigens). The fixed antigens were overlaid with fluoroscein isothiocyanate (FITC) - labeled globulin directed against that antigen. The antigens bind the labeled immune globulins, forming antigen-antibody complexes which will be rendered visible upon excitation of the FITC by ultraviolet light. When exposed to ultraviolet light (500nm), FITC emits longer wavelength of light in the yellow-green (500nm) portion of the colour spectrum, and the *Legionella* cells are observed as brilliantly fluorescent yellow-green rods.

3.2.5.4 **Preparation of DFA Staining buffers**.

A. *Phosphate buffered Saline (PBS), pH 7.6.* (0.01M buffer; 0.85% NaCl).

Concentrated stock solution of this buffer was prepared by dissolving three salts.

Na_2HPO_4 (anhydrous reagent grade)	12.36g
NaH_2PO_4 (reagent grade)	1.80g
and NaCl (reagent grade)	85.00g
in distilled water to make final volume	1,000ml

Working solution (pH 7.6: 0.01M buffer; 0.85% NaCl) was prepared by adding 100ml of concentrated stock solution to 900ml of distilled water to make 1,000ml. This final volume was dispensed in a wash bottle for use.

B. *Glycerol Mounting Medium Preparation*:

Buffered saline (carbonate-Bicarbonate)

Buffer pH 9.0 [0.5M]	1 part
Glycerol (glycerin) neutral	9 parts.

Carbonate-Bicarbonate buffer was prepared by mixing 4.4 ml 0.5M Na_2CO_3 (or 5.3g in 100ml distilled water) with 100ml 0.5M $NaHCO_3$ (or 4.2g in 100ml distilled water). The pH of the mixture was pH 9.0. Addition of 0.5M Na_2C0_3 may be required to adjust the pH.

3.2.5.5 **Direct fluorescent Antibody (DFA) Staining Procedure**.

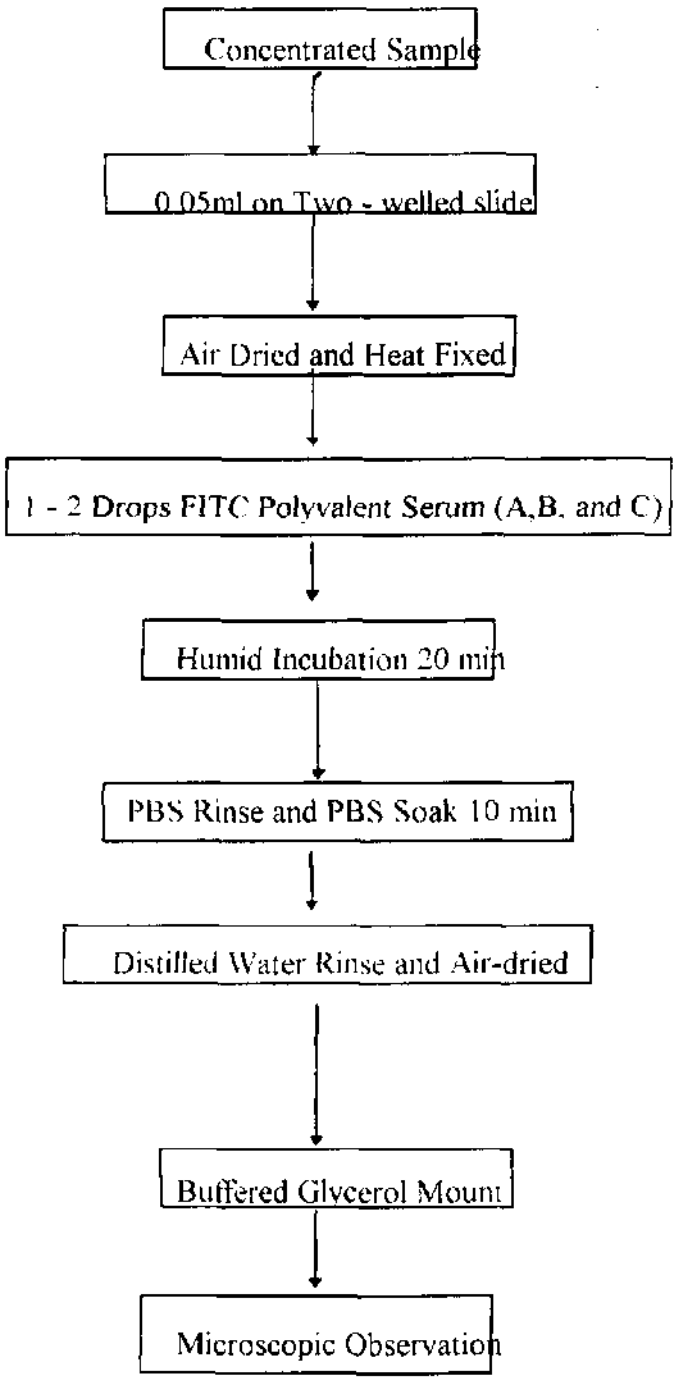
This most widely accepted, convenient and rapid laboratory test for confirming a suspected *Legionella* isolate (McKinney *et al*, 1979; Cherry and McKinney, 1979; Koneman

et al., 1992) employs *Legionella* polyvalent antibody conjugates. Polyvalent pool A identifies *L. pneumophila* serogroups 1,2,3 and 4. Pool B detects *L. pneumophila* serogroup 5 and 6 as well as *L. dumoffii* serogroup 1, and *L. longbeachae* serogroup 1. Pool C on the other hand detects *L. micdadei* serogroup 1, *L. bozemanii* sergroup 1, *L. gormanii* serogroup 1, and *L. longbeachae* serogroup 2. Pre-immune serum conjugate with FITC was used as a negative control. Each conjugate was tested against its specific antigen as a positive control. A water sample containing *L. pneumophila* serogroup 1 (ATCC 33152, American Type Culture Collections, Rockville, Maryland) was included as an additional positive control. (DFA conjugates were kindly supplied by the Biological Product Division, CDC, Atlanta, USA).

All preparations including antigen controls were stained by covering the smear nearest the frosted/labeled end of the slide with 1-2 drops (0.05ml) of the negative (control) conjugate. The remaining smears were covered or stained with 1 - 2 drops of the dilution of each of the polyvalent conjugates to be tested. Then the slides were incubated in moist chamber, at room temperature for 20 minutes. At the end of the incubation period, excess conjugate was removed by gently tapping the slide edge against a tissue towel.

The smears were washed with a stream of PBS from a wash bottle while slides were held horizontally with the long edge tipped downward. Then slides were immersed in PBS in separate coplin jars for 10 minutes. Then the slides were briefly washed with running stream of distilled water, air-dried and mounted with buffered glycerol. The mounted slides were examined with a fluocrescence microscope. Each sample preparation showing specific staining was tested with the negative control conjugate to ensure that the observed reaction

was serologically specific. This protocol was followed for all preparations of samples for type specific (monovalent) staining. The procedural outline for the staining process is presented in a flow-chart, Figure 3 - 2.



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Figure 3-2 : Direct Fluorescent Antibody Staining Procedure Flow Chart

3.2.5.6 **Microscopic Examination of Stained Slides**:

Slides were examined under the 10x to 40x objective of a binocular fluorescence microscope (AO Vertical Illuminator for Incident Light Fluorescence Microscopy, Model 2070, American Instrument Division, Buffalo, NY) for visible uniformly sized dots. Upon selection of areas of the smear where organisms were present, the objective was switched to 63x oil immersion, for rapid screening. The bacteria became visible as short to long rods (0.3µm to 0.9µm by 2µm and up to 20µm) or clumps of cells showing strong peripheral staining with darker centres. The observation was confirmed using 100x oil immersion objective whereby the bacteria fluoresced yellow-green around the entire periphery, thus confirming the observation. A sample was considered positive if a smear contained brightly fluorescing bacilli that were compatible morphologically with *Legionellae* (Evans and Winn, W.C. 1981; Yakubu and Pass, 1983; Negron-Alvira *et al.*, 1988). In some samples the number of fluorescing cells with morphological characteristics of *L. pneumophila* was counted in the smear or per well (of 0.05ml of sample concentrate), and the number of staining *L. pneumophila* per litre of unconcentrated sample was determined.

Scoring of fluorescence intensities was based on CDC standard (Wilkinson, 1986), and recorded thus:

4+ = brilliant yellow-green staining of bacterial cells (very strong fluorescence)

3+ = bright yellow-green staining (strong fluorescence)

2+ = definite but dim staining (weak fluorescence)

1+ = barely visible staining (very weak fluorescence)

\- = no staining

PBS control without conjugate was used as a guide

3.2.5.7 **Pre-treatments**

Acid and heat pre-treatments were used to reduce unwanted microbial flora. Acid pre-treatment was based on relative resistance of *Legionella* to low pH of 2.2 on short period exposure (Wang *et al.*, 1979). The heat pre-treatment relies on the greater tolerance *Legionella pneumophila* has to a temperature of 50°C in comparison to other microorganisms isolated from environmental water sample (Dennis *et al.*, 1984). Both methods were used as a matter of convenience and to determine which was the most effective.

A. *Acid Pretreatment Method - Acid Buffer (pH2.2)*

Preparation of the reagents:

Reagent A.

0.2M KCl (14.9gm/L in Distilled water).

Reagent B.

0.2M HCl (16.7ml/L in Distilled water).

To 18 parts of solution (A) 1 part of solution (B) was added to form KCl - HCl buffer, pH 2.2. The pH of the solution was checked against a pH 2.0 standard buffer. A 0.5ml sample aliquot was added to 4.5ml diluent containing a 0.2M KCl - HCl buffer (pH 2.2) to achieve a 1:10 dilution (Bopp *et al.*, 1981; Buesching *et. al.*, 1983). This was then mixed with a vortex mixer until a homogenous suspension was effected. The suspension was incubated at

room temperature to allow a total contact time of 15 minutes with the acid solution (Gorman and Feeley, 1982). The suspension was then neutralized with 0.1N KOH so that the final pH was 6.9 (Gorman and Feeley, 1982). The samples were then inoculated onto agar plates as described earlier.

B. *Preparation for Neutralizer - 0.1N KOH:*

To 1L of distilled water, 6.46gm of KOH was added (weight may vary with purity of the reagent). The working solution was prepared by diluting 10.7ml of 0.1N KOH with distilled water to yield 100ml. The solution was dispensed into screwcap tubes in convenient volumes and sterilized by autoclaving (at 121 °C for 15 minutes). A 1.0ml of neutralizer (KOH), was then added to 1.0ml of acidified buffer that had been mixed with, and incubated for 15 minutes, 1.0ml of vortexed specimen suspension.

C. *Heat Pretreatment Method:*

From sample concentrate, immediately after sonication 10ml was taken and placed in a 50 °C water bath for 30 minutes. The heated sample was mixed well and 0.1ml aliquot was spread on to agar plates. Both pretreatment methods were employed to enhance isolation from "high count" (Dennis *et al.*, 1984) and from well water samples.

3.2.6 **Preparation of Buffered Charcoal Yeast-Extract (BCYE) Agar:**

The BCYE agar used for this research was procured from GIBCO. Laboratories, Life Technologies, Inc., Madison, Wisconsin, USA, and GIBCO, Ltd. Paisley, Scotland,

England. The final concentrations per litre of the medium is shown in Appendix E. The medium was prepared as described by REMEL technical information manual (1982) by adding 39.0 grams into one litre of deionized water. This was dissolved by boiling and. neutralized by adding 40ml 1N KOH prior to autoclaving. The boiling was gentle with frequent swirling to keep charcoal in suspension and prevent charring.

The medium was autoclaved at 121 °C, 15Lb pressure for 15 minutes and cooled to 50 °C in a water bath. Then 5.0ml of sterile deionized water was added to 1 vial of L-cysteine HCl and 1 vial of Ferric pyrophosphate. Each was swirled to dissolve. The contents of the L-cysteine-HCl vial was added slowly to the cooled BCYE-alpha first. followed by the contents of the Ferric Pyrophosphate vial. The vial of the lyophilized triple antibiotics PAV (polymyxin-B, anisomycin, and vancomycin), was reconstituted with 5.0ml of sterile deionized water and added into the flask of BCYE-alpha medium. The final pH was adjusted, (using sterile 1N KOH) to pH 6.9 ± 0.05. The cooled medium was dispensed (20ml/plate) aseptically under the biological safety cabinet (LABCONCO Corporation. Glove Box filter, Model 50014, Kansas City, Missouri) into sterile 15 x 100mm petri dishes while periodically swirling the contents of the flask to keep the charcoal particles suspended.

3.2.7 **Plating and Incubation**

Each bottle of concentrated sample was swirled several times to mix the suspension. Both acid and heat-pretreated samples were plated by inoculating 0.1ml aliquot, with sterile pasteur pipette onto two plates each of BCYE-alpha with L-cysteine and BCYE-alpha without L-cysteine, and antibiotics (PAV). The inoculum was spread evenly over the

surface of each plate using a sterile spreader. A PAV plate without L-cysteine was used as a negative control.

Plates were then incubated at 36 °C in candle extinction jars in a humidified incubator (NAPCO Controlled Environment, Model 5100, Portland Oregan USA) and about 2.5 percent carbondioxide (Gorman *et al.*, 1983; Remel, 1982). Two uninoculated plates were also incubated after being momentarily opened to check protocol and possible contamination and ensure purity.

3.2.8 **Quality Control**:

The performance of the BCYE-alpha medium can only be ascertained by the growth characteristics of the Legionnaaires" disease bacterium (Gorman and Feeley, 1983; Remel 1982). In this work it was demonstrated by the growth of *L. pneumophila* serogroup 1 (ATCC 33152) used as a quality assurance control to evaluate protocol. *L. pneumophila* (American Type Culture, Parklawn Drive, Rockcastle, Maryland USA) was obtained in a freeze-dried preparation. It was rehydrated under strict safety precaution, and transferred into half-strength BCYE-alpha broth (5ml BCYE-alpha agar + 5ml deionized sterile water). The procedure was carried out under maximum safety precaution and in a biological safety cabinet. From the broth, a sample was taken and processed the same way, as water samples, then inoculated onto one plate each of BCYE-alpha, unsupplemented (without L-cysteine HCl), BCYE-alpha supplemented with antibiotics, and blood agar plate.

3.2.9 **Interpretation of Plates**:

Plates were examined after 48 hours of incubation and every 24 hours thereafter for 7 to 12 days. BCYE-alpha plates were observed microscopically with the aid of stereomicroscope (Olympus, VT-II, 211892, Tokyo, Japan), and oblique light for colonies with morphology similar to that of *Legionella* bacteria white, glistening, convex, circular, entire, and from about 2 to 3mm in diameter with a 'ground glass' appearance. Colonies with these morphological characters were further examined with long wave ultra violet light for blue-white fluorescence. At the time of each reading, all colonies that were morphologically consistent with *Legionella* species were sub-cultured, using heavy inocula to triplicate plates, BCYE-alpha with and without L-cysteine, and blood agar. . Those isolates showing growth on BCYE-alpha but no growth on blood agar after 2 - 5 days of incubation were presumptive *Legionella* species. Repeated subculture was made of suspect colonies which consistently grew on both BCYE-alpha with and without L-cysteine, but fail to grow on blood agar plates. *Legionella* characteristically fails to grow on blood agar, and require L-cysteine HCl for growth. However, *L. jordanis* and *L. oakridgensis* are known to lose their requirement for L-cysteine on subculture (Orrison *et al*. 1983b).

Suspect colonies were further examined with species-specific antibody conjugates as a final confirmation of the organisms. However reaction of the isolates with the DNA probe was also attempted but hampered by the lack of adequate Gamma Counter, and a short-half-life of the kit.

CHAPTER FOUR

4.0 RESULT

A total of 663 water samples from various sources of both raw and potable water were processed and screened for the presence of *Legionellaceae*. Samples collection map (Figure 4-1) shows the major rivers, lakes and dams, towns, and cities in and around which samples were collected. From these sources, including creeks, streams and springs, 246 raw water samples, (37.1%) were collected, processed and screened for *Legionellae*. However, the map does not include potable water sample collection sites for the 417 samples (62.9%) collected.

4.1 Results of Potable Water Samples.

Four hundred and seventeen potable water samples (417) were processed and screened for *Legionella* bacteria. *Legionella*-like cells were detected in all (100%) waters from public and private taps, all air-conditioning systems, and all well water samples. Water samples from hotels and swimming pools were 96% (23 of 24) positive for *Legionellae* while samples from hospitals were 94% (50 of 53) positive. However, borehole water samples were only 42% (10 of 24) positive for *Legionella*-like bacteria.

Table 4-1 gives a summary of the parameters of the samples recorded from the various sources. The mean pH was slightly acidic in all "habitats," (pH 5.4-6.4). The range was equally narrow at between pH 4.5 to 7.5. On the other hand, temperature profile provided a wider range of 20 to 33 °C, while the mean range was 21.8 to 32.4 °C. Most swimming pools had warmer temperatures and slightly less acidic water than most other sources. The mean pH for borehole water was more acidic (pH 5.4), than those of other sources. The lower pH range for both borehole and well water was equally acidic (pH 4.5).

Fig. 4-1

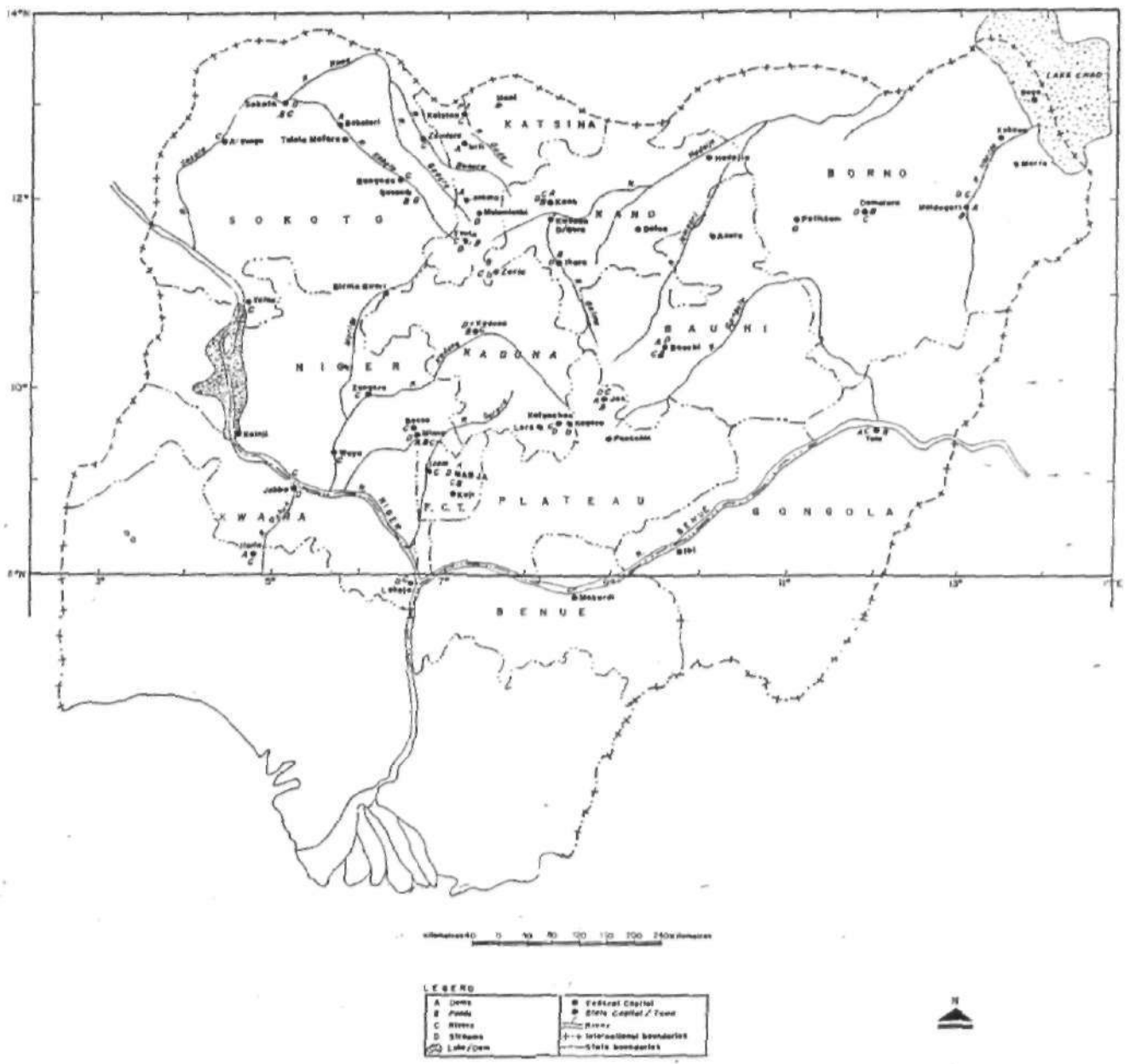


Fig.4-1 DISTRIBUTION OF SAMPLE SITES FOR LEGIONELLA STUDIES IN NORTHERN NIGERIA

Table 4 - 1 Results Summary of Physico-chemical Characteristics and % of Potable* Water Habitats Showing the presence of LEGIONELLAE by Direct Immunofluorescence.

Sample Source	No. of samples (& %)**	Mean pH	Mean Temp.	Mean Conductivity	No. of Samples Positive	% Positive Samples
Taps (Public and Homes)	118 (28.3)	5.5	28.5	NT	118	100
Hospitals Water	53 (12.7)	6.2	28.5	NT	50	94
Hotel water	51 (12.2)	6.0	28.0	NT	49	96
Air-Cond. System Water	42 (10.1)	NT	NT	NT	42	100
Swimming Pools	24 (5.8)	6.4	32.4	NT	23	96
Borehole Water	24 (5.8)	5.4	29.0	NT	10	42
Well Water	105 (25.2)	6.2	29.4	NT	105	100

Total Samples = 417

Note: *Potable water in this study for convenience, included all sources listed on table.

**Percentages of the total samples are shown in brackets

NT = Not tested.

4.1. .1.1 Potable Water Sources and Relative Abundance of *Legionella* Species by Polyvalent Pool Reaction

The results of polyvalent pool conjugate reactions of selected water samples are summarised on Table 4-2. All the pool conjugates A,B, and C fluoresced with cells of *Legionella* characteristics in all the samples tested. However, pool B, and C had 4 and 3 samples respectively, which did not exhibit any cells of *Legionella* morphology.

With polyvalent pool A conjugate, all samples were tested positive with fluorescence activity ranging from 2+ to 4+ intensity. Six results of 4+ fluorescence intensity were recorded one each from Abuja Hotel A tap, Bauchi public tap, Borno Rest House tap, Kaduna Hotel A cooling tower, Katsina College ancient well, and Jos public well. There were 13 (3+) strong reactions recorded in Abuja Hotel B bathroom, Bauchi Hotel B reservoir, Borno 1 well, 1 tap, and a hotel shower, Kaduna college tap, Kano public well, Katsina College well, Ilorin public well, Minna public tap, and Jos Hotel A and B taps. The rest of the reactions (21) were of 2+ intensity (Table 4-2). Thus *L. pneumophila* serogroup 1,2,3, and 4 prevailed in all sampled sites.

Under polyvalent pool B conjugate all samples, except a hotel shower in Maiduguri, a college tap in Kaduna , a public well in Tegina and a borehole in Sokoto, fluoresced with *Legionella*-like cells. The samples from Hotel B reservoir in Bauchi and a college tap in Yola recorded fluorescence activity of 4+ intensity. Eleven other samples, 3 from Abuja, 3 from Bauchi, 1 from Kaduna, 2 from Katsina, 1 from Jos, and 1 from Sokoto fluoresced at 3+ level. Most of the samples however, recorded weaker fluorescence activity. There were 14 2+ and 9 1+ reactions observed. The positivity rate was 90% representing largely *L. pneumophila* serogroup 5 and 6, in combination with *L. dumoffii*, and *L. longbeachae* serogroup 1. Four samples from four states did not show fluorescing *Legionella*-like cells.

Similarly, for polyvalent pool C, the species of the group occurred in 92.5% of the samples. These species are *L. bozemanii, L. gormanii, L. micdadei* and *L. longbeachae* serotype 2. Fluorescence activity observed could be attributable to anyone or all of the members. Strong fluorescence of 3+ and 4+ level were observed less frequently. Only one public tap fluoresced 4+ level in Borno state. At 3+ intensity level 10 samples were observed. These were samples from Hotel B bathroom tap in Abuja , a hotel shower in Maiduguri, Hotel A and B cooling tower and bathroom tap respectively, and a college tap in Kaduna; Katsina college well, a public tap in Offa, Hospital B shower and a public well in Jos, and a public well in Sokoto. The majority of the samples however, gave weaker fluorescence intensity of 2+ (13) and 1+ (13). Three samples from Bauchi did not show any fluorescence of *Legionella* morphology (Table 4.2).

Table 4-2. Results of *Legionella* Polyvalent Pool* Reaction on Selected Potable Water Samples by Direct Immunofluorescence Technique.

Sample Source	State	Pool		
		A	B	C
Hotel A hot tap	FCT	4+**	3+	1+
Hotel B Bathroom tap	FCT	3+	2+	3+
Hotel C lobby tap	FCT	2+	3+	2+
Air-port tap	FCT	2+	3+	2+
Public tap	BA	4+	3+	2+
Hotel A Swimming pool	BA	2+	1+	0
Hotel B Reservoir	BA	3+	4+	1+
Hotel C tap	BA	2+	3+	0
Hotel C shower	BA	2+	3+	0
Public tap	BN	2+	2+	1+
Public well	BN	2+	1+	2+
Public well	BO	3+	2+	1+
Public tap	BO	3+	1+	4+
Rest House tap	BO	4+	2+	2+
Hotel shower	BO	3+	0	3+
Hospital tap	GG	2+	2+	1+
College tap	GG	2+	4+	1+
Public well	GG	2+	2+	1+
Hotel A Cooling tower	KD	4+	3+	3+
Hotel B Bathroom tap	KD	2+	1+	3+
College tap	KD	3+	0	3+
Nursing home tap	KD	2+	1+	2+
Public tap	KN	3+	1+	2+
Public well	KN	3+	1+	1+
Hospital tap	KN	2+	2+	1+
Market tap	KN	2+	2+	2+
Ancient well	KT	4+	3+	2+
College well	KT	3+	2+	3+
Hospital tap	KT	2+	3+	1+
Public well	KW	3+	1+	2+
Public tap	KW	2+	2+	3+

Table 4 - 2 (Continued)

Sample source	State	Pool		
		A	B	C
Public well	NG	2+	0	1+
Public tap	NG	3+	1+	2+
Hospital A tap	PL	3+	2+	1+
Hospital B shower	PL	2+	3+	3+
Public well	PL	4+	2+	3+
Hotel tap	PL	3+	2+	2+
Borehole	SO	2+	0	1+
Public tap	SO	2+	3+	2+
Public well	SO	2+	2+	3+

States: FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

*Poly. Pool A: = *L. pneumophila* serogroups 1, 2, 3 and 4

B: = *L. pneumophila* serogroups 5 and 6,

L. dumoffii serogroup 1, and *L. logbeachae* serogroup 1.

C: = *L. micdadei* serogroup 1, *L. bozemanii* serogroup 1,

L. gormanii serogroup 1, and *L. longbeachae* serogroup 2.

** On a scale of 0 - 4+.

0 = no fluorescence

4+ = brilliant yellow - green staining of bacterial cells.

3+ = bright yellow - green staining

2+ = definite but dim Staining

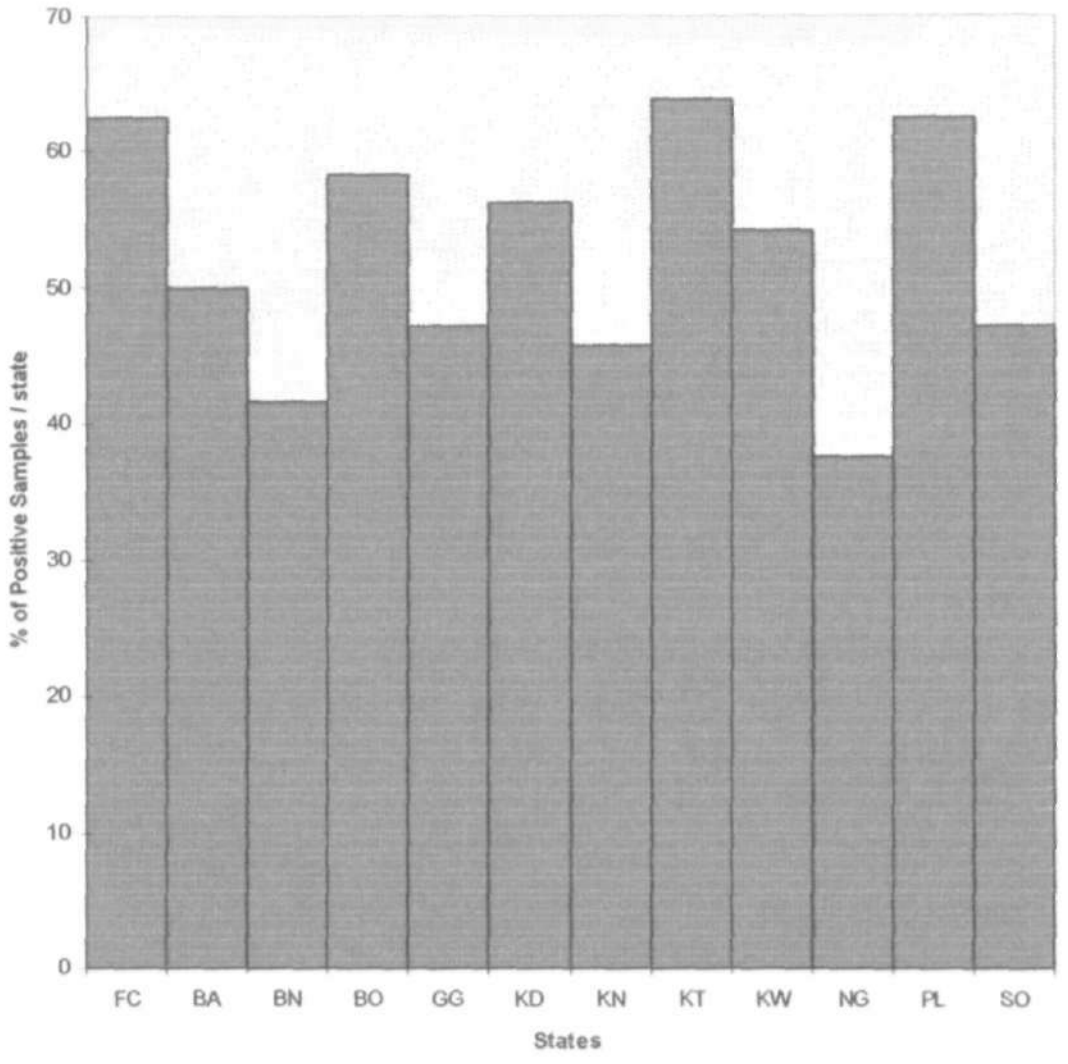
1+ = barely visible staining.

4.1.2 Ubiquity of *Legionella* spp as Shown by the Results of Polyvalent Pool Conjugate Reactions.

Figure 4-2 illustrates the ubiquity and mean occurrence of *Legionella* species in all the states, and the degree of fluorescence activity by polyvalent pool conjugates. Katsina State had the highest mean occurrence of 63.9%, followed closely by Abuja and Plateau with 62.5% each. Four other States, Borno, Kaduna, Kwara and Bauchi, had 58.3%, 56.3%, 54.2% and 50% occurrence respectively. The rest of the States had less than 50% mean occurrence with Niger State having the lowest mean of 37.5%, followed by Benue with 41.7% mean occurrence. Others are Kano State with 45.8% and Gongola and Sokoto States with 47.2% mean occurrence each.

Fig. 4 - 2.

Summary of mean occurrence of *Legionella* species in Potable water systems as shown by the Results of Polyvalent Pool Conjugate Reactions.



FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto

4.1.3 Population Densities of *Legionella* Species By Polyvalent Pool Conjugates

Some samples from most States (8) and Abuja were screened and analysed by DFA to microscopically quantitate *Legionella* species and serogroups identifiable by the three polyvalent pool conjugates. Of the 39 samples that were screened by Pool A conjugate, 22 samples (56.4%) had cell densities of 10^5 cells per ml (Table 4-3). This number comprised of 13 taps, 3 swimming pools, 2 showerheads, 2 wells, 1 fountain, and 1 reservoir. Cell densities of 10^6 /ml were observed in 6 taps, 4 wells, 1 showerhead, and 1 air-conditioning (A/C) line, while another sample from a tap, a well, and an A/C cooling tower recorded 10^7 cells/ml. Yet another A/C cooling tower had the highest cell density of 10^8. The lowest cell density of 10^4 was recorded in one sample from a tap in Offa, Kwara State.

Similarly polyvalent pool B conjugate recorded cell densities of 10^5 cells/ml in 23 samples (59.0%) from 10 taps , 5 wells, 3 swimming pools, 2 A/C cooling towers, 1 shower, 1 reservoir, and 1 decorative water fountain. Seven samples (17.9%) from 6 taps and 1 shower-head had cell densities of 10^4 cells/ml while the remaining five samples of 2 wells, 2 taps, and 1 A/C line showed cell densities of 10^6/ml. Four samples of 3 taps, and a showerhead apparently did not have fluorescing cells of *Legionella* morphology .

With pool C, 19 samples (48.7%) from 7 taps. 4 wells, 3 swimming pools, 2 showerheads, 1 decorative fountain, 1 cooling tower and 1 reservoir had cell densities of 10^5 /ml. The lowest density of 10^4 cells per ml was recorded in 8 taps and 1 shower. Highest cell densities of 10^6 were recorded in 8 samples from 3 taps, 3 wells and 2 A/C system. However, 3 samples from 3 taps, 1 in Argungu, and 2 in Jos did not fluoresce with cells of *Legionella* morphology.

Table 4 - 3 *LEGIONELLA* Population Densities by DFA Polyvalent Pool Conjugates of Selected Water Distribution System and well water

Sample Source	State	Polyvalent Pool* Conjugates		
		A	B	C
Hotel A fountain	FC	2.4×10^5	2.2×10^5	2.0×10^5
Hotel B S/Pool	FC	1.2×10^5	1.5×10^5	2.4×10^5
Hotel C S/Pool	FC	1.3×10^5	2.3×10^5	2.6×10^5
Hotel B tap	BA	3.1×10^5	1.8×10^4	1.2×10^4
Hotel B shower	BA	3.6×10^5	1.4×10^4	1.3×10^4
Hotel B Reservoir	BA	4.5×10^5	3.5×10^5	2.8×10^5
Hotel A S/Pool	BA	3.4×10^5	1.6×10^5	1.4×10^5
Hotel A tap	BA	2.7×10^5	2.5×10^5	1.1×10^4
Hotel C A/C line	BA	3.8×10^6	2.6×10^6	2.9×10^6
Hotel C shower	BA	3.6×10^5	1.9×10^5	2.5×10^5
Town well	BN	6.0×10^6	1.2×10^5	4.2×10^6
Hotel Shower	BO	2.6×10^6	-	3.4×10^5
Damaturu tap	BO	1.2×10^5	1.0×10^5	4.2×10^6
Potiskum Townwell	BO	2.1×10^6	1.2×10^5	1.4×10^5
Rest House tap	BO	3.5×10^6	1.3×10^5	1.0×10^5
College Tap	GG	2.4×10^6	4.2×10^6	1.4×10^5
Briyal well	GG	2.1×10^5	2.3×10^5	1.3×10^5
Garkida Townwell	GG	2.8×10^6	2.6×10^6	2.2×10^6
Hosp. Tap	GG	2.1×10^5	2.3×10^5	1.3×10^5
Biu well	GG	2.3×10^5	2.4×10^5	1.4×10^5
Hotel A C/Tower	KD	7.9×10^8	2.5×10^5	2.1×10^5
Hotel B C/Tower	KD	8.0×10^7	1.1×10^5	2.5×10^6
College tap	KD	3.6×10^6	-	3.4×10^6
Orth. Ward tap	KD	1.8×10^5	1.2×10^4	2.6×10^5
Ilorin tap	KW	2.0×10^5	1.2×10^4	1.4×10^4
Offa tap	KW	1.3×10^4	-	2.1×10^4
Shao well	KW	2.6×10^6	1.0×10^5	2.2×10^6
Hotel C tap	PL	1.1×10^5	1.5×10^5	3.6×10^6
Hosp A tap	PL	3.2×10^6	1.5×10^5	1.6×10^5
Hosp. B tap	PL	1.6×10^5	3.2×10^6	-
Hosp. C tap	PL	2.7×10^6	1.3×10^4	-

Table 4 - 3 Continued

	State	Polyvalent Pool* Conjugates		
Hosp. D tap	PL	2.4×10^5	-	1.4×10^4
Town well	PL	4.6×10^7	1.1×10^5	3.4×10^5
Company tap	PL	2.3×10^6	1.6×10^4	1.2×10^4
Hotel tap	SO	3.6×10^7	2.8×10^5	1.6×10^5
Hosp. tap	SO	1.4×10^5	2.2×10^4	2.7×10^4
Argungu tap	SO	2.2×10^5	1.1×10^5	-
Jega tap	SO	1.2×10^5	1.6×10^5	1.0×10^4
Yelwa tap	SO	2.4×10^5	2.6×10^5	1.5×10^5

*Polyvalent Pool A = *L. pneumophila* serogroups 1, 2, 3, & 4.

B = *L. pneumophila* serogroup 5 and 6; *L. dumoffii* serogroup 1, and *L. longbeachae* serogroup 1.

C = *L. micdadei* serogroup 1, *L. bozamanii* serogroup 1, *L. gormanii* serogroup 1, and *L. longbeachae* serogroup 2.

** States: FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno; GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina; KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

*** Counts were based on microscopic observation of fluorescing cells of *Legionella* morphology in 0.05ml. sample concentrate and reported as the number of fluorescing cells/ml.

4.1.4. Monovalent Conjugate Reactions and Relative Abundance of LEGIONNELLA Bacteria:

These results by DFA (Table 4-4) illustrate the ubiquity of *Legionellaceae* in domestic water systems. The prototype species of the genus *Legionella*, *L. pneumophila*, was predominant in all of the sites sampled. All of its first 6 serotypes were detected in 67.9% of the samples. The other 3 species, namely, , *L. bozemanii L. gormanii*, and *L. micdadei* were detected in 61.7% of all the samples tested.

On each species, *L. pneumophila* serotype 1 was the most predominant. It was detected in all the samples tested. It demonstrated the strongest staining (4+) in six samples: a public well and a rest house tap in Borno, Hotel A cooling tower, a college tap and a Nursing Home tap in Kaduna, and a public well in Plateau. Staining reactions of 3+ intensity were observed in 14 samples, one at least from every State except Kaduna. Public taps had the highest number of 7 samples, followed by 3 hotels, 2 hospitals and 2 wells. Weak staining reactions of 2+ intensity were recorded from 17 samples collected from 7 hotels, 6 wells, and 1 each from a hospital, reservoir, swimming pool and a public tap. There were however, 3 very weak staining reaction of 1+ intensity observed in 2 public taps and a borehole.

L. pneumophila serotype 3 was second in abundance; it had the least (4) number of negative samples. Two very strong staining reaction of 4+ intensity were observed, one from Hotel C in Bauchi, and the other from a public tap in Kano. Ten of its strong staining (3+) came from 3 hotels, 2 hospitals, 2 wells, 1 cooling tower, 1 reservoir, and a tap. However, the greater number of samples (24) stained weakly (2+) or very weakly (1+). Of the 2+ staining reactions, 6 came from hotels, 4 from public taps, 3 from wells, and one each from borehole water, swimming pool water and nursing home tap water. The remaining 7 samples

that were of 1+ fluorescence staining were drawn mainly from public taps (4), two wells and a hotel tap. The total number of positive samples were 36 or 90%. Four samples that did not show any cells of *Legionella* characteristics came from two wells, and a hotel and a public tap (Table 4-4).

L. pneumophila serogroup 4 was third in relative abundance even though it had five more strong reactions than serogroup 3. Very strong staining fluorescence reaction was recorded for a tap in Bauchi and a well in Benue and Katsina. Strong staining (3+) was recorded for 15 samples: 5 public taps, 3 wells, 3 hotels taps, 2 hospitals taps, 1 cooling tower and a swimming pool. The 2+, weak staining fluorescence cells were observed in 10 samples from 3 hotels, 2 hospitals, 2 taps, 2 wells and a reservior. Lastly, 5 weaker (1+) staining reactions comprising of 3 wells, 1 tap, and a hospital shower-head were also recoreded, thus giving this serotype a total of 33 positive samples (82.5%)as recorded in Bauchi Hotel C showerhead. Three strong (3+) reactions were recorded also. Two of these samples came from Bauchi Hotels B and C, while the remaining one was recorded from a market tap in Kano. Most of the results were weak staining (2+ and 1+). The 2+ fluorescence were recorded in 14 samples, 8 of which were from public taps, 2 from wells, 2 from hotels, 1 from a hospital and 1 from a cooling tower. Eight samples fluoresced at 1+ level. Four of these samples came from wells, and two each from hospitals and hotels. The rest of the 14 samples (35.0%) tested negative.

Serogroup 6 showed 12 samples which fluoresced 3+ level. This serotype seemed to predominate in Katsina (3), Sokoto (2) and Abuja (2). The remaining 5 strong reactions came one each from Bauchi, Benue, Gongola, Kaduna and Kwara. Four other samples stained 2+ intensity, one each from Bauchi, Borno, Kwara and Niger States. A well water sample in Jos stained weakly (1+). Majority of the samples, 23 out of 40 (67.6) were tested

negative although over half (15 of 23) were positive at polyvalent pool B conjugate reactions.

The least positive reactions were recorded in *L. pneumophila* serotype 5 (Table 4-4), where only 11 samples (27.5%) tested positive. Of this number, one 4+ fluorescence staining was observed in Borno well water sample, while a market tap in Kano and a college well in Katsina stained 3+ level. The other 8 positive results were weak; 2+ fluorescence was recorded for a hotel sample from Abuja and Bauchi, a hospital sample from Gongola, and a well sample from Jos. Similarity, 1+ staining reactions were recorded in Abuja (2 samples), a hotel in Bauchi, and a well in Katsina. Of the majority of samples which tested negative (29 or 72.5%), over half (15 of 29) of them were tested positive with polyvalent pool B conjugate. *L. longbeachae* serogroup 1 and *L. dumoffii* were not tested due to lack of type-specific antisera (Table 4-2).

Of the other *Legionella* spp that were tested, *L. gormanii* predominated. It was observed in 85.0% (34 of 40) of the water samples tested from all the States (Table 4-4). There were 12 strong staining reactions observed in 9 States. Kaduna had the highest number of 3 with one college tap staining 4+ and, Hotel A and B 3+ level. Niger and Plateau states had 2 samples each. A well and a tap samples stained 3+ in Niger while Hospital A and a well in Plateau fluoresced 3+ intensity. Other States had one sample each: Bauchi State Hotel B reservoir stained 3+, Borno State hotel shower was remarkably positive (4+), Gongola hospital tap fluoresced 3+, a market tap in Kano and a well in Sokoto also stained 3+ intensity. The remaining 22 positive samples stained either 2+ (18) or 1+ (4) intensity. The 2+ level were from 7 public taps, 5 wells, 3 hotel taps, 2 hospital taps and a nursing home tap. The 1+ level reactions were observed in Hotel A sample in Abuja, a well in Benue, and a well and a tap in Kwara State. Of the 6 samples that tested negative, 2 samples

from Hospital B shower in Plateau and a borehole from Sokoto were positive at Polyvalent pool C conjugate reactions, (Table 4-2).

L. micdadei was second in relative abundance; 82.5% of the water samples tested positive (Table 4-4). Seven of its strong staining reaction (3+) came from Abuja Hotel B and Airport tap, Borno rest house and a public tap, a well in Benue with very strong reaction (4+), a nursing home in Kaduna and a College well in Katsina. The remaining 26 positive samples stained 2+ (22) and 1+ (4) fluorescence. Of the 22 fluorescing samples, there were 8 public taps, 3 hospital taps, 8 wells, 2 hotel taps and a reservoir. The 1+ staining reactions were observed in Abuja (1), Kaduna (1) and Kano State (2). Seven other samples tested negative; however 2 of the samples were positive previously at pool C conjugate reactions (Table4-2). The least prevalent of all *Legionella* species, *L. bozemanii* was detected in only 17.5% (7 of 40) of the water samples with fluorescence intensity of 2+ in only 1 hotel sample from Borno, while the remaining 6 samples stained 1+ level. The majority of the samples (82.5% 0r 33 of 40) tested negative (Table 4-4).

Table 4 - 4: RESULTS OF *LEGIONELLA* SEROSPECIFIC REACTIONS ON SELECTED POTABLE WATER SAMPLES BY DIRECT IMMUNOFLUORESCENCE TECHNIQUE.

Sample Source	State	*Legionella pneumophila* serogroups						Other *Legionella* Species				
		1	2	3	4	5	6	*L. bozemanii*	*L. gormanii*	*L. longbeachae*	*L. micdadei*	*L. dumoffii*
Hotel A hot water	FCT	3+	-	-	2+	2+	3+	-	1+	NT	2+	NT
Hotel B Bathroom	FCT	2+	2+	3+	-	1+	3+	1+	2+	NT	3+	NT
Hotel C lobby tap	FCT	2+	1+	2+	3+	X	X	-	2+	NT	1+	NT
Air-port Hotel	FCT	2+	2+	1+	2+	1+	-	1+	2+	NT	3+	NT
Public tap	BA	3+	2+	2+	4+	-	3+	-	2+	NT	2+	NT
Hotel A Swim./ Pool	BA	2+	-	2+	3+	X	X	-	-	NT	-	NT
Hotel B reservoir	BA	2+	3+	3+	2+	X	X	-	3+	NT	2+	NT
Hotel C tap	BA	3+	3+	3+	2+	1+	-	-	-	NT	-	NT
Hotel C shower	BA	2+	4+	4+	3+	2+	2+	-	-	NT	-	NT
Public tap	BN	3+	2+	1+	2+	-	3+	-	2+	NT	2+	NT
Public well	BN	2+	2+	2+	4+	X	X	-	1+	NT	4+	NT
Public well	BO	4+	-	2+	3+	4+	2+	-	2+	NT	2+	NT
Public tap	BO	3+	2+	3+	-	X	X	-	2+	NT	3+	NT
Rest House tap	BO	4+	2+	2+	3+	X	X	1+	-	NT	3+	NT
Hotel B tap	BO	2+	2+	2+	3+	-	-	2+	4+	NT	-	NT
Hospital tap	GG	2+	2+	3+	2+	2+	-	1+	3+	NT	2+	NT
College tap	GG	3+	-	2+	3+	X	X	1+	2+	NT	2+	NT
Public well	GG	2+	1+	1+	2+	-	3+	-	2+	NT	2+	NT
Hotel A Cooling tower	KD	4+	2+	3+	3+	X	X	-	3+	NT	1+	NT

Table 4 - 4 Continued

Sample Source	State	*Legionella pneumophila* serogroups						Other *Legionella* Species				
		1	2	3	4	5	6	*L. bozemanii*	*L. gormanii*	*L. longbeachae*	*L. micdadei*	*L. dumoffii*
Hotel B Bathroom tap	KD	2+	-	2+	-	X	X	-	3+	NT	-	NT
College tap	KD	4+	-	2+	3+	-	-	-	4+	NT	2+	NT
Nursing Home	KD	4+	-	2+	-	-	3+	-	2+	NT	3+	NT
Public tap	KN	1+	2	4+	-	X	X	-	2+	NT	2+	NT
Public Well	KN	2+	-	2+	3+	X	X	-	2+	NT	2+	NT
Hospital tap	KN	3+	-	2+	2+	X	X	1+	2+	NT	1+	NT
Market tap	KN	1+	3+	1+	1+	3+	-	-	3+	NT	1+	NT
Ancient Well	KT	3+	1+	2+	4+	1+	3+	-	2+	NT	2+	NT
College Well	KT	3+	1+	1+	2+	3+	3+	-	2+	NT	3+	NT
Hospital tap	KT	2+	1+	3+	3+	-	3+	-	2+	NT	2+	NT
Public Well	KW	3+	-	3+	1+	-	2+	-	1+	NT	2+	NT
Public tap	KW	3+	2+	-	3+	-	3+	-	1+	NT	2+	NT
Public Well	NG	2+	2+	-	3+	-	-	-	3+	NT	2+	NT
Public tap	NG	3+	2+	3+	-	-	2+	-	3+	NT	2+	NT
Hospital A tap	PL	2+	-	2+	3+	X	X	-	3+	NT	2+	NT
Hospital B Shower	PL	3+	1+	2+	1+	X	X	-	X	NT	X	NT
Public Well	PL	4+	-	3+	1+	2+	1+	-	3+	NT	2+	NT
Hotel tap	PL	3+	1+	1+	2+	X	X	-	2+	NT	2+	NT
Borehole	SO	1+	-	2+	-	-	-	-	X	NT	X	NT
Public tap	SO	3+	-	1+	3+	-	3+	-	2+	NT	2+	NT
Public Well	SO	2+	1+	-	1+	-	3+	-	3+	NT	2+	NT

NOTE: scoring of fluorescence intensities on a diminishing scale

4+ = brilliant yellow - green staining of bacterial cells

3+ = bright yellow - green staining

2+ = definite but dim staining

1+ = barely visible staining of bacterial cells of *Legionella* morphology

\- = no staining of *Legionella* morphology

x = negative type - specific reaction where pool reactions was positive.

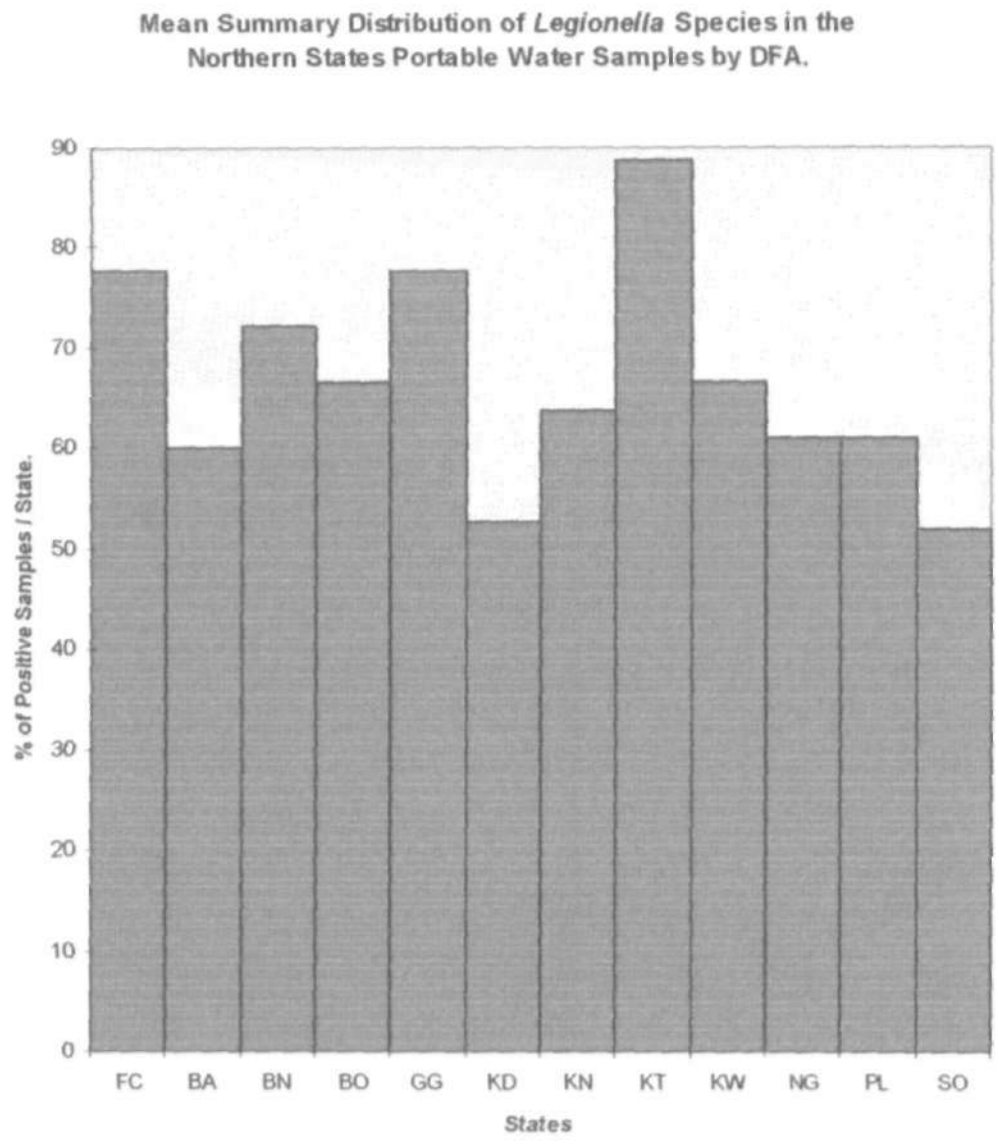
NT = not tested, reagent not available.

States: FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;
GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;
KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

4.1.5 Summary Distribution of *Legionella* species in the Northern State's Potable Water Samples by Species Specific Conjugates.

A summary of the mean occurrence of all *Legionella* species per State is shown in Figure 4-3. The highest percentage of occurrence of all spp (except *L. bozemanii*) was recorded in Katsina State with the mean occurrence of 88.9%. This is followed by Gongola and the Federal Capital Territory, Abuja with occurrence of 77.8% each; also 72.2% occurrence was recorded in Benue State. Six other States with frequency of occurrence ≥ 60% were as following: Borno and Kwara 66.7% each, Kano, 63.9%, Niger and Plateau, 61.1% each, and Bauchi State which recorded 60.0% mean occurrence. The last two States, Kaduna and Sokoto, with the least occurrence recorded 52.8% and 51.9% respectively.

Fig. 4 -3



FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

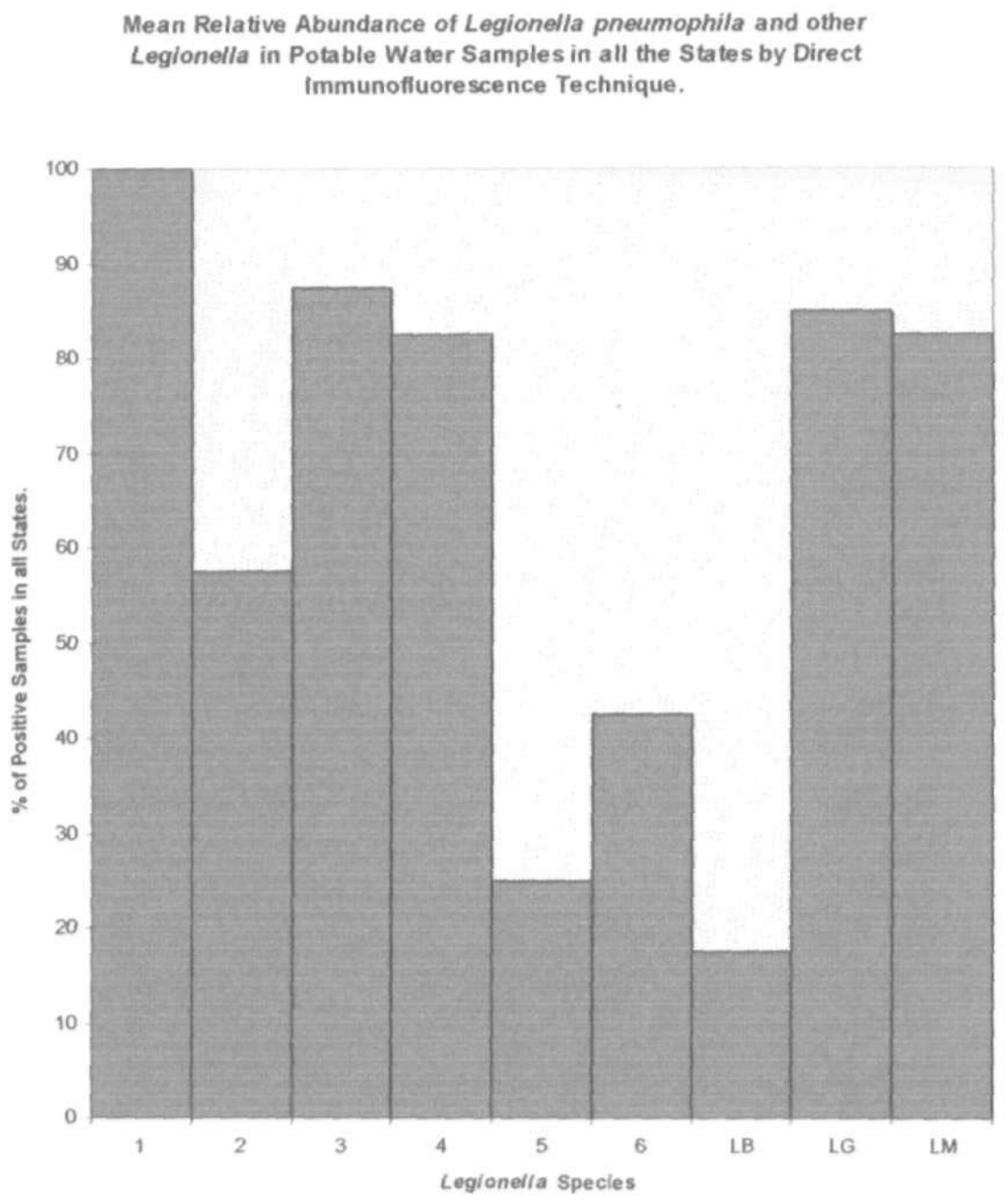
DFA = Direct Fluorescence Assay.

4.1.6 Relative Abundance of *Legionella* Species and Serogroups in all the States:

A comparison of the relative abundance of *L. pneumophila* serogroup 1 through 6 and 3 serogroups of other legionellae in all the Northern States is illustrated on Figure 4 - 4. *L. pneumophila* serogroup 1, the prototype serogroup was detected in all samples from all the States (100%). It was followed by serogroup 3, detected in 35 of 40 samples (87.5%); serogroup 4 was present in 33 of 40 samples (82.5%). In decreasing order of occurrence, serogroup 2 was detected in 23 of 40 samples (57.5%), followed by serogroup 6 which was detected in 42.5% of the samples. The least detected of all, serogroup 5 was only positive in 10 samples (25%).

Of the other legionellae, *L. gormanii* was the most abundant species, with 85% positivity; followed by *L. micdadei* with a positivity rate of 82.5% (33 of 40), while the least dominant species, *L. bozemanii* was detected only in seven samples, representing 17.5%.

Fig. 4 - 4.



*LP 1 - 6 = *Legionella pneumophila* Serogroups; LB = *Legionella bozemanii*

LG = *Legionella gormanii*; LM = *Legionella micdadei*.

4.1.7 Distribution of *L. pneumophila* Serogroup 1 - 6 in Potable Water Samples of Northern States of Nigeria by DFA.

Figure 4-5 gives a summary distribution of *Legionella pneumophila* serogroup 1 - 6. Examination with immunofluorescence antibody conjugates has revealed a remarkable random interstate - intraserotypic distribution and percentage occurrence rate of each serotype in potable water sample in all the sampled areas. The prototype serogroup 1 was detected in every sample in all the states and Abuja. This type serogroup alone predominated in the Federal capital, Abuja and also in Sokoto State.

In every other state a combination of serogroups featured predominantly. In Abuja, while serogroup 1 was detected in all the samples, serogroup 2,3,4, and 5 were present in 75% of the samples. Serogroup 6 was the least (50%) detected. In Bauchi State, serogroup 1,3, and 4 prevailed in all the samples, whereas serogroup 2 was present in 80%, of the samples. The least detected serogroup 5 and 6 were identified in 40% of the samples. In Benue state however, serogroup 1,2,3,and 4 were detected in all the samples (100%). Serogroup 5 was not detected in any of the samples, whereas serogroup 6 was identified in 50% of the samples.

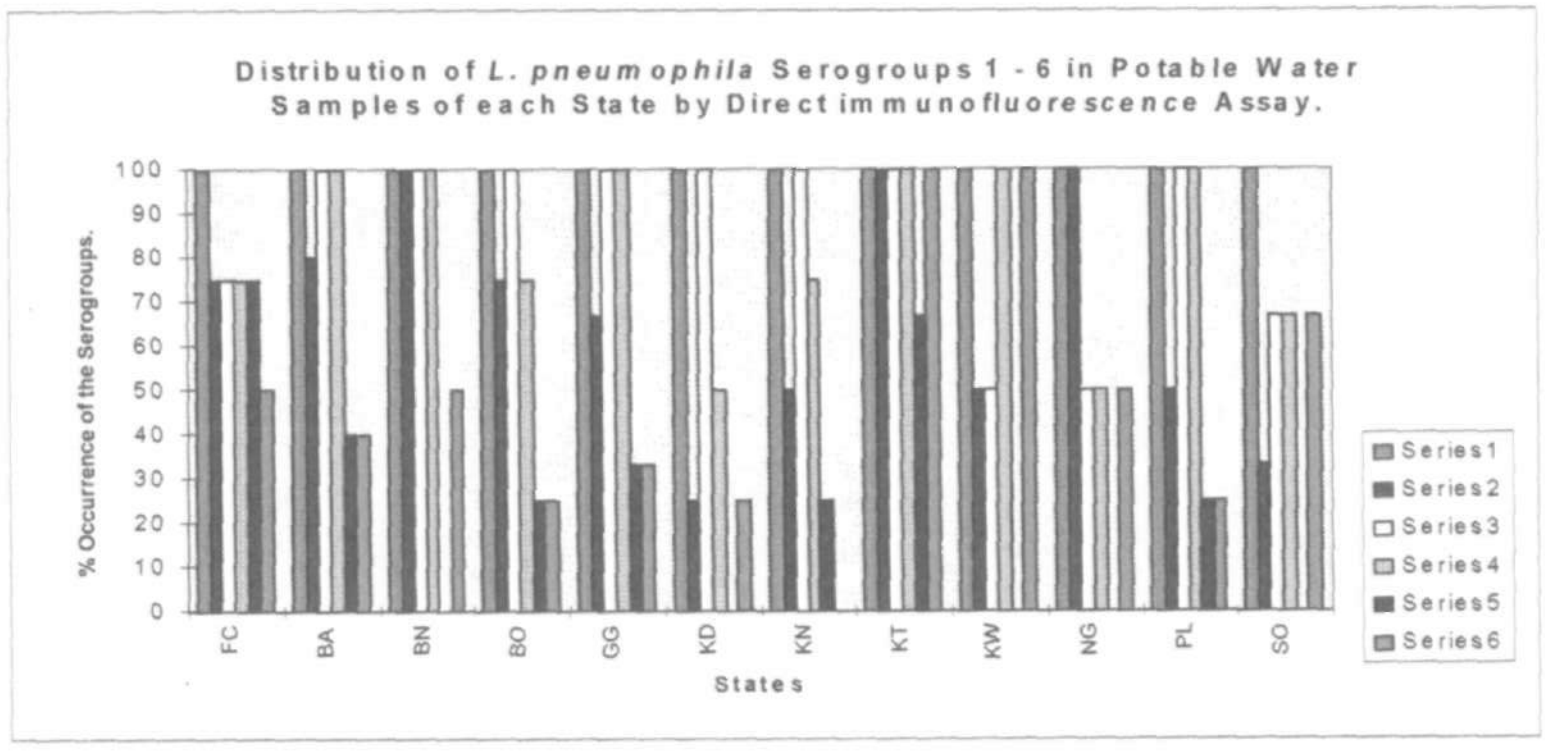
Borno state water samples were varied in percentage positivity rate. Serotype 1 and 3 were detected in every sample, while serogroup 2 and 4 were present in 75% of the samples. The least detected serogroup 5 and 6 were detected in only 25% of the samples. Gongola State water samples on the other hand were 100% positive for serogroup 1,3 and 4, while 67% of the samples were positive for serotype 2. The least detected serogroup 5 and 6 were found in 33% of the samples. Serogroup 1 and 3 were detected in all potable water samples in Kaduna State.

Serotype 2 and 6 were present in 25% of the samples. while serotype 4 was detected in 50% of the samples. However, serotype 5 was not detected in any of the samples. Kano State similarly had serotype 1 and 3 as the predominant serogroups; they were detected in all the samples. Serotype 2 was detected in 50% of the samples. whereas serotype 4 was present in 75% of the samples. Serotype 5 was found in 25% of the samples while serotype 6 was not detected at all.

Katsina State had the highest percentage occurrence rate. Serogroup 5 that had been low or absent in detection rate, except in Abuja, was found in 67% of the samples. All other serogroups were detected in all samples (100%). In Kwara State, serotype 1,4, and 6 were detected in all samples, whereas serotype 2 and 3 were present in 50% of the samples. Serotype 5 however was not detected in any sample. Serotype 1 and 2 prevailed in all samples in Niger State while serotype 3, 4 and 6 were detected in 50% of the samples. Serotype 5 was again not detected in any sample.

Plateau State potable water samples were all positive for serotype 1,3, and 4. However, serotype 2 was positive in 50% of the samples while serotype 5 and 6 were detected in only 25% of the samples. Sokoto state water samples were tested positive for the prototype serogroup 1. While serogroup 2 was detected in only 33% of the samples, serotype 3,4, and 6 were positive in 67% of the samples. However, serotypes 5 was not detected. In all, serotype 5 was not detected in five states' potable water samples. These states are Benue, Kaduna, Kwara, Niger and Sokoto State. Serotype 6, on the other hand was not detected in one state (Kano) water samples.

Fig. 4 - 5



Series 1 - 6 = Serogroups 1 - 6 respectively

FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

4.1.8 Results of Isolation of *Legionella* Bacteria from Potable water samples.

Table 4-5 gives a summary of water samples from distribution systems, wells, and boreholes that were screened by immunofluorescence technique prior to direct plating. All samples from wells, public taps and hotels showed DFA positive *Legionella*-like cells. All samples from hospitals except one were similarly DFA positive. However, 5 out of 12 borehole samples (41.7%) were DFA positive.

Ten well water samples were found to be DFA positive. Six of the ten samples (60%) had culturable legionellae. These samples comprised of a well each from Ikara, Ilorin, Potiskum, Bauchi, Makurdi and Jos.

Ten water samples that were drawn from public taps were 20% positive by culture ; these came from Ilorin and Borno taps. On the contrary, of the 12 hotel's related samples which all had *Legionella* - like cells by DFA, eight samples (66.7%) were culture positive. These samples comprised of 5 A/C cooling towers from Hotels A, B, and C in Abuja, and Hotels A and B in Kaduna; Hotel B swimming pool also in Abuja, Hotel B tap in Kano, and Hotel A tap in Bauchi.

Twenty-one hospital's related samples (19 hospitals and 2 clinics) were 95.2% (20 of 21) positive by immunofluorescence assay. However only 8 samples (38.1%) had culturable *Legionella* bacteria. The lone DFA negative sample came from one operating theatre in Kano, while the eight culture positive samples came from Kaduna Hospital A and G, Katsina Hospital A, Kano Hospital A, Plateau Hospital A, D, and a down town clinic, and Bauchi Hospital A.

There were 12 boreholes samples analysed. Only 5 samples from Ikara, Dange, Sabon Fage, Mashi and Saminaka gave DFA positive cells, thus giving this source the lowest number of DFA positive samples. Culturable legionellae were isolated in 3 of the

5 DFA positive samples. One each of these 3 culture positive samples was recorded in Ikara, Dange and Saminaka.

In summary, 87.7% (57 of 65) of the samples tested by DFA assay were positive. However, less than half of this positive samples (27 of 57 or 47.4%), were culture positive. In terms of the percentage positivity in decreasing order of the categories, Hotel water samples were most culture positive, (66.7%), followed by wells water samples, (60.0%). Hospitals water samples, were third in culture positivity (38.1%), followed by Boreholes water samples (25.0%). And lastly, tap water with 20% culture positive samples(Figure 4-6).

Table 4 - 5. Isolation of *LEGIONELLA* Bacteria From potable Water Samples By Direct Immunofluorescence and Buffered Charcoal Yeast-Extract (BCYE) media.

Sample Number	Date	Sample Source	Direct Analysis of Samples	
			DFA*	BGYE
WS700	17-06-91	Well water. Ikara	+	+
WS701	17-06-91	Well water. Funtua	+	-
WS702	17-06-91	Well water. Samaru	+	-
WS703	20-06-91	Well water. Ilorin	+	+
WS704	31-06-91	Well water. Kano	+	-
WS705	21-06-91	Well water. Ciromawa	+	-
WS706	23-06-91	Well water. Potiskum	+	+
WS707	23-06-91	Well water. Bauchi	+	+
WS708	24-06-91	Well water. Makurdi	+	+
WS709	24-06-91	Well water. Jos	+	+
WS800	26-06-91	Well water. Horin	+	+
WS801	28-06-91	Tap water. Borno	+	+
WS802	18-06-91	Tap water. Kaduna	+	-
WS803	02-05-91	Tap water. Abuja	+	-
WS804	02-05-91	Tap water. Minna	+	-
WS805	26-05-91	Tap water. Jos	+	-
WS806	26-05-91	Tap water. Bauchi	+	-
WS807	18-05-91	Tap water. Kano	+	-
WS808	31-05-91	Tap water. Sokoto	+	-

Table 4 - 5 Continued.

Sample Number	Date	Sample Source	Direct Analysis of Samples	
			DFA*	BGYE
WS809	17-06-91	Tap water, Zaria	+	-
WS860	02-05-91	Hotel A A/C, Abuja	+	+
WS861	02-05-91	Hotel A Swimming pool, Abuja	+	-
WS862	02-05-91	Hotel B A/C, Abuja	+	+
WS863	02-05-91	Hotel B Swimming pool, Abuja	+	+
WS864	07-05-91	Hotel C A/C, Abuja	+	+
WS864b	02-05-91	Hotel C Swimming pool, Abuja	+	-
WS865	18-05-91	Hotel A A/C, Kaduna	+	+
WS866	18-05-91	Hotel A Swimming pool, Kaduna	+	-
WS867	18-05-91	Hotel B A/C, Kaduna	+	+
WS868	18-05-91	Hotel B Swimming pool, Kaduna	+	-
WS869	18-05-91	Hotel B, Kano	+	+
WS870	17-06-91	Hotel A, Bauchi	+	+
WS900	18-06-91	Hospital A, Kaduna	+	+
WS901	18-06-91	Hospital B, Kaduna	+	-
WS902	18-06-91	Hospital C, Kaduna	+	-
WS903	18-06-91	Hospital D, Kaduna	+	-
WS904	18-06-91	Hospital E, Kaduna	+	-
WS905	18-06-91	Hospital F, Kaduna	+	-
WS906	18-06-91	Hospital G, Kaduna	+	+
WS907	19-06-91	Hospital A, Katsina	+	+

Table 4 - 5 Continued

Sample Number	Date		Direct Analysis of Samples	
			DFA	BCYE
WS908	19-06-91	Hospital B, Katsina	+	-
WS909	19-06-91	Hospital C, Katsina	+	-
WS910	23-04-91	Hospital A, theatre, Kano	-	-
WS911	23-04-91	Hospital A, Ward, Kano	+	+
WS912	23-04-91	Hospital B, Kano	+	-
WS913	23-04-91	Hospital B, Ward, Kano	+	-
WS914	29-05-91	Hospital A, Plateau	+	+
WS915	26-05-91	Hospital B, Plateau	+	-
WS916	26-05-91	Hospital C, Plateau	+	-
WS917	26-05-91	Hospital D, Plateau	+	+
WS918	26-05-91	Down Town Clinics, Jos	+	+
WS919	26-05-91	Hospital A, Bauchi	+	+
WS920	26-05-91	Clinics DS, Bauchi	+	-
BWS910	29-05-91	Borehole, Ikara	+	+
BWS911	31-05-91	Borehole, Tungun Giwa	-	-
BWS912	31-05-91	Borehole, Minna	-	-
BWS913	31-05-91	Borehole, Dange	+	+
BWS914	31-05-91	Borehole, Trata	-	-
BWS915	31-05-91	Borehole, SabonFage	+	-
BWS916	03-06-91	Borehole, Mashi	+	-
BWS917	03-05-91	Borehole, Giwa	-	-

Table 4- 5 Continued

Sample Number	Date	Sample Source	Direct Analysis of sample	
			DFA	*BCYE*
BWS918	03-06-91	Borehole. Lere	-	-
BWS919	03-06-91	Borehole. Saminaka	+	+
BWS920	05-06-91	Borehole. Kono	-	-
BWS921	06-04-91	Borehole. Bauchi	-	-

* DFA = Direct immunofluorescence assay

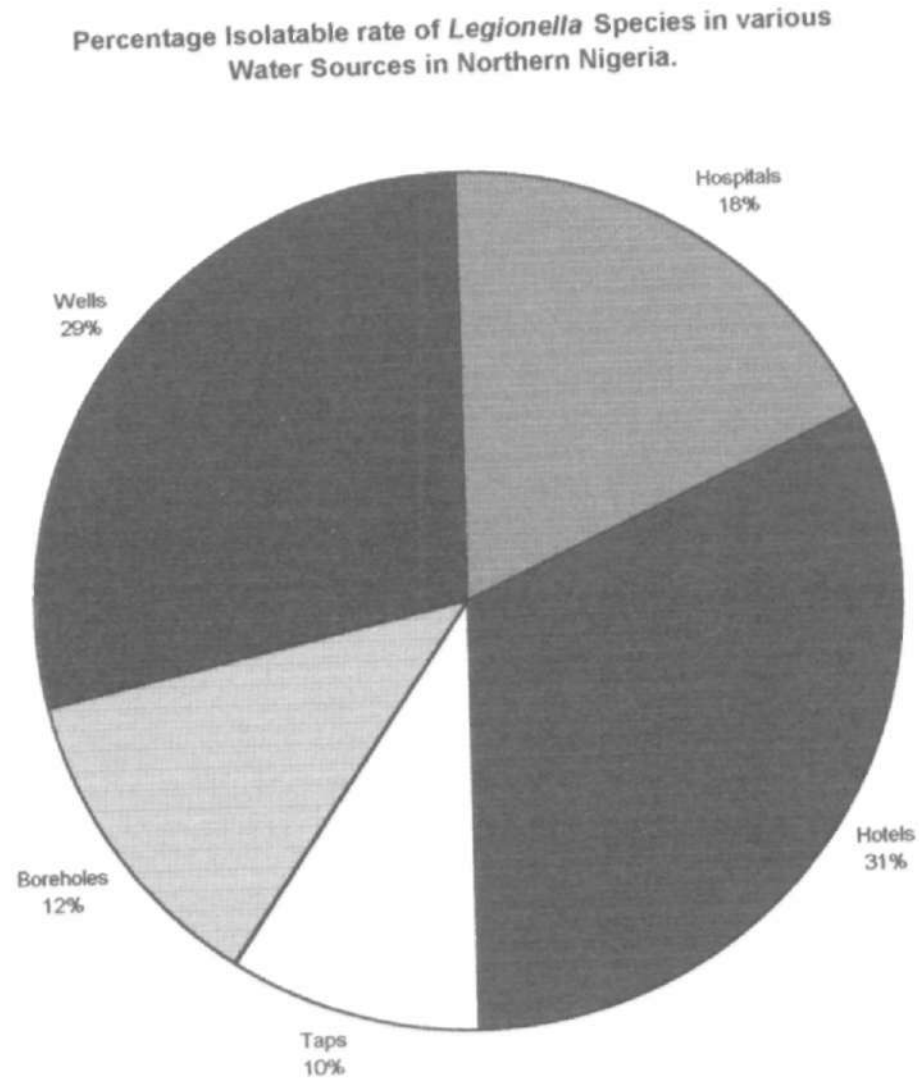
+ = Presence of *Legionella*-like cells or colonies

- = Absence of *Legionella*-like cells or colonies.

WS = Water sample.

BWS = Borehole water sample.

Fig. 4 - 6.


Percentage Isolatable rate of *Legionella* Species in various Water Sources in Northern Nigeria.
Hospitals 18%
Wells 29%
Hotels 31%
Boreholes 12%
Taps 10%

4.2.0 Results of Raw Water Samples

4.2.1 Results of Physical and Biological Characteristics of Raw Water Samples.

A total of 246 (37%) water samples from various sources were screened by DFA for *Legionella* spp. Figure 4-1 is a map showing the sampling areas as well as indicating the major rivers, towns and cities. Selected physical, chemical and biological characteristics of the habitats are summarised on Table 4-6. It was observed that there was not much difference in mean water temperatures between rivers, streams, dams, lakes and ponds. However, the mean temperature for springs was much lower (21.7 °C). For all the microenvironments the temperature ranged 21.7 to 28.5 °C). The highest mean temperature (28.5 °C) was recorded from river samples, followed by dams/lakes (27.4 °C). Streams/creeks samples followed closely with a mean temperature of 27.3 °C.while ponds recorded 26.7 °C.

It was also observed that the pH of raw water samples was acidic with a very narrow range of pH 4.8 recorded in pond samples to pH 5.9 in streams/creeks samples. Critical analysis of Table 4-6 shows that the physical characteristics of the raw water from the various sources had little or no effect on bacterial density as seen by the data. The streams/creeks recorded a mean cell density of 2.7 X 10^5 l^{-}, while rivers and dams/lakes recorded 2.3 X 10^5 l^{-} each. One hot spring had cell density of 3.4 X 10^5 l^{-}.

Table 4 - 6: A Summary of some Mean Physical and Biological Characteristics of Raw Water Samples Screened for *Legionella* Species

Sample Source	Number of samples (%)	Mean Temp. °C	Mean pH	Mean Conductivity µs/cm.	DO (mg/l)	Mean bacterial density/ml
Rivers	112 (45.5)	28.53	5.84	163	NT	2.2×10^5
Streams/Creeks	41 (16.7)	27.33	5.91	142	NT	2.7×10^5
Springs	3 (1.2)	21.7	NT	NT	NT	3.4×10^5*
Dams/Lakes	73 (29.7)	27.42	5.34	119	NT	2.3×10^5
Ponds	17 (6.9)	26.72	4.83	NT	NT	NT

NT= not tested

*=only one hot spring was quantitated.

4.2.2 Raw Water Samples Results of the Polyvalent Pool Conjugate Reactions

The results of 51 raw water samples are illustrated on Table 4-7. *Legionellaceae* were detected in virtually all the natural habitats sampled in all the States. However, polyvalent pool B and C had some samples which did not fluoresce with *Legionella* - like cells.

At polyvalent pool conjugate reactions, only *L. pneumophila* was found in every sample tested. Under pool A which identified *L. pneumophila* serotype 1, 2, 3 and 4, there were 21 strong reactions. Wikki spring in Bauchi and one unnamed river in Benue State fluoresced 4+ intensity. The remaining 19 strong reactions came from 11 rivers, 6 dams, and 2 streams. The rest of the 29 reactions were weak (2+) with the weakest intensity of 1+ recorded for River Upa in Abuja. There were 14 dams, 12 rivers and 3 streams for the remaining 2+ reaction.

Polyvalent pool B had the least number of positive results (39 or 76.5%) with only 4 strong (3+) reactions with samples from Wikki spring in Bauchi, Lake Chad in Borno, Saminaka river in Kaduna and River Niger in Kwara State. The remaining 35 positive samples gave different levels of intensity reaction. Twenty-four were of 2+ level, whereas 11 exhibited 1+ fluorescence intensity. The 24 that fluoresced 2+ comprised of 11 rivers, 9 dams and 4 streams. Six rivers, 4 dams and 1 creek fluoresced 1+ intensity. However, 12 samples of 6 rivers and 6 dams did not have detectable cells of *Legionella* morphology. These twelve samples spread across all the States except Niger and Plateau.

Polyvalent pool C reactions were second in prevalence: 94.1% of the samples tested positive. Only 3 samples from Udei in Benue, and Bosso in Niger, and river Osun

in Kwara State tested negative . The remaining 48 samples tested positive with fluorescence activities ranging from very weak (1+) to very strong (4+) intensity. One sample from one unnamed river in Benue State was strongly positive (4+), while 5 other samples from Gulbi dam, River Dindima and Toro river (all in Bauchi). Asa dam in Kwara State. and river Lafiya in Plateau State recorded 3+ intensity reactions. Majority of the samples (33) fluoresced 2+ level. These were from 17 rivers, 14 dams. 1 stream and 1 spring. The remaining 9 samples of 3 rivers 4 dams and 2 streams gave fluorescence activity of 1+ level. In summary, Polyvalent pool A conjugate exhibited the highest number (21) of strong reactions of 4+ (2 samples) and 3+ (19 samples) intensity. Pool C conjugate had six strong reactions. and lastly pool B which had only 4 strong reactions. There were more river water samples with strong reactions (12, 6. and 2 samples each from pool A. C, and B respectively) than dam water samples (6, 2. and 1 respectively).

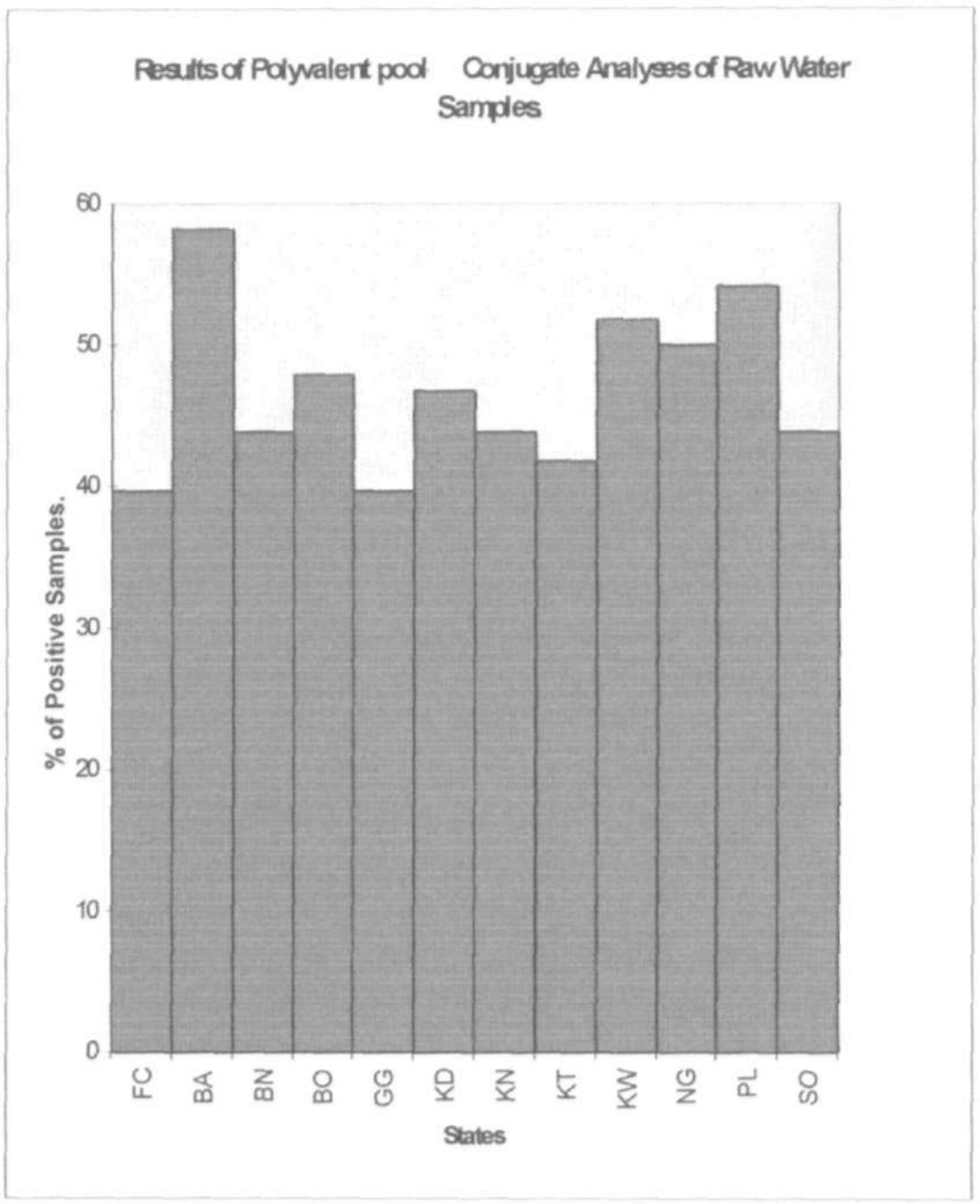
Table 4 - 7: *LEGIONELLA* Polyvalent Pool* Reactions On Selected Raw Water Samples

Sample Source	State	Polyvalent Pool Conjugates		
		A	B	C
Airport Creek	FCT	2+**	1+	2+
River Gwagwalada	FCT	2+	1+	2+
River Jabi	FCT	2+	0	2+
River Upa	FCT	1+	2+	2+
Gulbi Dam	BA	3+	0	3+
River Dindima	BA	2+	1+	3+
River Toro	BA	3+	2+	3+
River TuldenFulani	BA	2+	2+	2+
Wikki Spring	BA	4+	3+	2+
Yandev Dam	BN	2+	0	2+
River Ogi	BN	2+	1+	2+
River Q.	BN	4+	0	4+
Stream Undei	BN	3+	2+	0
Lake Alao	BO	3+	0	2+
Lake Chad	BO	2+	3+	2+
Lake Canal	BO	2+	2+	1+
River Ngada	BO	3+	2+	1+
River Benue	GG	2+	0	2+
River Ibi	GG	2+	2+	1+
Kiri Dam	GG	2+	0	1+
River Ngada	GG	3+	2+	2+
River Kono	KD	3+	0	2+
River Saminaka	KD	2+	3+	2+
River Jere	KD	2+	2+	2+
University Dam	KD	2+	1+	2+
Tarau Dam	KD	2+	1+	2+
River Ciromawa	KN	3+	1+	2+
Bagauda Dam	KN	2+	0	2+
Kadawa Dam	KN	2+	2+	2+
Tiga Dam	KN	2+	1+	2+
Ajiwa Dam	KT	2+	0	1+

4.2.3 Summary Results of Polyvalent Pool Analysis of Raw Water Samples.

Illustrated in Figure 4 - 7 is the polyvalent pool reaction demonstrating the prevalence of *Legionella* bacteria in all of the sampled sites in Northern Nigeria. Under the polyvalent pool conjugate analyses, the prevalence percentage rates were much lower than that recorded in the monovalent reactions. Bauchi State recorded the highest prevalence rate of 58.3%. Three other States recording 50% and above prevalence rate were: Plateau, 54.2%; Kwara .51.7%; and Niger State, 50.0%. In 6 other states the prevalence rates were below 50%. These were observed in Borno State, 47.9%; Kaduna State, 46.7%; Benue, Kano, and Sokoto States all recorded 43.8% each; and lastly, Katsina State which recorded 41.7%. The last two sampling areas, Abuja and Gongola States with the lowest prevalence rates recorded 39.6% each.

Fig. 4 - 7.



FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

4.2.4. Population Densities of *LEGIONELLA* Species in Raw Water By Polyvalent Pool Conjugates

Raw Water samples from various rivers (23), dams and lakes (17) streams and creeks (9), a spring and a pond were analysed by DFA assay. *Legionella* spp and serogroups were microscopically quantitated from 51 samples using *Legionella* polyvalent pool conjugates. Table 4-8 shows that although *Legionella* spp are ubiquitous and coexist in virtually all the habitats, their densities vary slightly from one conjugate to the other, thus reflecting fluorescence activities of the species identifiable by their respective pool conjugate.

Polyvalent pool A conjugate recorded the highest number of positive samples (50 of 51), 98.0% as well as the highest cell densities per millilitre. Cell densities of 10^7 were recorded from 2 samples, one in Bakalori dam, Sokoto, and the other from one unnamed river in Benue State. There were 21 samples (41.2%) which recorded cell densities of 10^6. These were 9 dams and lakes, 7 rivers, and 5 creeks and streams. The highest number (27 of 51) or 52.9% of samples had call densities of 10^5. There were more rivers (15) in this group of samples followed by dams and lakes (7), creeks and streams (4), and one spring. The only pond examined surprisingly had no fluorescing calls of *Legionella* characteristics.

At polyvalent pool B conjugate where 80.4% of the samples were positive, only 7 samples had cell densities of 10^6. This number comprised of 4 dams, 2 rivers and one creek. However, the majority of the samples, 56.9% or 29 of 51 recorded cell densities of 10^5. These samples comprised of 15 rivers, 8 dams/lakes, 5 streams/creeks and Wikki spring. Lowest cell densities of 10^4 were observed in 5 samples from Abuja (2)

and one sample each from Bauchi, Borno and Kaduna. However, there were 10 samples which did not fluoresce with *Legionella* - like cells.

Polyvalent pool C fluorescence activities of 82.4% positivity rate were second to pool A. There was one sample from one unnamed river in Kaduna with cell density of 10^7. Twelve other samples of 7 rivers, 4 dams and a stream recorded cell densities of 10^6. Of the 7 rivers, 3 (rivers) from Benue State had the same cell densities of 10^6 at both pool A and C, while they also recorded 10^5 at pool B. Four other rivers, 2 from Kaduna, one each from Kano and Kwara State exhibited similar cell densities (10^6) at both pool A and B as well. Majority of the remaining samples (23) had cell densities of 10^5. They were 9 rivers, 7 creeks and stream, 6 dams and lakes, and one spring. There were more rivers (7) tested from Sokoto State. The 4 rivers that fluoresced at pool C had similar cell densities (of 10^5) with pool B, although slightly lower than at pool A. The remaining 5 rivers samples were from Bauchi, Borno, Kaduna, Niger and Plateau State. Three rivers recorded cell densities of 10^4 cells per millilitre. Incidentally, one of these rivers in Benue state, recorded the highest cell density of 10^7 at pool A reaction while recording no fluorescing cells at pool B. Ngada river in Borno State similarly did not show fluorescing cells at pool C although cell density of 10^5 was observed at Pool A. The third river, Gulbi in Sokoto demonstrated cell densities of 10^5 at both pool A and B while recording 10^4 at pool C. On the other hand 9 samples of 4 dams, 3 rivers, a stream and a pond did not record any (fluorescence) cells of *Legionella* morphology.

Table 4 - 8: *Legionella* Population Densities by DFA Polyvalent Pool* Conjugates on Selected Raw Water Samples.

Sample Source	State**	Polyvalent Pool Conjugates		
		A	B	C
Airport Creek	FC	5.0×10^{6}***	4.0×10^{6}	4.5×10^{5}
River Usman	FC	1.8×10^{5}	1.0×10^{4}	-
Sheraton Creek	FC	2.0×10^{5}	1.0×10^{4}	2.3×10^{5}
Wikki Spring	BA	4.6×10^{5}	3.0×10^{5}	2.5×10^{5}
Tulden Fulani River	BA	2.3×10^{5}	2.1×10^{5}	1.4×10^{5}
Gulbi Dam	BA	3.5×10^{5}	1.0×10^{4}	2.7×10^{5}
River *Ogi*	BN	2.6×10^{6}	1.0×10^{5}	2.7×10^{6}
River Ogoli	BN	1.2×10^{6}	1.0×10^{5}	2.5×10^{6}
River Benue	BN	2.8×10^{6}	1.2×10^{5}	2.6×10^{6}
River Q1	BN	3.5×10^{7}	-	3.8×10^{4}
River Q2	BN	9.0×10^{5}	4.0×10^{5}	-
Lake Chad	BO	2.6×10^{6}	1.6×10^{5}	1.4×10^{5}
River Ibeji	BO	1.8×10^{5}	2.2×10^{5}	1.3×10^{5}
River Ngada	BO	4.1×10^{5}	1.2×10^{5}	1.5×10^{4}
Lake Alao	BO	1.8×10^{5}	-	1.6×10^{4}
Kiri Dam	GG	1.7×10^{6}	-	1.2×10^{4}
River Benue	GG	3.4×10^{5}	-	-
Karewa Pond	GG	-	-	-
River Jere	KD	8.0×10^{5}	1.2 x 106	2.6×10^{5}
River Q1	KD	1.8×10^{5}	2.1×10^{5}	6.3×10^{7}
Tarau Dam	KD	2.5×10^{5}	4.0×10^{4}	8.0×10^{4}
Zaria Dam	KD	2.6×10^{6}	1.8×10^{5}	-
River Q2	KD	5.5×10^{6}	1.1×10^{6}	1.5×10^{6}
Saminaka Creek	KD	3.5×10^{6}	1.0×10^{5}	1.4×10^{5}
River Kono	KD	1.2×10^{5}	-	2.4×10^{6}
Bagauda Lake	KN	2.3×10^{6}	-	1.2×10^{5}
Kadawa Dam	KN	1.3×10^{5}	1.2×10^{5}	2.4×10^{6}
Tiga Dam	KN	2.7×10^{6}	1.7×10^{5}	1.3×10^{5}
River Ciromawa	KN	1.0×10^{5}	1.8×10^{5}	2.6×10^{6}
River Niger	KW	2.8×10^{5}	1.8×10^{5}	2.6×10^{6}
River Kainji	KW	1.5×10^{5}	2.8×10^{6}	-

Table 4 - 8 Continued

Sample Source	State	Polyvalent Pool Conjugates		
		A	B	C
Asa Dam	KW	1.0×10^5	2.4×10^5	1.2×10^5
Offa Dam	KW	1.2×10^5	2.0×10^6	2.6×10^6
River Wuya	NG	3.6×10^6	1.2×10^5	1.5×10^5
Bosso Stream	NG	2.7×10^6	1.6×10^5	-
Jebba Dam	NG	2.2×10^6	1.8×10^6	2.7×10^6
Shiroro Dam	NG	2.3×10^6	1.2×10^5	2.4×10^6
Suleja Stream	NG	2.6×10^5	-	2.5×10^5
Liberty Dam	PL	3.5×10^6	2.2×10^5	2.4×10^5
Rock Dam	PL	2.5×10^6	1.8×10^5	-
Gada Biyu Stream	PL	3.3×10^6	1.2×10^5	3.5×10^6
Farin Gada Stream	PL	3.4×10^6	1.5×10^5	1.7×10^5
River Lafiya	PL	3.8×10^5	2.6×10^5	3.2×10^5
Jos-Bukuru Stream	PL	1.9×10^5	3.8×10^5	1.1×10^5
River Gulbi	SO	1.3×10^5	1.4×10^5	8.0×10^4
River Bungudu	SO	3.5×10^6	-	1.8×10^5
Bakalori Dam	SO	3.7×10^6	2.4×10^5	-
River Jega	SO	2.1×10^5	1.6×10^5	1.2×10^5
River Argungu	SO	3.4×10^6	1.2×10^5	2.7×10^5
River Malando	SO	3.0×10^5	2.2×10^5	2.8×10^5
University Creek	SO	2.2×10^5	-	1.3×10^5

.* Polyvalent Pool A = *L. pneumophila* serogroups 1, 2, 3 and 4.

B = *L. pneumophila* serogroups 5 and 6; *L. dumoffii* serogroup 1, and *L. longbeachae* serogroup 1.

C = *L. micdadei* serogroup 1, *L. bozemanii* serogroup 1, *L. gormanii* serogroup 1, and *L. longbeachae* serogroup 2.

**States: FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

*** Counts were based on microscopic observation of fluorescing cells of *Legionella* morphology in 0.05ml of sample concentrate and reported as the number of fluorescing cells/ml.

- = absence of *Legionella*-like cells

Q = Name of river unknown.

4.2.5 Results of Type-Specific Antisera Reactions

L. pneumophila, the prototype species and serogroup1, was detected in 50 of 51, (98.0%) of the samples. One site, Ajiwa dam did not fluoresce with cells of *Legionella* morphology (Table 4-9). This serogroup predominated and exhibited more strong reactions (3+ fluorescence), 49.0% (25 of 50) than the other five serogroups. These 25 (3+ reaction), comprised 12 river samples, 10 dams/lakes samples and 3 samples from creeks/streams. The remaining 25 samples comprising of 13 rivers, 9 dams/lakes, 2 streams and a spring exhibited 2+ intensity (Table 4-9). Serogroup 4 closely followed the prototype of the species in relative abundance. It was detected in 94.1% (48 of 51) of the samples with 43.1% (22 of 51) of the samples fluorescing at 3+ intensity. These 22 samples were drawn from 11 rivers, 10 dams/lakes and a stream. Majority of the positive samples (25 of 48) fluoresced at 2+ level, and comprised of 13 rivers, 8 dams/lakes and 4 streams. A very weak (1+) reaction was observed in one sample from Kiri dam while the remaining 3 samples did not have fluorescing *Legionella* - like cells (Table 4-9).

L. pneumophila serogroup 2 was third in abundance. It exhibited forty-three out of the 51 samples (84.3%), with fluorescence cells of *Legionella* morphology, however, only 3 samples (5.9%), one each from Bauchi, Kano and Niger fluoresced at 3+ intensity. Most samples (30 of 51) however, gave fluorescence activity of 2+ level. Of this number, 17 came from various rivers from all the States sampled. Except for Abuja, the Capital Territory and Kano State with only one 2+ samples each, every other State had 2 to 3 samples with such fluorescence activity. There were 10 samples, (4 rivers and 6 dams) of 1+ intensity fluorescence. However, the remaining 8 samples did not show fluorescing cells of *Legionella* morphology.

L. pneumophila serogroup 3 had 9 negative samples from 5 rivers, 2 dams and 2 streams. However, fluorescence activity of 3+ intensity was observed in 3 other rivers, one each from Bauchi, Benue and Kaduna. Another river yet with higher fluorescence activity of 4+ level was recorded in Borno State. The remaining positive samples of 2+ level were recorded from 31 sites comprising -12 rivers, 16 dams, 2 streams and a spring. The rest of the samples (7) of 3 rivers, 3 dams and a stream fluoresced faintly at 1+ intensity. In all, 82.4% (42 of 51) of the samples tested positive for serogroup 3.

L. pneumophila serogroup 6 had 33 (64.7%) positive samples. Six of these samples (4 rivers and 2 dams) fluoresced at 3+ intensity whereas the remaining 27 samples fluorescence was at 2+ level. These were 13 rivers, 10 dams, and 4 streams. No samples fluoresced at 1+ level. Of the 18 (35.3%) that did not exhibit fluorescence activity, 4 samples were positive at polyvalent pool B reaction: Airport creek, Wikki spring, Ngada river and Tiga dam. Serogroup 5 had the least positive samples (26 of 51) with fluorescence activity ranging from 1+ to 2+ intensity. Nine of the positive samples were from 3 rivers, 4 dams and 2 streams while the remaining 17 positive samples were drawn from 10 rivers and 7 dams. However, 25 samples did not show any detectable fluorescence activity (Table 4-9).

Of the other *Legionella* species that were screened, *L. gormanii* was the predominant species. It was detected in 86.3% (44 of 51) of the samples. Fluorescence activity was observed as follow: 3+ for 4 samples of 2 rivers and 2 dams, and 2+ intensity for 35 samples of 17 rivers, 15 dams/lakes, 2 streams and a spring. Four samples (1 river and 3 dams) fluoresced at 1+ intensity. However, the remaining 7 samples, (4 rivers and 3 streams) did not fluoresce with *Legionella* - like cells.

L. micdadei second in ubiquity was detected in 82.4% (42 of 51) of the samples. There were however more samples fluorescing at 2+ level. Nine samples fluoresced at 3+ intensity while Wikki spring sample fluoresced 4+ level, the highest intensity in the scale. The 9 samples which fluoresced with 3+ intensity level comprised of 6 rivers, 2 dams and a stream. Of the remaining 32 positive samples, 30 samples of 15 rivers, 14 dams and a stream exhibited fluorescence activity of 2+ level. Two dams fluoresced faintly at 1+ intensity. Nine samples however, did not show any cells of *Legionella* morphology.

Fluorescence activity of *L. bozemanii* was shown only in 24 of the 51 samples (47.1%). Intensity of 2+ level was observed on 12 samples (7 rivers, 4 dams and a spring), while another 12 samples (8 rivers, and 4 dams), fluoresced at 1+ level. Majority of the samples (27) comprising of 10 rivers, 12 dams and 5 streams were tested negative for fluorescence activity. (Table 4-9).

Table 4 - 9: Results of *Legionella* Serospecific Reactions on Selected Raw Water Samples by Direct Immunofluorescence Assay

Sample Source	State	*Legionella pneumophila* serogroups						Other *Legionella* Species				
		1	2	3	4	5	6	*L. bozemanii*	*L. gormanii*	*L. longbeachae*	*L. micdadei*	*L. dumoffii*
Airport Creek	FCT	3+	-	1+	2+	X	X	-	2+	NT	2+	NT
River Gwagwalada	FCT	2+	1+	-	2+	1+	2+	-	2+	NT	2+	NT
River Jabi	FCT	2+	2+	2+	3+	-	-	-	2+	NT	2+	NT
River Upa	FCT	2+	-	-	2+	1+	2+	1+	-	NT	3+	NT
Gulbi Dam	BA	3+	-	2+	3+	-	-	2+	2+	NT	1+	NT
River Dindima	BA	2+	2+	2+	2+	2+	-	2+	3+	NT	-	NT
River Toro	BA	3+	2+	3+	3+	-	2+	-	2+	NT	2+	NT
River Tulden Fulani	BA	3+	2+	1+	2+	-	2+	2+	2+	NT	3+	NT
Wikki Spring	BA	2+	3+	2+	3+	X	X	2+	2+	NT	4+	NT
Yandev Dam	BN	3+	2+	2+	3+	-	-	-	1+	NT	2+	NT
River Ogi	BN	2+	2+	-	2+	-	3+	-	2+	NT	2+	NT
River Q1	BN	3+	2+	3+	3+	-	-	2+	2+	NT	-	NT
Stream Undei	BN	3+	-	2+	2+	2+	2+	-	-	NT	-	NT
Lake Alau	BO	3+	2+	2+	2+	-	-	1+	2+	NT	2+	NT
Lake Chad	BO	3+	2+	2+	3+	1+	2+	1+	2+	NT	3+	NT
Lake Canal	BO	3+	1+	2+	2+	2+	3+	1+	2+	NT	2+	NT
River Ngada	BO	3+	2+	4+	-	X	X	2+	X	NT	X	NT
River Benue	GG	2+	1+	2+	2+	-	-	2+	2+	NT	2+	NT
River Ngada	GG	2+	2+	-	2+	1+	2+	1+	2+	NT	3+	NT

Table 4 - 9 Continued

. Sample Source	State.	*Legionella pneumophila* serogroups						Other *Legionella* Species				
		1	2	3	4	5	6	*L. bozemanii*	*L. gormanii*	*L. longbeachae*	*L. micdadei*	*L. dumoffii*
Kiri Dam	GG	2+	1+	2+	1+	-	-	1+	2+	NT	2+	NT
River Benue at Ibi	GG	3+	2+	2+	3+	1+	2+	2+	3+	NT	2+	NT
River Kono	KD	2+	2+	2+	3+	-	-	-	-	NT	2+	NT
River Saminaka	KD	3+	1+	3+	2+	1+	2+	1+	1+	NT	2+	NT
River Jere	KD	3+	2+	-	2+	1+	2+	1+	2+	NT	2+	NT
University Dam	KD	2+	1+	2+	3+	1+	2+	-	2+	NT	2+	NT
Tarau Dam	KD	3+	2+	1+	2+	1+	2+	-	1+	NT	2+	NT
River Ciromawa	KN	2+	3+	2+	3+	1+	2+	-	2+	NT	2+	NT
Bagauda Dam	KN	2+	1+	-	3+	-	-	-	2+	NT	2+	NT
Kadawa Dam	KN	2+	2+	2+	-	2+	2+	2+	3+	NT	2+	NT
Tiga Dam	KN	3+	1+	2+	3+	N	N	-	2+	NT	2+	NT
Ajiwa Dam	KT	-	2+	2+	3+	-	-	-	2+	NT	-	NT
Kankara Dam	KT	2+	-	2+	2+	1+	-	-	2+	NT	-	NT
Mairuwa Dam	KT	3+	2+	1+	2+	-	2+	-	1+	NT	2+	NT
Zobe Dam	KT	2+	-	2+	-	1+	2+	-	2+	NT	1+	NT
River Osun	KW	2+	1+	2+	3+	-	-	-	-	NT	-	NT
River Niger	KW	3+	2+	2+	2+	-	3+	1+	2+	NT	2+	NT
Asa Dam	KW	3+	1+	1+	3+	2+	2+	-	3+	NT	2+	NT
Offa Dam	KW	2+	2+	2+	3+	2+	2+	2+	2+	NT	2+	NT
Lake Kainji	KW	2+	2+	-	2+	1+	2+	-	2+	NT	2+	NT

Table 4 - 9
Continued

	State	*Legionella pneumophila* serogroups						*Other Legionella Species*				
		1	2	3	4	5	6	*L. bozemanii*	*L. gormanii*	*L. longbeachae*	*L. micdadei*	*L. dumoffii*
River Wuya	NG	2+	3+	2+	2+	1+	3+	1+	2+	NT	3+	NT
Stream Bosso	NG	3+	2+	-	2+	-	2+	-	-	NT	-	NT
Gurara Falls	NG	3+	2+	1+	3+	2+	2+	2+	2+	NT	3+	NT
Shiroro Dam	NG	2+	2+	2+	3+	1+	2+	-	2+	NT	2+	NT
River Lafiya	PL	3+	2+	2+	3+	1+	2+	1+	3+	NT	2+	NT
Farin Gada Stream	PL	2+	-	-	2+	2+	2+	-	x	NT	x	NT
Gada Biyu Stream	PL	2+	2+	2+	3+	-	2+	-	2+	NT	3+	NT
Liberty Dam	PL	3+	2+	2+	2+	1+	3+	2+	2+	NT	3+	NT
River Argungu	SO	3+	2+	2+	2+	2+	2+	1+	2+	NT	2+	NT
River Bungudu	SO	2+	2+	2+	3+	-	-	-	2+	NT	3+	NT
River Gulbi	SO	3+	2+	2+	2+	-	3+	-	2+	NT	2+	NT
River Jega	SO	2+	-	1+	2+	-	2+	-	2+	NT	2+	NT

Note: Scoring of fluorescence intensities on a diminishing scale:

4+ = brilliant yellow-green staining of bacterial cells

3+ = bright yellow-green staining of bacterial cells

2+ = definite but dim staining of bacterial cells

\+ = barely visible staining of bacterial cells of *Legionella* morphology.

\- = absence of *Legionella*-like cells

x = no staining with type-specific antisera where pool reaction was positive.

NT = not tested- reagents were not available

Q1 = Name of river unknown.

States: FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

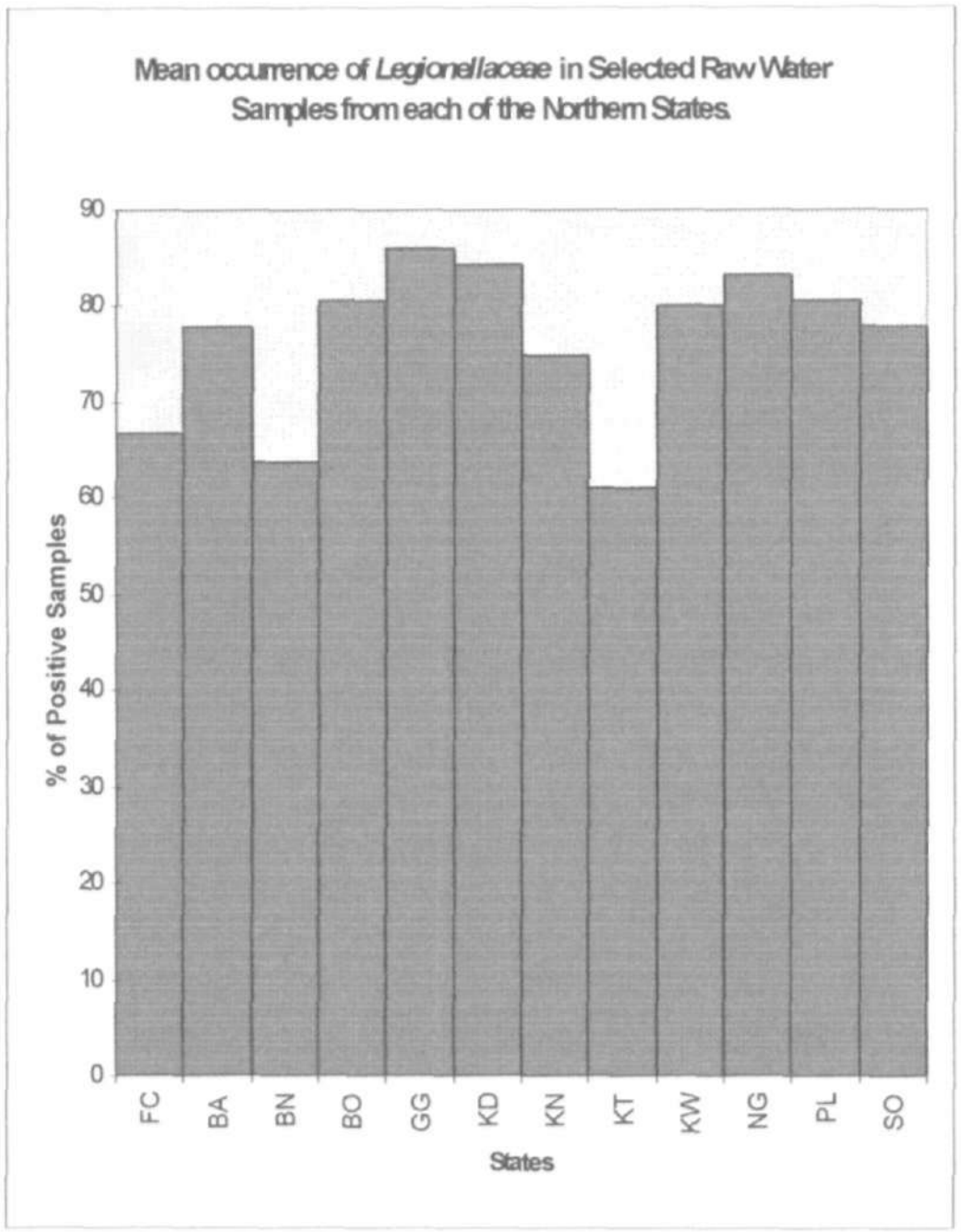
GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger ; PL = Plateau; SO = Sokoto.

4.2.6 Relative Abundance of *Legionellaceae* in Northern Nigeria Raw Waters

The mean occurence of *Legionella*ceae for each State is illustrated in Figure 4-8. Legionellae under study included *L. pneumophila* and its first 6 serotypes, *L. bozemanii*, *L. gormanii*, and *L. micdadei*. Under monospecific reactions, each State had exhibited a high degree of positivity rate that did not always coincide exactly with its corresponding polyvalent pool reactions (Figure 4-6). The ubiquitous nature of *Legionella* spp. could not be better demonstrated: even the sampling areas (Abuja and Gongola) with the lowest number (39.6%) of positive samples were still over 60% positive for legionellae. Second in lower positivity rate was Benue State which recorded 63.9% (23 of 36) positive samples. Adjoining to and east of Benue State is Gongola State which had the highest number (31 of 36 or 86.1%) of positive samples. Second in high percentage occurrence of positive samples was Kaduna State with 84.4% (38 of 45), then Niger State with 83.3% (30 of 36) positive samples. Other states in decreasing order of positive samples were: Plateau, 80.6% (29 of 36); Kwara, 80.0% (36 of 45); Borno and Sokoto, 77.8% each; Bauchi, 75.6%, Kano, 75.0% (27 of 36); and lastly, Abuja the Capital Territory with 66.7% (24 of 36) positive samples. There were more samples (45) screened from three States - Bauchi, Kaduna, and Kwara - than in the rest of the States where 36 samples each were analysed.

Fig. 4 - 8.



FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

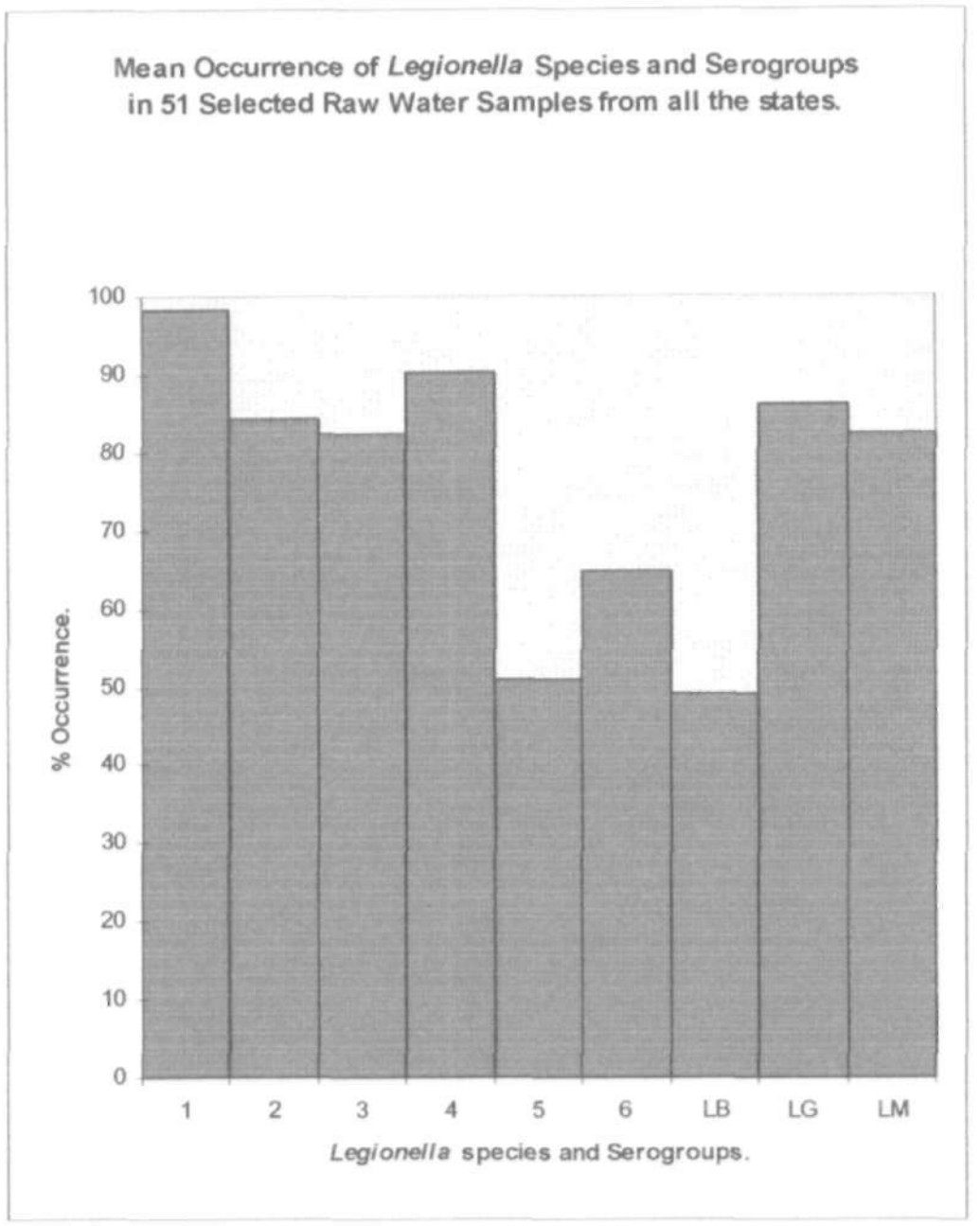
GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

4.2.7 Results Distribution of *Legionella* Species and Serogroups in Raw Water Samples in Northern Nigeria

Figure 4-9 gives a summary of the percentage occurrence of the 4 major species of *Legionella* including 6 serogroups of *L. pneumophila*. *L. pneumophila* serogroup 1, the prototype species and serogroup was detected in 50 of the 51 samples (98.0%). Serogroup 4 of the species was second in relative abundance, 90.0% (46 of 51) of the samples were positive. Third was species *L. gormanii*, which was detected in 86.3% (44 of 51) of the samples screened. Serotype 2 of *L. pneumophila* was fourth in occurrence, with 43 of 51 (84.3%) samples positive under type-specific antisera analysis. *L. pneumophila* serotype 3 and *L. micdadei* occurred in equal number of samples (42 of 51); both were detected in 82.4% of the samples. Of the remaining less frequently detected serogroups 5 and 6 of *L. pneumophila*, serogroup 5 was the least encountered species. It occurred in 51.0% (26 of 51) of the samples while serogroup 6 was detected in 64.7% (33 of 51) of the samples screened. *L. bozemanii*, the least in occurrence of all the *Legionella* species was detected in only 24 of 51 (47.1%) of all the samples.

Fig. 4 - 9.



L. pneumophila (Lp) Serogroups 1 - 6

L. bozemanii (LB) Serogroup 1.

L. gormanii (LG) Serogroup 1.

L. micdadei (LM) Serogroup 1.

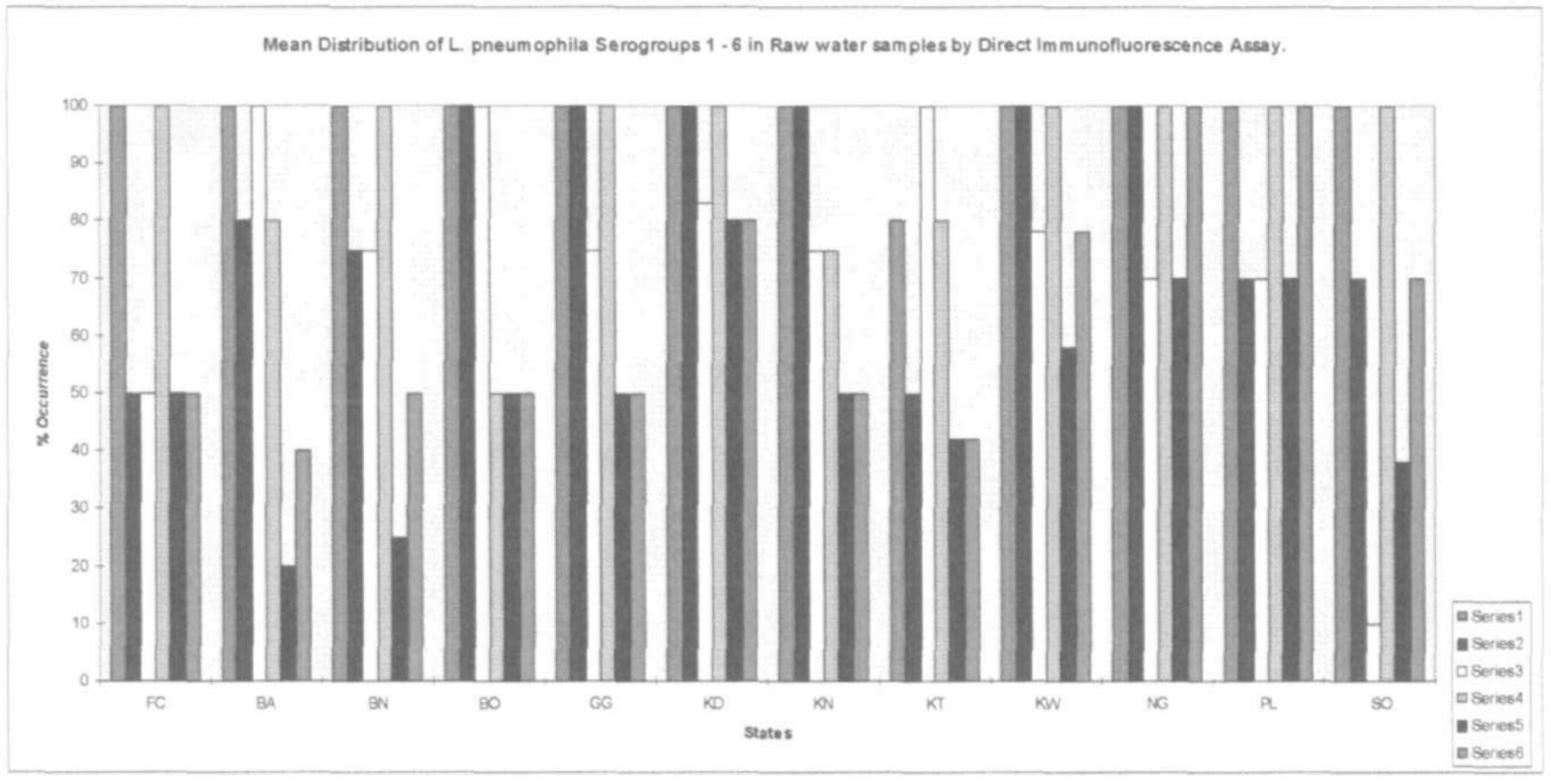
4.2.8 Distribution of *Legionella pneumophila* Serogroup 1-6 in Raw Water Samples in Northern Nigeria

Summary distribution of *L. pneumophila* serotypes 1-6 in Raw water samples is illustrated in Fig.4-10. *L. pneumophila* interserotypic distribution difference was not observed. Rather, all six serotypes appeared to coexist in all habitats in all the States and Abuja and was distributed in a random fashion. There was no predictive trend, except perhaps serotype 1 that predominated in every State other than Katsina where it was in position two together with serotype 4. In the rest of the intrasampling areas, serotypes 2,3, and 4 either interchanged positions in predominance, as well as in frequency occurrence, or have occurred at equal frequency rate from one State to the other. Only in one State (Katsina) did one serotype (3) predominated. In all other States, a combination of two or more serotypes occurred at the same frequency rate. In Abuja, Bauchi, Benue, and Kano State sampling areas, only two serotypes combination, viz. 1 and 4, 1 and 3, 1 and 4, and 1 and 2 respectively, featured prominently. Of the remaining seven sampling areas, six States (Borno, Gongola, Kaduna, Kwara, Plateau, and Sokoto) had three serogroups each that were detected in all samples. The three serotypes for each State were as follows: Borno, serotypes 1, 2, and 3; Gongola, 1, 2 and 4 ; Kaduna, 1, 2, and 4; Kwara, 1, 2, and 4; Plateau, 1,4, and 6; and Sokoto, 1,3 and 4. The most frequent serotype combination was 1,2, and 4. Other combinations occurred once. Only in one State (Niger) did four serogroups (1,2,4, and 6) occurred in all samples while the remaining two serotypes (3 and 5) occurred also at the same frequency rate.

Except in Niger and Plateau States where serogroup 6 was detected in every sample assayed, this serogroup 6, and serogroup 5 were least detected in Gongola, and Kano States. Serotype 5 was the least detected of all. In Bauchi State it was detected in

20% of the samples, in Benue 25%, Kwara 60% and Sokoto 25%. The sampling areas of Bauchi and Benue, had the most heterogeneous intrasampling area variation while Abuja, Borno, Kaduna, Niger and Plateau could be considered relatively homogeneous in terms of the intraserotypic distribution. The remaining five States, Gongola, Kano, Katsina, Kwara, and Sokoto were relatively less homogeneous. In spite of the grouping of the sampling areas there was not a single area or State with similar intraserotypic distribution pattern or variation. Rather inter-state-intraserotypic differences in distribution were observed in every sampling area. Thus, emphasizing intrasampling area variation. However, unlike potable water samples, all the serotypes of *L. pneumophila* (1-6), were detectable to a greater or lesser extent in every State raw water samples.

Fig. 4 - 10.



FC = Abuja; BA = Bauchi; BN = Benue; BO = Borno;

GG = Gongola; KD = Kaduna; KN = Kano; KT = Katsina;

KW = Kwara; NG = Niger; PL = Plateau; SO = Sokoto.

4.2.9 Isolation of *LEGIONELLA* from Raw Water Samples by Culture Media.

Table 4-10 gives a summary of the results from 50 selected raw water samples from all the Northern States examined by DFA test and culture method. Analysis of the 50 samples by DFA revealed that nearly all (49 of 50 or 98%) of the samples were positive by immunofluorescence assay. However, by the use of culture media only 46% (23 of 50) of the samples were culture positive. These positive samples comprised of 5 rivers, 11 dams, 3 lakes, 2 creeks, 1 stream and 1 pond.

Of the 20 rivers that were analysed, 5 (25%) were culture positive. These were Toro river in Bauchi State, Lere river in Kaduna State, Wuya river and Gurara river both in Niger State, and Malando river in Sokoto State. Samples from dams and lakes were richer in isolatable *Legionella* bacteria. Of the 18 samples examined, 11 samples (61.1%) contained culturable legionellae. These samples were drawn from the following dams: Jabi dam in Abuja, Gulbi dam in Bauchi, Zaria and A.B.U. dams in Kaduna, Ajiwa and Kankara dams in Katsina, Tiga and Kadawa dams in Kano, Offa dam in Kwara, and lastly, Shiroro dam in Niger State.

There were four samples from the lakes, Lake Chad and its canal mid way interland, and Lake Alao in Borno, and Lake Kainji in Kwara State, all of which tested positive by culture. Similarly there were two creeks, Airport and Shereton in Abuja, one stream, Tulden Fulani in Bauchi State, and one tin mine pond in Plateau State that also contained culturable *Legionella* species.

Table 4 - 10: Results of Isolation of *Legionella* Bacteria from Raw Water Samples by Direct Immunofluorescence and Buffered Charcoal Yeast – Extract (BCYE) Media

Sample Source	State	Date	Direct Analysis of Samples	
			DFA*	BCYE
Airport Creek	Abuja	23-03-90	+	+
Shereton Creek	Abuja	23-03-90	+	+
River Upa	Abuja	23-03-90	+	-
River Gwagwalada	Abuja	23-03-90	+	-
Jabi Dam	Abuja	23-03-90	+	+
TuldenFulani Stream	Bauchi	19-03-89	+	+
Gulbi Dam	Bauchi	19-03-89	+	+
Dindima River	Bauchi	19-03-89	+	-
Toro River	Bauchi	19-03-89	+	+
River Benue	Benue	15-03-89	+	-
Q River	Benue	15-03-89	+	-
River Ogoli	Benue	15-03-89	+	-
Lake Chad	Borno	12-03-89	+	+
Lake Canal	Borno	12-03-89	+	+
Lake Alao	Borno	12-03-89	+	+
River Ngada	Borno	12-03-89	+	-
Kiri Dam	Gongola	13-03-89	+	-
Karewa pond	Gongola	13-03-89	-	-

Table 4-10 continued

Sample source	State	Date	Direct Analysis of Samples	
			DFA*	BCYE
Ibi River	Gongola	13-03-89	+	-
Lere River	Kaduna	20-04-90	+	+
Rigachukum Stream	Kaduna	20-04-90	+	-
Zaria Dam	Kaduna	06-05-92	+	+
A.B.U. Dam	Kaduna	06-05-92	+	-
Kangimi Dam	Kaduna	06-05-92	+	+
Kagoro Stream	Kaduna	13-05-92	+	-
Ajiwa Dam	Katsina	19-05-92	+	+
Kankara Dam	Katsina	19-05-92	+	+
Mairuwa Dam	Katsina	19-05-92	+	-
Zobe Dam	Katsina	19-05-92	+	-
Tiga Dam	Kano	03-03-92	+	+
Bagauda Dam	Kano	03-03-92	+	-
Kadawa Dam	Kano	03-03-92	+	+
Ciromawa River	Kano	03-03-92	+	-
Asa Dam	Kwara	04-04=92	+	-
Offa Dam	Kwara	04-04-92	+	+
River Osun	Kwara	04-04-92	+	-
Kainji Lake	Kwara	04-04-92	+	+
Bosso Stream	Niger	05-04-92	+	-

Table 4-10 continued

Sample source	State	Date	Direct Analysis of Samples	
				BCYE
Shiroro Dam	Niger	05-04-92	+	+
Wuya River	Niger	05-04-92	+	+
Gurara River	Niger	05-04-92	+	+
Liberty Dam	Plateau	08-04-92	+	-
Lafiya River	Plateau	08-04-92	+	-
Assop Falls	Plateau	08-04-92	+	-
Tin Mine pond	Plateau	08-04-92	+	+
Bakalori Dam	Sokoto	06-05-92	+	-
Argungu River	Sokoto	06-05-92	+	-
Malando River	Sokoto	06-05-92	+	+
Gulbi River	Sokoto	06-05-92	+	-
Jega River	Sokoto	06-05-92	+	-

*DFA = Direct Immunofluorescence Assay

Q River = Unnamed river

+ = presence of *Legionella* - like cells or colonies

- = absence of *Legionella* - like cells or colonies.

4.2.10 Identification of *Legionella* Species

Tables 4-5 and 4-10 show the analyses of *Legionella* spp. from 65 samples of distribution water, well water and borehole water systems; and 50 raw water sources respectively. Isolation rates were 41.5% for distribution water and 46.0% for raw water samples. Isolates from each set of samples were identified according to CDC recommendations which include growth and cultural characteristics of the isolates.

Colonies that were consistent with and characteristic of *Legionella* were visible under dissecting microscope on the third and fourth day of humid incubation. They were small, pin-pointed initially, convex, gray, circular and with entire edge. Glistening and coloured iridescence along the periphery of the colonies were particularly helpful in identifications. And a typical "ground-glass" appearance even when the colonies were visible only microscopically proved equally useful (CDC, 1994, Pasculle, 1994). Older colonies had typically white centre, often bordered with blue, purple, green or red iridescence.

Those isolates that failed to grow on BCYE-α without L-cystein (BCYE-cysteine) and blood agar and were small, slow-growing, poorly stained, Gram-negative rods were presumptively identified as legionellae. Some isolates that repeatedly grew on BCYE-cysteine but failed to grow on blood agar were preserved for further examination. (*L. oakridgensis* and *L. jordanis* are the only species known to lose their requirement for L-cysteine on subculture, Orrison *et. al.*, 1983b).

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

The *Legionellaceae* are agents of Legionnaires' disease and Pontiac fever. Legionnaires' disease, a pneumonic illness affecting human is of major public health interest because it can, and does occur as an epidemic, often resulting in high morbidity and mortality rate. Also *Legionella pneumophila*, the type species has emerged as an important cause of endemic and epidemic nosocomial *Legionella* pneumonia (England and Fraser, 1981).

Since the discovery of *Legionella pneumophila* in Philadelphia in 1976, the disease it caused and still causes, and the subsequent outbreaks, sporadic cases of, and nosocomial Legionnaires' disease, the mode of transmission has remained less definitely identifiable. Although for the past almost two decades, information about Legionnaires' disease and the methods of isolating the etiologic gent, *L. pneumophila*, has accumulated, the exact mode of transmission has eluded definition, and there is only insufficient data regarding the mode of transmission of other *Legionella* species (Muder *et. al.*, 1986). This information gap is thought-provoking and a matter of great concern.

The prevalence of *Legionella pneumophila* in natural and man-made aquatic environs has been well documented (Fliermans *et. al.*, 1979 and 1981; Tobin *et. al.*, 1981; Dennis *et. al.*, 1982; Stout *et. al.*, 1982; Wadowsky *et. al.*, 1982; Hanbleton, *et al.*, 1983; Yakubu and Pass, 1983; Joly *et al.*, 1986; States *et al.*, 1987; Paszko-Kolva *et al.*, 1990) and has also been demonstrated in this work. This wide spread of *Legionella* spp. in diverse

natural aquatic habitats underscores the human health hazards and the need to better understand the agents' physiological ecology.

The results of this work show the ubiquity of *Legionellaceae* in all water habitats. This widespread occurrences are not unexpected. Leginollae are found throughout the natural and man-made environment, surviving in soils and collections of water (Brown *et al.*, 1982; Tobin *et al.*, 1981; Steel *et al.*, 1990a and 1990b; Watson and O'Mahony, 1990). The high percentage detection of the organisms by direct fluorescence antibody (DFA) method (Table 4-1) was a reflection of their abundance, and also due to the high degree of specificity of DFA. But borehole water samples were surprisingly least positive. This raises some questions, especially when the mean pH and temperature were about the same as other sources. Evidently the temperature and pH of the water were not critical factors regulating *Legionella* bacteria in these habitats.

Since legionellae have been found in upland waters, rivers, and bore-hole water, as has also been demonstrated in this research, the question is whether *Legionella* is a contaminant of ground waters or a native bacterium. It is easier to explain the former: activities such as well drilling practice, sampling protocols, and fractured rock strata may introduce these organisms into high quality ground-water (Jakubowsky *et. al.*, 1984). But how *Legionella* survives in borehole water under perpetual darkness is a question yet to be addressed.

5.1.1 **Polyvalent Pool Conjugate Reactions**.

On DFA polyvalent pool reactions, polyvalent pool A Conjugate, consisting of antisera for *L. pneumophila* serogroup 1 - 4 had 13 strong, and 6 very strong (3+ and 4+

intensity) reactions. Five states in the Middle Belt area. Abuja, Bauchi, Borno, Kaduna and Plateau showed 4+ intensity reaction. However, Katsina, further north central state also had one 4+ level reaction. All the states, except Benue, Gongola, and Sokoto exhibited 1+ to 3+ strong (3+ intensity) reactions. This further underscores the ubiquitousness of *L. pneumophila* within water habitats. Each state, except Borno, also demonstrated one or more 2+ intensity reaction. All the states were positive for pool A conjugate, indicating the likelihood of isolating the organisms in every state water sample under study. Table 4-2 and Figure 4-2 give a summary of the results for potable water samples.

On polyvalent pool B conjugate which stains for *L. pneumophila* serogroup 5 and 6, and *L. dumoffii* serogroup 1, and *L. longheachae* sergroup 1, Borno, Kaduna, Niger, and Sokoto states samples were negative. Samples negative included two taps (Borno and Kaduna), one well (Niger), and one borehole (Sokoto). There were 13 ≥ 3+, 13 ≤ 1+, and, 14, 2+ staining reactions. This makes pool B conjugate reactions relatively weak and partly explains the paucity of *Legionella* spp detected in Table

4 -2 and figure 4-2 by this conjugate.

Polyvalent pool C conjugate has three negative reactions, all from Bauchi State hotel's samples. By this reaction the three sample sites did not have the corresponding *Legionella* spp., namely, *L. micdadei* serogroup 1, *L. bozemanii* serogroup 1, *L. gormanii* sergroup 1, and *L. longbeachae* serogroup 2. Of the remaining reactions, 11 samples were of 1+ intensity, 15 other samples also were of 2+ intensity, while only 11 samples demonstrated (10) strong and (1) very strong reactions (Table 4-2).

Therefore, based on the number of strong and weak reactions, the species of *L. pneumophila* was the most predominant. It exhibited 13 strong (3+ staining), and 6 very

strong (4+ staining) reactions, while the remaining 21 reactions were weak. There were no negative-nor 1+-fluorescence staining. This was followed by pool B conjugate which exhibited 11 strong and 2 very strong staining reactions, while 27 reactions stained weakly. Lastly, pool C conjugate had the most number (29) of weak and least number (11) of strong reactions. Thus indicating the relative abundance of the corresponding *Legionella* spp. of each of the pool conjugates (Table 4-2).

The summary of polyvalent pool reactions is demonstrated on Figure 4-2. *Legionella* was detected more frequently in waters from the middle belt states. Percentage occurrence of ≥ 50% was recorded in such states, except Katsina, further north central, which was highest of all (64%). This was as a result of fluorescence intensity of the species in the three polyvalent conjugates (Table 4-2). However, most of the low percentage ocurrence also occurred in the same region. Niger state had the least degree of fluorescence followed by Benue and Gongola states, all in the Middle Belt. Evidently the measured parameters did not seem to be critical; the ranges, although rather narrow were about the same across all the states. Thus, by this study, *Legionella* spp. seem to be indeed common environmental bacteria in all the Northern States waters (Figure 4-2 and Table 4-2).

However, the few exceptions which are not so easily explainable are the very low occurrences of *Legionella* spp in borehole waters (Table 4-1) and the rather curious negative results at polyvalent pool B (four results) and C (three results) conjugate reactions (Table 4-2). With the four or more species of *Legionella* identifiable by each of the pool conjugates, it is more likely that experimental error could be partly responsible for the negative findings. Possible contributing factors to the findings also are long transportation

distances, fluctuating temperature during storage and extended time periods from specimen collection, processing, and microscopic observation.

The ubiquitous occurrence of *Legionella* and the distribution of the various serogroups was further demonstrated in this work. Both Table 4-4 and Figure 4-2 illustrate the fact that this organism is a natural component of water distribution system, and wells, as is of freshwater aquatic communities (Skarliy and McEachern, 1979; Solomon *et. al.*, 1984; Spino et al., 1984; Fliermans *et. al.*, 1989). All samples tested were positive for *L. pneumophila* serogroup 1. Other studies reported similar ubiquity (Macrae *et. al.*, 1979; Storch *et. al.*, 1979; Helms *et. al.*, 1980; Desplaces *et. al.*, 1984; Saito *et. al.*, 1984; Chen *et. al.*, 1984; Ortiz-Roque and Hazen, 1987). This serogroup of the environmental isolates is also reported to be the same as that of pathogenic isolates. Thus the most common cause of Legionnaires' disease is *L. pneumophila* serogroup 1 (Stout *et. al.*, 1982; Reingold *et. al.*, 1984; Wilkinson, 1986; Reingold *et al.*, 1984).

On polyvalent conjugates, the positivity rate was highest for pool A (100%) which consists of *L. pneumophila* serogroup 1, 2, 3, and 4. Pool C comprising of *L. bozemanii*, *L. gormanii*, *L. micdadei*, and *L. lonbeachae* serogroup 2 was second with the positivity rate of 92.5%. Pool B with *L. pneumophila* serogroup 5 and 6, and two other species: *L. dumoffii* and *L. longbeachae* serogroup 1 was third with 90% of the samples positive. It could partly be explained that pool B had 4 samples that did not exhibit any fluorescence activity even though it was second in the number of strong fluorescence activity (Table 4-2).

Both pool B and C did have samples - 4 and 3 respectively - that exhibited no fluorescence activity. Of these 7 water samples, 5 samples were from potable source, while the remaining 2 samples came one from a well and the other from a borehole. This result

seems to suggest that potable water may have undetectable concentrations of pool B and C *Legionella* species, possibly due to effective water treatment regimen. However, more work on a larger number of samples need to be conducted to ensure a more valid conclusion. To the best of my knowledge, the existing literature has not recorded this aspect of the work on water samples except on clinical specimens.

5.1.2 **Serogroup Specific Antisera Reactions**

The serogroup - specific antisera reactions underscored the startling finding that *Legionella* were ubiquitous in the water distribution system of our hospitals, hotels and public taps, as well as in our wells and, to some extent in the boreholes waters. However, one species, *L. bozemanii* was less frequently encountered. One possible explanation is that this species may either be present in less detectable numbers or that it does not compete favourably with other species. Difficult to explain is the fact that this species was detectable only in potable water samples in Abuja (2), Borno (2), Gongola (2), and Kano (1) (Table 4-4).

It was also observed with serogroup-specific reactions for *L. pneumophila* serogroup 5 and 6 in Abuja (1), and seven states: Bauchi (2), Benue (1), Borno (2), Gongola (1), Kaduna (2), Kano (3), and Plateau (3) that these states' samples stained negative. Similarly, one hospital sample in Plateau and one borehole sample in Sokoto stained negative for both *L. gormanii* and *L. micdadei*. These negative reactions were positive at polyvalent pool reactions; thus suggesting that those species identifiable by poly B group conjugate, but for which type specific antisera were not available (i.e., *L. longbeachae* serogroup 1 and/or *L. dumoffii* serogroup 1) might have been responsible for

the positive reactions observed. There may also be a possibility of the presence of (a) new species, or (b) that other non-*Legionella* bacteria with serologically related antigens may have been present, or both assumptions may be true. There were also two samples, hospital B tap in Plateau and a borehole in Sokoto (Table 4-2) which exhibited fluorescence at pool C conjugate reactions, suggesting either the presence of *L. longbeachae* serogroup 2 for which reagent was not available, or other serologically related bacteria or both. This borehole sample was particularly low in the positivity rate. Only *L. pneumophila* serogroup 1 and 3 fluoresced dimly (1+ and 2+ fluorescence). Other *L. pneumophila* serogroups and other *Legionella* spp. were not detected, therefore suggesting either the non-presence or presence below detectability limit of these *Legionellae*. A sensitivity limit of 10^3 to 10^4 cells per ml of environmental samples was recorded by Gilbart (1984). However, Solomon *et. al.* (1984) were able to demonstrate *L. pneumophila* by DFA staining in 265 of 270 samples, in which the minimum cell density detectable was approximately 3 organisms per ml. Both figures 4-2 and 4-3 show the ubiquitousness of these species of pathogenic bacteria - *Legionella* - in all the Northern states of Nigeria.

Table 4-4 and Table 4-9 showed wide distribution of *L. pneumophila* serotypes as well as *Legionellaceae* in all sample sources. There was no *L. pneumophila* interserotypic distribution difference. Rather, there was a randomized distribution pattern in both potable and raw water sources. Similarly there was no *Legionellaceae* population density distribution difference in both potable and raw water sources (Table 4-3 and Table 4-8). However, the high percentage frequent occurrence of serogroups of *L. pneumohila*, especially, serotypes 1, 3, and 4 was still randomized and in line with its predominance at all sites in this study and others recorded earlier elsewhere (Desplaces *et. al.*, 1984; Ortiz-

Roque and Hazen, 1987; Negron-Alvira *et. al.*, 1988; Kusnetsov *et. al.*, 1993). In spite of the low percentage occurrence of serotypes 5 and 6, all the six serotypes seem to be distributed in a random fashion because this distribution is said to be determined by *L. pneumophila* characteristics that are species specific but not clearly understood. Figure 4-4 and Figure 4-9 both illustrated the predominance of *L. pneumophila* at all sites in potable and raw water sources. Similarly, Figures 4-5 and 4-10 stressed the predominance of these species in all water sources in Northern Nigeria.

Similarly, *L. pneumophila* predominated in Kaduna State, followed also by L. *gormanii*. *L. pneumophila* still predominated in Plateau State, with *L. gormanii* and *L. micdadei* occurring at the same rate. This spatial and interspecific heterogeneities in distribution suggests habitat adaptation rather than wholly random spatial distribution, although there are no data currently available to support or explain habitat variabilities. The absence of interserotypic distribution difference also suggests that (a) DFA counts are indeed independent serotypes of the same species and/or (b) the serotype, as a surface antigen plays no environmental adaptive role in these samples from both potable and non-potable water sources.

However, on percentage occurrence of 4+ and 3+ intensity reactions (very strong and strong fluorescence), potable water samples in Borno and Kaduna States exhibited 31.1% positive fluorescence activities each. Whereas, the corresponding raw water samples were only 28% and 27%. In Bauchi and Plateau States on the other hand, raw water samples predominated in strong (4+ and 3+ fluorescence) reactions (31.1%, and 29%) over potable water samples (26%, and 27%) respectively. *L. pneumophila* was still the most prevalent in all the samples both in intensity reactions and frequency occurrence.

Potable water samples relatively exhibited more 4+ staining intensity reactions than the raw water samples. There were 15 such very strong fluorescence reactions - 12 from *L. pneumophila* serogroup 1 to 6, 2 from *L. germanii* and one from *L. micdadei*. On the contrary only 2 very strong (4+) reactions were recorded from raw water samples, one from *L. pneumophila* and the other from *L. micdadei*. Generally, other fluorescence activities were weaker in raw water samples as a whole. Coincidentally the weakest conjugate tested in both sample sources was *L. bozemanii* which was detected in more of the raw water samples and with more 2+ fluorescence reactions than in potable water samples. This may seem to suggest that *L. bozemanii* species is more adapted to natural aquatic environment than in potable water systems. However, the over all weaker staining intensities of all the species and serotypes in raw water samples pose a difficult challenge in explanation. Reasons for weaker fluorescence include: (1) the greater number of microflora in samples, (2) more serologically related or cross-reacting microbes, (3) inadequate conjugates or understaining due to higher concentration of microbiota in the samples, and (4) weak cross - reactions reported among environmental isolates (Cherry *et. al.*, 1978 and 1982; Cherry and McKinney, 1981). More data and work on this area will help to elucidate as well as proffer solution to the problem.

5.1.3 **Growth Supporting Factors in Potable Water**.

Potable water supply has long been observed as a reservoir for both Legionnaires' disease and Pontiac fever bacteria. Hot water cylinders and tanks in particular have provided reservoirs for the growth of *L. pneumophila* and an incubator-like environment for the proliferation of these organisms (Colbourne *et. al.*, 1984; Bartlett *et. al.*, 1983 and 1984;

Thackey *et al*., 1981). This explains in part why the number of *Legionellaceeae* appears to increase rather than decrease in potable water. Other growth stimulating factors for *L. pneumophila*, and other organism in potable water systems include thermal stratification, scale formation, corrosion, and any operational manaeuvers which cause sudden changes in the direction of water flow.

There seem to be other not-so-well defined physical factors which appear to encourage the growth of *L. pneumophila* in tap water systems. These have been known to include elevated temperature (35 to 45 °C), stagnation, sediment formation, and the presence of biodegradable substances including rubber washers (Wadowsky *et al*., 1982; Yee and Wadowsky, 1982; Plouffe *et al*., 1983; Ciesielski *et al*., 1984; Colbourne *et al*., 1984; Stout *et. al* 1985). These physical conditions are operative in the tropics where the sun heats storage water tanks and pipe lines to comfortable temperatures for the legionellae. Stagnation, irregular or fluctuating water flow in pipes, sediment formation, and breakdowns in the distribution systems are common problems, especially in this part of Nigeria. So conditions for supporting legionallae in our water distribution systems are ripe and prevalent. Coexistence of other microflora goes a long way to explain partly too, the ability of *Legionella* to proliferate in potable water distribution systems containing lower concentrations of nutrients than are found in the complex BCYE medium (Edelstein, 1985). Other associations of public health interest are explained briefly hereafter.

5.1.3.1 **Association of *Legionella* with other Microbiota**

Protozoa may be responsible for supporting multiplication of *L. pneumophila* in drinking water systems. Rowbotham (1980) first demonstrated that free living amoebae of

the genera. *Acanthamoeba* and *Naegleria* can be infected with *L. pneumophila*. *L. pneumophila* may also be used as food source for these amoebae (Tyndall and Domingue, 1982; Tyndall *et al.*, 1982, Holden *et al.*, 1984; Anang *et al.*, 1983). Consequently since these free-living amoebae are frequently present in drinking water systems, they may be responsible for *L. pneumophila* contamination in plumbing systems (Henke and Seidel, 1986). It was also shown by Fields *et al.*, (1984 and 1986) that *L. pneumophila* multiplies within the ciliated protozoan, *Tetrahymena pyriformis* which occurs in both plumbing systems as well as cooling towers (Barbaree *et al.*, 1986). Additionally, growth-supporting activity for *L. pneumophila* is also known to be provided by hartmannellid amoebae (Wadowsky *et al.*, 1988); thus *Hartmannella* spp may be important protozoans in the ecology of *L. pneumophila* in hot-water plumbing systems. These amoebae in particular have the ability to revert from the trophozoite stage to the cyst stage and vice versa, as environmental conditions change. Hence they can provide an environment for legionellae within them to survive more adverse conditions than they could as free bacteria (Anand *et al.*, 1984; Barbaree *et al.*, 1986). This protective mechanism may explain in part how legionellae survive the winter in cooling towers water of temperate countries and reappear in the spring to propagate readily in favourable condition.

Furthermore, *Legionella* spp are known to be ingested by some fresh water protozoans, especially *Acanthamoeba*, *Naegleria*, and *Tetrahymena spp*. Once inside these amoebae and ciliates, bacterial growth occurs, as the protozoans provide a conducive environment for intracellular multiplication of the bacteria. Eventually the host cells' membranes rupture, thus releasing the bacteria into the water (Rowbotham, 1980a; Tyndal *et. al.*, 1983; Anand *et. al.*, 1983; Fields *et. al.*, 1984; Holden *et. al.*, 1984). This

relationship is contributory to the recalcitrance of legionellae in water systems. Its intra-amoebal growth has a profound effect on the physiological status and antimicrobial susceptibility of *L. pneumophila* as this enhances greatly its resistance to chlorine (States *et al.*, 1989; Barker *et al.*, 1992), a chemical widely used in the control measures. Its ability to growth within an adherent biofilm comprising myriads of other bacterial species, protozoa and ciliates (Lee and West, 1991) has been suggested to account for the recalcitrance of this organism - *Legionella*- to conventional water treatment (Barker *et. al.*, 1992). It will be interesting and informative to document the incidence of amoebae and ciliates in the water distribution systems covered by this study. The high rate of detection of legionellae in our distribution systems may not be unconnected with the presence of amoebae.

5.1.3.2 Association with Blue-Green Algae and Green Algae

Tison *et al.* (1980) proposed that association between *L. pneumophila* and certain blue-green algae (cyanobacteria) suggests an explanation for the apparent widespread distribution of the bacterium in nature. Rowbotham (1984a) went further to affirm that some cyanobacteria excrete amino acids and peptides into the surrounding environment, and that since *L. pneumophila* has been grown in defined amino acid media by Tesh and Miller (1981), such compounds might be utilized by legionellae for growth. *L. pneumophila* is also known to grow better in mixed cultures with green algae (Chlorophyta) than when grown alone (Hume and Hann, 1984). This apparent relationship or symbiosis with algae appears to support the ubiquity of legionellae in both the natural and man-made water habitats as demonstrated by this work. And indeed, this symbiotic relationships if expanded will lead to

a better understanding of the ecological distribution of this relatively abundant group of bacteria of the genus *Legionella*.

5.1.3.3 **Association with Heat Rejection Systems**.

Not only do such systems as cooling towers provide the right ecological micro-environment for the amplification of *Legionella* pathogens, but the dissemination of the bacteria through aerosol drift is likewise achieved. In his experiment, Berendt (1981) demonstrated that the survivability of *L. pneumophila* in an aerosolized form was enhanced when the organism was associated with the blue-green algae of the species, *Fisherella*. Thus suggesting that the dissemination of the legionellae away from a source, as a cooling tower could be enhanced by their association with an algal component. Berendt went on to suggest that this enhancement may be due to a physical protection of *Legionella* organisms from desiccation since *Fisherella spp* will produce a mucilagenous matrix. "If this protection occurs in heat rejection systems with the formation of aerosols", continued Berendt, "then the dissemination of viable *Legionella* particles away from a point source may be quite significant; this would increase considerably the job of epidemiologists, since the source of infection may be 'miles' away". Thus making the source of an outbreak rather difficult to locate.

Hambleton *et al.* (1984) reported that the survival rate of airborne microorganisms with low metabolic activity is such that significant numbers of viable organisms can be recovered from bacterial clouds even after 2 hours. Contaminated air-conditioning system such as evaporative condensers, for example, might be anticipated continuously to generate aerosols containing viable *L. pneumophila* bacteria, so that exposed unsuspecting

susceptible individuals might readily inhale and retain sufficient viable organisms to acquire an infection. Because the air-conditioning cooling towers are generally exposed to either direct or indirect lighting, are warm, moist habitats, often receive make up water from sources that contain algae, and are efficient scrubbers of the microorganisms from the air, these systems are logically important in the amplification of *Legionella* bacteria (Fliermans, 1984). It follows therefore that cooling towers simply provide the right ecological niche for both the amplification and the dissemination of all types of microorganisms, including legionellae found in the air (Adams *et al.*, 1980). From this environment of myraid microorganisms, aerosols seeded with legionellae, algal cells containing legionellae (Hume and Hann, 1984) and amoebal cysts also containing legionellae (Anand *et al.*, 1984; Rowbotham, 1984a) might be inhaled by unsuspecting humans. The inhaled aerosols, algal cells or amoebal cysts may contain an infective dose sufficient to cause disease as such particle size if inhaled could reach the alveoli of the lung (Rowbotham, 1984a). Thus cooling towers and evaporative condensers contamination present an important public health hazard (Glick *et al.*,1978; Cordes *et al;* 1980, Garbe *et al.*,1985; Addis *et al.*,1989), as they constantly expose tremendous volumes of air to circulating water, creating aerosols (Miller, 1979).

However, heat rejection systems are still in developmental stage in most parts of Nigeria, especially the northern States. Only large hotels of international 'standard' have central air-conditioning with cooling towers. No hospital has any central cooling system. For the most part, and in many other establishments small air-conditioners, one for each room or office are installed. In the banking sector, many banks still use the office type air-conditioners, while in some major banks, larger A/C systems are used but without cooling

towers, rather fan cooling system where water for cooling the engines is never exposed to open air. This development does not necessarily reduce legionellae in the environment since amplifying factors still exist in the system, however, the means of dissemination is strictly curtailed. This may be advantageous in some way.

5.1.4 **Isolation of Legionellae**

While DFA procedure detected *Legionella*-like bacteria in virtually all potable water samples as well as in well water, less than half (5 of 12) of borehole water samples fluoresced with cells of *Legionella* morphology (Table 4.5). However, fewer water samples in all had isolatable legionellae. Because potable water received chlorination regimen, fewer samples still had culturable *Legionella* bacteria. This set of samples was divided into three categories: domestic public water tap samples, hospital related water samples, and hotel related water samples. The last two sample sources had air-conditioning systems with their inherent cooling towers, evaporative condensers and hot water tanks. These water-recirculating systems often possess favourable growth conditions for *L. pneumophila* such as elevated temperatures and mineral deposits (Stout *et al*, 1985), which can serve as a source of nutrients for biomasses, and in turn, support the growth of legionellae (Muraca *et al*., 1988). Consequently these two sources had more culturable *Legionella* bacteria. Incidentally hotels had more typical heat-rejection systems and so 66.7% (8 of 12) of the samples were positive by culture for *Legionella* bacteria (Table 4-5). This figure is slightly higher than those recorded in other parts of the world, particularly Europe, America and Japan where cooling towers in Finland were 50% positive for legionellae (Kusnetsov et. at, 1993), England and Wales 54% (Colbourne and Trew 1986). Cooling towers in the U.S.A

varied in positivity rate for *Legionella* from 8.5% to 54% (Witherell *et al.*, 1986, England, III *et al.*, 1982) respectively, and in Japan, up to 73% rate (Yabuuchi *et al.*, 1989).

Hospitals in this study had no elaborate heat-rejection systems. The only domestic type air-conditioners were located in offices and operating theaters. There were few water heaters, thus limiting elevated temperatures required by *Legionella* for proliferation and subsequent dissemination. Consequently, the number of isolatable *Legionella* decreased considerably. Only 38.1% (8 of 21) of the samples had culturable *Legionella* bacteria (Table 4-5). Domestic/public tap water samples were least positive by culture. Only two out of ten samples (20%) had cultrurable *Legionella* bacteria (Table 4-5). This isolation rate is probably high considering the non-epidemic nature of the sample source. The isolation rate of *L. pneumophila* reported in the literature has been variable. Desplaces *et al.* (1984) reported 21% rate in tap water to 72% in hot tanks, whereas Stout *et al.*, (1984) reported 4 - 68%. Colbourne *et al.* (1984) found that water had lower recovery rate (11%) than from the components such as gaskets or rubber washers (30%). These isolation rates were much lower than their corresponding DFA detection rates. As was the case in this work, the possibility exists that cross-reactions with some unknown or untested environmental bacteria may have been responsible for the high DFA detection rate (Dr. B. Fields, CDC. Personal communication 1995). It is also possible for *L. pneumopila* to be viable but non-culturable (Hussong, *et al.*, 1987). However, the high rate nonetheless still highlighted the difficulties in the prevention and control of *Legionella* in water distribution systems, especially in large buildings.

Untreated well water samples had culturable *Legionella* in 60% (6 of 10) of the samples, while borehole samples that were 41.7% (5 to 12) DFA positive turned out to have

a comparable low 25% culturable *Legionella* samples too. Understandably, they were both 'raw' water from two different sources; however, well water samples were more open to assaults and contamination than the less exposed underground water of boreholes. This isolation rate may still be lower because of possible experimental errors and other hinderances from contaminating non-*Legionella* organisms, over growth of fungi, yeast, and other bacteria some of which may inhibit growth of *Legionella* bacteria.

That *Legionella spp.* were isolated in potable water is a reminder of the fact that this group of bacteria are difficult, if not impossible, to eradicate from potable water system. It underscores the various associations and biofilm formation discussed earlier which make these pathogenic bacteria recalcitrant to preventive measures and conventional water treatment regimen. The data also suggest at least three things: (i) that legionellae coexist in all the habitats tested, (ii) that these samples which were obtained from areas where *Legionella* and legionellosis have not been reported are unique and cannot easily be compared with those previously published from both epidemic/endemic and non-epidemic areas where *Legionella* and legionellosis have been widely reported, and (iii) that the number of samples needs to be increased to allow for a wider definitive conclusion about the presence and behaviour of *Legionella* bacteria in these environments. Evidently chlorination treatment of pipe-borne water does not prevent the proliferation of this hardy bacterium in potable drinkable water.

5.1.4.1 **Raw Water Samples**

The hardiness and widespread occurrence of *Legionellaceae* was further demonstrated by the survival ability of this group of bacteria in natural and man-made

aquatic environment. In spite of the fastidiousness of *Legionella* and the super abundance of other microbiota, these bacteria appear to survive and compete well in the often substrate-limiting conditions of aquatic ecosystems. The results of this work show that *Legionella* are not doing badly at all in all the sample sources, and yielding a mean cell density of 10^5/ ml (Table 4-3 and Table 4-8).

5.1.4.2 **Polyvalent Pool Conjugates Reactions**

On polyvalent pool reaction, pool A has the highest number of strong ($\geq$ 3+) staining reactions. This may suggest that *L. pneumophila* sergroups 1 to 4 is the most widely spread species of *Legionella* and may equally be easily isolatable. Rivers and dams in particular, exhibited most of the 3 + intensity reactions. Figure 4-7 shows the ubiquity of these free-living and pathogenic bacteria. Bauchi State recorded the highest average percentage occurrence rate (58.3%) followed by Plateau (54.2%), Kwara (51.7%) and lastly, Niger State (50.0%). These four States have in common the geographical location in the 'Middle Belt' region. Curiously however, recorded lowest percentage rate also occurred in this region. Federal Capital, Abuja, and Gongola exhibited fluorescence in 39.6% of the samples each. The remaining six other states recorded in the lower and mid-fourties. There are no immediate reasons to explain, based on limited measured parameters, why Middle Belt States recorded both highest as well as lowest percentage occurrence of fluorescing *Legionella*- like bacteria. Of the remaining States recording average percentage occurrence rate, three States, Benue, Borno and Kaduna are in the Middle Belt zone while the remaining three are extreme north, and all bordering the Sahara Desert. None of these

geographical locations seems to explain the spatial distribution of *Legionella* bacteria in the raw water samples of Northern States.

5.1.4.3 *Legionella* Population Densities at Polyvalent Conjugate Reactions

The population densities of fluorescing *Legionella*-like cells by group conjugates reactions ranged from 12 x 10^5 to 3.7 x 10^7 cells/ml in the pool A, 1.0 x 10^4 to 4.0 x 10^6 cells/ml in pool B, and 1.2 x 10^4 to 6.3 x 10^7 cells/ml in pool C (Table 4-8). These densities were all above the lower limit of detectability (9.1 x 10^3 bacteria per litre), established by Fliermans *et al.*, (1979). Based on this, all States' samples can be considered to contain potentially culturable densities of *Legionella* bacteria. These densities also were higher than those from temperate countries reported by Fliermans *et al.* (1981) in natural aquatic habitats in the Southeastern United States (10^1 to 10^3 cells per ml). Many of the densities were within the range 10^5 to 10^6 cells/ml, that is considered potentially pathogenic to humans (Fliermans *et al.*, 1981), especially, the susceptibles. Thus, there were no samples with densities below the detectabale limits. This may be due to the fact that each group conjugate was detecting at least four serogroups or four different species of *Legionella* and, the high specificicity rate of the DFA.

However, there were some samples which did not contain observable densities of *Legionella* bacteria at all. There were some with observable densities in only one or two of the group conjugates. This observation was rather puzzling. The ubiquity of *Legionellaceae* in the environment makes it easy for DFA to detect some of the cells, life or dead, and possibly other cross-reacting bacteria. Perhaps their complete absence goes to confirm the high specificity of the DFA. Since environmental samples are known to contain

numerous and diverse microbiota, and *Legionella* population densities are extremely low compared to the total bacterial population (Fliermans *et al*., 1981), their detection may be obscured, especially in under-staining. The presence of only one negative sample under polyvalent pool A conjugate further underscores the ubiquity and the predominance of *L. pneumophila*, especially serogroups 1 to 4 in the samples tested.

5.1.4.4 **Species-Specific FA Reactions**

From the natural water related environments, DFA casts further light on the universality of *Legionella* organisms. Every possible niche examined showed *Legionella*-like cells under both group-specific polyvalent pools of fluorescent antibody and type-specific antibody tests. *L. pneumophila*, the type species, again occupies its first position among *Legionella* species. It showed a strong presence in both population densities (Table 4-8), and fluorescence intensities (Table 4-9). This predominance was also observed under potable water samples (Table 4-3, and Table 4-4).

The widespread occurrence of *Legionella* in natural aquatic environment is not as surprising as in potable drinkable water. In potable water where low microbial load and low nutrient contents exist and persist for the most part, the various associations of *Legionella* and other microbiota was observed to be supportive of *Legionella* growth and survival (Stout *et al*., 1985). Obviously, the conditions which exist in potable water systems for instance, tip the ecological balance in favour of *L. pneumophila*, the prototype species. In the raw water (natural environment), many microorganisms such as *Flavobacterium*, *Pseudomonas*, *Alcaligenes*, and *Acinetobacter* which support the growth of *L. pneumophila* (Pope *et al*., 1982a; Stout *et al*., 1985), are found more freely in larger numbers than in

potable water. Thus, the increase in *Legionella* species and their distribution in the natural environment of Northern Nigeria. However, the challenge to establish the abundance of these *Legionella* - stimulating/supporting bacteria in our natural aquatic environment will need to be under taken in the future. Only then can we have data on our local environment to support the findings in our environment.

5.1.5 **Comparison of Group-Specific and Type-Specific Reactions (from Potable and Raw Water Samples).**

The correlation between polyvalent pool conjugates and serogroup - specific fluorescent antibody conjugates reactions was, most of the time positive. The number of reactions were fewer on polyvalent pool conjugates, whereas, there were three times as many reactions at group-specific conjugates. Consequently, the percentage occurrence of *Legionella* was higher with group-specific antibody reactions.

In potable water samples (Fig 4-2), most of the percentage ocurrence of polyvalent conjugate reactions correlated positively to group - specific reactions. Katsina State waters had the highest number of samples with detectable cells of *Legionella* morphology. The percentage of fluorescing cells was 64%. At monovalent conjugate reaction (Figure 4-3), the percentage rate went even higher (89%), a very good positive correlation. Abuja (FC) and Plateau were both second in percentage positivity rate. However, while Abuja maintained a positive and higher percentage rate correlation at monovalent reaction, Plateau gave a negative correlation percentage rate (-1.4). The reasons for this downward trend becomes obvious looking at Table 4-4. There were very low or no fluorescence activity for *L. pneumophila* serogroups 2, 4, 5, and 6. There were no detectable cells of *L. bozemanii*.

L. gormanii exhibited 3+ fluorescence in 50% of the samples but weak staining reaction (2+) or complete absence in 50%. *L. micdadei* showed only weak staining reaction and was not detected in one of the samples at group-specific conjugate reaction. The 3+ fluorescence at pool B and C observed earlier (Table 4-2), may have been caused by either *L. dumoffii* and /or *L. longbeachae* serogroup 1, pool B; or *L. longbeachae* serogroup 2, pool C, or other antigenically related non-*Legionella* bcteria. Similarly a negative correlation (of -3.5) was observed between polyvalent and monovalent pool conjugates reactions in Kaduna State. Table 4-4 shows that *L. pneumophilla* serogroups 2, 4, 5, and 6 fluoresced in one or two samples only, while *L. bozemanii* was not detected at all. The observed pool B and C fluorescence activity of 3+ level in Hotel A cooling tower and (of 1− level) in Hotel B bath-tap (Table 4-2) may have resulted from *L. dumoffii* and *L. longbeachae* serogroup 1, or any other antigenically related non-*Legionella* bacteria. The remaining States all exhibited positive correlation ranging from 4.7 in Sokoto to 30.6 in Gongola State (data not shown).

Polyvalent pool conjugate reactions in raw water samples all correlated positively with monovalent fluorescent antibody conjugate reactions. The correlation rates ranged from 17.3 in Bauchi waters to 46.5 in Gongola. Rivers and dams were particularly rich in most reactions. Contributive to these high positive correlation rates is the fact that *L. pneumophila* serogroups, especially serogroups 5 and 6, were more readily detectable than they were in potable water. Particularly, *L. bozemanii* species that was probably present below detectability limits, or was almost absent in potable water, suddenly became abundant in raw water samples.

Reasons for this strong presence of this species may be that natural environment provides a 'less competitive' habitat because of its abundance of food sources, and less demands for adaptation and nutritional stresses. Only in Katsina was *L. bozemanii* below detectability limit or was lacking completely. In few other states (Kano, Benue, and Abuja) the organism was present in only one sample each (Kadawa dam, Q. river, and River Upa respectively). These reasons together with the fact that more symbiotic associations can be easily established in natural environment of diverse microflora than in chemically treated water, may also support the wide-spread and high concentrations of all species in all environments in this study.

The high densities of all species in all the states are clearly illustrated on Figures 4-3, and 4-8. Nearly all the sample sources were off city limits, or dotted by villages. Therefore the possibility for heavy water pollution was remote. Neither was there any significant recreational activities in, or around any of the sampled sites. So without any apparent pollutants such as detergents to cause algal growth and cyanobacterial proliferation (Ortiz-Rogue and Hazen, 1987), *Legionella* organisms were still relatively abundant and widespread in the Northern States. This was not surprising because of the various associations (discussed earlier) these bacteria have with other aquatic microflora. Fresh water amoebae and ciliates of the genera *Acanthamoeba, Echinamoeba, Hartmanella, Naegleria, Tetrahymena,* and *Cyclidium* spp. support the intracellular growth of *Legionella* and consequently constitute a mechanism for the amplification of these bacteria in aquatic habitats (Rowbotham, 1980a; Fields *et al*., 1989; Wadowski *et al*., 1988 and 1991).

There were a number of sample sources that were located within the towns or cities and open to limited human activities. Such sample sources include rivers running through

the cities like Kaduna, Zaria and Jos; and dams such as A.B.U. Dam, Zaria Dam, Shika Dam, Birnin Gwari Dam, and Saminaka Dam, all in Kaduna State; Liberty and Rock Fountain Dams in Jos, Bagauda and Tiga Dams, in Kano, Mairuwa and Kankara Dams in Katina State, and Kainji Dan in Kwara State. Pollution was limited to human activities and as such did not affect significantly the measured parameters. However, chemical parameters were not tested, and the densities of *Legionella* were not different from those of other sample sources. Heterotrophic Plate counts were slightly higher (data not shown).

5.1.6 *Legionella* Species and Serogroups from all the States.

The occurrence of *L. pneumophila* serogoups 1 to 6, *and L bozemanii*, *L. gormanii* and *L. micdadei* (Figure 4-4, and Figure 4-9) further highlights the seeming interserotypic as well as interspecific distribution of *Legionellaceae* in all natural aquatic habitats in Northern Nigeria. The heterogeneity in species distribution seems to suggest habitat adaptation; since the pattern is similar, if not exactly the same, in every State. Why *L. bozemanii* was the least detected member of *Legionellaceae* in every environment is not known. In potable water samples it was detected only in Abuja, Borno, Gongola and Kano States. It was not detected at all in A/C water samples nor in wells or boreholes water samples (Table 4-4). The answer to the question may be suggested from the analyses of raw water samples (Table 4-9). In this natural environment, there was a remarkable increase in the numbers of *L. bozemanii* positive samples. Only Katsina State did not have any *L. bozemanii* positive water sample; the remaining ten States and the Federal Capital had 1 to 4 positive samples each (Table 4-9). This may be that (a) *L. bozemanii* does not adapt well in man-made or man-altered environment, (b) the organism is more subject to environmental and nutritional

stresses than other members of the *Legionellaceae* or (c) the bacterium was present below detectable limits, or all of the above may be true. However, this still does not answer the whole question because the first two answers could not explain why *L. bozemanii* was not detected in wells and boreholes water samples. Neither was the bacterium detected in A/C cooling tower samples, although Negron-Alvira *et al.* (1988) were able to detect and isolate *L. bozemanii* in one out of four cooling tower water in tropical Southern America.

In potable water samples, *L. pneumophila* serogroups 5 and 6 were detected less frequently than the first 4 serogroups in almost all the states except Katsina State (Serogroup 6) and Kwara State (Serogroup 6). All other states, except Katsina, Kwara, Niger and Sokoto States have between one and three negative results (Table 4-4) that were positive at polyvalent conjugate (Table 4-2). This gives about 38% of the samples negative but which were positive at pool B conjugate reactions, suggesting that about 38% of potable water samples may contain *L. dumoffii* serogroup 1 and *L. longbeachae* serogroup 1. In raw water samples however, only about 8% of the samples which were positive at pool B conjugate reactions gave negative results at species - specific conjugate reactions. This findings, if further investigated may throw some light on whether *L. dumoffii* serogroup 1 and *L. longbeachae* serogroup 1 are more adapted (curiously) to man-made environment or that they are simply overshadowed by numerous other bacteria in the natural environment. In other reported non-epidemic studies (Filermans *et al.*, 1979, and 1981; Orrison *et al.*, 1981; Bopp *et al.*, 1981; Yakubu and Pass, 1983; Ortiz-Roque and Hazen, 1987; Negron-Alvira *et al.*, 1988) there was no emphasis at this length on individual species or serogroups. Most work has been as a result of epidemic outbreaks, of Legionnaire's disease (LD), consequently *L. pneumophila*, the most common cause of LD, since Philadelphia 1976, has

frequently been the species commonly isolated. *L. micdadei* has been the species most commonly implicated in pneumonia after *L. pneumophila*, then followed less frequently in isolated cases, by *L. bozemanii*. Implicated even less frequently have been *L. dumoffii* and *L. gormanii* (Fang *et al*., 1989), although *L. dumoffii* was among the three most common isolates in a French hot spring spa studies (Bornstein *et al*., 1989).

5.1.7 *Legionella pneumophila* Serogroups 1 - 6 in every State.

Approximately 85% of the documented cases of Legionellosis have been caused by *L. pneumophila*, while serogroup 1 and 6 have by themselves, accounted for up to 75% of the *Legionella* reported to cause human illness in the United States of America (Reingold *et al*., 1984). Figure 4-5 and Figure 4-10 show the widespread and distribution of these serogroups of *L. pneumophila* in all the Northern States of Nigeria. All six serotypes can be seen to be distributed in a random fashion from one state to the other. The common or predominant serotypes, after serotype 1, in decreasing order of frequent and high percentage occurrences were serotype 3, 4, 2, 6, and 5, for potable water samples (Figure 4-5). In the raw water sources the interserotypic distribution pattern observed slightly varied. *L. pneumophila* serotype 1 still maintained the lead, though losing position in Katsina State samples where serotype 3 predominated. Similarly, of the remaining serotypes, the frequent and high percentage occurrence in decreasing order was serotype 4, 2, 3, 6, and 5. Serotypes 2, 3, and 4 often traded positions (Figure 4-10). In this study, the intraserotypic difference in distribution and the intrasampling area variation were distinctive in all sampling areas. In potable water samples analysis for example, *L. pneumophila* serotype 1 alone predominated in Abuja and Sokoto State samples. However, the intraserotypic distribution

of the remaining five serotypes in these sampling areas was remarkably different. Abuja constituted a homogeneous sampling area, whereas Sokoto was more heterogeneous and lack serotype 5 (Figure 4-5). At a French hot spring spa sampling area, *L. pneumophila* serotype 5 was not detected among the fifteen different species or serogroups that were identified by Bornstein *et al.* (1989).

In other findings elsewhere in the world, *L. pneumophila* serotype 1 has always maintained predominance, followed by serotype 3 in the tropics, whereas serotypes 2, 3 and 4 in temperate climate have often traded positions. (Negron - Alvira *et al.*, 1988, Ortiz-Roque *et al.*, 1987; Bopp *et al.*, 1981; Fliermans *et al.*, 1979, and 1981).

More research work need to be carried out in Nigeria, and Africa as a whole to supply more data upon which future findings will be based. Currently, there is insufficient data to permit any conclusion as to which *Legionella* spp or serotype is predominant in tropical Africa. Only the distribution of *Legionella* in the tropical habitats could tell (us) whether the aquatic parameters are distinctively different from those in the temperate and tropical regions thus far studied.

5.1.8 Isolation of *Legionella* from the Environment.

Bacteria of the family *Legionellaceae* can be found in both natural and man-made environments. And although only a handful of studies relatively, have examined natural aquatic habitats for the presence of these bacteria, numerous investigations on the presence of *Legionella* in artificial environments have been made. These man-made environments are thought to act either as amplifiers or disseminators of *Legionella* and are thus of great importance in understanding the epidemiology of Legionnaires' disease. The relatively

higher percentage occurrence and higher population densities observed in this study are in agreement with the role artificial environment plays in the amplification of *Legionella* bacteria in the environment.

Although *Legionella* species were wide-spread in both artificial and natural environments, isolation rates were below 50% (41.5% and 46.0%) respectively. These low rates were not unexpected. *Legionella* bacteria, in spite of their ubiquitousness in aquatic environments, constitute a minute minority among microbiota found in water. Additionally, they are very fastidious and slow growing, taking up to 3 - 7 days or more to form visible colonies, consequently they are readily outgrown by faster growing contaminants, even on selective media. This is because the selective media at best are still sub-optimum and the numerous and diverse microflora found or contained in environmental specimens make it impossibly difficult for any single combination of antibiotics to be effective in suppressing competing flora in all specimens (Bopp *et al*, 1981). Even the acid buffer procedure used in combination with selective medium, were able to isolate *Legionella* from only 45% of water samples known to contain this group of bacteria (Bopp *et al*., 1981). Other important reasons for the failure to isolate *Legionella* from a higher percentage of DFA-positive water samples include: (i) Lack of optimum selective isolation medium (long storage under warm and humid conditions including product quality may contribute to this factor), (ii) the presence of nonviable but intact cells, (iii) competition and antagonism from the natural biota and, (iv) the presence of 'injured' or non-culturable cells as a result of long transportation distances, long storage period under fluctuating temperatures (electricity failures), impaction on the filter membrane or because of toxicity of the membrane itself. Many of these militating factors are common problems in the isolation of *Legionella* from

environmental samples (Fliermans *et al.*, 1981; Cherry and Milan, 1981; Yakubu and Pass, 1983).

5.1.9 Mode of Transmission of *Legionella pneumophila*

L. pneumophila is a well-known cause of respiratory illness called Legionnaires' disease (LD), while some strains of the species cause a non-pneumonic illness called Pontiac fever (Fraser *et al.*, 1977; Glick *et al.* 1978). While several outbreaks of the disease have been epidemiologically linked to air-borne route (Arnow *et al.*, 1982; Band *et al.*, 1981; Kirby *et al.*, 1980; Zuravleff *et al.*, 1983), other outbreaks and sporadic cases have been difficult to explain by this mechanism (Plouffe *et al.*, 1986). The pioneer, Fraser (1984) of the CDC has suggested that other potential routes of acquiring *L. pneumophila* should be explored. And as the exact mode of transmission of this life-threatening disease has eluded definition, the following theories of transmission have been proposed (Muder *et al.*, 1986).

5.1.9.1 Aerosolization as a Mode of Transmission.

Aerosols are produced by such sources as cooling towers and evaporative condensers, showers, whirlpools, humidifiers, and respiratory care equipment. These aerosol-producing devices are all man-made and operate with water. Under pressure water passing through them is atomized into small droplets, drift or aerosols and are entrained in the air-stream, carrying along with them microorganisms in the water. These aerosols of A/C and water distribution systems can be spread or dispersed over a relatively large area. Such A/C cooling tower drift are known to reach up to 20 to 50 km downwind (Tyndall, 1982). *Legionella* cells, amoebal cysts or algal cells containing legionellae are carried on

these fine drift, which are in respirable size (1 to 5 μm), and capable of lodging far down into the alveoli and terminal bronchioles (Fitzgeorge *et al*., 1983). Susceptible individuals may then inhale these cells, or legionellae-carrying amoebal cysts or algal cells in sufficient number to start the disease. Fitzgeorge *et al*. (1983), and Baskerville *et al*. (1981), have also demonstrated the development of fatal infection in guinea-pigs exposed to aerosols of *L. pneumophila* to support this mode of transmission. This makes the inhalation of *L. pneumophila* from aerosol-producing sources the most likely route of transmission of LD (Breiman *et al*., 1990). These sources include cooling towers and evaporative condensers (Dondero *et al*., 1980; Cordes *et al*., 1980; Garbe *et al*., 1985), showers (Breiman *et al*., 1990), whirl pools (Vogt *et al*., 1987), humidifiers (Zuravleff *et al*., 1983), and respiratory care equipment (Arnow *et al*., 1982). This phenomenon of aerosols production from cooling tower systems would seem to limit legionellosis to industrialized countries where heat rejection systems abound. This would appear to spare the developing world the menace of this dreaded disease.

But this is not the whole picture. Apparently there is no recorded literature on aerosols generation from natural aquatic environment, such as water falls, wave motions in open bodies of water (lakes, dams, ponds, and rivers). However, there is evidence to suggest that aerosols are generated. Researchers at Mount St. Helens Lakes (USA), often contracted flulike symptoms after retrieval of water samples from the lakes (Fliermans *et al*., 1984). Isolation of *L. pneumophila* from the lakes confirmed the fear that LD can be contracted from natural bodies of water, although research has not turned on a serious search light there yet. The occurrence of LD among patients and therapists at a French hot spring spa (Bornstein *et al*., 1989) further highlights the phenomenon of aerosols, the

ecology of *Legionellaceae* and the epidemiology of human exposure to them. Even though only mild cases of infection were encountered, the findings have important implications for the maintenance of adequate standards of hygiene, bacteriological sampling and clinical surveillance in this and similar establishments.

5.1.9.2 **Ingestion as a Mode of Transmission.**

Although there was a significant association with acquisition of LD and the consumption of water in the implicated hotel at the Philadelphia 1976 outbreak of LD, evidence to support ingestion did not come easily. However, more evidence has since emerged to support this theory. The demonstrable evidence include:

(i) infection of guinea-pigs with *L. pneumophila* via the oral route (Plouffe *et al*, 1986, Katz and Matus, 1984).

(ii) Gastric lavage using contaminated tap water has led to Legionnaires' disease (Dournon *et al*, 1982).

(iii) Identification of antacid use as a risk factor (Jones *et al*., 1984), suggesting that ingestion of potable water contaminated with *L. pneumophila* may be a mode of entry of the organism. This putative mode of transmission brings the disease even closer to rural settings, because *Legionella* bacteria were detected in well water, creeks and streams, and spring water, all of which are used for drinking and domestic purposes in most developing countries.

5.1.9.3 **Aspiration as a Mode of Transmission**

This has not been fully established. However, bacteria associated with aspiration pneumonia tend to be normal flora capable of colonizing the oropharynx in the situation of prolonged hospitalization. Many such bacteria are endowed with organelles such as pili that mediate adherence to pharyngeal epithelial cells; these organelles are possessed by *Legionella* bacteria (Rodgers *et al.* 1980). Additionally, the only parameter that could be linked statistically to acquisition of the disease in the Philadelphia 1976 original outbreak was ingestion of water (Fraser *et al.*, 1977). Affected individuals had a high incidence of conditions that could predispose to subclinical aspiration, such as alcohol ingestion, or impede pulmonary clearance of aspirated microorganisms, such as cigarette smoking (Fraser *et al.*, 1977). Given this new information that water distribution systems of large buildings may be contaminated by *Legionella*, it now seems plausible that acquisition of the organism can occur not via the A/C system alone, but also via the water distribution system with aspiration of contaminated water as the mode of entry into the lungs. This has underscored the need for alcoholics and smokers to reduce this additional risk factor.

5.1.9.4 **Direct Instillation via Respiratory Devices.**

In clinical setting, some respiratory devices have the potential to disseminate pathogenic microorganisms because of the high pressures and volumes with which they deliver gases and may generate rather intensive aerosols. This pressurized aerosols may be capable of overwhelming or bypassing the normal defence mechanisms of the respiratory tree and lodging infective aerosols in alveoli. The practice of rinsing respiratory devices with tap water carries along this inherent risk because of the widespread distribution of

Legionella in tap water. Rather, distill water should be used in rinsing respiratory devices. Nosocomial Legionnaires' disease has been linked to the use of such devices as jet nebulizers filled with contaminated water (Arnow *et al.*, 1982)

5.1.9.5 **Non Pulmonary Portals of Entry.**

Pneumonia is the presenting clinical manifestation in more than 95% of cases of Legionnaires' disease (Muder *et al.*, 1986). However, Brabender *et al.* (1983) reported a convincing evidence of nonpulmonic portal of entry of *L. pneumophila* in wound infection of a post-operative patient who had undergone daily immersion in a Hubbard tank. Both the wound and the water supply of the tank yielded *L. pneumophila* serogoup 4; the patient did not develop any evidence of pulmonary infection. Therefore, contact with contaminated water may, on rare occasion, lead to soft-tissue infection with *L. pneumophila* (Muder *et al.*, 1986). However, in both forms of legionellosis, person-to-person transmission has not been documented (Dondero *et al.*, 1979, Yu *et al.*, 1983).

From the foregoing theories therefore, Legionnaires' disease may be contracted through any one of the 'modes' above. There has also been two reports of pericarditis followed by seroconversion to *L. pneumophila* in the absence of pneumonia (Harris, 1981; Spano *et al.*, 1982). This, together with the fact that *Legionella* spp are ubiquitously distributed within water related environments further lends credence to the fact that many people daily come in contact with *Legionella* bacteria in one environment or the other.

Incidentally, data are lacking with regard to the magnitude of *Legionella* contamination in home potable water and hot water systems and any potential association with legionellosis. However, the ubiquity of these organisms in nature gives a high

probability that large numbers of healthy persons are frequently exposed to *Legionella* in homes, in the workplace, and in private and public buildings of all sorts. It will appear then that many *Legionella* bacteria in natural and municipal waters are either not highly virulent, that exposure to the organisms is minimal in extent, or that most persons are not susceptible hosts; or perhaps all are true (Fraser, and Mcdade 1979).

So far the predominance of *L. pneumophila* serogroup 1 was adequately demonstrated in this work as was also attested by Mckinney *et al.*, (1979), who showed that most strains of LD bacteria belonged to serogroup 1. This fact may reflect the ability of this particular serogroup to adapt to diverse demographic conditions and hence its superior ability to survive, compete, and multiply to a concentration easily detectable and to maintain such concentration overtime as repeat sampling has shown in this work. Fliermans (1985) reported that *L. pneumophila* serogroup 1 (Knoxville strain) multiplied and survived longer than representative strains of serogroups 2,3,and 4 in diffusion chambers at 55 °C. Giving its prominent role in legionellosis and its survivance, it will be interesting to know if this perdurability is a virulence characteristic. Giving also the wide interspecies and interserotypic distribution of *Legionella*, it was rather puzzling that direct immunofluorescence tests failed to elucidate serogroup 5 in five states' portable water samples, although serogroup 6 was not sighted either in one state (Kano) samples. Ortiz-Roque and Hazen (1987) report shows that serogroup 3 and 4 were not detected in one of their sample sites while species *L. dumoffii* similarly was not sighted in the same site.

5.2 CONCLUSION

L. pneumophila and other *Legionella* species can be isolated from both natural and man-made water related environmental sites of the tropics. Fliermans, Cherry, and co-workers long established that *L. pneumophila* was present in non-disease associated natural waters, often in high concentrations and at wide temperature ranges. However, in tropical Africa where for the most part, disease surveillance is poor or non-existent, disease - association in this present findings is difficult to rule out. Furthermore, there have been many sporadic and often epidemic outbreaks of diseases resulting in many unexplainable deaths in various parts of Northern Nigeria. Symptoms of many of these diseases have not been unconnected to chest or respiratory problems, commonly referred to simply, as "pneumonia".

The concentration of *Legionella* bacteria in the water distribution systems, especially in hotels and hospitals has once again highlighted the public health significance of this study, and lends further support for the ubiquity of *Legionella* species, in the water distribution systems as well as natural aqueous environment. It also concurs that the etiological agent of human LD well resides in natural and artificial aqueous habitats. And with the high rate of *Legionella* contamination of residential tap water, and the frequent exposure of residents to aerosol from cooling towers of hotels, and probably from showers, it would be needful to carry out serological test to demonstrate in the immunocompetent population the incidence of legionellosis in Northern States of Nigeria.

My data also indicate that *L. pneumophila* and other *Legionella* bacteria contamination of tap water in homes, offices, hospitals, and hotels occurs commonly and unimpeded. This observation is compatible with previous reports of *Legionella* species

contamination of potable water. Hot potable water systems maintained at a temperature below 55 °C are more prone to such contamination. The potential does exist therefore for *Legionella* to contaminate distribution water through one of several routes. These include (1) breakdowns in the treatment process (Tobin *et. al*., 1980), (2) seeding with low number of organisms from the supplying reservoir (Stout *et al*., 1982), (3) contamination of pipes and tanks exposed to dirt and surface water which is likely to be contaminated with this ubiquitous environmental organism and (4) burst pipe lines during constructions, repairs, and / or high pressure changes. The knowledge of these possible contamination routes is important and should be mandatory rather than voluntary. Hence the importance of ensuring that people responsible for water - systems in large public buildings have an adequate understanding of water-supply hygiene.

Infection with *L. pneumophila* is caused by the inhalation of the organism in a water aerosol with particle size between 2 and 8 microns. This particle size can travel deep into the tracheobronchial tree (Bartlett and Macfarlane, 1986), and cause the disease (LD). The disease which occurs in sporadic or epidemic outbreaks has been heralded by a chain of five conditions. (1) *Legionella* of a strain virulent to man, must be present in an environmental reservoir. (2) Environmental factors must exist that promote the growth of *Legionella* in the reservoir to high concentrations. (3) A mechanism must exist for creating an aerosol of the water from the reservoir. (4) Atmospheric conditions must permit the *Legionella*, suspended in microscopic water particles to remain viable. And (5) a susceptible human host must inhale the vapour containing viable organism (Watson and O'Mahony, 1990). Outbreaks have occurred (and still are occurring) in developed countries of the world, and have been associated with cooling water systems used for air-conditioning and

industrial purposes. domestic hot water systems. whirlpool-spas and respiratory therapy equipment. Since domestic hot water systems have been implicated in outbreaks of LD in both hospitals and hotels. this has further underscored the health significance of legionellosis and calls for even stringent measures at prevention.

5.2.1 **Preventive Measures**

A good knowledge of *Legionella* bacteria and its ubiquity in water related environment. especially in commercial air-conditioning systems. and its predilection for hot water systems where it may be sprayed as an aerosol, will aid in its prevention. One area of priority is the epidemiology. the reservoir and possible means of control of this group of pathogens. The clear message *Legionella* bacteria seem to be sounding is that microorganisms can exist even in the most developed medical centres and most sophisticated hotels. can produce human disease and even death and still go undetected. Every epidemic in our modern times therefore reminds the scientific world of the stark reality of just how difficult the identification of a pathogenic microorganism can be and just how obscure its method of spread can be. The complexity of water containing services and higher temperature prevailing in modern buildings undoubtedly increases both the risk and severity of Legionnaires' disease outbreaks. Thus. all water systems in buildings must be regarded as potential breeding grounds for *Legionella* bacteria (Burberry. 1992).

It is important therefore to develop standards for air - handling equipment. standards for air-conditioning equipment. including a rigorous programme of cleaning. disinfection and testing. These procedures should be monitored and kept up to date with the best current practice (Burberry. 1992). It is important also to identify biocidal agents effective against

Legionella and amoebae in air-conditioning systems (Elanore, 1986). This is needful because: (i) *Legionella* has been shown to be a common environmental contaminant. (ii) Air-conditioning cooling tower system contamination occasionally present an important public health hazard. (iii) cooling towers constantly expose tremendous volumes of air to recirculating water, thus creating aerosols, and (iv) the established associations between *L. pneumophila* and fresh water protozoans amoebae in air conditioning cooling system must be tackled and discouraged so that to reduce the proliferation of these bacteria in cooling tower system (Elanore, 1986).

Another preventive measure worth contemplating, but for the very expensive operational cost is that of replacing wet cooling system with dry system. This system does not use water and consequently eliminates the risk of Legionnaires' disease spread from this source. In the absence of the dry system, a new device, known as the "Bug Barrier", could provide an answer to the problem of LD. This device developed by the British scientists is designed to sterilize both internal and external emission from large air conditioning systems by subjecting the air flow to high energy ionic radiations. The installation of such a simple device in hospitals, hotels, factories and large office blocks would completely eliminate the risk of infections agents being distributed among unsuspecting public. However, our level of development may not permit the use of some of these sophisticated measures. Prevention of *Legionella* infections can therefore be accomplished best by minimizing exposure of environmental *Legionella*, especially for individuals who are at risk of developing the disease. Thus surveillance of patients rather than the environment is expedient.

If all the above measures fail, then the diagnosis of *Legionella* pneumonia is

highly desirable because timely administration of specialized, diagnosis-dependent treatment can dramatically affect patient outcome. It also calls for hospital epidemiologists, clinicians, and laboratory microbiologists to include this genus in the differential diagnosis of clusters of pneumonia, even if cases have not been documented previously. Erythromycin has been effective in reducing the case-mortality rate and it is still the drug of choice of legionellosis. Primary treatment with two to four grams per day, administered for three weeks is required. Rifampin could be given in addition to erythromycin to some patients who are seriously ill or fail to respond to erythromycin alone, but rifampin should not be given alone. If erythromycin cannot be given doxycyclines in combination with rifampin have been recommended. Additional alternative agents are trimethoprim-sulphamethoxazole, imipenem, and ciprofloxacin. However, cephalosporins, aminoglycosides, and penicillins are not effective for legionnaires' disease, several species of *Legionella* produce β-lactamase(s). Patient prognosis is linked to the time required for a diagnosis to be reached and erythromycin therapy initiated. This piece of information may be useful for our medical practitioners and clinicians.

REFERENCES

Adams, A.P., Garbett M., Rees H.B., and Lewis, B.G. (1980). Bacterial Aerosols produced from a cooling Tower Using Wastewater Effluent as Make-up water. *Jour. Weter Pollut Control. Fed.* **25**.498- 501.

Addis, D.G., David J.P., Laventure, M., Wand, P.J., Hutchinson, M.A. and McKinnery, R.M. (1989).Community - acquired Legionaires' disease associated with a cooling tower: Evidence for Longer distance transport of *Legionella pneumophila*. *Am. J. Epidem* **130**: 557-568.

APHA (1985). Standard Methods for the Examination of Water and Wastewater, 16th Edition. American Public Health Association, Washington, D.C.

Anand, C. M., Skinner, A.R., Malic, A., Kurtz, J.B. (1983). Interaction of *L. pneumophilia* and a free - living amoeba (*Acanthamoeba palestinensis*). *J. Hyg.* **91**: 167-178

Anand, C.M., Skinner, A.R., Malic A, Kurtz, J.B. (1984). Intracellular Replication of *Legionella pneumophila* in *Acanthamoeba palestinensis*, pp 330 - 332. In *Legionella*: Proceedings of the 2nd International Symposium. C. Thornsberry, A. Balows, J. C. Feeley and W.Jakubowski (Eds). *Am. Soc. Microbiol.* Washington, D. C.

Ampel, N.M., Ruben, F.L., Norden, C.W. (1985). Cutaneous abscess caused by *L. micdadei* in an immunosuppressed patient. *Ann. Intern Med.* **102**: 630 - 2.

Arnow, P. M., Chou, T., Weip, D., Shapiro, E. N., and Kretzeschmar, C. (1982). Nosocomial Legionaris' disease caused by aerosolized tap water from respiratory divices. *J. infect Dis.* **146**: 460-467.

Balows, A. (1984). Microbiology of *Legionella*. Pp 109 - 112. In C. Thornsberry, A. Balows, J. C. Feeley, W. Jakubowski (Eds.). *Legionella*: Proceedings of the 2nd International Symposium. *Am. Soc. Microbiol.* Washington, D. C.

Band, J.D., and Fraser, D.W. (1981). LEGIONELLOSIS: (Legionnaires' Disease and Pontiac Fever) p. 958 - 971. In Medical Microbiology and Infectious Diseases. A Braude, C.E. Doris and J. Fierea (Eds). International test-book of Medicine. W.B. Sannders company.

Band, J. D., LaVenture, M., Davies, J. P., Mallison, G. F., Skaliy, P., Hayes, P. S., Schell, W. L., Weiss, H., Greenberg, D. J., and Fraser, D. W. (1981). Epidemic Legionnaires' Disease: Air-borne Transmission down a Chimney. *JAMA* **245**: 2404 - 2407

Baine, W. B., and Rasheed, J. K., (1979). Aromatic Substrate Specificity of Browning by Cultures of the Legionnaires' Disease Bacterium. *Am. Intern. Med.* **90**: 619 -620.

Barbaree, J.M., Fields, B.S., Feeley, J. C., Gorman, G.W., and Martin, W.T. (1986). Isolation of protozoa from water associated with Legionellosis out-break and demonstration of intracellular multiplication of *Legionella pneumophila*. *Appl. Environ. Microbiol.* **51**: 422 - 424.

Barbaree, J.M., Gorman, G.W., Martin, W.T., Fields, B.S., and Morrill, W.E. (1987). Protocol for sampling environmental sites for *Legionellae*. *Appl. Environ. Microbiol.* **53**: 1454 - 1458.

Barker, J., Brown, M.R.W., Collier, J.P., Farrell, J., and Gilert, P. (1992). Relationship between *Legionella pneumophila* and *Acanthamoeba polyphaga*: Physiological status and susceptibility to chemical inactivation. *Appl. Environ. Microbiol* **58**: 2420 - 2425.

Bartlett, C.L.R. (1984). State of the art lecture: Potable water as reservoir and means of transmission, pp. 210-2315. In *Legionella*: Proceedings of the 2nd International Symposium. C. Thonsberry, A. Balows, J. C. Feeley and W. Jakubowski (Eds). *Am. Soc. Microbiol.* Washington, D.C.

Bartlett, C.L.R., Kratz, J. B., Hutchinson, J.G.P., Turner, G.C., and Wright, A.E. (1983). *Legionella* in Hospital and Hotel water supplies. *Lancet* **ii**: 1315.

Barlett, C.L.R., Macre, A. D., and Macfarlane, J. T., (1986). *Legionella* Infections. Edwards Arnold. London.

Bartlett, C.L.R., Swan, R.A., Casual, J., Canada, R. L., Taylor, A (1984) Recurrent Legionnaires' disease from a hotel water system. In *Legionella*: Proceedings of the Second International Symposium. C. Thornsberry, and J.C. Feeley (Eds). Pp. 235 - 239 *Am Soc. Microbiol.* Washington, D.C.

Baskerville, A., Conlan, J. W., Ashworth, L. A., and Dowsett, A. B., (1986). Pulmonary Damage caused by a Protease from *L. pneumophila*. *Br. J. Exp. Pathol.* **67**: 527 - 536.

Baskerville, A., Fitzgeorge, R.B., Brewster, M., Hambleton, P., and Dennis, P.J. (1981). Experimental transmission of Legionnaires' disease by exposure to aerosols of *Legionella pneumophila*. *Lancet* **ii**: 1389 - 1390.

Beaty, H.N. (1984). State of the art lecture. *Clinical features of Legionellosis*. pp. 6 - 10. In *Legionella*. Proceeding of the 2nd International Symposium. C.Thornsberry, A. Bellows, J. Feeley and W. Jakubowski (Eds). *Am. Soc. Microbiol.* Washington, D.C.

Benson, R.F., Thacker, W. L, Walters, R.P. (1989). *Legionella quinlivanii*. sp. nov. a new species of *Legionella* isolated from water. *Curr. Microbiol.* Is: 195-197.

Benson, R.F., Thacker, W.L., Lanser, J.A., Sangstir, N., Mayberry, W.R. Brenner, D.J. (1991). *Legionella adelaidensis*. a new species isolated from cooling tower water. *J. Clin Microbiol* **29**: 1004-6.

Bercovier, H., Steigerwalt, A.G. (1986). Isolation of Legionellae from oxidation ponds and fish ponds in Isreal and description of *L. israelensis* sp. nov. *Int. J. Syst. Bact.* **36:** 368 - 371.

Berendt., R.F. (1981). Influence of Bleu-green algae (Cyanobacteria) on survival of *Legionella pneumophila* in aerosols. *Infect. Immun.* **32:** 690-692.

Best, M., Yu, V.L., Sout, J., Goetz, A., Muder, R.R., Taylor, F. (1983). *Legionellaceae*. In the hospital water supply - Epidemiological link with disease and evaluation of the methods of control of nosocomial legionnairs' disease - and pittsburgh pneumonia. *Lancet* **ii**. 307-310.

Blackmon, J. A., Chandler, F. W., and Cherry, W. B. (1981). Legionellosis. *Am. J. Pathol.*, 103: 429 - 465.

Bohach, G. A., and Snyder, I. S., (1983). Cyanobacterial Stimulation of Growth and Oxygen uptake by *L. pneumophila*. *Appl., Environ. Microbiol.*, **46**: 528 - 531.

Bollin, G.E., Plouffe, J. F., Para, M.F., and Hackman, B. (1985). Aerosols containing *Legionella pneumophila* generated by shower heads and hot water faccet. *Appl. Environ Microbiol.* **50:** 1128 - 1131.

Bopp, C.A., Summer, J.W., Morris, C.K., Wells, J. G., (1981) Isolation of *Legionella* spp. from environmental water sample by low-pH treatment and use of a selective medium. *J. Clin. Microbiol.* **13**: 714-719.

Bornstein, N., Marmet, D., Surgot, M., Nowicki, M., Arslan, A., Esteve, J., and Flourette. J. (1989a). Exposure to *Legionellaceae* at a hot spring spa: a prospective clinical and serologicl study. *Epidem. Inf.* **102**: 31-36.

Bornstein, N., Marmet, T.D., Surgot, M. Nowicki, M. and Meugnier, H. (1989b). *Legionella gratiana* sp.nov. isolated from French spa water. *Res. Microbiol.* **140**: 541-552.

Bornstein. N., Vielly. C., Marmet. D., Surgot. M. Fleuretts. J. (1985). Isolation of *Legionella anisa* from a hospital hot water system. *Europ. J. Clin Microbiol.* **4:** 447 - 459.

Bozeman. F. M., Humpries. J.W., Campbell. J.M. (1968). A new group of ricketsia - like agents recovered from guinea - pigs pp 659-661. In A. Balows and D.W. Fraser (ed.). *Annals of Internal Medicine*. Vol. **90**. No. 4. 1979.

Brabender. W., Hinthorn. D., and Asher. M., (1983). *Legionella pneumophila* wound infection. *JAMA* **250:** 3091-3092.

Breiman. R.F., Fields. B.S., Sanden. G.N., Voimer. L., and Meier. (1990). Association of shower use with Legionnaires' disease: Possible role of amoebae. JAMA **263**: 2924 - 2926.

Brenner. D.J., Steigerwalt. A.G., and McDade. J.E. (1979). Classification of the Legionnaires' disease bacterium: *Legionella pneumophila* genus novum species nova of the family *Legionellaceae*, familia nova. *Ann. Intern. Med.* **90**: 656-658

Brenner. D.J., (1984). Classification of legionellae. Pp. 55 - 60. In *Legionella:* Proceedings of the 2nd International Symposium. C. Thornsberry, A. Balows, J. C. Feeley, and W. Jakubowski (eds) *Am. Soc. Microbiol.* Washington D.C.

Brenner. D.J. (1986). Classification of *Legionellaceae*.Current status and remaining questions. *Int. J. Med. Sci.* **22**: 620 - 632.

Brenner. D.J., Feeley. J.C., Weaver. R. E. (1979). Family vii. *Legionellacea* in Bergey's Manual of Systemic Bacteriology Vol. 1. Brenner. Streigerwalt. McDade (eds). Baltimore. Willians and Wilkins. 1984. p. 658.

Brenner. D.J., Steigerwalt. A.G., Gorman. C.W., Weaver. R.E. Feeley. J.C., Cordes. L.G. Wilkinson. H.W., Patton. C., Thampson. B.M., Sasseville. K.R.L. (1980). *Legionella bozemanii* sp. nov. and *Legionella dumoffii* sp. nov. Classification of two additional species of *Legionella* associated with human pneumonia. *Curr. Microbiol.* **4**: 111-116.

Brenner. D.J., Steigerwalt. A.G., Gorman. W.G., Wilkinson. H.W., Bibb. W.E., Hackol. M., Tyndall. R.L., Campbell J., Peeley. J.C., Thacker. W. L., Skeliy. P., Martin. W.T., Drake. B.J., Fields. B.S., McEachern. H.V., Corcoran. L.K. (1985). Ten New species of *Legionella. Int. J.Syst. Bacteriol.* **35**.(1): 50 - 59.

Brenner, D.J., Steigerwalt, A.G., Weaver, R.E., McDade, J.E., Feeley, J.C., and Mandel, M. (1978). Classification of Legionnaires' disease bacterium: an interim report. *Curr. Microbiol.* **1**: 71-75.

Brenner, D.J. Steigerwalt, A.G., Pohl, S., Behrens, H., Mannhein, W., and Weaver, R.E. (1981). Lack of relatedness of *Legionella pneumophila* to *Cytophagaceae: "Pasteurrellaceae"* and *Kingella. Int. J.Syst. Bacterial.* **31**: 89-90.

Broome, C.V., (1984). State of the art lecture: Current Issues in Epidemiology of Legionellossis, 1983. Pp. 205 -209. In C. Thornsberry, A. Balows, J. C. Feeley, and W. Jakubowski (eds). *Legionella*, Proceedings of the 2nd International Symposium. *Am. Soc. Microbiol.* Washington D.C.

Broome, C.V., Cherry, W.B., Winn, W.C., and Macpherosn, B.R., Jr. (1979). Rapid diagnosis of Legionnaires' Disease by direct immunofluorescent staining. *Ann. Intern. Med.* **90**:1-4.

Broome, C. V., and Fraser, D. W., (1979). Epidemiologic aspects of Legionellossis. *Epidemiol. Rev.*, 1: 1 - 16.

Brown, A. B., Yu, V. L., Magnussen, M. H., Vickers, R. M., Garrity, G. M., and Elder, E. M.(1982). Isolation of Pittsburgh pneumonia agent from a hospital shower. *Appl.Environ.Microbiol.* **43**:735-736.

Brown, A., Garrity, G.M., and Vickers, R.W. (1981). *Fluoribacter dumoffii* (Brenner *et al.*) Comb. nov. and *Fluoribacter gormanii* (Morris *et al*) comb. nov. *Int. J. syst. Bacteriol.* **31**: 111-115.

Bryan., and Enns, (1986). The Gen-Probe Rapid Diagnostic system for *Legionella*. Clinical Studies Summary Report pp 1 - 4 Gen-Probe, Inc., San Diego, California.

Buesching, W.J., Brust, R.A., and Ayers, L.W. (1983). Enhanced primary isolation of *Legionella pneumophila* from clinical specimens by low-pH treatment. *J. Clin. Microbiol.* **17**: 1153 - 1155.

Burberry, P. (1992). Environment and Services (7th ed), p 54 - 55. Mitchell's Building Series, Longman Scientific and Technical

Campbell, J., Bibb, W. F., Lambert, M. A., Eng, S., Steigerwalt, A. G., Allard, J., Moss, C. W., and Brenner, D. J. (1984). *Legionella sanithelensi*: a new species of *Legionella* isolated from water near Mt. St. Helens. *Appl. Environ. Microbiol.*, **47**: 369 - 373.

Chandler, F.W., Hicklin, M.D., Backmon, J.A., (1977) Demonstration of the agent of Legionnaires' disease in tissue. *N. Engl. J. Med.* **297**:1218-1220.

Chen, M.M.G., Yang, Y.R., and Shen, H.T. (1984). Prevalence of antiboidies to *Legionella* species in young men in Taiwan pp. 254-255. In C. Thronsberry, A. Balows, J.C. Feeley, W. Jakubowski (eds.) *Legionella*: Proceedings of the 2nd International Symposium. American society for Microbiology, Washington, D. C.

Cherry, W.B., Pittman, B., Harris,P.P., Herbert, G.A., Thomason, B.M., Thacker, L., Weaver, R.E. (1978). Detection of Legionnaires' disease bacteria by direct immunofluorescent staining. *J. Clin. Microbiol.* **8:** 329 -338.

Cherry, W.B., Gorman, G. W., Orrison, L.H., Moss, C.W., Steigerwalt, A.G., Wilkison, H.W., Johnson, S.E., Mckinney, R.M, Brenner, D.J. (1982). *Legionella jordanis*: a new species of *Legionella* isolated from water and sewage. *J. Clin. Microbiol.* **15**: 290 - 297.

Cherry, W.B., and McKinney, R.M., (1979). Detection of Legionnaires' disease bacteria in clinical Specimens by Direct Immunofluorescence. P 91 - 103. In G.L. Jones and G.A. Hebbert (eds). "Legionnaires" the Disease, the Baterium and Methodology. U.S. Department of Health Education and Welfare Public Health Service, Centers for Disease control, Atlanta, Georgia.

Cherry, W.B., and Mckinney, R.M. (1981). Direct immunofluorescence test for diagnosis of legionellosis. *Am. J. Pathol* **103:** 448 - 454.

Ciesielski, C.A., Blaser, M.J., Laforee, F.M., and Wang, WL. (1984). Role of stagnation and obstruction of water flow in isolation of *Legionella pneumophila* from hospital plumbing. pp 307 - 307. In C. Thinsberry, A. Ballows, J.C. Feeley and W. Jakubowski (eds). *Legionella*: Proceeding of the 2nd International Symposium. *Am. Soc. Microbiol.* Washington, D.C.

Cohen, M. L., Broome, C. V., Paris, A. L., Martin, W. T., and Allen, J. R., (1979). Fatal nosocomial legionnaires' Disease: Clinical and Epidemiologic Characteristics. *Ann. Intern. Med.* **90**: 611 - 613.

Colbourne, J.S., and Trew, R.M. (1986). Presence of *Legionella* in Londons' water supplies. *Israel J. Med. Sci.* **22:** 633 - 639.

Colbourne, J.S, Pratt, D.J., Smith, M.G., Fisher - Hoch,S.P., Harper, D. (1984). Water fitting as sources of *Legionella pneumophila* in Hospital plumbing system.. *Lancet* **I:** 210 - 213.

Cordes, L.G., Fraser, D.W., Skaliy, P., Perlino, C.A., Elsea, W.R., Mallison, G.F., and Hayes, P. S. (1980). Legionnaires' disease outbrek at an Atlanta, Georgia, country club: Evidence for spread from an evaporative condenser. *Am. J. Epidemiol.* **111**: 425 - 431.

Cordes, L.G., Weisenthal, A.M., Gorman, G.W., Phair, J.T., Sommer, H.M., Brown, A., Yu, V.L., Mangnussen, M.H., Meyer, R.D., Wolf, J.S., Shands, K. N., Fraser, D.W. (1981a). Are showers a source of nosocomial Legionnaires' diseases? *Ann. Intern. Med.* **94:** 195-197.

Cordes, L.G., Wiesenthal, A.M., Gorman, G.W., Phair, J.T., Sommer, H.M., Brown, A., Yu, V.L., Mangnussen, M.H., Meyer, R.D., Wolf, J.S., Shands, K. N., Fraser, D.W, (1981b). Isolation of *Legionella pneumophila* from hospital shower heads. *Ann. Intern. Med.* **94**: 195 - 197.

Dennis, J.P., Taylor, J.A., Fitzgeorge, R., Bartlett, C., and Barrow, G.I. (1982). *Legionella pneumophila* in water plumbing systems *Lancet* **ii:** 949-951.

Dennis, J. P., Bartlett, L. R., and Wright, A. E. (1984a). Comparison of Isolation methods for *Legionella* spp., Pp. 294 -296. In C. Thinsberry, A. Ballows, J.C. Feeley and W. Jakubowski (eds). *Legionella*: Proceedings of the 2nd International Symposium. *Am. Soc. Merobiol.* Washington, D.C

Dennis, J. P., Wright, A.E., Rutter, D.A., Death, J.E. and Jones, B.P. (1984b) *Legionella pneumophila* in aerosols from shower - baths. *J. Hyg.* **93**: 349 - 353.

Desplaces, N., Bure, A., Dournon, E. (1984). *Legionella* spp. in environmental water samples in Paris, pp. 3220 - 321. In C. Thornsberry, A. Balows, J.C., Feeley, W. Jakubowski (ed.), *Legionella:* Proceedings of the 2nd International Symposium. American Society for Microbiology, Washingtron, D.C.

Dondero, T. J., Clegg, H. W., Tsai, T. F., Weeks, R. M., Duncan, E., Strickler, J. Capman, C., Mallison, G. F., Politi, B., Potter, M. E., and Schaffner, W., (1979). Legionnaires' Disease in Kingspot Tennessee. Ann. Intern. Med. 19: 569 - 573.

Dondero, T.J., Rendtorff, R.C., Mallison, Weeks, R.M., Levy, J.S., Wong E.W., Schaffner, W. (1980). An outbreak of Legionnaires' disease associated with a contaminated airconditioning cooling tower. *N. Eng. J. Med.* **302**: 365-370.

Dournon, E., Bure, A., Desplaces, N., Carette, M. F., and Mayaud, C., (1982). Legionnaires' disease related to Gastric lavage with tap water. *Lancet* i: 797 - 798.

Dowling, J. N., Pasculle, A. W., Frola, F. N., Zaphyr, M. K., and Yee, R.B., (1984). Infections caused by *L. micdadei* and *L. pneumophila* among renal transplant recipients. *J. Infect. Dis.*, **149**: 703 - 713.

Edlstein, P.H. (1986). Evaluation of the Gen-probe DNA probe for the detection of *Legionella* in culture. J. Clin. Microbiol. **24**: 556 - 558.

Edelstein, P.H., (1981). Improved semi selective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. *J. Clin. Microbiol.* **14**: 298 - 303.

Edelstein, P.H. (1984). Legionnaires' disease Laboratory Manual. National Technical Information Service, Publication number P.B. 84 - 156827, U.S. Dept. of Commerce, Springfield, V.A.

Edelstein, P.H. (1985). Environmental aspect of *Legionella*. *ASM News* 51: 460-466.

Edelstein, P.H. (1987). Laboratory Diagnosis of infections caused by Legionellea. *Eur. J. Clin. Microbiol.* 6: 4 - 10.

Edelstein, P.H., and Finegold, S. N., (1979). Isolation of *Legionella pneumophila* from blood. *Lancet* **i**: 750 - 751.

Edelstein, P.H., and Meyer, R.D. (1980). Susceptibility of *Legionella pneumophila* to twenty antimicrobial agents. *Antimicrob. Ag. Chemother.* **18:** 403-408.

Edelstein, P.H., Snitzer, T.B., and Finegold, S.M. (1982). Isolation of *Legionella pneumophila* from hospital potable water specimens: Comparison of direct plating with guinea pig inoculation. *J. Clin. Microbiol.* **15**: 1092 - 96.

Elamore, R. (1986). Biocidal control of Legionellae. *Isreal J. Med. Sci.* **22:** 647 - 654.

England, A.C. III, and Fraser, D.W., (1981). Sporadic and Epidemic nasocomial Legionellosis in the United States. Epideomiologic features. *Am. J. Med.*, **70**: 707 - 711.

England, A.C. III, Fraser, D.W., Mallison, G.F., Mackel, D.C., Skaliy, P. and Gorman, G.W. (1982). Failure of *Legionella pneumophila* sensitivities to predict culture results from disinfectant - treated air - conditioning cooling towers. *Appl. Environ. Microbiol.* **43**: 240 - 244.

Evans, C. P. and Winn, W. C., (1981). Extrathoracic Localization of *Legionella pneumophila* in Legionnaires' pneumonia. *Am. J. Clin. Pathol.*, **76**: 813 - 815.

Fallon, R.J., Rowbotham, T.J. (1990). Microbiological investigations into an outbreak of Pontiac fever due to *Legionella micdadei* associated with use of a whirlpool. *J. Clin. Pathol.* **43**: 479-483.

Fang, G. D., Fine, M., and Orloff, J., (1990). New and emerging etiologies for community - acquired pneumonia with implications for therapy: A perspective multicenter study of 359 cases. *Medicine*, **69**: 307 - 316.

Fang, G.D., Yu, V.L., Vickers, B.S. (1989). Disease due to the *Legionellaceae* (other than *Legionelle pneumophila):* Historical, microbiological , clinical and epidemiological review pp 351-369. In E.W. Koneman, S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Win, Jr. (eds), Color Atlas and Testbook of Diagnostic Microbiology, 4th ed. (1992). J.B. Lippincott Company Philadelphia.

Fay, D., Baird, I. M., Aquirre, A., Haegren, V., Werling, K. and Bell, T.W. (1980). Unrecognized Legionnaires' disease as a cause of fatal illness. *J. Am. Med. Assoc.* **243:** 2311 - 2313.

Feeley, J. C. (1984). State of the art Lecture: Current microbiological methods used in the analysis of environmental specimens for *Legionella* spp. Pp 283 - 284. In C. Thornsberry et al., (Eds) Legionella: proceeding of the 2nd Ingernational symposium. *Am. Soc. Microbiol.* Washington, D.C.

Feeley, J.C., Gibson, R.J., Gorman, G.E., Langford, N.C., (1979). Charcoal yeast-extract agar: Primary isolation medium for *Legionella pneumophila*. *J. Clinical Microbiol.* **10:** 436 - 441.

Feeley, C.W., Gorman, C.W., Weaver, R.E., Mackel, D.C., Smith, U. H. (1978). Primary isolation media for Legionnaires' disease bacterium. *J. Clin. Microbiol* **8**: 320 - 325.

Fields, B.S., Shotts, Jr. E.B., Feeley, J.C., Gorman, W.G., Martin, W.T., (1984). Proliferation of *Legionella pneumophila* as an intra cellular parasite of the ciliated protozoan *Tetrahymena pyriformis*. *Appl. Environ. Microbiol.* **47**: 467 - 471.

Fields, B.S., Barbaree, J. N., Shotts, E. B., Feeley, J. C., Morrill, W. E., Sanden, G. N., and Dykstra, M. J., (1986). Comparison of guinea-pig and protozoan models for determining virulence of *Legionella* species. Infec. Immun. **53**: 553 - 559.

Fields, B.S., Barbaree, J.M., Martin, W.T., Morrill, W.E., Sanden, G.N. (1989). Intracellular multiplication of *Legionella pneumophila* in amoebae isolated from hospitial hot water tanks. *Curr. Microbiol.* **18:** 131 - 137.

Fisher - Hoch, S.P., Bartlett, CLR, Tobin, M. B, Gillett, MB.., and Gillett, M. B., (1981). Investigation and control of an outbreak of Legionnaires' disease in distric general hospital. *Lancet* **i:** 937 - 936.

Fitzgeorge, R.B., Baskerville, A., Broster, M., Hambleton, P., and Dennis, P.J., (1983). Aerosol infection of animals with strains of *Legionella pneumophila* of different virulence: comparison with intraperitoneal and intranasal routes of infection. *J. Hgy. Comb.* **90**: 81-89.

Fliermans, C.B. (1984). Philosophical ecology. *Legionella* in historical perspective, p. 285 - 289. In C. Thornsberry, A. Balows, J.C. Feeley, and W. Jakubowski (eds), *Legionella:* Proceedings of the 2nd international symposium. American Society for Microbiology, Washington, D.C.

Fliermans, C.B., (1985). Ecological niche of *Legionella pneumophila*, Pp. 75 - 116. In S. M. Katz (ed.), Legionollosis: CRC Press Inc., Boca Raton, Fla.

Fliermans, C.B., Cherry, W.B., Orrison, L.H., Thacker, L. (1979). Isolation of *Legionella pneumophila* from non epidemic related aquatic habitats by water filtration. *Appl. Environ. Microbiol.* **37:** 1239 - 1242.

Fliermans, C.B., Cherry, W.B., Orrison, L.H., Smith, S.J., Tison, D.L., Pope, D.H., (1981). Ecological Distribution of *Legionella pneumophila*. *Appl. Environ. Microbiol.* **41**: 9 - 16.

Fox, J.L. (1985). Michigan's Spring Legionnaies' disease outbreak. *ASM. News* **51**: 463.

Fraser, D.W. (1980). Legionellosis: evidence of airborne transmission. *Ann. N.Y. Acad. Sci.* **353**: 61 - 66.

Fraser, D. W. (1984). Sources of Legionollosis. In *Legionella*: Proceedings of the 2nd International Symposium on *Legionella*. (Edited by C. Thornsberry, A. Balows, J.C. Feeley, and W. Jakubowski), Pp. 277 - 280. ASM Publication, Washington, D. C.

Fraser, D.W. (1985). Potable water as a source of Legionellosis. Swarthmore College, Swarthmore P.A. **62.**337 - 341.

Fraser, D.W., and McDade, J.E., (1979). Legionellosis. *Sci. Am.* **241**: 82 - 99.

Fraser, D.W., Tsai, T.F., Orensten, W., Parkin, W. E., Beecham, H. J., Sharral, R. G., Harris, J., Mallison, G. F., Martin, S. M., McDade, J. E., Shepard, C. C., and Brachman, P. S., (1977). Legionnaires' disease: description of an Epidemic of pneumonia. *N. Engl. J. Med.* **297**: 1184 - 1197.

Friedman, R. L., Iglewski, B. H., and Miller, R. D., (1980). Identification of a cytotoxin produced by *Legionella pneumophila*. *Infect. Immun.* **29**: 271 - 274.

Fumarola, D., Marcuccio, C., and Miragliotta, G., (1984). In vitro influence of lipopolysaccharide from two species of *Legionella* on human blood leukocyte procoagulant activity. PP. 192 - 193. In C. Thornsberry, A. Balows, J.C. Feeley, and W. Jakubowski (ed.). Legionella: Proceedings of the 2nd International Symposium. *Am. Soc. Microbiol.*, Washington, D. C.

Garbe, P. L., Davis, B.J., Weisfield, J.S., and Markowitz, L., (1985). Nosocomial Legionnaires' disease: Epidemiologic demonstration of cooling towers as a source. *J. Am. Med. Assoc.* **254:** 521 - 524.

Garrity, G.M, Brown, A., Vickers, R. M. (1980). *Tatlockia* and *Fluoribacter:* two new general of organisms resembling *Legionella pneumophila*. *Int. J. Syst. Bacteriol.* **30**: 609 - 614.

Genetic systems Corporation (1985). Recognition and Diagnosis of Legionnaires' Disease. Genetic systems, Lit. No. 149 - T83, Seattle, Washington, D. C.

Gilbert, J., (1984). Detection of Legionella spp In water samples by electron capture gas chromatography. Pp. 296 - 298. In C. Thornsberry, A. Balows, J.C. Feeley, and W. Jakubowski (ed.) Leginella: Proceedings of the 2nd International Symposium. *Am. Soc. Microbiol.* Washington, D.C.

Glick, T.H., Gregg, M.B., Berman, B., Mallison, G., Rhodes, Jnr. W.W., Kassanoff, I. (1978). Pontiac fever: an epidemic of unknown etiology in a health department. *Am. J. Epidemiol.* **197**: 149-160.

Gorman, G. W. and Feeley, J. C., (1982). Procedures for the recovery of *Legionella* from water. Developmental Manual. CDC, Atlanta, GA.

Gorman, G.W., Barbaree, J.M., Feeley, J.C. (1983) Developmental mannual: procedures for the recovery of *Legionella* from water. Respiratory and special pathogens Laboratory Branch, Field investigation section, Division of Bacterial Disease. Centers for Diseases Control, Atlanta, G.A.

Gorman, G.W., Feeley, J.C., Steigerwalt, A., Edelstein, P.H., Moss, C.W. and Brenner, D.J. (1985). *Legionella anisa*: a new species of *Legionella* isolated from potable water and cooling tower. *Appl. Environ. Microbiol.* **49**: 305 - 309.

Haley, C.E., Cohen, M.L., Halter, J., Meyer, R.D. (1979). Nosocomial Legionnaires' disease: a continuing common-source epidemic at Wadsworth Medical Center. *Ann. Int. Med.* **90**: 583 - 586.

Hambleton, P., Broster, M.C., and Dennis, P.J., (1983). Survival of virulent *Legionella pneumophila* in aerosols. *H.J. Hyg.* (Camb.) **90**: 451-460.

Hambleton, P., Dennis, P.J., and Fitzgeorge, R. (1984). Survival of airborne *Legionella pneumophila*, pp. 301 - 311. In C. Thornsberry et al (Eds). *Legionella*. Procedings of the 2nd International symposium. *Am. Soc. Microbiol.* Washington, D.C.

Harris, L. (1981). Legionnaires' disease associated with massive pericardial effusion. *Arch. Interm. Med.* **141**: 385.

Harrison, T. G., and Taylor, A. G., (1988). A laboratory mannual for *Legionella*. A Wiley International Publication. John Wiley and Sons. New York.

Hassung, D., Colwell, R.R., O'Brien, M., Weiss, A., Person, A.D., Weiner, R., Burge, W. (1987). Viable *Legionella pneumophila* not detectable by culture on agar media. *Bio Technology* **5**: 947 - 950.

Hebert, G.A. Callaway, C.S, Ewong, E. P., (1984). Comparison of *Legionella pneumophila*, *L. bozemanii*, and *L. dumoffii* by transmision electron microscopy. *J. Clin. Microbiol.* **19**: 116 - 121.

Hebert, G.A., Moss, C.W., McDougal, L.K., Bozeman F.M., McKinney, R.M. Brenner, D.J. (1980). The rickettsia-like organisms TATLOCK (1943) and HEBA (1959): bacteria phenotypically similar to but genetically distinct from *Legionella pneumophila* and the WIGA bacterium. Ann. Intern. Med. **92**:45-52.

Helms, C.M., Marsanari, R. M., and Zeitler, R., (1983). Legionnaires' disease associated with hospital water system: A cluster of 24 nosocomial cases. *Ann. Inter. Med.* **99**: 172 - 178.

Helms, C.M., Renner, E.D., Viner, J.P., Hierholzer Jnr. W.J., Wintermeyer, L., Johnson, W. (1980). Indirect immunofluorescence antibodies to *Legionella pneumophila*: frequency in a rural community. *J. Clin. Microbiol.* **12**: 326-328.

Heltberg, I., Jepsen, O. B., Larsen, S. O., and Lind, K., (1988). Seroepidemiological study of *Legionella* infection in Denmark. *Dan. Med. Bull.* 35: 95 - 98.

Henke, M., and Siedel, K.M. (1986). Association between *Legionella pneumophila* and amoebae in water, pp. 2627 - 2682. In R.M. Wadowsky et al. (Eds) Applied and environmental Microbiology (Vol. 54. No. 11). *Am Soc. Microbiol.* Washington, D.C.

Herwaldt, L. A., Gorman, G. W., McGrath, T., Tomas, S., Brake, B., Hightower, A. W., Jones, J., Reingold, A. L., Baker, P. A., Tang, P. W., Moss, C. W., Wilkinson, H., Brenner, D. J., Steigerwalt, A. G., Broome, C. V., (1984). A new Legionella species, *Legionella feeleii* species nova, causes pontiac fever in an automobile plant. *Ann. Intern. Med.* 84: 333 - 338.

Holden, E. P., Wood, D. O., Winkler, H. H., and Leinbach, E. D. (1984). Intracellular growth of *Legionella pneumophila* within frshwate amoeba. Pp. 329 - 330. In C. Thornsberry, A. Balows, J.C. Feeley, and W. Jakubowski (ed.), Legionella: Proceedings of the 2nd International Symposium. *Am. Soc. Microbiol.*, Washington, D. C.

Hume, R.D., and Hann., W.D., (1984). Growth relationships of *Legionella pneumophila* with Green algae (Chlorophyta) pp 323-324. In C.Thornsberry, A. Balow, J.C. Feeley, and W. Jakubowski (ed), *Legionella*. Proceeding of the 2nd International Symposium. American Society for Microbiology, Washington, D.C.

Isenberg, H. D., (1979). Microbiology of Legoinnaires' disease bacterium. *Ann. Intern. Med.* **90**: 502 - 505.

Jackson, E.B., Crocker, T.T., Smadel, J.E. (1952). Studies on two rickettsia-like agents. Probably isolated from guinea pigs. In McDade J.E. Brenner, D.J., Bozeman, F.M. (eds) Legionnaires' Disease Bacterium isolated in 1947. *Ann. Intern. Medicin. April*, 1979. Vol. **90** number 4.

Jakubowski, W., Broome, C.U., Geldreich, E.E., Dufour A.P. (Modrators). (1984). Roundatable discussion. Transmission and control, pp. 351 - 355. In C. Thornsbery et al., (Eds) *Legionella*. Proceedings of the 2nd International Symposium. *American Society for Microbiology*. Washington, D.C.

Johnson, J.D., Raff, M.Y., Van Arsdali, J.A., (1984). Neurologic manifestions of Legionnaires' disease. *Medicine* **63**: 303 - 310.

Joly, J. (1985): *Legionella* and domestic water heaters in the Quebec City area. *Can Med. Assoc. J.* Vol. **132.**

Joly, J.R., Dery, P., Gauvreau, L., Cote L., Trepanier, C. (1986). Legionnaires' disease caused by *Legionella dumoffii* in distilled water. Clinical and Community Studies *CMAJ* **135:** 1274 - 1277.

Jones, E., Checko, P. Dalton, A., Cape, J., Boreharee, J., Klein, G., Martin, W., Broome, C. (1984). Nosocomial Legionnaires' disease associated with exposure to respiratory therapy equipment, Connecticut, pp 225 - 227. In C. Thornsberry, A. Balows, J. C., Feeley, and W. Jakubowski (ed.), *Legionella:*

Proceedings of the 2nd International Symposium. American Society for Microbiology. Washington. D.C.

Katz. s., and Matus. J., (1984). Febile illness produced in guinea pigs by oral inoculation of *Legionella pneumophila* In C.Thornsberry. A Balow. J.C. Feeley. and W. Jakubowski (ed). *Legionella* in the Proceeding of the 2nd International Symposium. Washington. D. C., Am. Soc., Microbiol., 1984. Pp. 124 -126.

Kaufman. A.F., McDade. J.E., Patton. C.M., Bennett. J.V., Skaliy. P. et al., (1981). Pontiac fever: isolation of the etiologic agent (*Legionella pneumophila*) and demonstration of its mode of transmission. *Am. J. Epidemiol.* **114:** 337 - 347.

Keathly. J.D. and Winn. W.C., Jnr. (1984). Comparison of the media for recovery of *Legionella pneumophila* clinical isolate. pp 19-21. In C. Tromsberry. A. Ballows et al., (Eds) *Legionella*: proceedings of the 2nd Ingernational symposium. *Am. Soc. Microbiol.* Washington. D.C.

Keel. J.A., Finnerty. W.R. Feeley. J.C. (1978). Fine structure of the Legionnaires' disease bacterium: In-vitro and in-vivo studies of four isolates. *Ann. Intern. Med.* **90**:652 - 655.

King. C.H., Shotts. E.B., *et al.* (1988). Survival of coliforms and bacterial pathogens within protozoa during chlorination. *Appl. Environ. Microbiol.* **54**:3023 - 3033.

Kirby. B.D., Snyder. K. M., Meyer. R.D., Finegold. S.M. (1980). Legionnaires diseases: report of sixty-five nosocomially acquired cases and review of the literature. *Medicine*, **59:** 188 - 205.

Kohne. D.E. (1986). Application of DNA Probe technology in clinical microbiology. Clinical Lab. Products pp 21 - 24. March, 1986.

Koneman. E.W., Allen. S.D., Janda. W.M., Schreckenberger P.C., Winn. W.C. (1992). Color Atlas and Textbook of Diagnositic Microbiology (4th Eds). pp. 351 - 368. J. B. Lippincott Company Pailadelphia.

Kunsnetsor. J. M. Martjkainen. P. J., Jousimies - somer. H.R. (1993). Physical. chemical and microbiological water characteristics associated with the occurrence of *Legionella* in cooling tower systems. *J. Int. Assoc. Water Quel. Water Research* Vol. 27. No. 1 pp . 85 - 90.

Lambert. M.A., and Moss. C.W. (1989). Cellular fatty acid composition and isoprenoid quinone contents of 23 *Legionella* species. *J. Clin. Microbiol.* **27:** 465 - 473.

Lee, T. C., Stout, J., and Yu, V. L., (1988). Factors predisposing to *Legionella pneumophila* colonization in residential water systems. *Arch. Environ. Health* **43**: 59 - 62.

Lee, J.V., and West, A.A., (1991). Survival and growth of Legionella spices in the environment. *J. Appl. Bacteriol* (symp. Suppl) **70**: 121-1295.

Lui, F., and Wright, D.N., (1984). Gram stain in Legionnaires' disease. *AM. J. Med.* **77**: 549 - 550.

Macrae, A.D., Appleton, P.N., 2nd Laverick, A. (1979). Legionnaires' disease in Nottingham, England. *Ann. Intern. Med.* **90:** 580 - 583.

Mangione, E. J., Remis, R. S., Tait, K. A., McGee, H. B., Gorman, G. W., Wentworth, B. B., Baron, P. A., Hightower, A. W., Barbaree, J. M., and Broome, C. V., (1985). An outbreak of pontiac fever related to whirlpool use, Michigan 1982. *J. Am. Med. Assoc.* **253**: 535 - 539.

Mauff, A.C., and Koornhof, H. J. (1984). Legionellosis in Southern Africa. In C. Thornsberry, A., A., Balows, J. C. Feeley, W. Jakubowski, (eds.). *Legionella*: Proceedings of the 2nd International Symposium. pp 247 - 249 Washington, D.C. *America society for Microbiology*.

McDade, J.E., Shepard, C.C., Fraser, D.W., Tsai, T.F., Redus, M.A., Dowdle, W.R., Laboratory Investigation Team (1977). Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory diseases. *N. Engl. J. Med.* **297**: 1197-1203.

McDade, J.C., Brenner, D.J., Bozeman, F.M. (1979). Legionnaires' Disease bacterium isolated in 1947. *Ann. Intern. Med.* **90:** 667-670.

McKinney, R.M., Thacker, L., Harris, P. P., Lewallen, K.R., Hebert, G.A., Edelstein, P.H., Thomason, B.M. (1979). Four serogroups of Legionnaires' disease bacteria defined by direct immunofluorescence. *Ann. Intern. Med.* **90**: 621 - 624.

Meyer, R.D. (1983). *Legionella* infections: a review of five years of research. *Rev. Inf. Dis.* **5**: 258 - 278.

Meyer, R.D., and Finegold, S. M. (1980). Legionnaires' Disease. Annual Rviews Inc. (USA). *Ann. Rev. Med.* P. 219 - 231.

Miller, R.P. (1979). Cooling towers and evaporative Condensers. *Ann. Intern. Med.* **90:** 667 - 670.

Morris, G.K., Patton, C.M., Feeley, J.C., Johnson, S.E., Gorman, G., Martin, W.T., Skaliy, P., Mallison, G.F., Politi, B.D., Mackel D.C. (1979). Isolation of the Legionnaires' disease bacterium from environmental samples. *Ann. Intern. Med.* **90**: 664-666.

Morris, K.G., Steigerwalt, A.G., Feeley, J.C., Wong, E.S., Martin., W.T., Patton, C.M., Brenner, D.J. (1980). *Legionella gormanii sp.* nov. *J. Clin Microbiol.* **12:** 713 - 721.

Moss, C.W., Weaver, R. E, Dees, S.B., Cherry, W.B. (1977). Cellular fatty acid composition of isolates from Legionnaires' disease. *J. Clin. Microbiol.* **6:** 140 - 143.

Moss, C.W. (1981). Gas liquid chromatography as an analytical tool in Microbiology. *J. Chromatogr* **203:** 337-346.

Muder, R. R., Yu, V. L., and Woo, A. H., (1986). Mode of transmission of *Legionella pneumophila*: a critical review. *Arch. Intern. Med.* **146**: 1607 - 1612.

Muraca, P.W., Yu. V. L., and Stout, J.E. (1988). Environmental aspects of Legionnaires' disease. *J. Am. Water Works Assoc.* **80**: 78 - 86.

Nagington, J., Wreghitt, T.G., Tobin, J.D., and Macrae, A.D., (1979). The antibody response in Legionnaires' disease. *J. Hyg.* **83:** 377 - 381.

Negron-Alvira, A., Perezuarez, I. and Hazen, T.C. (1988). *Legionella* spp. In Puerto Rico cooling tower. *Appl. Environ. Microbiol.* **54**: 2331 - 2334.

Newsome, A.L., Baker, R.L., Miller, R.D., and Arnold, R.R. (1985). Interactions between *Naegleria fowleri* and *Legionella pneumophila*. *Infect. Immun.* **50**:449 - 452.

Niedeveld, C.J., Pet, F.M., and Meenhorst, P.L. (1986). Effect of rubbers and their constituents on proliferation of *Legionella pneumophila* in naturally contaminated hot water. *Lancet* **ii:** 180-184.

Orrison, L. H., Cherry, W. B., Fliermans, C. B., Dees, S. B., McDougal, L. K., Dodd, D. J. (1981a). Characteristics of environmental isolates of *Legionella pneumophila*. *App. Environ. Microbiol.* **42**: 109 - 115.

Orrison, L. H., Cherry, W. B., Tyndall, R. L., Fliermans, C. B., Gough, S. B., Lambert, M. A., McDougal, L. K., Bibb, W. F., and Brenner, D. J. (1983). *Legionella oakridgensis*: unusual new species isolated from cooling tower water. *App. Environ. Microbiol.* **45**: 536 - 545.

Ortiz-Roque, C.M., and Hazen, T. C. (1987). Abundance and distribution of *Legionellaceae* in Pue rto Rican waters. *Appl. Environ. Microbiol.* **53**: 2231 - 2236.

Pasculle, A. W. (1993). *Legionella*, pp 461 - 466. In Barbara J. Howard, John F. Keisek, Thomas F. Smith, Allice S. Weissfield, and Richard C. Ticton, (eds), Microbiology: Clinical and Pathogenic. Mosby Year Book Inc. St. Louis M.O.

Pasculle, A.W., Feeley, J.C., Gibson, R.J., Cordes, L.G., Myerowtz, R. L., Patton, C.M., Gormon, G.W., Carmack, C. L., Ezzell, J.W., Dowling, J.N. (1980). Pittsburgh pneumonia agent: direct isolation from human tissue. *J. Infect. Dis.* **141**: 727 - 732.

Paszko-Kolva, C., Yamamoto, H., Shahamat, M., Sawyer, T.K., Morris, G., and Colwell, R. R. (1991). Isolation of *Amoeba* and *Pseudomonas* and *Legionella* spp. from eyewash stations. *Appl. Environ. Microbiol.* **37**: 163 - 167.

Philips, E., and Nash, P. (1985). Culture media, pp. 1051-1092. In E.H. Lennette, A. Balows, W.J. Hausler, Jr., and H.J. Shadomy (ed). Manual of clinical Microbiology, 4th ed. *Am. Soc. Microbiol.*, Washington, D.C.

Pine, D., George, J. R., Reeves, M.I.N., Harrel, W.K. (1979). Development of chemically defined liquid medium for growth of *Legionella pneumophila*. *J. Clin. Microbiol.* **9**: 615-626.

Plouffe, J.F., Webster, L.R., Hackman, B. (1983). Relationship between colonization of Hospital buildings with *Legionella pneumophila* and hot water temperatures. *Appl. Environ. Microbiol* **46**: 769-770.

Plouffe, J.F., Para, M.F., Fuller, K.A., and Bollin, G.E. (1986). Oral ingestion of *Legionella pneumophila*. *J. Clin. Lab. Immunol.* **20**: 113 - 117.

Politi, B. D., Fraser, D. W., mallison, G. F., Moheet, J. V., Morris, G. K., Patton, C. M., Feeley, J. C., Telle, R. D. and Bennett, J. U. (1979). A major focus of Legionnaires' disease in Bloomington, India. *Ann. Intern. Med.* **90**: 587 - 591.

Pope, D.H., Soracco, R. J., Gili, H. K. Fliermans, C.B. (1982). Growth of *Legionella pneumophila* in two-membrane cultures with green algae and cyanobacteria. *Curr. Microbiol.* **7**: 319-322.

Pope, T. L. Jr. Amstrong, P., Thompson R., and Donowitz, G.R. (1982). Pittsburgh pneumonia agent: Chest film manifestations. *Am. J. Roentgenol.* **138**: 237 - 241.

Rashed, K., Mason, A., Gibson, J., Francis, J., Fairfax, A., and Daggett, P. (1986). Legionnaires' disease in Stafford: management of an epidemic *Lancet* **ii**: 197-199.

Reingold, A. L., Thomson, B.M., Brake, J. B., Thacker L., Wilkinson, H.W., Kuritsky, J.N. (1984a). *Legionella* pneumonia in the United States: The distribution of serogroups and species causing human illness. *J. Infect. Dis.* **149:** 819.

Reingold, A. L., Thomson, B.M., and Kurtisky, J. (1984b). Results of Legionnaires' disease testing at the Centers for Disease Control 1980 - 1982, Pp. 21 - 22.In C. Thornsberry, A., A., Balows, J. C. Feeley, W. Jakubowski, (eds.). *Legionella*: Proceedings of the 2nd International Symposium,. *America society for Microbiology*: Washington, D.C.

Remel (1982). Technical information: BCYE Agar base (Dehydrated) and supplements. TI No. 45070. Remel. Lenexa, K.S.

Rihs, J D., Yu, V.L., Zuravleff, J.J., Goetz, A., and Muder, R.R. (1985). Isolation of *Legionella pneumophila* from blood with the BACTEC system: a prospective study yeilding positive results. *J. Clin. Microbiol.* **22**: 422 - 424.

Rodgers, F.G., Greaves, P.W., MacRae, A.D., and Lewis, M.J. (1980). Electron microscopic evidence of Flagella and pilli on *Legionella pneumophila*. *J. Clin. Pathol.* **33**: 1184-1188.

Rodgers, F.G., Pasculle, A.W. (1991). *Legionella*. In Balows, A. Hauslex, W.J., Hermann, K. L., *et al.*, (eds). Manual of Clinical Microbiology, pp 442 - 453. *Am. Soc. Microbiol.* Washington, D.C.,.

Rowbotham, T. J., (1980a). Preliminary report on the pathogenicity of *Legionella pneumophila* for fresh water and soil amoebae. *J. Clin. Pathol.* **33**: 1179 - 1183.

Rowbotham, T.J., (1980b). Pontiac fever explained? *Lancet* **ii**: 969.

Rowbothan, T.J. (1984). *Legionella* and Amoebae, pp. 325-327. In C. Thornsberry, A. Balows, J.C., Feeley, and Jakubowski (eds) *Legionella:* Proceedings of the 2nd International Symposium. *Am. Soc. Microbiol.* Washington, D.C.

Saito, A., Ito, N., Fujita, K., Suzuyama, Y., Yamaguchi, K., and Hara, K. (1984). Distribution of *Legionella* species in Japan and cases of Legionnaires Disease, pp 251 - 252. In C. Tharnsberry, A. Balows, J. C., Feeley, and W. Yakubowski (ed). *Legionella*: Proceedings of the 2nd. International Symposium. *American Society for Microbiology*, Washington, D.C.,

Seidler, R. J. (1984). Ecology, transmission and control. Pp. 356 - 362. In C. Tharnsberry, A. Balows, J. C., Feeley, and W. Yakubowski (ed). *Legionella*. Proceedings of the 2nd. International Symposium. *American Society for Microbiology*, Washington, D.C.,

Shands, K. N., Ho, J.L., Meyer, R.D., Gorman, G.W., Edelstein, P. H., Mallison, G.F., Finegold, S.M., Fraser, D.W. (1985). Potable water as a source of Legionnaires' disease *J. Am. Med. Assoc.* **253**: 1412 - 1416.

Sherwood, M. (1985). When the Legion Marched on Stafford. *A.S.M. News*. **51**: 462.

Skarliv, P., and McEachern, H.V. (1979). Survival of the Legionnaires' disease bacterium in water. *Ann. Intern. Med.* **90**: 662 - 663.

Solomon, J. A., Christensen, S. W., Tyndal, R. L., Fliermans, C. B., and Gough, S. B. (1984). Distribution of *L. pneumophila* in power plant environments. Pp. 309 - 311. In C. Tharnsberry, A. Balows, J. C., Feeley, and W. Yakubowski (ed). *Legionella*. Proceedings of the 2nd. International Symposium. *American Society for Microbiology*, Washington, D.C.,

Sompolinsky, D., Boldur, I., Goldwasser, R.A., Kahana, H., Kazak, R., Kapary, A., and Pik, A. (1986). Serological cross-reactions between *Rickettsia typhi, Proteus vulgaris* OX19, and *Legionella bozemanii* in a series of febrile patients. *Jnr. J. Med. Sci.* **22:** 745 - 752.

Spino, D.F., Rice, E.W. Geldrich, E.E., (1984). Occurrence of *Legionella* spp. and other aquatic bacteria in chemically contaminated groundwater by aeration, pp 318 - 320. In C. Thornsberry, A. Balows, J. C. Feeley, and W. Jakubowski (eds). *Legionella*. Proceedings of the 2nd International symposium. American society for Microbiology Washington, D. C.

States, S. J., Conley, L.F., Ceraso, M., Stephenson, T.E., Wolford, R.S., Wadowsky, R. M. and McNamara, A.M. (1985). Effects of metals on *Legionella pneumophila* growth in drinking Water plumbing systems. *Appl. Environ. Microbiol.* **50**: 1149 - 1154.

States, S. J., Conley, L.F., Towner, S. G., Wolford, R. S., Stephenson, T. E., McNamara, A. M., Wadowsky, R. M., and Yee, R. B. (1987). An alkalin approach to treating cooling towers for control of *Legionella pneumophila*. *Appl. Environ. Microbiol.* **53**: 1775 - 1779.

States, S. J., Conley, L.F., Kuchta, J. M., Oleck, B.M., Lipovitch, M.J., Wolford, R.S., Wadowsky, R.M., McNamara, A.M., Sykora, J.L., Keleti, G., Yee, R.B. (1987) Survival and multiplication of *Legionella pneumophila* in municipal drinking water systems. *Appl. Environ. Microbiol.* **53:** 979 - 986.

States, S.J., Conley, L.F., Kuchta, J.M. Wolford, R.S., Wadowsky, R.M., Yee, R.B. (1989). Chlorine, pH, and control of *Legionella* in hospital plumbing systems (letter). *JAMA* **261**: 1882 - 1883.

Steele, T.W., Lanser, J., Sangster, N. (1990a). Isolation of *Legionella longbeachae* serogroup 1 from potting mixes. *Appl. Environ. Microbiol.* **56**: 49-53.

Steele, T.W., Moore, C.V., Sangster, N. (1990b). Distribution of *Legionella longbeachae* serogroup 1 and other Legionellae in potting soils in Australia. *Appl. Environ. Microbiol.* **56**: 2984-2988.

Storch, G., Hayes, P.S., Hill, D. L., and Baine, W.B. (1979). Prevalence of antibody to *Legionella pneumophila* in middle - aged and elderly Americans. *J. Infect. Dis.* **140:** 784 - 787.

Stout, J.E. Yu, V.L., Vicker, R.M., Zuravleft, J., Best, M., Brown, A., Yoe, R.B., and Wadowsky, R. M. (1982a). Ubiquitousness of *Legionella pneumophila* in the water supply of a hospital with endemic Legionnaires' disease. *N. Engl. J. Med.* **306:** 466 - 468.

Stout, J. E., Yu. V. L., Vickers, R. M., Shonnard, J. (1982b). Potable water supply as the hospital reservoir for Pittsburgh pneumonia agent. *Lancet* **i:** 471 - 472.

Stout, J. E., Yu, V. L., Muraca, P., (1984). Isolation of *L. pneumophila* from the cold water of hospital ice machines: Implications for origin and transmission of the organism. *Infect. Control.* **6**: 141 - 146.

Stout, J. E. Yu, K. L., Best, M.G., (1985). Ecology of *Legionella pneumophila* within water distribution systems. *Appl. Environ. Microbiol.* **49**: 221 - 228.

Tesh, M. J., and Miller, R.D. (1981). Amino acid requirements for *Legionella pneumophila* growth. *J. Clin. Microbiol.* **13**: 865 - 869.

Thacker, W.L., Benson, R.F., and Chifman, R.B., (1989). *Legionella tucsonensis* sp. nov. isolated from a renal transplant recipient. *J. Clin. Microbiol.* **27**: 1831 - 1834.

Thacker, W.L., Benson, R. F., Hawes, L., Brenner, D. J. (1990). Characterization of a *Legionella anisa* strain isolated from a patient with pneumonia. *J. Clin. Microbiol.* **28**: 122-123.

Thacker, W. L., Benson, R. F., Wilkinson, H.W., Am pel., N.M., Wing, E.J., Steigerwalt, A.G., Brenner, D.J. (1986). 11th serogroup of *Legionella pneumophila* isolated from a patient with fatal pneumonia. *J. Clin. Microbiol.* **23**: 1146 - 1147.

Thacker, W.L. Plikaytis, B.B., Wilkinson, H.W. (1985). Indentification of 22 *Legionella* species and 33 serogroups with the slide agglutination test. *J. Clin. Microbiol.* **21**: 779-782.

Thornsberry, C., Baker, C.M., Kirvin, L.A. (1978a). *In vitro* activity of antimicrobial agents of Legionnaires' disease bacterium. Antimicrob. *Agents Chemother.* **13**: 78-80.

Thornsberry, C., and Kirvin, L.A. (1978b). β-lactamases of the Legionnaires' bacterium. *Curr. Microbiol.* **1**: 51-54.

Tison, D. L., Hope, D.H., Cherry, W.B., Fliermans, C.B., (1980). Growth of *Legionella pneumophila* in association with blue - green algae (cyanobacteria). *Appl. Environ. Microbiol.* **39**: 456-459.

Tison, D.L., and Seilder, R. J. (1983). *Legionella* incidence and density in potable drinking water supplies. *Appl. Environ. Microbiol.* **45**: 337 - 339.

Tobin, J.O., Bartlett, C.L.R., and Waitkins, S. A., (1981). Legionnaires' disease: Further evidence to implicate storage and distribution systems as sources. *Br. Med. J.* **282**: 573.

Tobin, J. O., Beare, J., Dunhill, M.S., Fisher-Hoch, S., French, M., Mitchell, R. G., Morris, P. J., and Muers, M. F., (1980). Legionnaires' disease in a transplant Unit: isolation of the causative agent from shower baths. *Lancet* **ii:** 118 - 121.

Tobin, J. O., Swann, R.A., Bartlett, C.L.R. (1981). Isolation of *Legionella pneumophila* from water systems: Methods and preliminary results. *Br. Med. J.* 282: 515 - 517.

Turner, W. E., Nash, R., Harada, G. F., and Iampietro, L. M., (1984). Detection of L. pneumophila and L. micdadei in water collected from a rainfall in Southeastern Pennsylvania, Pp. 321 - 322. In C. Tharnsberry, A. Balows, J. C., Feeley, and W. Yakubowski (ed). *Legionella*. Proceedings of the 2nd. International Symposium. *American Society for Microbiology*, Washington, D.C.,

Tyndall, R. J., (1982a) Concentrations, serotypic profiles and infectivity of Legionnaires' disease bacteria populations in cooling towers. *J. Cooling Tower Inst.* **3**: 25 - 33.

Tyndall, R. L. and Domingue, E.L. (1982b). Cocultivation of *Legionella pneumophila* and free-living amoebae. *Appl. Environ. Microbiol.* **44**: 954 - 959.

Tyndal, R. L., Christenson, S. W., Solomon, J. A., Fliermans, C. B., and Gough, S. B., (1984). Thermally altered habitats as a source of known and new *Legionella* species. Pp. 311 - 313. In C. Tharnsberry, A. Balows, J. C., Feeley, and W. Yakubowski (ed). *Legionella*. Proceedings of the 2nd International Symposium. *American Society for Microbiology*, Washington, D.C.,

Vickers, R.M., Brown, A., and Garrity, G.M. (1981). Dye - containing buffered charcoal-yeast extract medium for differentiation of members of the family *Legionellaceae*. *J. Clin. Microbiol.* 13: 380-382.

Vogt, R. L., Hudson, P.J., Orciari, L., Heun, E. M., Woods, T.C. (1987). Legionnaires' disease and a whirlpool-spa (letter). *Ann. Intern. Med.* **107**: 596.

Wadowski, R.M., and Yee, R. B. (1981). Glycine-containing selective medium for isolation of *Legionellaceae* from environmental specimens. *Appl. Environ. Microbiol.* **42:** 768 - 772.

Wadowski, R. M., Yee, R. R., Mezmar, L., Wing, E. J., Dowling, J. N. (1982) Hot water systems as sources of *Legionella pneumophila* in hospital and non hospital plumbing fixtures. *Appl. Environ. Microbiol.***43**: 1104 - 1110.

Wadowski, R.M., and Yee, R.B. (1983). Satellite growth of *Legionella pneumophila* with an environmental isolate of *Flovobacterium breve*. *Appl. Environ. Microbiol.* **46**: 1447 - 1449.

Wadowski, R.M., Wolford, R., McNamara, A.M., Yee, R.B. (1985a). Effect of temperature, pH, and oxygen level on the multiplication of naturally occurring *Legionella pneumophila* in potable water. *Appl. Environ. Microbiol.* **49**: 1197 - 1205.

Wadowski, R. M. and Yee, R.B. (1985b). Effect of non *Legionellaceae* bacteria on the multiplication of *Legionella pneumophila* in potable water. *Appl. Environ. Microbiol.* **49:** 1206 - 1210.

Wadowski, R. M., Butter, L.J., Cook, M.K., Verma, S.M., Paul, M.A., Fields, B.S., Keleti, G., Sykora, J.L., Yee, R.B. (1988). Growth supporting activity for *Legionella pneumophila* in tap water cultures and implication of hartmannellid amoebae as growth factors. *Appl. Environ. Microbiol* **54**: 2677 - 2682.

Wadowski, R.M., Wilson, T.M., Kapp, N.J., West, A.J., Kuchta, J.M., States, S.J., Dowling, J.N., and Yee, R.B. (1991). Multiplication of *Legionella* spp.in tap water containing *Hartmanella*.

Wang, W.L., Blaser, M.J., Cravens, J., Johnson, M.A., (1979). Growth survival and resistance of Legionnaires' disease bacterium. *Ann. Intern. Med.* **90**: 614 - 618.

Watson, J.M., and O'Mahony, M. (1990). Legionnaires' disease - a recurring problem. *Health and Hygiene* **11:** 10 - 14.

Wentworth, B.B., Baselski, V.S., Doern, G.V., McArthur, C.P., Miller, J.M., Needham, C.A. Sewell, D.L. (1987). Diagnostic procedures for bacterial infections. (7th ed.). American Public Health assoc. Inc: Washington, D.C.

Wilkinson, H. W., (1982). Serodiagnosis of Legionellosis. *Lab. Med.* **13**: 151 - 157.

Wilkinson, H.W. (1986). *Legionella:* Ten years later. Clinical Microbiology Newsletter. Vol. **8,** 7: 45-48 .Elsevier Science Publishing Co. Inc.

Wilkinson, H.W., Sampson, J.S., Plikaytis, B.B. (1986). Evalutation of commercial Gene-Probe for identification of *Legionella* cultures. *J. Clin. Microbiol.* **23:** 217 - 220.

Wilkinson, H.W., (1986). Serodiagnosis of *Legionella pneumophila* disease, pp 395 - 398. In N.R. Rose, H. Friedman, J.D., Fahey (eds). Manual of Clinical Laboratory Immunology 3rd ed. American Society for Micobiology. Washinton, D.C.

Wilkinson, H. W. (1987). Hospital - laboratory diagnosis of *Legionella* infections, pp. 5 - 18. Centres for Disease Control laboratory manual. U.S. Department of Health and Human Services, Centres for Disease Control, Atlanta.

Wilkinson, H.W., Drasar, V., Thacker, W.L., Benson, R.F., Schinder, J., Potuznikova, B., Mayberry, W.R. Brenner, D.J. (1988). *Legionella moravica* sp. nov. and *Legionella brunensis* sp. nov. isolated from cooling tower water. Ann Inst. Pasteur/Microbiol. (Paris). 139: 393-402.

Winn, W.C., Jr. (1985). *Legionella* and Legionnaires' disease: a review with emphasis on environmental studies and laboratory diagnosis. *CRC Crit. Rev. Clin. Lab. Sci.* **21**: 323 - 381.

Winn, W.C. Jr. (1987a). Legionellosis. In Wentworth B.B. (ed): Diagnostic Procedures for Bacterial Infections, pp 318 - 334. Washington, D.C., American Public Health Association.

Winn, W.C., (1987b). Legionellosis. In Wentworth B.B., Baselski V.S., Doern, G.U., McArthur, C.P., Miller, J.M. Needham, C.A., Sewell D.L., (eds): Diagnostic procedures for Bacterial Infections (7th Ed.), pp. 317 - 334. American public Health Association, Inc., Washington, D.C.

Win, W.C., (1988). Legionnaires' Disease: Historical Perspective. *Clin. Microbiol. Reviews* **1**: 60 - 81.

Winn, W.C., Jr., Glavin, F.L., Perl, D.P., Keller, J.L., Andres, T.L., Brown, T.M., Coffin, C.M., Sensecqua, J.E. Roman, L.N., and Craighead, J.E. (1978). The pathology of Legionnaires' disease. Fourteen total cases from the 1977 outbreak in Vermont. *Arch. Pothol. Lab. Med.* **102:** 344 - 350.

Witherell, L. E., Novick, L. E., Stone, K. M., Duncan, R. W., Orciari, L. A., Kappel, S. J., and Jillson, D. A., (1986). *Legionella* in coolingtowers. *J. Environ. Hlth* **49**: 134 - 139.

World Health Organization. Epidemiology, prevention and control of Legionellosis: Memorandum from a WHO meeting. Bull WHO **68**: 155 - 164, 1990.

Yabuuchi, E., Ikado, M., Yamamoto, H. (1989). Ecology of legionellae in cooling tower water. Poster Presentation at the Fifth International Symposium on Microbiol. Ecology, 28 August - 1 September, 1989, Kyoto. Abstract Book, 186.

Yakubu , S. E., and Pass, T., II, (1983). The isolation and identification of *Legionella* species from environmental sites in Rowan County, Kentucky. A Masters' Thesis presented to the Faculty of the School of Sciences and Mathematics, Morehead State University, Morehead, Kentucky, USA.

Yee, R. B., and Wadowsky, R.M. (1982). Multiplication of *Legionella pneumophila* in unsterilized tap water. *Appl. Environ. Microbiol.* **43**: 1330 - 1334.

Yu, V.L. (1990). *Legionella pneumophila* (Legionnaires' disease). In principles and practice of Infectious Diseases, 3rd ed, pp 1764. New York, John Wiley and Sons.

Yu, V. L., Kroboth, F. J., Shonnard, J., Brown, A., McDearman, S., and Magnussen, M., (1982). Legionnaires' disease: new clinical perspective from a prospective pneumonia study. *Am. J. Med.* **73**: 257 - 361.

Yu, V.L., Zuravleff, J. J., Gavlik, L., and Magnussen, M.H., (1983). Lack of evidence for person - to - person transmission of Legionnaires' disease. *J. Infect. Dis.* **147**: 362.

Zimmerman, S.E. French, M.L., Allen, S.D., Wilson, E., and Kohler, R.B. (1982). Immunoglobin M Antibody titers in the diagnosis of Legionnaires' disease *J. Clin. Microbiol.* **16**: 1007 - 1011.

Zuravleff, M.S., Yu, V.L., Shonnard, J. W., Davies, B K., and Rihs, J D., (1983). Diagnosis of Legionnaires' disease: an update of laboratory methods with new emphasis on isolation by culture *JAM. Med. Assoc.* **250:** 1981 - 1985.

Zuravleff, J. J., Yu, V.L., Shornard, J.W., Rihs, J.D., Best, M. (1983). *Legionella pneumophila* contamination of a hospital humidifier: demonstration of aerosol transmission and subsequent subclinical infection in exposed guinea pigs. *AM. Rev. Repir. Dis.* **128:** 657-66.

APPENDIX A.

Possible Division of Legionellae into Multiple genera on the basis of DNA Relatedness Levels*

Levels of Relatedness %	Genus Designation	Species included
40	I	*L. rubrilucens, L. erythra*
	II	*L. parisiensis, L. cherrii*
		L. steigerwaltii, L. bozemanii
		L. dumoffii, L. gormanii, L. anisa
	III	*L. sainthelensi, L. santicrusis*
	IV	*L. spiritensis*
	V	*L. wadsworthii*
	VI	*L. longbeachae*
	VII	*L. micdadei*
	VIII	*L. maceachernii*
	IX	*L. pneumophila*
	X	*L. jordanis*
	XI	*L. oakridgensis*
	XII	*L. geestiae*
	XIII	*L. jamestowniensis*
	XIV	*L. haeckeliae*
30	I	*L. rubriluceans, L. erythra, L. spiritensis*
	II	*L. parisiensis, L. cherri*
		L. steigerwaltii, L. bozemanii
		L. dumoffii, L. gormanii, L. anisa
		L. wadsworthii, L. santicrusis
		L. sainthelensi

APENDIX A (Continued)

Levels of Relatedness %	Genus Designation	Species included
	III	*L. saintheleusi, L. sanmerusis*
		L. longbeachae, L. steigerwaltii
		L.cherrii
	IV	*L. micdadei*
	V	*L. maceachernii*
	VI	*L. pneumophila*
	VII	*L. jordanis*
	VIII	*L. oakridgensis*
	IX	*L. feeleii*
	X	*L. jamestowniensis*
	XI	*L. hackeliae*
25	I	*L. rubrilucens, L. erythra,*
		L. spiritensis.
	II	*L. parisiensis, L. cheerii*
		L. steigerwaltii, L. bozemanii
		L. dumoffii, L. gormanii, L.
		anisa
		L. sainthelensi, L. longbeachae
	III	*L. micdadei, L. maceachernii*
	IV	*L. pneumophila*
	V	*L. jordanis*
	VI	*L. oakridgensis*
	VII	*L. feeleii*
	VIII	*L. jamestowniensis*
	IX	*L. hackeliae*

From Garrity *et. al.*, 1988.

APPENDIX B

LEGIONELLA SPECIES, SOURCE AND PLACE OF ISOLATION.

Species	Source and Place	Year of Isolation
L. pneumophila	Lung tissue, human pneumonia, USA	1977
L. bozemanii	Lung tissue, human, USA	1979
L. micdadei	Blood, human, USA	1979
L. dumoffii	Water, cooling tower, USA	1978
L. gormanii	Soil, creek bank, USA	1978
L. longbeachae	Transtracheal aspirate, USA	1980
L. jordanis	Water, River, USA	1978
L. oakridgensis	Water, cooling tower, USA	1981
L.wadsworthii	Sputum, human pneumonia, USA	1981
L. feeleii	water, USA	1981
L. sainthelensi	Water, Spring, USA	1981
L. anisa	Hot water, sink, USA	1981
L. maceachernii	Water, home evaporator cooler, USA	1979
L. maceachernii	Potable water cistern, USA	1982
L. jamestowniensis	Wet soil, USA	1979
L. rubrilucens	Tap water, USA	1980
L. erythra	Water, cooling tower, USA	1981
L. hackeliae	Bronchial biopsy, USA	1981
L. spiritensis	Water, lake, USA	1981
L. parisiensis	Water, cooling tower, USA	1981
L. cherrii	Water, thermally altered, USA	1982
L. steigerwaltii	Tap water, USA	1981
L. sainticrusis	Tap water, USA	1982
L. brunensis	Water, cooling tower, Czech	1987
L. moravica	Water, cooling tower, Czech	1987
L. gratiana	Water, Spa water, France	1988 or 89

APPENDIX B Continued

Species	Source and Place	Year of Isolation
L. adelaidensis	Water, cooling tower, Adelaide (Australia)	1987
L. quinlivanii	Evaporative A/C system of a bus Australia	1986
L. birminghamensis	I. N. A.	
L. tucsonensis	I. N. A.	
L. cincinnatiensis	I. N. A.	
L. israelensis	I. N. A.	
L. fairfieldensis	I. N. A.	
L. londiniensis	I. N. A.	
L. geestiae	I. N. A.	
L. quarteiraensis	I. N. A.	
L. nautarum	I. N. A	
L. worsleiensis	I. N. A.	

INA = Information not available.

Modified from Brenner et al., 1985.

APPENDIX C

Pathogenic and Non-pathogenic Species of the Genus *Legionella**

L. pneumophila	*L. parisiensis*
L.micdadei	*L. steigerwaltii*
L. bozemanii	*L. santicrusis*
L. dumoffii	*L. israelensis*
L. longbeachae	*L. gratiana*
L. gormanii	*L. fairfieldensis*
L. jordanis	*L. moravica*
L. feeleii	*L. brunensis*
L. anisa	*L. quinlivanii*
L. oakridgensis	*L. adelaidensis*
L. cincinnatiensis	*L. jamestowniensis*
L. wadsworthii	*L. londiniensis*
L. hackeliae	*L. geestiae*
L. mesonensis	*L. quateirensis*
L. maceachernii	*L. nautarum*
L. birminghamensis	*L. worleiensis*
L. cherrii	*L. erythra*
L. rubrilucens	*L. spiritensis*
L. sainthelensi	

* Species in the left column have been implicated in human illnesses

Modified from Koneman *et al*., 1992.

APPENDIX E

The Composition of Buffered Charcoal Yeast- Extract Medium.

Type of Constituents	Amount/Litre.
Yeast Extract	10.0g
Activated Charcoal (Norit SG)	2.0g
L-cysteine HCl. $2H_2O$	0.4g
Ferric Pyrophosphate, soluble	0.25g
Agar (GIBCO)	17.0g
ACES Buffer (n-2-acetamido-2- aminoethane sulfonic acid)	10.0g
Alpha-ketogluterate	1.0g
Polymyxin B (selective)	40.000U
Vancomycin (selective)	0.1mg
Anisomycin (selective)	80.0mg
1M KOH	40ml
Deionized water	1L

APPENDIX D

Clinical Manifestation in Two Types of Legionellosis

	Legionnaires' disease	Pontiac fever
Attack rate	1 - 5%	95 - 100%
Mortality	15 - 30%	0%
Incubation Period	2 - 10 days	5-6 hr up to 1-2 days
Symptoms	Fever, chills, cough myalagia, headache, chest pain, sputum and diarhoea (and confusion or other mental states on some)	Similar to influenza, fever, chills, and myalgia (and chest pain, and confusion in some)
Effects on Lungs	Pneumonia and pleural effusion (lung abscess in some)	Pleuritic pain; no pneumonia; no lung abscess
Effects on Kidneys	Renal failure (proteinuria, azotemia, and heamaturia in some)	No renal manifestation
Effects on Liver	Modest liver function abnormalities	No liver function abnormalities
Gastrointestinal Tract	Watery diarrhea, abdominal pain and nausea and vomiting	No abnormalities
Central Nervous System	Somnolence, delirium disorientation, confusion and obtundation (rare)	No manifestations.

Modified from Fraser and McDade, 1979; Win, 1988; WHO, 1990; Yu, 1990 and, Koneman *et al*., (1992).

APPENDIX C

Pathogenic and Non-pathogenic Species of the Genus *Legionella**

L. pneumophila	*L. parisiensis*
L. micdadei	*L. steigerwaltii*
L. bozemanii	*L. santicrusis*
L. dumoffii	*L. israelensis*
L. longbeachae	*L. gratiana*
L. gormanii	*L. fairfieldensis*
L. jordanis	*L. moravica*
L. feeleii	*L. brunensis*
L. anisa	*L. quinlivanii*
L. oakridgensis	*L. adelaidensis*
L. cincinnatiensis	*L. jamestowniensis*
L. wadsworthii	*L. londiniensis*
L. hackeliae	*L. geestiae*
L. tucsonensis	*L. quarterraensis*
L. maceachernii	*L. nautarum*
L. birminghamensis	*L. worleiensis*
L. cherrii	*L. erythra*
L. rubrilucens	*L. spiritensis*
L. sainthelensi	

* Species in the left column have been implicated in human illnesses

Modified from Koneman *et al.*, 1992.