Master of Public Health

Master international de Santé Publique

Identification of environmental factors involved in legionella development.

Kshitij KARKI

Class and year of the Master: MPH (M2)

Location of the practicum: LERES, Rennes, France

Professional/Academic advisor: Pierre Le CANN, EHESP

Acknowledgements

I am thankful to European commission, Europubhealth team, Ecole des hautes etudes en santé publique (EHESP), LERES, ScHARR, The University of Sheffield and other many institutions without which this study would be impossible.

First and foremost, my deepest gratitude goes to my thesis advisor professor Pierre Le CANN for his continued guidance, encouragement, motivation and support throughout the whole research process. Likewise, I would like to thank my first year supervisor Dr. Ravi Maheswaran for his guidance and support.

My special thanks go to Séverine Deguen for her guidance and support on statistical analysis. Similarly, my greatest gratitude goes to Prof. Paul Bissell, Prof. Petra Meier, Alan O'Rourke, Prof. Denis Zmirou Navier, William Sherlaw, MPH coordinator Martine Bellanger, Anke Grapenthin, Katel Le Floch and Francoise Cormerais for their continuous support.

I am indebted to my many student colleagues from EPH and MPH for providing a stimulating and fun environment in which to learn and grow.

I wish to thank my parents and brother for providing me a lovely environment, continuous encouragement and support from faraway.

At last, I would like to acknowledge all the known and unknown people for contributing directly or indirectly help to this study.

Cordially,

Kshitij KARKI

01/06/2011

Rennes, France

Table of contents

List of Tables

List of Figures

List of Acronyms

WHO	World Health Organization
LERES	Laboratoire d'étude et de recherche en environnement et santé
EHESP	Ecole Des Hautes Etudes En Sante Publique
ScHARR	School of Health and Related Researh
EWGLI	The European Working Group for Legionella Infections
ELDSNet	European Legionnaires' Disease Surveillance Network
INVS	Institut de veille sanitaire
HPA	Health Protection Agency
VFDB	Virulence Factors Database
GIS	Geographical information System
Lp1	Legionella pneumophila serogroup 1
CFU	C*olony*-forming Unit
NaCl	Sodium Chloride
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
NTU	Nephelometric turbidity units
HPC	Heterotrophic plate count
Cu	Copper
Fe	Iron
Pb	Lead
Zn	Zinc
Mn	Manganese
OR	Odds Ratio
CI	Confidence Interval
Adj.	Adjusted

Operational definitions

Iron: It is a metal in the first transition series. It is the most common element in the whole planet Earth, forming much of Earth's outer and inner core, and it is the fourth most common element in the Earth's crust. It is measured in mg/l.

Free Chlorine: It is a chemical element that uses in disinfection of drinking water. When chlorine is added to water, underchloric acids form. Then, underchloric acid (HOCl, which is electrically neutral) and hypochlorite ions (OCl^-, electrically negative) will form free chlorine when bound together. It is measured in mg/l.

PH: It is a measure of the acidity or basicity of an aqueous solution.

Temperature: It is a physical property of matter that quantitatively expresses the common notions of hot and cold. Much of the world uses the Celsius scale (°C) for most temperature measurements. Likewise, scientists use Kelvin scale and few countries like USA use Fahrenheit scale for the common purpose.

Hardness: It is water that has high mineral content (in contrast with *soft water*). Hard water has high concentrations of Ca^{2+} and Mg^{2+} ions. It is measured in French degrees (˚f).

Nitrate: Nitrate is the compound predominantly found in groundwater and surface waters. Nitrate containing compounds in the soil are generally soluble and readily migrate with groundwater. It is measured in mg/l.

Conductivity: The conductivity of an electrolyte solution is a measure of its ability to conduct electricity. It is measured in μS/cm.

Legionella: Legionella is a pathogenic Gram negative bacterium, including species that cause legionellosis or Legionnaires' disease, most notably *L. pneumophila*. It may be readily visualized with a silver stain.

Logistic Regression: In statistics, logistic regression (sometimes called the logistic model or logit model) is used for prediction of the probability of occurrence of an event by fitting data to a logit function logistic curve. It is a generalized linear model used for binomial regression.

P-Value: In statistical significance testing, the p-value is the probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true.

Odds Ratio: It is a measure of effect size, describing the strength of association or non-independence between two binary data values. It is used as a descriptive statistic, and plays an important role in logistic regression.

Chapter 1 Introduction

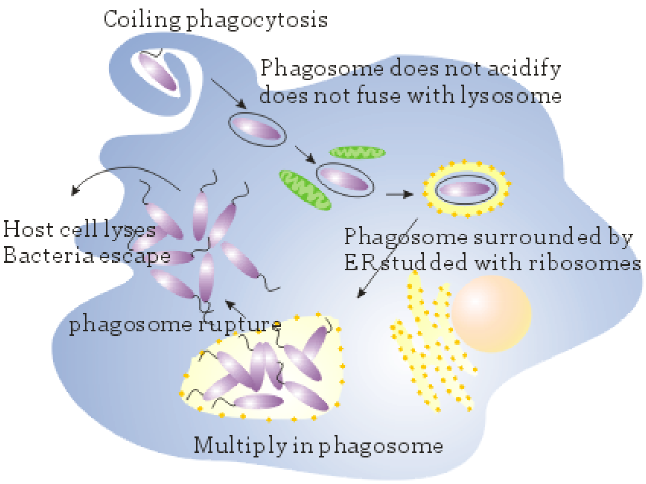
1.1) Legionella and Legionellosis

Legionella are aerobic, gram-negative bacteria which grow in natural aquatic environments such as lakes and rivers as well as artificial water systems; wet soil and compost. Mostly legionella species are motile and have lateral flagellae or one to three polar (Drozanski, 1991; Riffard et al., 1998). Amino acids are used by legionella for energy. However, *Legionella lytica* and other species of legionella get energy from their hosts. Currently, it has been proven that above 20 species are responsible for Legionnaires' disease among 52 species and higher than 70 serogroups of legionella (Lee et al., 2010; Fields et al., 2002).

a) Structure and lifecycle

Legionella are rod or coccoid shaped which do not form spores. It is known that life cycle of legionella is divided into two stages. In the first phase, the bacteria are non motile and have a low virulence. It is called the replicative phase. Likewise, in the second phase, the bacteria are thicker and shorter. They have well developed flagella and are very virulent which is called as infectious phase (Molofsky and Swanson, 2004).

Cell replication of legionella is totally intracellular, extracellular is not possible. When virulent legionella cells are ingested by amoebae or macrophages, a phagosome is established. Then, the phagosome is surrounded by endoplasmic reticulum and becomes entirely isolated from the endosomal pathway. Likewise, bacteria are converted into the acidic and relicative form without virulence factors inside the protective vacuole and later merged with lysosomal compartment. Then after, amino acid is gradually depleted, and the bacteria are changed into the virulent form and dispersed into the environment and ready for another phagocyte (Tison and Seidler, 1983; Helbig et al., 2002; Swanson and Hammer, 2000).



Source: (VFDB, 2011)

Figure 1 L. pneumophila cycle in macrophages

b) Ecology

Legionella species are everywhere in freshwater environments but can also be found in wet mud. However, the main environments for some species like *Legionella longbeachae* are soil and composting site (Steele et al., 1990).

Currently, it is not clearly known about formation of biofilm and legionella colonization. It is argued that the possibility of producing biofilms is not possible without another prokaryote or eukaryote species. However, some studies confirmed that legionella is able to produce biofilms in a rich medium containing nutrients, temperature between 25 to 42 Celsius degrees and so on while it is depending on planktonic growth (Pécastaings et al., 2010).

The virulence of the legionella bacteria is developed from the parasitic interactions between amoebas and other eukaryotics. The ideal environment provided by amoebas is important for the reproduction and development of the bacteria. In addition, legionella can be more resisted to adverse environmental conditions. It is occurred in high amount in thermal water and enters the human hosts accidentally. Likewise, the bacteria can be grown anywhere from 5 to 63 Celsius degrees, whereas, maximum growth occurs between 25 and 40 Celsius degrees. The *Legionella micdadei* and amoeba host have been found in highly acidic geothermal environments (Kwaik et al., 1998).

c) Diseases

Legionella pneumophila is the organism that causes Legionnaires' disease and Pontiac fever (Steinert et al., 2002). Legionellae rarely cause human disease. More than half of legionella species have been concerned in human diseases. Among all species, *Legionella pneumophila* (Lp) consists of 95% of total legionellosis cases because of having greater virulence. Likewise, serogroup 1 is better adapted to human beings that accounts for up to 80% of cases among fifteen serogroups (Sg) of *Legionella pneumophila* (Yu et al., 2002).

Legionnaires' disease is a severe pneumonia that can be accompanied by extrapulmonary manifestations, such as renal failure, encephalopathy, and pericarditis. The symptoms of Legionnaires' disease start with low fever, malaise, a mild cough, muscle aches and gastrointestinal symptoms. Similarly high fever, alveolitis and bronchiolitis are the later development of the disease (Winn, 1988). The Legionnaires' disease has the average of two to ten days of incubation periods. The route of disease transmission is strictly by inhalation of legionella contaminated water droplets but not by ingestion. The transmission between human beings has never been observed to this date. Therefore, it is focused on the elimination of legionella pathogens of hot water systems and cooling towers for the prevention and control of Legionellosis (WHO, 2004). Likewise, the other disease caused by legionella is Pontiac fever. It is clinically different, milder and non-pneumonic as well as influenza-like type of disease (Fields et al., 1990). However, disease determinants of milder form are not well known and are rarely detected.

The disease is mainly diagnosed by four methods that are antibody determination, immunoflurescent microscopy (tissues or body fluids demonstration), antigenuria detection, lung radiography and culture media. The mortality rate of the disease ranges from 5 to 80% if not treated. Immunocompetent individuals are more likely to survive from an infection than immunocompromised patients. Cigarette smoking, AIDS, lung cancer, immunosuppression, renal failure, above 50 years of age, and hematologic malignancies are the risk factors for Legionnaires' disease. Likewise, it is not determined yet but alcohol abuse may be a risk factor. In addition, women are 2.5 times less likely to be infected by *Legionella pneumophila* than men (Fields et al., 2002). Outbreaks occur usually from purpose-built water systems where temperatures are warm enough to encourage growth of the bacteria, e.g. in cooling towers, whirlpool spas, hot water network and their topology, and water used for domestic purposes. This is due to symbiotic associations of legionella with amoebas (Diederen, 2008).

1.2) Epidemiology of Legionella

Legionella was first recognized in 1976 as human pathogens after an outbreak of pneumonia among veterans attending a convention in Philadelphia (Bartram et al., 2007). More than 221 persons were sickened and 34 deaths occurred at that time (Lawrence, 2006). Among all community acquired pneumonias, Legionellosis that needs hospitalization is accounted for 2% to 15% (Carratala et al., 1994; Marston et al., 1994). Legionellosis outbreak was remained remarkable but only 5% cases or below fit the community-acquired legionnaires' disease (Marston et al., 1994). It was estimated that about 20,000 cases of Legionnaires' diseases were annually occurred but only 1000 cases were reported in the United States (Montalbano MA, 1996). The case fatality rate of legionnaires' disease can be increased with risk factors like alcoholism, cigarette smoking, age and cancer (HSE, 2003).

Cases of legionellosis have been repeatedly detected in a small cluster of tourists staying in the hotels, mainly in Mediterranean regions. A study in certain European holiday hotels by Starlinger and Tiefenbrunner (1996) showed that about 55% of hotels had legionella contaminated hot water network and 73% had amoebae in their water distribution systems. Legionella Infections reported an average rate across Europe of 9.8 per million populations in 2003. Likewise, legionella infection caused by Legionella pneumophila serogroup 1 is approximately 70%, other serogroups is 20 to 30%, and nearly 5 to10% is affected by non-pneumophila species (Joseph, 2002).

Surveillance of travel-associated legionellosis by EWGLI (ELDSnet from 2010) showed that the source of the legionellosis infection is mainly related to large buildings in which 77.1% are from hotels and 6.7% are from apartment buildings (Joseph et al., 1996).

In France, almost 1500 cases of legionellosis are detected each year. The incidence rate of legionnaires' disease was about 1.9 per 100 000 population in 2009 (Campese et al., 2010). About fifty four sporadic isolates were gathered and seventeen epidemic isolates were found in recent five outbreaks in France. These epidemic isolates are i) one (Montparnasse strain) from Paris outbreak, 1999; ii) seven (two strains – Rennes a and Rennes b) from Rennes outbreak, 2000; iii) four (Lyon strain) from Lyon outbreak, 2001; iv) three from Sarlat nosocomial outbreak, 2002 and v) two from Besancon nosocomial outbreak, 2002. Forty nine endemic isolates designated Paris was isolated from various parts of France (Aurell et al., 2005).

Paris strain is the most common endemic strain in France since 1998, which were accountable for 12.2% of legionellosis cases, confirmed by culture from 1998 through 2002 (Aurell et al., 2003). It has been found in clinical and in environmental samples from other European countries such as Sweden, Switzerland, Spain and Italy as well (Fields et al., 2002; Lawrence et al., 1999). As an epidemiological surveillance of legionellosis, all 259 strains of legionella collected from patients were sent by 57 French departments to the National legionella reference center (NRCL) of France between 2001 and 2002. Among all, 58 % were acquired from community, 16% were acquired from hospital, and 26% were obtained from undetermined source. The distribution of the legionella species and serogroup was comparable to that stated in another place (Swanson and Hammer, 2000; Yu et al., 2002).

A total of 13 patients were dead due to two outbreaks in 2000 and 2005 respectively in the city of Rennes (Tessier, 2009). Between the end of July and 15 November 2000, nineteen cases of legionnaires' disease (among them five were fatal) were reported in Rennes, in north west France. Seven cases were caused by Legionella pneumophila serogroup 1 (the seven strains have the same genomic profile) which has reported by the French National Reference Centre (Decludt, 2000).

1.3) International guidelines and Surveillance of Legionella

A number of international and national guidelines and control strategies have been implemented for the prevention and limitations of legionellosis in building water systems.

These guidelines incorporate the occurrence of virulent legionella in fresh water and purposed built water environment, bacterial multiplication to an unknown infectious dosage, and susceptibility of infection to a human host through spread of the bacteria via aerosol (HSE, 2000).

World Health Organisation prepared a guideline named ‘WHO Guidelines for Drinking-water Quality’ for water quality and public health protection. The main principle of the guidelines are controlling of infectious and hazardous elements from the water distribution system with the development of risk management and implementation strategies. According to the WHO guidelines, contamination of drinking water distribution systems should be prevented by applying secured roof with external waste management system for the security of storage tanks (WHO, 2006). Likewise, European guidelines for legionella named ‘The European Guidelines for Control and Prevention of Travel Associated Legionnaires’ disease’ also gives the full procedures on the controlling, reporting and response of the legionellosis which is applied for European Union countries. Similarly, risk assessment procedures, water treatment methods, outbreak investigations in hotels and community, disease surveillance and coordination among state members are also included (EWGLI, 2002).

Changes in the epidemiological triad such as host, agent and environment could be monitored by the surveillance, a significant tool, for the future planning. In England and Wales, Legionnaires disease surveillance was begun from 1979 but the complete annual data was recorded from 1980 (Bartlett and Bibby, 1983; HPA, 2008). Similarly, in France, a compulsory notification system of Legionnaires’ disease based on clinical diagnosis was established in 1987. Infectious disease surveillance systems in France were re-evaluated in 1995 and prioritized the surveillance of Legionnaires’ disease (Hubert and Haury, 1996). Public hospital laboratories were surveyed and estimated the under reporting and feasibility of reporting systems based on laboratory. In 1995, the surveyed cases, notified cases and cases from National reference Laboratory were matched to estimate the total Legionella cases in France (Hubert and Haury, 1996).

1.4) Factors involved in Legionella development

a) Microbiological factors

The necessary growth requirements for the development and protection of microbial communities as well as legionella are provided by biofilms. It is difficult to remove biofilms from the complex water distribution systems but the prevention of biofilms is necessary to control the proliferation of legionella bacteria (Rogers and Keevil, 1992). On the one hand, persistence and adherence of *Legionella pneumophila* is favoured by the presence of *Mycobacterium species* or *Empedobacter breve* under biofilms in the artificial water environments. On the other hand, different micro-organisms such as *Corynebacterium glutamicum*, *Pseudomonas species*, and *Klebsiella pneumoniae* have been accelerated for the detachment of Legionella pneumophila from the biofilms and reduced the adherence of bacteria (Mampel et al., 2006). *Legionella pneumophila* can be proliferated in vivo up to 6.3 log CFU cm^2 cell density within protozoan hosts in the presence of aquatic biofilms (Kuiper et al., 2004). It is more resistant to the biocides and chemical disinfectants when the *Legionella pneumophila* bacteria are within protozoa than in vitro-grown legionella (Barker et al., 1992; Barker et al., 1993; Barker et al., 1995).

b) Physical and chemical factors

Legionella species are freshwater bacteria and few studies showed that sea water is also the potential reservoir for the bacteria. A laboratory analysis found the survival of Legionella pneumophila in salt solutions up to 3% NaCl at temperatures between 4 °C and 20 °C. These researches indicated that *Legionella pneumophila* can be survived in sea water as the marine temperature rarely rise above 20 °C in temperate region (Heller et al., 1998). The motility and virulence of the cultured *Legionella pneumophila* is also affected by temperature. More flagella have been assembled and legionella cells express additional proteins and flagellin RNA incubated at 30 °C than at 37 °C (Heuner et al., 1995; Ott et al., 1991).

The factors associated in the survival of *Legionella pneumophila* species in the microcosms of hot spring water and tap water were studied by examining metabolic activity and cultivability. In all microcosms at 42°C temperatures, *Legionella pneumophila* could survive by maintaining metabolic activity but was non cultivable in all microcosms, exception in the case of pH of <2.0 for one microcosm (Ohno et al., 2003). Survival of bacteria is also supported in lower temperatures without loss of cultivability. The metabolic activity was examined at temperatures up to 45°C whereas, the cultivability decreased with increasing temperatures. The maximum range of pH for survival is between 6.0 and 8.0. Even in microcosms with high concentrations of salt, the metabolic activity of bacteria could be maintained for long periods. Randomly amplified polymorphic DNA analysis of microcosms where metabolic activity was maintained but cultivability was lost showed an effect on the amplified DNA band pattern by production of stress proteins, although no change compared to cells grown freshly (Ohno et al., 2003).

Bacterial populations released in an aqueous environment are frequently exposed to stresses due to limitations and changes in nutrient availability, temperature, salinity, oxygen, and pH. Bacteria often enter a “temporarily non-cultivable state,” to adapt to such a stressful environment and then resuscitate when environment is favourable of growth. This physical change is generally referred to as “viable but non cultivable,” and some bacteria use this strategy (Kell et al., 1998). *Legionella pneumophila* that entered a non cultivable state in tap water were also reportedly revived by injection into embryonated eggs (Hussong et al., 1987). Cultivability was declined faster at pH 5.0 and 10.0 than at pH 6.0 and 8.0; however, metabolism of micro-organisms was sustained at pH 5.0. It was proved that a hot water micro-organism best survive at the adjusted pH 8.0 (Ohno et al., 2003). However, *Legionella pneumophila* have been declined 2-log after one month incubation periods in tap water at pH from 4.0 and 7.0, and 6-log at pH 8.0 (Katz and Hammel, 1987).

An experimental study demonstrated that cell numbers can be significantly reduced at the combination of high temperatures of 30 °C and 37 °C with the mixture of over 1.5% NaCl. However, the addition of 0.1% to 0.5% NaCl had suggested the positive effects of NaCl that increased the survivability of *Legionella pneumophila* (Heller et al., 1998). The CO_2 have been increasingly produced up to 45 °C temperature, where after it reduced the production of CO_2 and the multiplication rate of cell is also decreased. Thus, the metabolic activity and cell multiplication of Legionella have been retained beyond the utmost temperature (Kusnetsov et al., 1996).

A study by Leoni et al.(2001) showed that the lower temperatures, the greater concentration of chlorine and mix contamination in the pool are responsible to inhibit the growth and development of any Legionella which decreases the level to have health risks. However, this micro-organism is also known to live as a free organism for long periods in very low-nutrient environments under appropriate conditions (Lee and West, 1991). It is suggested that Legionella can exist for a long time periods in a low concentrations of chlorine (Kuchta et al., 1983).

Legionella were not found in the concentration of Heterotrophic Plate Counts (HPCs) less than 27 CFU per ml and 150 CFU per ml at 22 ˚C and 37 °C respectively as well as manganese less than 6 mg/l, though copper (Cu) concentrations above 50 mg/l and temperature above 55 °C were contributed to the absence of contamination. The HPCs at both 22 and 37 °C and concentrations of Zn, Fe, and Mn were positively correlated with legionella, while total hardness and temperature were negatively correlated (Bargellini et al., 2011). Growth of *Legionella species* are supported by the temperature of water, stagnation, flow, deterioration of pipe, materials used in pipe, flushing and water stresses (Exner et al., 2005), while water characteristics such as hardness and concentrations of chemical elements have been only proposed (Borella et al., 2005a). The important factors that involved in *Legionella pneumophila* growth are iron, potassium and zinc in lower concentrations, while toxic in high minerals concentrations (States et al., 1985). Similarly, the growth and development of Legionella pneumophila is inhibited by the appearance of copper in water networks (Leoni et al., 2005).

A study of Legionella in hot water networks of Italian hotels showed that lower hardness and high concentration of free chlorine were positively related with colonization of *Legionella pneumophila* serogroup 1, but colonised samples of *Legionella pneumophila* serogroup 2 to 14 had opposite trend (Borella et al., 2005b). Replication of Legionella pneumophila in the mammalian host is depended on Iron (Fe); and multiple pathways has been detected (Cianciotto, 2007). It is not well known whether the presence of minerals are responsible for the development of *Legionela pneumophila* in a nutrition deficient environment or not (States et al., 1985). The proper use of tanks, showers and heaters have decreased the water stagnation and Legionella proliferation (Tobin et al., 1980; Tison and Seidler, 1983). The major outbreaks are from cooling towers which can be controlled by the strengthening of regulation for the management of Legionella and its sources (Campese et al., 2008).

1.5) Significance of the study

Very few researches were conducted on environmental factors identification. It is difficult to control the growth of bacteria and prevention of disease caused by bacteria without knowing the environmental factors. Only half of the cases are elucidated, partly due to the fact that environmental factors involved in Legionella development are not well characterized. This research is important to identify such growth factors for the development of *Legionella species* and correlation between physical chemical parameters and *Legionella species* before and after the evolution of the water distribution system in Rennes in 2006.

Chapter 2 Methods

2.1) Objectives of the study

The general objective of the study was - to identify the environmental factors involved in legionella development in the city of Rennes.

Specific objectives

- To select the physical and chemical parameters from the dataset.
- To show the yearly variations of physical and chemical parameters.
- To demonstrate the place wise variations of physical and chemical parameters.
- To identify the yearly incidence of legionella bacteria in water distribution systems.
- To correlate physical chemical factors with the presence or absence of Legionella.
- To determine the correlation between legionella positive samples and physical chemical parameters before and after 2006.
- To indicate the sampling point and presence of legionella on GIS map.

2.2) Data Sources

Secondary sources of data from the database of Laboratoire d'Etude et de Recherche en Environnement et Santé (LERES), EHESP were used for this research. Water sources were from the cooling towers, swimming pools and water networks of Rennes, France, as the LERES carry out the regulatory surveillance of these sources for the presence of legionella. Likewise, laboratory diagnoses of water samples for physical chemical parameters and legionella were already performed and recorded as a database. It was the normal surveillance of water sources from 2000 to February 2011.

2.3) Physical, chemical and bacteriological parameters

Different parameters such as faecal streptococci densities (/100ml), prometryne, coliform densities (/100ml), aerobics micro-organisms (/ml), aluminium, copper, ammonium, nitrite, total alkalinity (°f), chloride, lead, sulfate, colour, total organic carbon, terbutryn, terbumeton, terbuthylazine, simazine, prometryn, metribuzin, desmetryn, cyanazine, atrazine, ametryne, turbidity (NTU), free chlorine, total chlorine, iron, total hardness of water, temperature of water, pH, nitrate and conductivity were diagnosed from water samples by technicians of LERES. Likewise, the bacteriological parameters like faecal streptococci, coliform, aerobic micro-organisms were not included in the study because of many zero values and absence of values. Free chlorine (mg/l), total hardness of water (°f), temperature of water (°C), iron (mg/l), pH, nitrate (mg/l) and conductivity (microS/cm) were selected for this study. Selection of these parameters was mainly based on the literatures, number of missing values and variability of data.

2.4) Sampling site

Water samples were collected from different parts of Rennes to find out physical chemical parameters and legionella. The available data was not sufficient for analysis and comparablee for all samples collected places. Therefore, sample places were divided into seven major places such as Villejean, Brequigny, Thabor-Saint-Helier, Nord Saint Martin, SUD Gare, Les Gayeulles and Patton. Places where water samples were taken for physical chemical parameters and for legionella are shown on the map below.

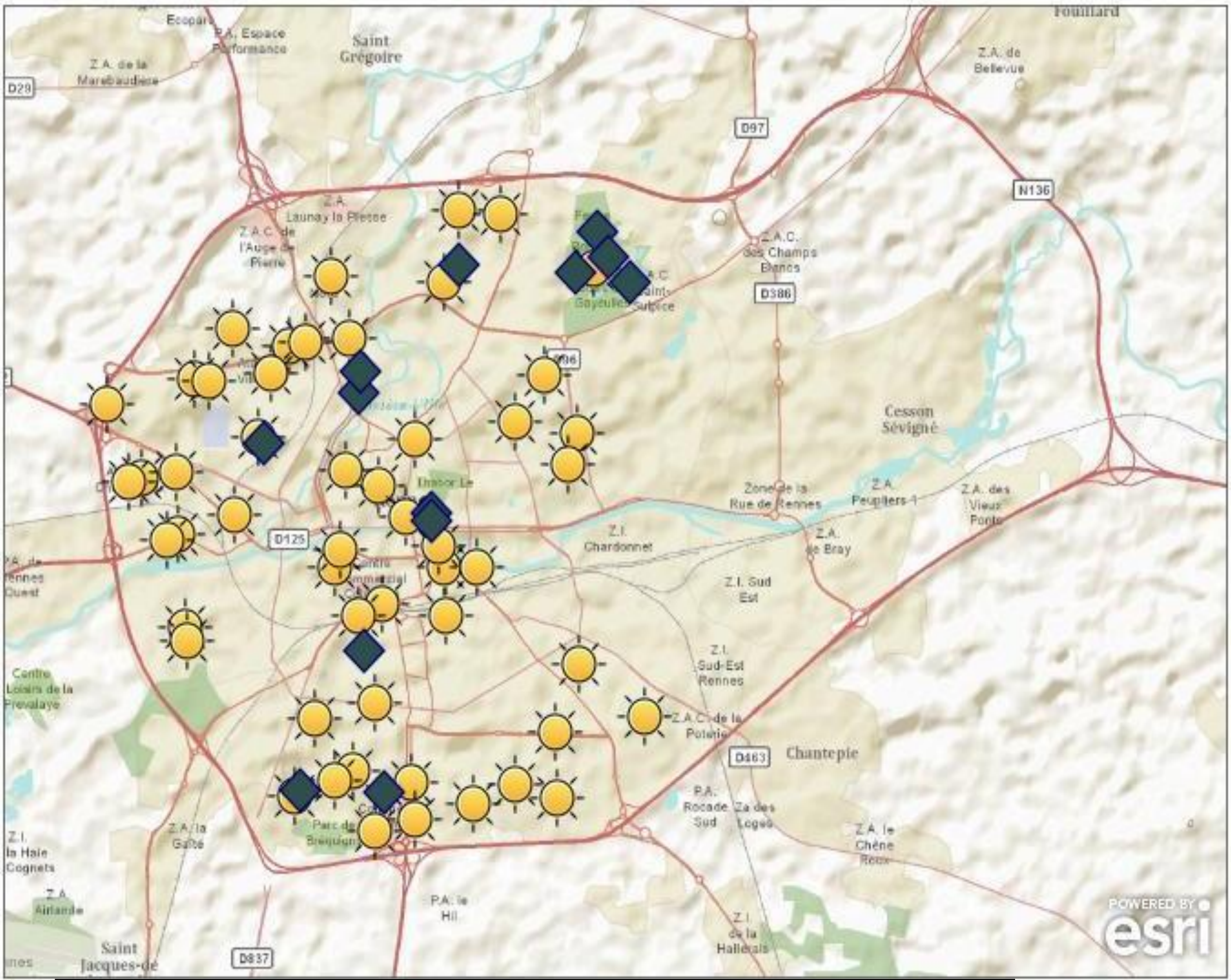


Figure 2 Water sampling points for physical chemical parameters (☼) and legionella (■)

2.5) Water networks of Rennes and modifications after 2006

Physical chemical parameters and legionella data from water samples (2000 to February 2011) were included in the database. Upper Rennes and lower Rennes were two water distribution systems of Rennes before 2006 (see Annex 1, Figure 10). It was modified at the end of 2006 and merged two water distribution systems into one (see Annex 1, Figure 11). As a consequence, there are variations in physical chemical parameters and legionella concentrations in water samples. Therefore, this study was also focused to find out the variation and effects of physical chemical parameters with legionella before and after 2006.

2.6) Statistical Analysis

Statistical analysis was done by using STATA 11 version and geographical information system (ArcGIS). Descriptive statistics (mean, median, variance, CI and skewness) of physical chemical characteristics from the database have been analysed and presented in the form of tables and graphs. Likewise, logistic regression analysis was done to correlate the presence of the legionella with the various physical and chemical parameters.

i) Coding of legionella positive and negative, and year

Presence and absence of legionella in water samples were the dependent variable. The concentration of legionella varied from place to place. The legionella values were converted into log 10 values to normalize the non normal values. Likewise, legionella negative ‘0’ for less than 250 (which is the regulatory positive threshold) and legionella positive ‘1’ for more than 250 were coded to convert the data into dichotomous form. Similarly, ‘0’ was coded for the year 2006 and before as well as ‘1’ for the year 2007 to February 2011.

ii) Modelling for logistic regression

Correlation matrix was estimated to know the correlation between two or more independent variables. The multivariate logistic regression was computed based on the correlation matrix and R^2 from univariate logistic regression. Mainly, the univariate logistic regression was carried out because of the high positive correlation between independent variables. Comparisons between dependent and independent variables were based on the odds ratio and 95% confidence interval (p-value less than 0.05).

iii) Spatial distribution of legionella and water sampling points in Rennes

Water sampling points for physico-chemical parameters and legionella were indicated in the map using ArcGIS software. It is the online geographical distribution system. The map of Rennes was used to point out the exact places of water sampling and the places, where positive legionella was found.

2.7) Validity and Reliability

Validity and reliability was highly maintained with confidentiality and security of data. Data were cross checked and excluded the parameter which had more than half missing values and no variations on values. Similarly, coding and analysis was performed by using the statistical software (STATA).

2.8) Ethical considerations

Ethical approval is necessary for every research which involved human subjects. This research is based on secondary data which does not include the human subjects. Ethical approval from the ScHARR, The University of Sheffield, United Kingdom was obtained to precede this study (Annex 2). This research did not need the ethical approval in France, whereas, the data was used after the approval of LERES, EHESP. Similarly, confidentiality of the data, data security and respecting previous works had been considered.

2.9) Limitations

This study has some limitations, whereas it is fully maintained the quality of the research. Below are some limitations:

i) Data quantity and quality

This research was based on secondary database. Therefore, water sample collection, laboratory diagnosis and data entry were done by LERES. Many parameters were removed from the study due to high number of missing values.

ii) Sampling site

It was difficult to compare legionella and physical chemical parameters because of different sampling locations. Therefore, the sampling locations were adjusted to the nearby place and quarters of Rennes.

iii) Positive legionella

Number of positive legionella was possibly influenced by the repeated examination of water samples where it was found previously.

iv) Generalization of results

This research can be generalised within Rennes, similar geographical areas and climates but not in whole nations, European countries or worldwide.

Chapter 3 Results

3.1) Descriptive statistics

The table (1) below shows that the characteristics of physical, chemical and microbiological parameters from 2000 to February 2011 which were tested and recorded in the database. Most of the parameters were either negatively or positively skewed from -0.09 to 13.42. Parameters such as lead, nickel, copper, aluminium, antimony, atrazine, benzo(11, 12)perylene, benzo(1, 12)fluoranthen, benzo(3, 4)fluoranthene, Benzo(3, 4)pyrene, cadmium, chrome, vinyl chloride, chlorites, dichloromonobromomethane, fluoranthene, indopyrine, monochlorodibromomethane, sum of 4 HPA, sum of T, H, M, tribromomethane and trichloromethane were excluded from the study because of very low number of observations. Likewise, other parameters which were not included in the study were total chlorine, sulphate, chloride, total organic carbon, colour, ammonium, thermotolerant coliform, faecal streptococci, aerobic microorganisms revivified at 37°C, aerobic microorganisms revivified at 22°C, nitrite, total alkali, total alkali complete and turbidity. It was due to the incomplete (data were available only before 2004) and absence of data.

S.N.	Parameters	Obs.	Median	Variance	Min.	Max.	Skewness	Unit
1	Iron	941	30	1648.18	0	326	2.77	mg/l
2	Water temperature	1021	16	30.28	5	65	1.39	°C
3	pH	1001	7.85	0.16	6.75	8.85	0.10	pH
4	Free Chlorine	998	0.1	0.08	0	2.5	4.33	mg/l
5	Total Chlorine	642	0.1	0.29	0	5	4.25	mg/l
6	Total Hardness	941	13	7.17	5.5	26.2	0.08	°f
7	Nitrate	941	26	113.79	1.3	50.2	-0.05	mg/l
8	Conductivity	1003	436	53482.13	238	1953	3.27	microS/cm
9	Ammonium	942	0.04	0.0003	0	0.17	0.09	mg/l
10	Lead	23	1	1.33	1	5	2.93	mg/l
11	Sulfate	358	35	158.94	11	71	0.46	mg/l
12	Nickel	23	3	2.33	2	8	1.43	mg/l
13	Chloride	371	40	310.91	0.5	109	0.59	mg/l
14	Copper	23	0.05	0.047	0.02	0.74	1.24	mg/l
15	Aluminium	23	10	49.54	0.02	21	-0.09	mg/l
16	Antimony	23	0.5	0	0.5	0.5		mg/l

17	Atrazine	132	0.02	0.0005	0	0.13	1.21	mg/l
18	Benzo(1,12)perylene	23	0.003	3.32e-07	0.003	0.005	2.93	mg/l
19	Benzo(1,12)fluoranthen	23	0.003	3.32e-07	0.003	0.005	2.93	mg/l
20	Benzo(3,4)fluoranthene	23	0.003	3.32e-07	0.003	0.005	2.93	mg/l
21	Benzo(3,4)pyrene	23	0.003	4.74e-07	0.003	0.005	2.19	mg/l
22	Cadmium	23	0.05	0.04	0.1	0.5	-0.64	mg/l
23	Colour	939	5	5.67	0	7.5	-0.62	mg/l
24	Chrome	23	5	0	5	5		mg/l
25	Vinyl chloride	13	0.5	0	0.5	0.5		mg/l
26	Chlorites	16	0.015	14.03	0.015	15	3.61	mg/l
27	Total organic carbon	940	1.7	0.23	0.3	4.3	0.66	mg/l
28	thermotolerant coliform	357	0	0.15	0	7	16.67	/100ml
29	Faecal streptococci	366	0	0.005	0	1	13.42	/100ml
30	Aerobicmicrorg,rev37°c	366	0	1021.43	0	301	7.49	/ml
31	Aerobicmicrorg,rev22°c	366	0	1911.11	0	301	5.56	/ml
32	Dichloromonobromomethane	23	10.6	14.38	4.9	20.9	0.49	mg/l
33	Fluoranthene	23	0.003	2.44e-06	0.003	0.01	3.32	mg/l
34	Indopyrene	23	0.003	3.32e-07	0.003	0.005	2.93	mg/l
35	Monochlorodibromomethane	23	14.6	9.09	8.4	19	-0.14	mg/l
36	Nitrite	941	0.02	0.00009	0	0.03	-0.49	mg/l
37	Sum of 4 HPA	13	0.012	0	0.012	0.012		mg/l
38	Sum of T, H, M,	23	41.9	99.45	24.4	67	0.65	mg/l
39	Total alkali	941	1	0.23	0	1	-0.46	°f
40	Total alkali complete	941	6.7	2.63	0	16.2	0.33	°f
41	Tribromomethane	23	5.3	11.63	2	16.7	1.25	mg/l
42	Trichloromethane	23	6.9	25.15	1.7	24.5	1.59	mg/l
43	Turbidity	939	0.5	0.03	0.1	1.3	0.10	NTU

Table 1 Descriptive statistics of different physical chemical parameters

Similarly, free chlorine, iron, total hardness of water, pH, water temperature, nitrate and conductivity were selected. This was due to many observations, variations on data, availability of data for all years (2000 to 2011), and also supported by previous literatures.

3.2) Values of physical chemical parameters responsible for legionella positive and negative

Out of total observations of free chlorine, 82 observations were associated to legionella positive samples and 194 were legionella negative samples. The average value of free

Parameters	***Legionella spp* from water samples.**							
	Legionella positive Samples				Legionella negative Samples			
	Obs.	Mean	[95% CI]		Obs.	Mean	[95% CI]	
Free Chlorine	82	0.51	0.38	0.64	194	0.22	0.17	0.27
Water Temp.	83	20.15	18.79	21.52	199	16.74	15.68	17.81
pH	70	8.27	8.17	8.38	181	7.99	7.93	8.05
Total Hardness	81	14.11	13.49	14.73	190	13.84	13.49	14.18
Iron (Fe)	82	36.04	29.03	43.05	190	34.22	30.09	38.35
Nitrate	49	25.67	21.89	29.44	124	27.12	25.23	29.02
Conductivity	77	796.5	686.70	907.19	152	592.74	540.01	645.47

Table 2 Values of physical chemical parameters responsible for legionella positive and negative

chlorine for positive samples was 0.51 mg/l (0.38 to 0.64, 95 % CI) and 0.22 mg/l (0.17 to 0.27, 95 % CI) for negative samples. Likewise, the average value of pH (8.27), total hardness of water (14.11°f), water temperature (20.15°C), iron (36.04mg/l) and conductivity (796.5 microS/cm) were found in legionella positive samples, which were higher than that of legionella negative samples. However, more than 27 mg/l (25.23 to 29.02, 95% CI) average nitrate was found in legionella negative samples which was higher than in positive legionella samples. The high 95% confidence interval in positive legionella samples showed that the distributions of data were more fluctuated.

3.3) Distribution of legionella positive water samples by year (percentages)

The highest percentage of legionella was found in 2007, which was about 22 % and lowest about 2% in 2008. Likewise, eighteen percent in 2006, 17 % in 2001, 15 % in 2009 and 14 % in 2004 was detected from different areas of Rennes.

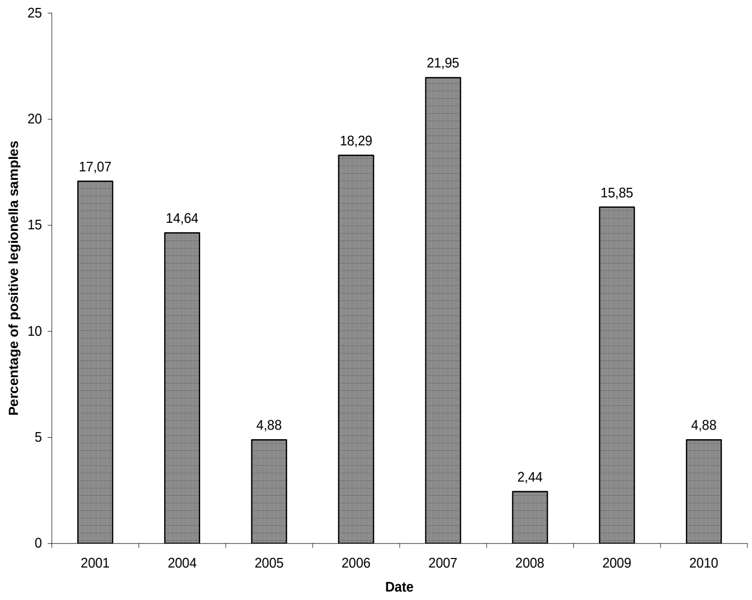


Figure 3 Distribution of legionella positive water samples by year (percentages)

3.4) Variation on physical chemical parameters

i)Yearly Variations

The trend of iron was decreasing from nearly 95 mg/l in 2000 to a little more than one third (33mg/l) in 2004 (figure 4). Then, it was slightly increased to 52 mg/l in 2005 and again followed the decreasing trend. Likewise, the mean water temperatures per annum were between 10 to 20°C from 2001 to 2010. The graph shows that it was nearly 16°C in 2003 while slightly declined in 2004.

Similarly, the value of pH was slightly increased from 7.6 in 2000 to 8.06 in 2007. However, the amount of free chlorine was higher (0.11mg/l) in 2002, 2003 and 2004 and 2010. Clearly the trend of total hardness was increasing from 2004 to 2008. The highest value of average total hardness of water sample was 16°f in 2008, whereas it was 10°f or less before 2005. Furthermore, the values of nitrate were fluctuated from 2000 to 2010. The maximum value was about 29 mg/l in 2007 and declined to 21 mg/l in 2010.

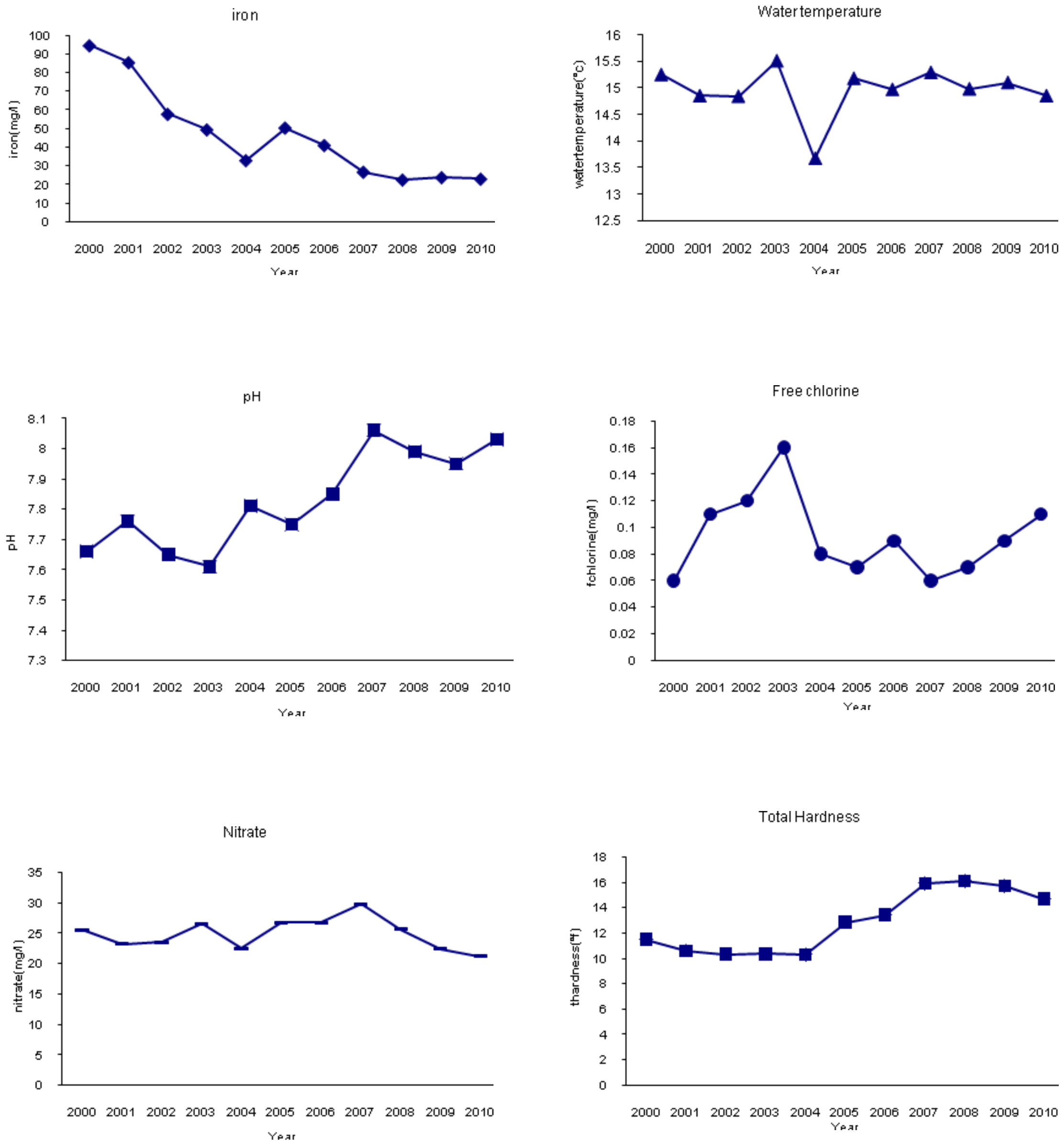


Figure 5 Yearly variations on physical and chemical parameters

Figure 5 shows that the average values of conductivity were higher after 2004. The highest value of conductivity was 480 microS/cm in 2010 and the lowest in 2001, which was 320 microS/cm.

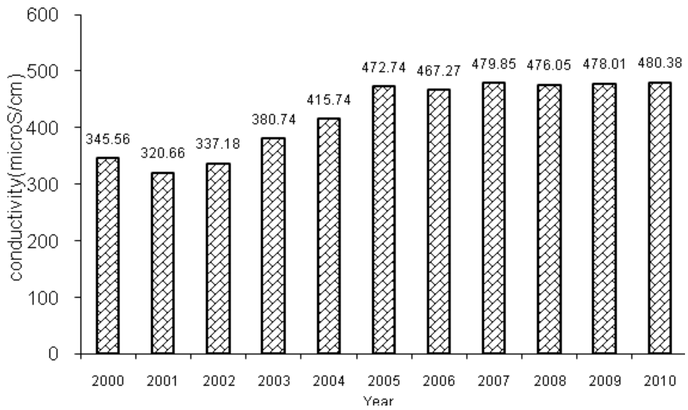


Figure 4 Yearly variations of conductivity

ii) Place wise Variation

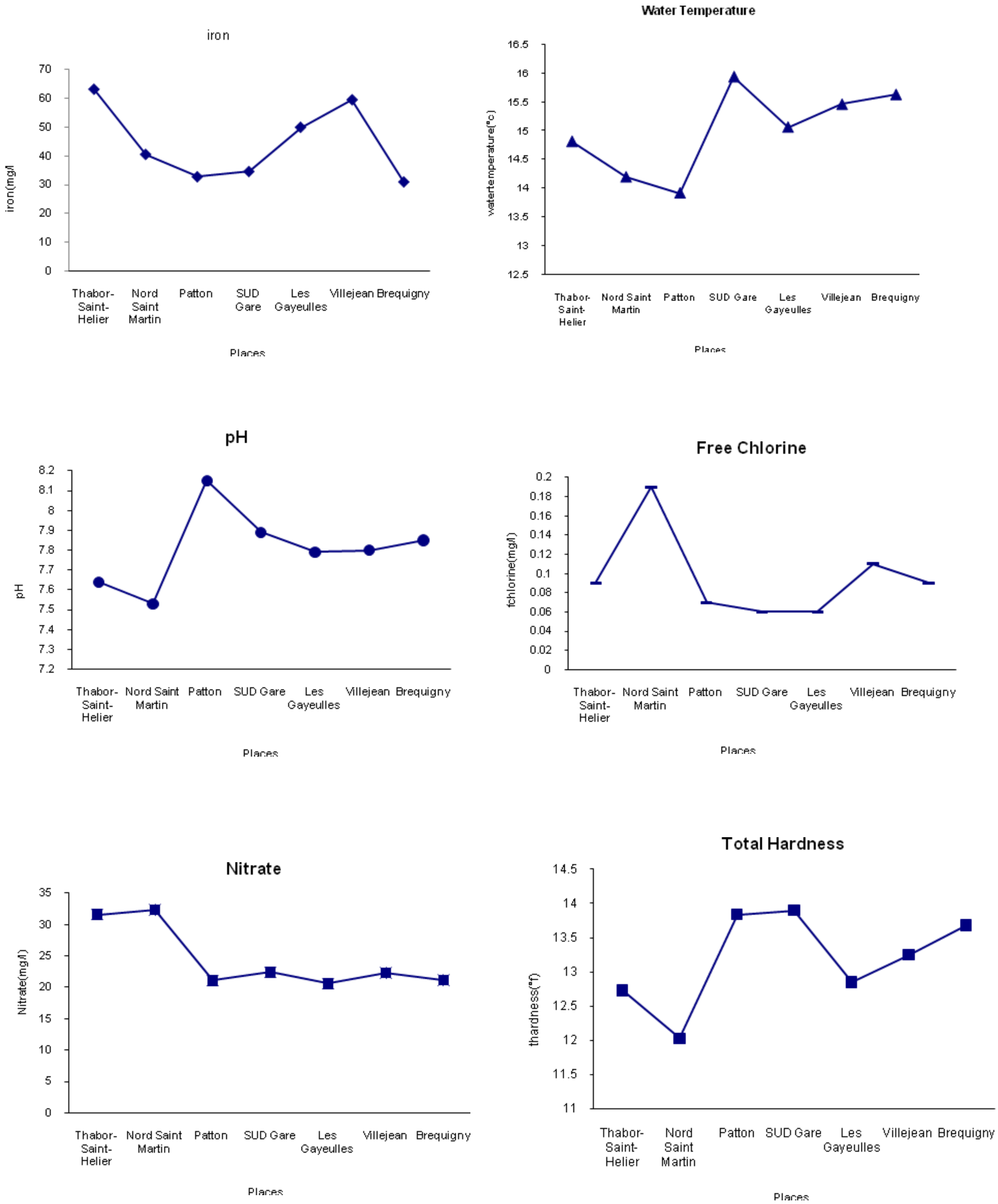


Figure 6 Place wise variations on physical and chemical parameters

The figure 6 shows that the average value of iron was observed more than 63 mg/l in Thabor-Saint-Martin which was followed by approximately 60mg/l in Villejean, 50 mg/l in Les Gayeulles and nearly two third in Nord Saint Martin. In the same way, the value was measured by nearly half in Brequigny, Patton and SUD Gare. More than 31 mg/l nitrate was detected in Nord Saint Martin and Thabor-Saint-Helier. However, it was 20 to 22 mg/l in Patton, SUD Gare, Le Gayeulles, Villejean and Brequigny. The utmost average value of free chlorine was 0.19 and 0.11 mg/l in Nord Saint Martin and Villejean respectively. Likewise, it was observed nearly half in Thabor-Saint-Helier and Brequigny as well as almost one third of free chlorine was examined in Patton, SUD Gare and Les Gayeulles. More than 15°C was found in SUD Gare, Villejean, Brequigny and Les Gayeulles. Likewise, nearly 14°C was detected in Thabor-Saint-Helier, Nord Saint Martin and Patton. Total hardness of water was measured more than 13°f in Patton, SUD Gare, Villejean and Brequigny. Similarly, 12 to 13°f total hardness of water was found in Thabor-Saint-Helier, Nord Saint Martin and Les Gayeulles. The value of pH was highest in Patton, which was more than 8. Then after, 7.9 in SUD Gare, followed by nearly 7.8 in Brequigny, Villejean and Les Gayeulles, and about 7.6 in Thabor-Saint-Martin was observed.

The maximum average value of conductivity was 449 microS/cm in SUD Gare and minimum 394 microS/cm in Nord Saint Martin. Additionally, it was nearly 444 microS/cm in Brequigny, which was followed by 438 microS/cm in Patton, 424 microS/cm in Les Gayeulles, 422 microS/cm in Villejean, 410 microS/cm in Thabor-Saint-Helier and 394 microS/cm in Nord Saint Martin (Figure 7).

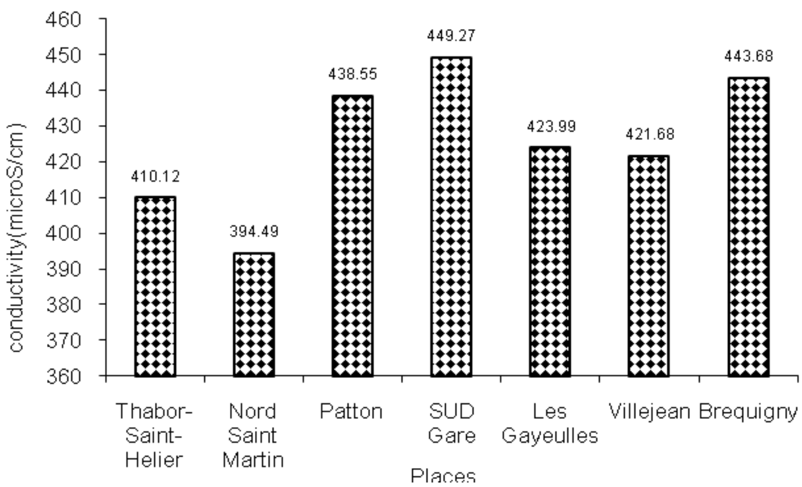


Figure 7 Place wise variations of conductivity

3.5) Trends of total hardness in Villejean and Brequigny from 2000 to 2010

The figure 8 shows that the comparison of total hardness of water between Villejean and Brequigny from 2000 to 2010. The maximum values of total hardness were more than 18°f in June 2007 in Villejean and January 2010 in Brequigny. Similarly, the trend was increasing from 2006 in both areas, whereas it was fluctuated. According to Kruskal – Wallis rank test, values of total hardness in both areas had been followed the same trends because the p-value was more than 0.05.

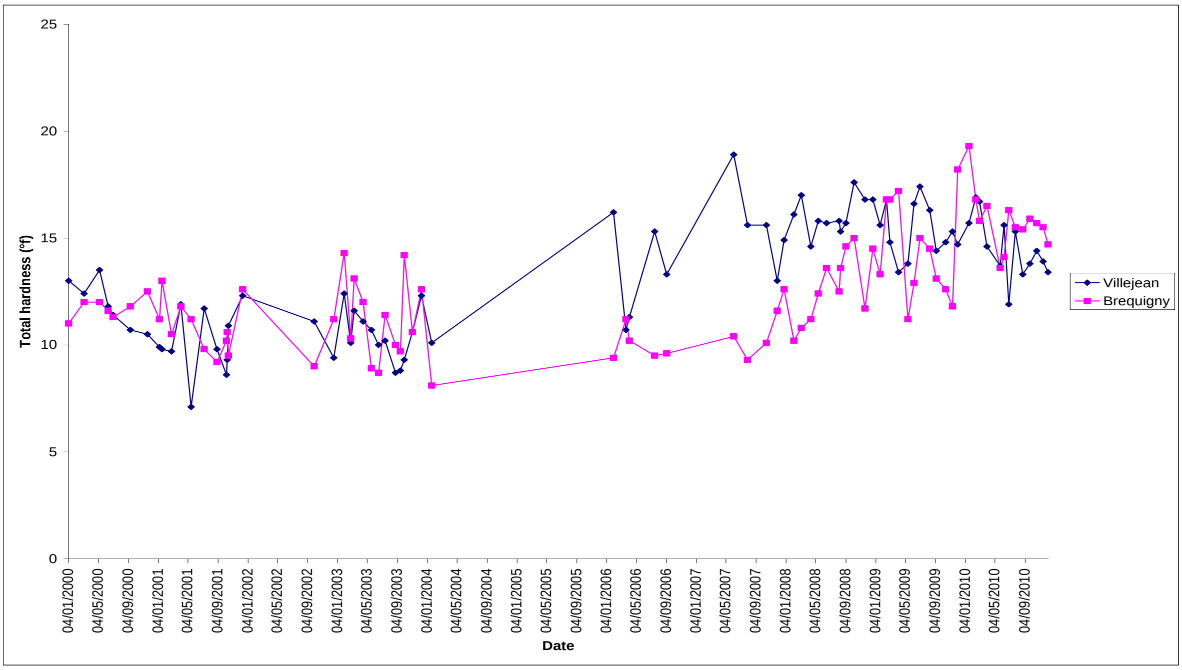


Figure 8 Trends of total hardness in Villejean and Brequigny from 2000 to 2010

3.6) Analytical Results

3.6.1) Correlation between the evolution of physical chemical parameters and presence of Legionella

i) Correlation Matrix

	Free chlorine	Nitrate	Fe	Conductivity	pH	Water temp.	Total hardness
Free chlorine	1.0000						
Nitrate	-0.1759	1.0000					
Fe	0.1352	-0.1066	1.0000				
Conductivity	0.8672	-0.1649	0.0713	1.0000			
pH	0.6574	-0.3509	0.0726	0.7845	1.0000		
Water temp.	0.5575	-0.3589	0.1894	0.6279	0.6800	1.0000	
Total hardness	-0.1088	0.1531	-0.2776	-0.0024	0.0195	-0.0387	1.0000

Table 3 Correlation matrix

Table 3 explains that the correlation between free chlorine, conductivity, pH and water temperature was high from 0.55 to 0.86. However, nitrate, iron (Fe) and total hardness of water were negatively correlated or very less correlation (-0.0024 to 0.1894).

i) Logistic regression of legionella and selected physical chemical parameters

Variables	OR crude	95% CI		P-value	OR adj.	95% CI		P-value adj.
Free Chlorine	3.38	1.93	5.91	0.000	*			
pH	5.05	2.55	10.03	0.000	*			
Water temperature	1.06	1.03	1.10	0.001	*			
Total Hardness	1.04	0.94	1.16	0.417	0.96	0.82	1.11	0.560
Nitrate	0.99	0.96	1.02	0.449	0.98	0.96	1.02	0.498
Iron (Fe)	1.002	0.99	1.01	0.645	1.003	0.99	1.01	0.595
Conductivity	1.001	1.001	1.002	0.000	*			

Table 4 Logistic regression of legionella and physical chemical parameters *P-value = <0.001

According to univariate logistic regression analysis, free chlorine [OR 3.38, CI (1.93 - 5.91), p<0.0000], pH [OR 5.05, CI (2.55 – 10.03), p<0.0000], conductivity [OR 1.001, CI (1.001 – 1.002), p<0.0000] and water temperature [OR 1.06, CI (1.03 – 1.10), p<0.0001] were significantly positively associated with the presence of legionella at 5% significance level.

However, total hardness of water [OR 1.04, CI (0.94 – 1.16), P<0.417], nitrate [OR 0.99, CI (0.96 – 1.02), p<0.449] and iron [OR 1.002, CI (0.99 – 1.01), p<0.645] were not correlated with the presence of legionella.

Likewise, multivariate logistic regression was applied for total hardness of water [OR 0.96, CI (0.82 – 1.11), p<0.560], nitrate [OR 0.98, CI (0.96 – 1.02), p<0.498] and iron [OR 1.003, CI (0.99 – 1.01), p<0.595], which were also not significant to the legionella.

ii) Relationship between legionella and physical chemical parameters before and after 2006

a) Logistic regression before 2006

Table 5 shows that free Chlorine [OR 9.43, CI (2.67 – 33.35), p<0.000], pH [OR 18.26, CI (5.22 – 63.84), p<0.000], water temperature [OR 1.28, CI (1.16 – 1.4), p<0.000] and conductivity [OR 1.002, CI (1.0006 –1.003), p<0.002] were significantly associated with the presence of legionella before 2006.

Variables	OR	95% CI		P-value
Free Chlorine	9.43	2.67	33.35	0.000
pH	18.26	5.22	63.84	0.000
Water temperature	1.28	1.16	1.4	0.000
Total Hardness	0.84	0.69	1.02	0.084
Nitrate	0.99	0.96	1.02	0.517
Iron (Fe)	1.005	0.99	1.01	0.286
Conductivity	1.002	1.0006	1.003	0.002

Table 5 Logistic regression before 2006

Whereas, total hardness [OR 0.84, CI (0.69 – 1.02), p<0.084], nitrate [OR 0.99, CI (0.96 – 1.02), p<0.517] and iron [OR 1.005, CI (0.99 – 1.01), p<0.286] were not correlated with legionella.

b) Logistic regression after 2006

Total hardness of water [OR 2.02, CI (1.05 – 4.16), p<0.000] was significantly associated to legionella. Similarly, free chlorine [OR 2.09, CI (1.05 – 4.16), p<0.036] was also correlated to the legionella positive samples at 5% significance level. Although, other parameters like conductivity [OR 1.001, CI (0.99 – 1.002), p<0.057], pH [OR 2.25, CI (0.88 – 5.72), p<0.089], water temperature [OR 1.01, CI (0.97 – 1.06), p<0.490], nitrate [OR 0.97, CI (0.87 – 1.08), p<0.618] and iron [OR 0.98, CI (0.92 – 1.05), p<0.528] were rejected at 5% significance level and not associated with legionella.

Variables	OR	95% CI		P-value
Free Chlorine	2.09	1.05	4.16	0.036
pH	2.25	0.88	5.72	0.089
Water temperature	1.01	0.97	1.06	0.490
Total Hardness	2.02	1.37	2.97	0.000
Nitrate	0.97	0.87	1.08	0.618
Iron (Fe)	0.98	0.92	1.05	0.528
Conductivity	1.001	0.99	1.002	0.057

Table 6 Logistic regression after 2006

3.7) Distribution of positive legionella samples in Rennes

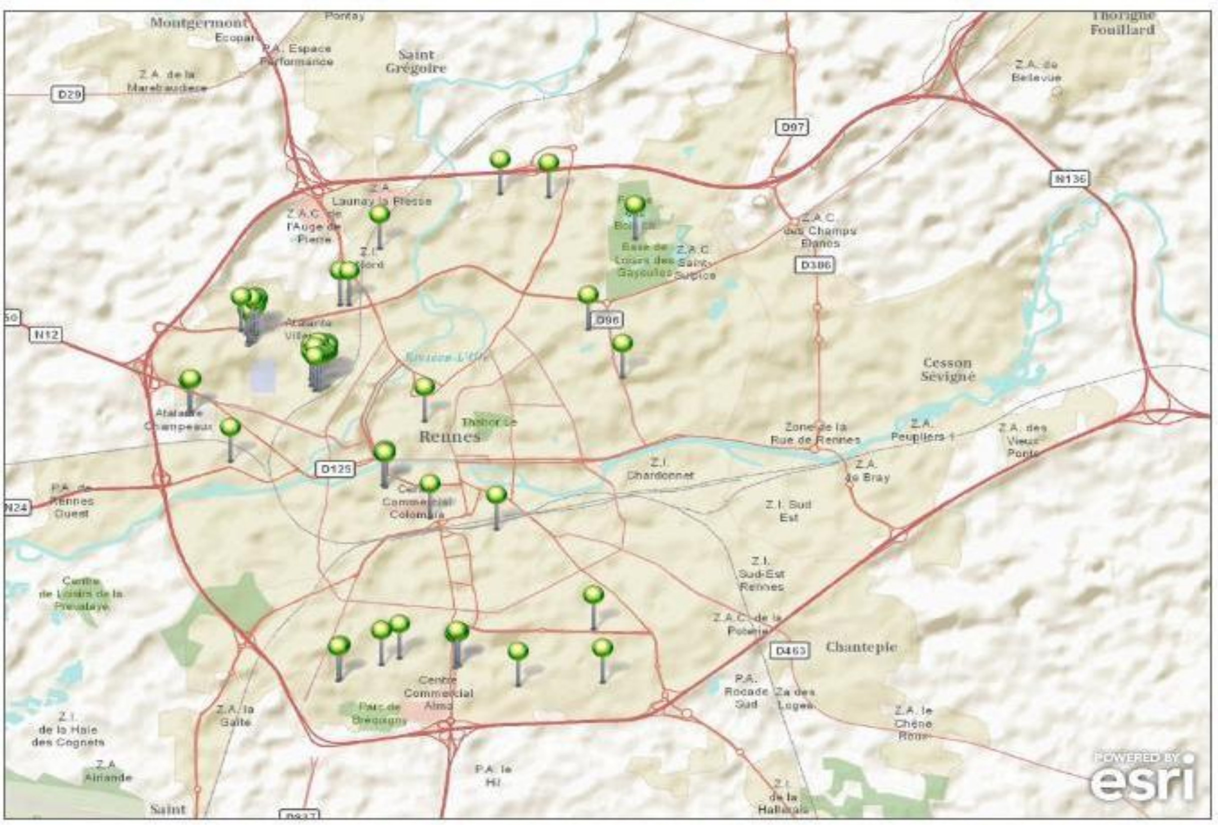


Figure 9 Distribution of positive legionella samples in Rennes

The highest numbers of legionella were found in Villejean and Brequigny. The water distribution system of both areas was same (upper line) before the integration at the end of 2006. Similarly, presence of legionella in SUD Gare and Patton were less than Villejean and Brequigny but higher than Les Gayeulles, Thabor-Saint-Helier and Nord Saint Martin.

Chapter 4 Discussions

This study shows the relationship between different physical and chemical parameters like free chlorine, pH, iron, water temperature, total hardness of water, nitrate and conductivity with the presence of legionella. Parameters were selected on the basis of total observations, availability of data for study time periods as well as previous researches (Table 1).

Legionella pneumophila were found in water samples where mean values of free chlorine, water temperature, pH, total hardness of water, iron and conductivity were higher than in legionella negative samples (Table 2). Likewise, nitrate was measured high in legionella negative water samples. However, in the literature similar result was found in the concentration of nitrate and legionella positive and negative samples in water systems (Kusnetsov et al., 1993). The presence of legionella in high average free chlorine (0.51 mg/l) value was possibly due to the existence of *Legionella pneumophila*. Similarly, high average pH environment was favorable to the growth of legionella but disinfectant chlorine was supported by low pH. However, *Legionella pneumophila* and *Legionella pneumophila* serogroup 2 to 14 were found positive with increases in pH but negative with other *Legionella spp*. (Mouchtouri et al., 2007). Our results for pH and legionella association were contrasted with the result of Katz and Hammel (1987). This might be because of their experimental research.

Water pipes were made up of cast iron so that the mean value was high in 2000 (Figure 4). However, it was in declining trends from few years due to replacing of pipes, changing into plastic pipes, but also modification on sampling techniques. Previously, stagnant water from first flush was collected for the sample but from few years onwards, sampling was doing after few minutes of first flushing. Minerals and metals were gathered in this stagnant water from the pipe and water sources. Different researches on drinking water supported the increases in concentration of metals like Fe are due to pipelines corrosion and stagnation (Sarin et al., 2004). Likewise, total hardness was slightly increased from 2004 and greater than before after 2006. The possibility of increased total hardness of water after 2006 was due to change in the water distribution system.

Iron was found high in Thabor-Saint-Helier, Les Gayeulles and Villejean compared to other places (Figure 6). This was possibly due to the existence of two different water distribution system (upper network – Villejean, Thabor-Saint-Helier, Les Gayeulles and Brequigny, and lower network – SUD Gare, Patton and Nord Saint Martin) in Rennes before 2007 (Annexes 1, Figure 10). It was the single water distribution system after 2006 (Annexes 1, Figure 11).

However, for other physical chemical parameters such as free chlorine, water temperature, total hardness of water, conductivity, nitrate and pH were not significantly different according to the water distribution system.

The results of this study prove the significant positive correlation between free chlorine [OR 3.38, CI (1.93 - 5.91), p<0.0000] and the presence of legionella. In another study, the concentration of free chlorine was found a major parameter for high contamination as previously. *Legionella pneumophila* serogroup 1 was significantly correlated (p<0.0008) with free chlorine concentrations above 0.2 mg/l but not (p<0.3) for other Legionella spp. (Fujimura et al., 2006; Mouchtouri et al., 2007).

Likewise, pH of water [OR 5.05, CI (2.55 – 10.03), p<0.0000] was significantly positively associated with the growth of legionella. Similarly, conductivity [OR 1.001, CI (1.001 – 1.002), p<0.0000] and water temperature [OR 1.06, CI (1.03 – 1.10), p<0.0001] were also highly positively related to the development of legionella. In contrary, pH and water temperature were only associated with the proliferation of legionella but not related to the occurrence of *legionella spp*. Legionella may be introduced by the presence of amoebae but it needs certain condition to multiply (Lasheras et al., 2006). These factors such as temperature and pH are important for the multiplication of legionella (Zanetti et al., 2000).

Corrosion level of pipes, stagnation and flow of water, flushing and temperature of water are also associated with the growth of *Legionella spp.* (Exner et al., 2005), while hardness of water and concentration of trace elements have been only recommended (Borella et al., 2005b). Recently, the investigation of correlation between legionella colonization and heterotrophic bacteria were also started (Edagawa et al., 2008; Völker et al., 2010).

According to our study, iron [OR 1.002, CI (0.99 – 1.01), P<0.645] was not correlated with legionella growth. Iron (Fe) and zinc (Zn) used in plumbing systems are possibly the cause of corrosion of pipes and high concentration of iron and zinc in water systems. Metallic pipes (cast iron) are often used in Italy (Borella et al., 2004) but also in France. Likewise, development and growth of legionella are the effects of metals leaked from water tanks and pipes (States et al., 1985). Iron is an important growth factor for legionella in laboratory and legionella infection. Similarly, a multicentric research in Italy by Borella et al. (2003) recommended that the occurrence of metals and relationship with legionella in water distribution systems are possibly due to geographical variations and many water treatment centers and sources. A recent study in Japan by Edagawa et al. (2008) reported a positive correlation between iron and *Legionella spp.*, while copper (Cu) and Zinc (Zn) were not associated. Experimental studies showed the growth of *Legionella pneumophila* was enhanced by concentrations of Fe and Zn whereas it became toxic in high concentrations (States et al., 1985).

Copper, silver and other bacteriological parameters were not included in our study because of high number of missing values. However, some studies showed that copper and silver have important role in the development of legionella and other pathogens in planktonic phase and biofilms (Hsiu-Yun and Yusen, 2010). Likewise, the pipes that made up of galvanized steels were less likely to be contaminated than copper pipes (Mathys et al., 2008).

Total hardness of water [OR 1.04, CI (0.94 – 1.16), p<0.417] and nitrate [OR 0.99, CI (0.96 – 1.02), p<0.449] were also not related to legionella development (Table 4). However, the result shows the association between total hardness [OR 2.02, CI (1.05 – 4.16), p<0.000] and positive legionella samples after 2006 (Table 6). This was possibly due to increased value of total hardness after 2006 due to modification of the water distribution system.

Furthermore, a study in Finnish hospital water systems showed occurrence of legionella was related to conductivity and HPC at 30˚C but not with hardness (Kusnetsov et al., 2003). The hygienic and sanitary conditions of water systems are supposed by the general indication of heterotrophic plate count (HPC) measurements (Bartram et al., 2003).

It is essential to know that biofilms and microorganisms such as Acanthamoeba are factors to support the development of legionella with other physical and chemical parameters. The heterotrophic organisms develop biofilms which is the prerequisite for the growth of legionella (Borella et al., 2005a). Similarly, the interaction between legionella and protozoa are favoured by biofilms (Lau and Ashbolt, 2009).

The reduction in concentrations of free chlorine and increased in concentrations of iron and HPC are due to stagnation of water which strongly supports the development of legionella. Prevention and control of legionella contamination and legionellosis might be achieved by preventing water stagnation in water systems.

The GIS map (Figure 9) shows that positive samples of legionella were accumulated in Villejean and Brequigny areas of Rennes. It was probably due to more water samples or changed in the water distribution system. Villejean and Brequigny had the same water distribution system before 2006 and after 2006 (Annexes1, Figure 10 and 11). However, the data demonstrated the presence of legionella after 2006 was higher than before in both places. The possibility of positive legionella was due to increased in physical chemical parameters in the integrated water distribution system.

Despite limitations presented in methodology, the strength of this study is the strong relationship between physical chemical parameters such as free chlorine, pH, water temperature and conductivity with the presence of legionella.

Chapter 5 Conclusion

The results of our study showed that the physical chemical parameters such as free chlorine, pH, water temperature and conductivity were associated with the growth of *Legionella spp*. in Rennes. However, total hardness of water was also correlated with legionella after the integration of water systems into one at the end of 2006. Previous studies have suggested more physical, chemical and bacteriological parameters than what it used in this research. Though, this study might be helpful to produce the water safety plan, prevention of legionella, diminishing of physical chemical parameters. It also suggests that the pipes should be checked time to time, maintenance of water treatment center and sources, use of tap water after few minutes of flushing (especially for showers in swimming pools) and planning of environmental sanitation and health education programmes for local populations. Likewise, maximum efforts were used to match the place and date because of separate samples for legionella, and physical chemical parameters. It should be necessary to test the legionella positive water samples, for physical chemical and biological parameters as well. Most of the physical chemical parameters (free chlorine, pH, water temperature and conductivity) which were studied in this research have significant positive correlation with legionella. Therefore, this study explained the environmental factors are responsible for the growth and development of *Legionella pneumophila*.

Chapter 6 References

Aurell, H., Farge, P., Meugnier, H., Gouy, M., Forey, F. o., Lina, G., Vandenesch, F. o., Etienne, J. & Jarraud, S. (2005) Clinical and environmental isolates of Legionella pneumophila serogroup 1 cannot be distinguished by sequence analysis of two surface protein genes and three housekeeping genes. *Applied And Environmental Microbiology,* 71**,** 282-289.

Bargellini, A., Marchesi, I., Righi, E., Ferrari, A., Cencetti, S., Borella, P. & Rovesti, S. (2011) Parameters predictive of Legionella contamination in hot water systems: Association with trace elements and heterotrophic plate counts. *Water Research,* In Press, Corrected Proof.

Barker, J., Brown, M. R., Collier, P. J., Farrell, I. & Gilbert, P. (1992) Relationship between Legionella pneumophila and Acanthamoeba polyphaga: physiological status and susceptibility to chemical inactivation. *Applied And Environmental Microbiology,* 58**,** 2420-2425.

Barker, J., Lambert, P. A. & Brown, M. R. (1993) Influence of intra-amoebic and other growth conditions on the surface properties of Legionella pneumophila. *Infection And Immunity,* 61**,** 3503-3510.

Barker, J., Scaife, H. & Brown, M. R. (1995) Intraphagocytic growth induces an antibiotic-resistant phenotype of Legionella pneumophila. *Antimicrobial Agents And Chemotherapy,* 39**,** 2684-2688.

Bartram, J., Chartier, Y., Lee, J. V., Pond, K. & Surman-Lee, S. (Eds.) (2007) *Legionella and the prevention of legionellosis,* Geneva, World Health Organization.

Bartram, J., Cotruvo, J., Exner, M., Fricker, C. & Glasmacher, A. (Eds.) (2003) *Heterotrophic plate counts and Drinking-water safety: The Significance of HPCs for Water Quality and Human Health,* World Health Organisation, Geneva, IWA Publishing, London, Uk.

Borella, P., Guerrieri, E., Marchesi, I., Bondi, M. & Messi, P. (2005a) Water ecology of Legionella and protozoan: environmental and public health perspectives. *Biotechnology Annual Review,* 11**,** 355-380.

Borella, P., Montagna, M. T., Romano-Spica, V., Stampi, S., Stancanelli, G., Triassi, M., Marchesi, I., Bargellini, A., Neglia, R., Paglionico, N., Spilotros, G., Moscato, U., Casati, G., Legnani, P. P., Sacchetti, R., Ossi, C., Moro, M. & Ribera, G. (2003) Relationship between mineral content of domestic hot water and microbial contamination. *Journal Of Trace Elements In Medicine And Biology: Organ Of The Society For Minerals And Trace Elements (GMS),* 17 Suppl 1**,** 37-43.

Borella, P., Montagna, M. T., Stampi, S., Stancanelli, G., Romano-Spica, V., Triassi, M., Marchesi, I., Bargellini, A., Tato, D., Napoli, C., Zanetti, F., Leoni, E., Moro, M., Scaltriti, S., Ribera D'Alcala , G., Santarpia, R. & Boccia, S. (2005b) Legionella contamination in hot water of Italian hotels. *Applied And Environmental Microbiology,* 71**,** 5805-5813.

Borella, P., Neglia, R., Marchesi, I., Fantuzzi, G., Montagna, M. T., Tat+Ý, D., Napoli, C., Stampi, S., Leoni, E., Stancanelli, G., Ossi, C., Moro, M., Triassi, M., D'Alcal+ , G. R., Quaranta, G., Laurenti, P., Romano-Spica, V. & De Luca, G. (2004) Legionella Infection Risk from Domestic Hot Water. *Emerging Infectious Diseases,* 10**,** 457-464.

Campese, C., Jarraud, S. & Che, D. (2008) *Characteristics of the most important outbreaks of legionnaire's disease in France from 1998 to 2007.* France, French Institute for Public Health Surveillance (Institut de veille sanitaire).

Campese, C., Maine, C. & Che, D. (2010) Les Cas de legionellose declares en France en 2009. *Institut de veille sanitaire* BEH 31-32**,** 334-335.

Carratala, J., Gudiol, F., Pallares, R., Dorca, J., Verdaguer, R., Ariza, J. & Manresa, F. (1994) Risk factors for nosocomial Legionella pneumophila pneumonia. *American Journal Of Respiratory And Critical Care Medicine,* 149**,** 625-629.

Cianciotto, N. (2007) Iron acquisition by Legionella pneumophila. *BioMetals,* 20**,** 323-331.

Decludt, B. (2000) Community Cluster of Legionnaires' disease in France. *Eurosurveillance,* 4**,** 1470.

Diederen, B. M. W. (2008) Legionella spp. and Legionnaires' disease. *Journal of Infection,* 56**,** 1-12.

Drozanski, W. J. (1991) Sarcobium lyticum gen. nov., sp. nov., an Obligate Intracellular Bacterial Parasite of Small Free-Living Amoebae. *International Journal of Systematic Bacteriology,* 41**,** 82-87.

Edagawa, A., Kimura, A., Doi, H., Tanaka, H., Tomioka, K., Sakabe, K., Nakajima, C. & Suzuki, Y. (2008) Detection of culturable and nonculturable Legionella species from hot water systems of public buildings in Japan. *Journal of Applied Microbiology,* 105**,** 2104-2114.

EWGLI (2002) European Guidelines for Control and Prevention of Travel Associated Legionnaires' disease. London, PHLS.

Exner, M., Kramer, A., Lajoie, L., Gebel, J., Engelhart, S. & Hartemann, P. (2005) Prevention and control of health care-associated waterborne infections in health care facilities. *American Journal of Infection Control,* 33**,** S26-S40.

Fields, B. S., Barbaree, J. M., Sanden, G. N. & Morrill, W. E. (1990) Virulence of a Legionella anisa strain associated with Pontiac fever: an evaluation using protozoan, cell culture, and guinea pig models. *Infection And Immunity,* 58**,** 3139-3142.

Fields, B. S., Benson, R. F. & Besser, R. E. (2002) Legionella and Legionnaires' Disease: 25 Years of Investigation. *Clinical Microbiology Reviews,* 15**,** 506-526.

Fujimura, S., Oka, T., Tooi, O., Meguro, M., Chiba, M., Kawamura, M., Maki, F., Takeda, H. & Watanabe, A. (2006) Detection of Legionella pneumophila serogroup 7 strain from bathwater samples in a Japanese hospital. *Journal Of Infection And Chemotherapy: Official Journal Of The Japan Society Of Chemotherapy,* 12**,** 105-108.

Helbig, J. H., Bernander, S., Castellani Pastoris, M., Etienne, J., Gaia, V., Lauwers, S., Lindsay, D., Luck, P. C., Marques, T., Mentula, S., Peeters, M. F., Pelaz, C., Struelens, M., Uldum, S. A., Wewalka, G. & Harrison, T. G. (2002) Pan-European study on culture-proven Legionnaires' disease: distribution of Legionella pneumophila serogroups and monoclonal subgroups. *European Journal Of Clinical Microbiology & Infectious Diseases: Official Publication Of The European Society Of Clinical Microbiology,* 21**,** 710-716.

Heller, R., Holler, C., Submuth, R. & Gundermann, K. O. (1998) Effect of salt concentration and temperature on survival of Legionella pneumophila. *Letters In Applied Microbiology,* 26**,** 64-68.

Heuner, K., der-Beck, L., Brand, B. C., Luck, P. C., Mann, K. H., Marre, R., Ott, M. & Hacker, J. (1995) Cloning and genetic characterization of the flagellum subunit gene (flaA) of Legionella pneumophila serogroup 1. *Infection And Immunity,* 63**,** 2499-2507.

HSE (2000) *Legionnaires' disease The control of legionella bacteria in water systems Approved Code of Practice and guidance Successful health and safety management HSG65* Third. Health and Safety Executive.

HSE (2003) Evaluation of HSC's ACOP and Guidance "Legionnaires disease: Control of legionella bacteria in water systems" (L8). Norwich, Environment for the Health and Safety Executive

Hsiu-Yun, S. & Yusen, E. L. (2010) Efficacy of Copper-Silver Ionization in Controlling Biofilm- and Plankton-Associated Waterborne Pathogens. *Applied & Environmental Microbiology,* 76**,** 2032-2035.

Hubert, B. & Haury, B. (1996) Orientations pour la révision des modalités de surveillance des maladies transmissibles en France. *Bulletin Epidémiologique Hebdomadaire,* 26**,** 115-7.

Hussong, D., Colwell, R., O'Brien, M., Wess, E., Pearson, A. & Weiner, R. (1987) Viable Legionella pneumophila not detectable by culture on agar media. *Bio/Technology,* 5**,** 947-950.

Joseph, C. (2002) Surveillance of Legionnaires disease in Europe. IN Marre, R. (Ed.). Washington DC, ASM Press.

Joseph, C., Morgan, D., Birtles, R., Pelaz, C., Martin-Bourgon, C., Black, M., Garcia-Sanchez, I., Griffin, M., Bornstein, N. & Bartlett, C. (1996) An international investigation of an outbreak of Legionnaires disease among UK and French tourists. *European Journal Of Epidemiology,* 12**,** 215-219.

Katz, S. M. & Hammel, J. M. (1987) The effect of drying, heat, and pH on the survival of Legionella pneumophila. *Annals Of Clinical And Laboratory Science,* 17**,** 150-156.

Kell, D. B., Kaprelyants, A. S., Weichart, D. H., Harwood, C. & Barer, M. (1998) Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. *Antonie Leeuwenhoek,* 73**,** 169-187.

Kuchta, J. M., States, S. J., McNamara, A. M., Wadowsky, R. M. & Yee, R. B. (1983) Susceptibility of Legionella pneumophila to chlorine in tap water. *Applied And Environmental Microbiology,* 46**,** 1134-1139.

Kuiper, M. W., Wullings, B. A., Akkermans, A. D. L., Beumer, R. R. & van der Kooij, D. (2004) Intracellular proliferation of Legionella pneumophila in Hartmannella vermiformis in aquatic biofilms grown on plasticized polyvinyl chloride. *Applied And Environmental Microbiology,* 70**,** 6826-6833.

Kusnetsov, J., Torvinen, E., Perola, O., Nousiainen, T. & Katila, M.-L. (2003) Colonization of hospital water systems by legionellae, mycobacteria and other heterotrophic bacteria potentially hazardous to risk group patients. *APMIS,* 111**,** 546-556.

Kusnetsov, J. M., Martikainen, P. J., Jousimies-Somer, H. R., Väisänen, M.-L., Tulkki, A. I., Ahonen, H. E. & Nevalainen, A. I. (1993) Physical, chemical and microbiological water characteristics associated with the occurrence of Legionella in cooling tower systems. *Water Research,* 27**,** 85-90.

Kusnetsov, J. M., Ottoila, E. & Martikainen, P. J. (1996) Growth, respiration and survival of Legionella pneumophila at high temperatures. *The Journal Of Applied Bacteriology,* 81**,** 341-347.

Kwaik, Y. A., Gao, L. Y., Stone, B. J., Venkataraman, C. & Harb, O. S. (1998) Invasion of protozoa by Legionella pneumophila and its role in bacterial ecology and pathogenesis. *Applied & Environmental Microbiology,* 64**,** 3127.

Lasheras, A., Boulestreau, H., Rogues, A. M., Ohayon, C., Labadie, J. C. & Gachie, J. P. (2006) Influence of amoebae and physical and chemical characteristics of water on presence and proliferation of Legionella species in hospital water systems. *American Journal of Infection Control,* 34**,** 520-525.

Lau, H. Y. & Ashbolt, N. J. (2009) The role of biofilms and protozoa in Legionella pathogenesis: implications for drinking water. *Journal Of Applied Microbiology,* 107**,** 368-378.

Lawrence, A. K. (2006) In Philadelphia 30 Years Ago, an Eruption of Illness and Fear. *New York Times.*

Lee, H. K., Shim, J. I., Kim, H. E., Yu, J. Y. & Kang, Y. H. (2010) Distribution of Legionella Species from Environmental Water Sources of Public Facilities and Genetic Diversity of L. pneumophila Serogroup 1 in South Korea. *Appl. Environ. Microbiol.,* 76**,** 6547-6554.

Lee, J. V. & West, A. A. (1991) Survival and growth of Legionella species in the environment. *Society for Applied Bacteriology symposium series,* 20**,** 121S-129S.

Leoni, E., De Luca, G., Legnani, P. P., Sacchetti, R., Stampi, S. & Zanetti, F. (2005) Legionella waterline colonization: detection of Legionella species in domestic, hotel and hospital hot water systems. *Journal Of Applied Microbiology,* 98**,** 373-379.

Leoni, E., Legnani, P. P., Bucci Sabattini, M. A. & Righi, F. (2001) Prevalence of Legionella spp. in swimming pool environment. *Water Research,* 35**,** 3749-3753.

Mampel, J., Spirig, T., Weber, S. S., Haagensen, J. A. J., Molin, S. & Hilbi, H. (2006) Planktonic replication is essential for biofilm formation by Legionella pneumophila in a complex medium under static and dynamic flow conditions. *Applied And Environmental Microbiology,* 72**,** 2885-2895.

Marston, B. J., Lipman, H. B. & Breiman, R. F. (1994) Surveillance for Legionnaires' disease. Risk factors for morbidity and mortality. *Archives Of Internal Medicine,* 154**,** 2417-2422.

Mathys, W., Stanke, J., Harmuth, M. & Junge-Mathys, E. (2008) Occurrence of Legionella in hot water systems of single-family residences in suburbs of two German cities with special reference to solar and district heating. *International Journal Of Hygiene And Environmental Health,* 211**,** 179-185.

Molofsky, A. B. & Swanson, M. S. (2004) Differentiate to thrive: lessons from the Legionella pneumophila life cycle. *Molecular Microbiology,* 53**,** 29-40.

Montalbano MA, K. C., Adams DA, Hall PA, Fagan RF (1996) Summary of Notifiable Diseases. *Morbid. Mortal. Wkly.* United States.

Mouchtouri, V., Velonakis, E., Tsakalof, A., Kapoula, C., Goutziana, G., Vatopoulos, A., Kremastinou, J. & Hadjichristodoulou, C. (2007) Risk factors for contamination of hotel water distribution systems by Legionella species. *Applied And Environmental Microbiology,* 73**,** 1489-1492.

Ohno, A., Kato, N., Yamada, K. & Yamaguchi, K. (2003) Factors influencing survival of Legionella pneumophila serotype 1 in hot spring water and tap water. *Applied And Environmental Microbiology,* 69**,** 2540-2547.

Ott, M., Messner, P., Heesemann, J., Marre, R. & Hacker, J. (1991) Temperature-dependent expression of flagella in Legionella. *Journal Of General Microbiology,* 137**,** 1955-1961.

Pécastaings, S., Bergé, M., Dubourg, K. M. & Roques, C. (2010) Sessile Legionella pneumophila is able to grow on surfaces and generate structured monospecies biofilms *Biofouling: The Journal of Bioadhesion and Biofilm Research,* 26**,** 809 - 819.

Riffard, S., Lo Presti, F., Normand, P., Forey, F., Reyrolle, M., Etienne, J. & Vandenesch, F. (1998) Species identification of Legionella via intergenic 16S-23S ribosomal spacer PCR analysis. *International Journal of Systematic Bacteriology,* 48 Pt 3**,** 723-730.

Rogers, J. & Keevil, C. W. (1992) Immunogold and fluorescein immunolabelling of Legionella pneumophila within an aquatic biofilm visualized by using episcopic differential interference contrast microscopy. *Applied And Environmental Microbiology,* 58**,** 2326-2330.

Sarin, P., Snoeyink, V. L., Bebee, J., Jim, K. K., Beckett, M. A., Kriven, W. M. & Clement, J. A. (2004) Iron release from corroded iron pipes in drinking water distribution systems: effect of dissolved oxygen. *Water Research,* 38**,** 1259-1269.

Starlinger, R. & Tiefenbrunner, F. (1996) Legionellae and Amoebae in European hotel water distribution systems. . *Proceedings of the 11th Meeting of the European Working Group on Legionella Infections.* Oslo, Norwegian Defence Microbiological Laboratory.

States, S. J., Conley, L. F., Ceraso, M., Stephenson, T. E., Wolford, R. S., Wadowsky, R. M., McNamara, A. M. & Yee, R. B. (1985) Effects of metals on Legionella pneumophila growth in drinking water plumbing systems. *Applied And Environmental Microbiology,* 50**,** 1149-1154.

Steele, T. W., Moore, C. V. & Sangster, N. (1990) Distribution of Legionella longbeachae serogroup 1 and other legionellae in potting soils in Australia. *Applied And Environmental Microbiology,* 56**,** 2984-2988.

Steinert, M., Hentschel, U. & Hacker, J. (2002) Legionella pneumophila: an aquatic microbe goes astray. *FEMS Microbiology Reviews,* 26**,** 149.

Swanson, M. S. & Hammer, B. K. (2000) LEGIONALLEA PNEUMOPHILA PATHOGENESIS: A Fateful Journey from Amoebae to Macrophages. *Annual Review of Microbiology,* 54**,** 567.

Tessier, F. (2009) Study of the risk of emergence of outbreaks of Legionella contamination in Rennes. Rennes, France, EHESP.

Tison, D. L. & Seidler, R. J. (1983) Legionella incidence and density in potable drinking water supplies. *Applied And Environmental Microbiology,* 45**,** 337-339.

Tobin, J. O., Beare, J., Dunnill, M. S., Fisher-Hoch, S., French, M., Mitchell, R. G., Morris, P. J. & Muers, M. F. (1980) Legionnaires' disease in a transplant unit: isolation of the causative agent from shower baths. *Lancet,* 2**,** 118-121.

VFDB (2011) *Legionella*, Available from: http://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus=Legionella [Accessed: 13/01/2011].

Völker, S., Schreiber, C. & Kistemann, T. (2010) Drinking water quality in household supply infrastructure—A survey of the current situation in Germany. *International Journal of Hygiene & Environmental Health,* 213**,** 204-209.

WHO (2004) Guidelines for Drinking Water Quality, Vol. 1. Recommendations. 3 ed. Geneva, World Health Organisation.

WHO (2006) Guidelines for drinking-water quality [electronic resource] : incorporating first addendum. Vol. 1, Recommendations. 3 ed. Geneva, World Health Organization.

Winn, W. C., Jr. (1988) Legionnaires disease: historical perspective. *Clinical Microbiology Reviews,* 1**,** 60-81.

Yu, V. L., Plouffe, J. F., Pastoris, M. C., Stout, J. E., Schousboe, M., Widmer, A., Summersgill, J., File, T., Heath, C. M., Paterson, D. L. & Chereshsky, A. (2002) Distribution of Legionella species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. *The Journal Of Infectious Diseases,* 186**,** 127-128.

Zanetti, F., Stampi, S., De Luca, G., Fateh-Moghadam, P., Bucci Sabattini, M. A. & Checchi, L. (2000) Water characteristics associated with the occurrence of Legionella pneumophila in dental units. *European Journal of Oral Sciences,* 108**,** 22-28.

Annexes

Annex 1 Various departments of the distribution of drinking water

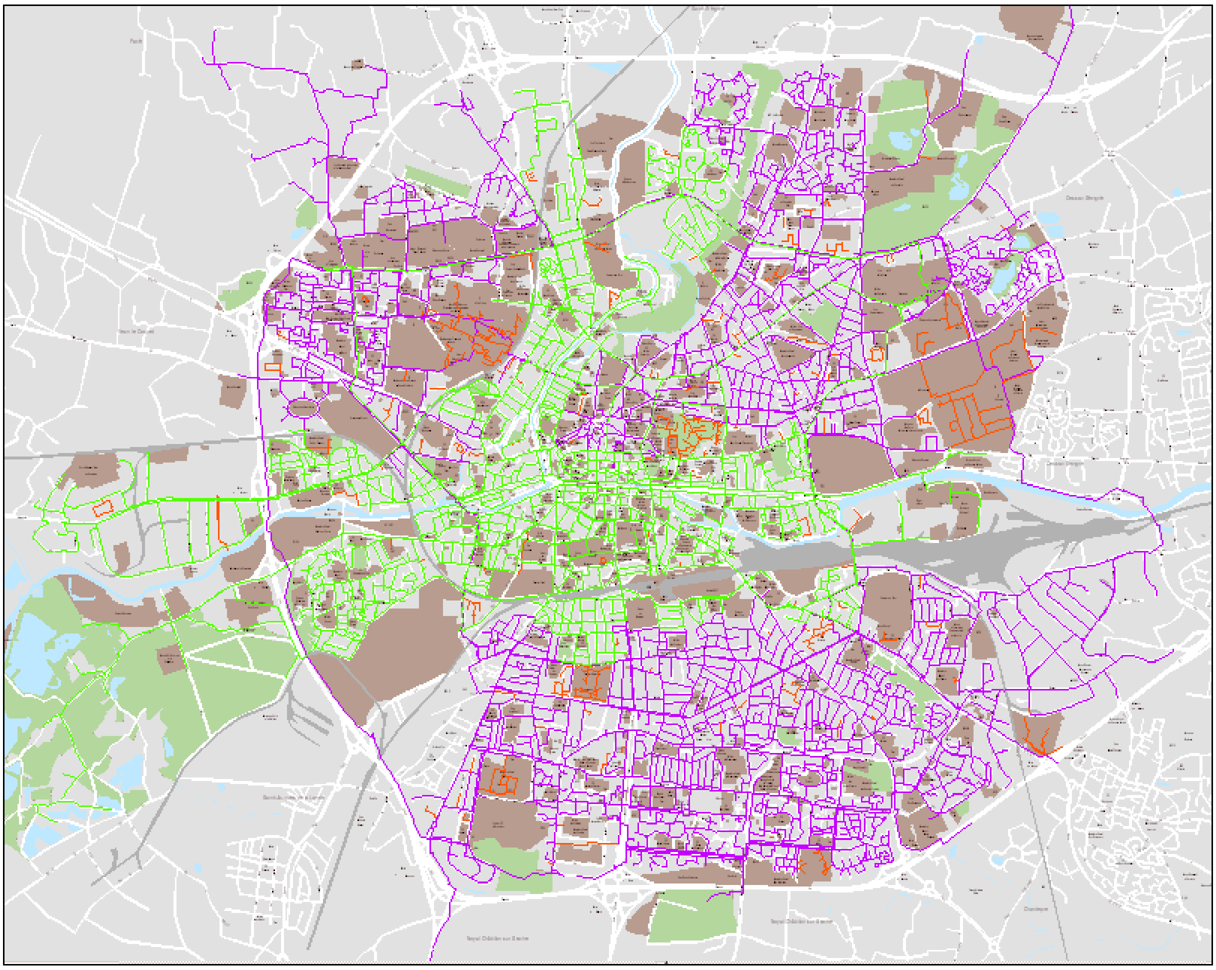


Figure 10 The distribution of drinking water for the city of Rennes before setting up the transfer Gallet - Villejean at the end of 2006

Legends	
(red line)	Private Network
(green line)	Down Network
(magenta line)	Top Network

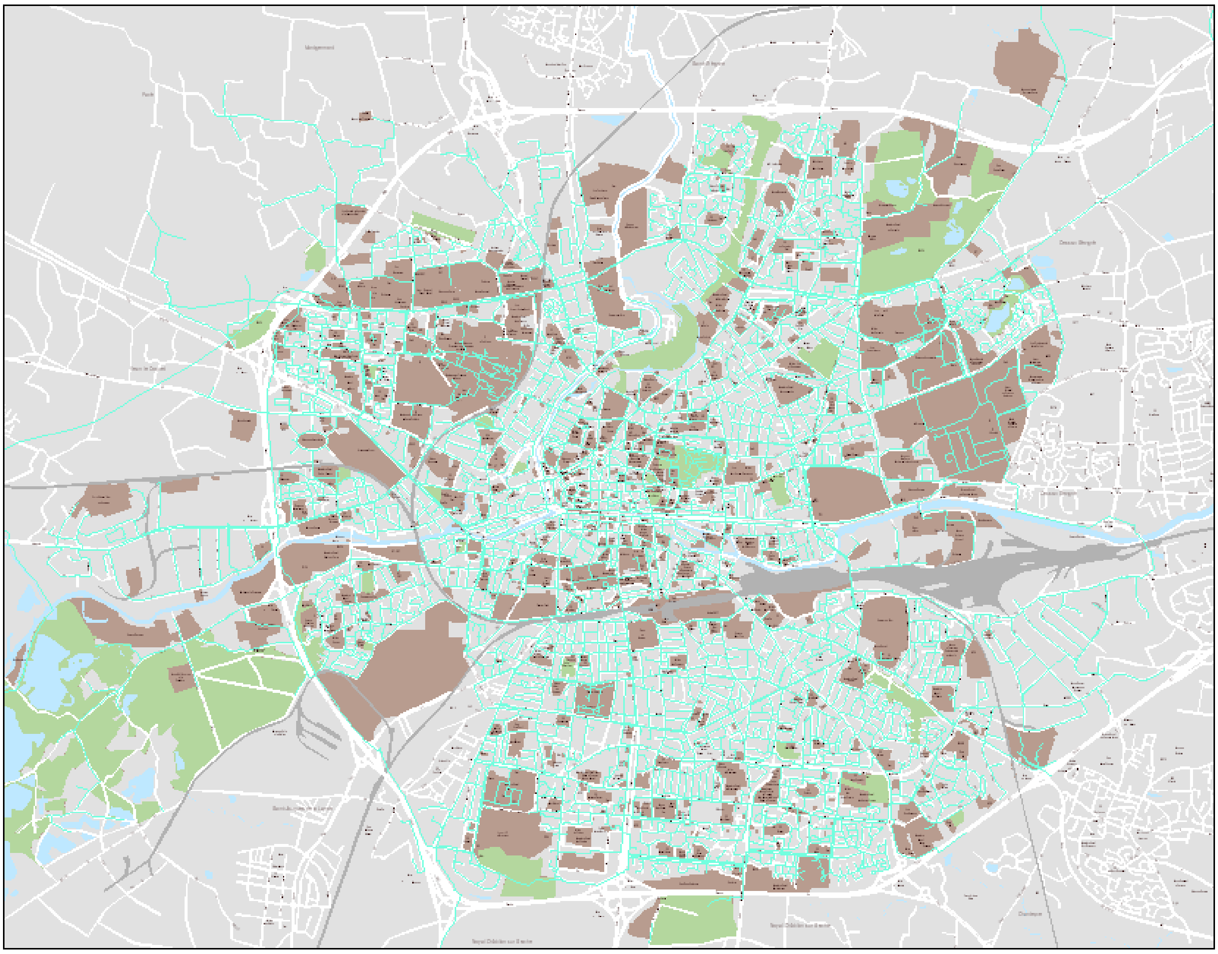


Figure 11 The distribution of drinking water for the city of Rennes after setting up the transfer Gallet - Villejean at the end of 2006

Legend	
	Single Network

Annex 2 Ethical Approval from The University of Sheffield, United Kingdom

The University Of Sheffield.

Cheryl Oliver
Ethics Committee Administrator

Regent Court
30 Regent Street
Sheffield S1 4DA

Telephone: +44 (0) 114 2220871
Fax: +44 (0) 114 272 4095 (non confidential)
Email: c.a.oliver@sheffield.ac.uk

17 March 2011

Kshitij Karki
ScHARR

Dear Kshitij Karki

Identification of environmental factors involved in legionella development

I am pleased to inform you your supervisor has reviewed your project and classed it as 'low risk' so you can proceed with your research. The research must be conducted within the requirements of the hosting/employing organisation or the organisation where the research is being undertaken.

I have received a hard copy of your student declaration together with your Supervisor's confirmation for research that does not involve human participants and that you will be undertaking research which involves analysis of already existing data ('secondary data').

Yours sincerely

Cheryl Oliver
Ethics Committee Administrator

Cc: Dr Ravi Maheswaran

Appendix

1) Distribution of total hardness by year

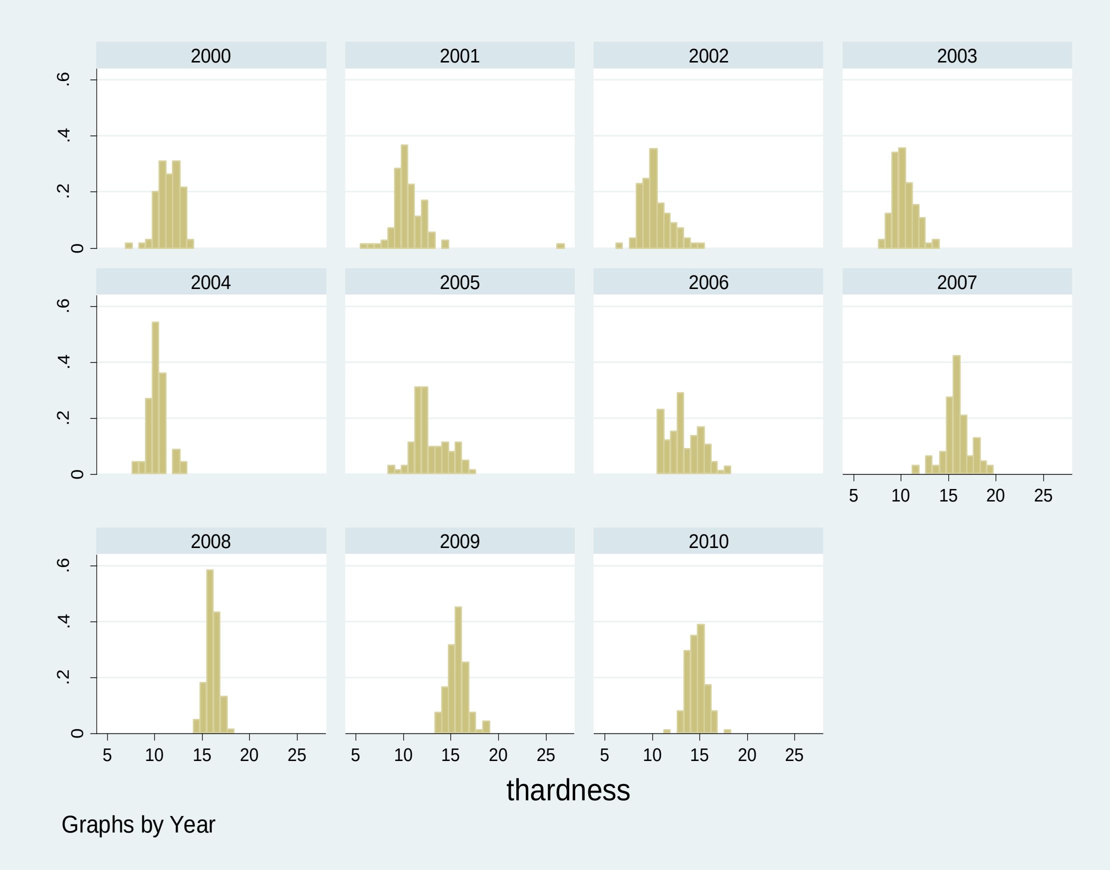


Figure 12 Distribution of total hardness of water by year

Abstract in English

Identification of Environmental factors involved in legionella development

Context: Legionella are aerobic, gram-negative bacteria which grow in natural aquatic environments such as lakes and rivers as well as artificial water systems; wet soil and compost. Nearly 1500 people are affected by legionella each year in France. Only half of the cases are elucidated, partly due to the fact that environmental factors involved in Legionella development are not well characterized.

Objectives: To identify environmental factors involved in legionella development.

Methods: Physical chemical parameters and legionella data were obtained from the database of LERES, Rennes, France. Certain parameters such as iron, free chlorine, water temperature, pH, total hardness, nitrate and conductivity were selected on the basis of literatures and missing values. Logistic regression was done to correlate the legionella and physical chemical parameters.

Results: The mean value of free chlorine, pH, iron, water temperature, total hardness and conductivity were found high in legionella positive samples except nitrate. According to univariate logistic regression analysis, free chlorine [OR 3.38, CI (1.93 - 5.91), p<0.0000], pH [OR 5.05, CI (2.55 – 10.03), p<0.0000], conductivity [OR 1.001, CI (1.001 – 1.002), p<0.0000] and water temperature [OR 1.06, CI (1.03 – 1.10), p<0.0001] were significantly associated with the presence of legionella at 5% significance level. However, total hardness of water (p<0.417), nitrate (p<0.449) and iron (p<0.645) were not correlated with the presence of legionella. Adjustment was done for nitrate, total hardness and iron using multivariate logistic regression but there was no correlation with legionella.

Conclusion: Physical chemical factors such as free chlorine, pH, water temperature and conductivity are associated with the presence of legionella. Hence, various environmental factors are involved in legionella development.

Key Words: Legionella species, *Legionella pneumophila*, water samples, Rennes France, environmental factors, Fe, total hardness of water, water temperature, free chlorine, nitrate, pH, conductivity, correlation and regression, GIS

Résumé en Français

Identification des facteurs environnementaux impliqués dans le développement légionelles

Contexte: Les légionelles des bactéries gram-négatives qui se développent dans les milieux aquatiques naturels comme les lacs et rivières ainsi que des systèmes d'eau artificiels; sol humide et compost. Près de 1500 cas de légionellose sont diagnostiqués en France chaque année. Seulement la moitié des cas sont élucidés, notamment du fait que les facteurs environnementaux impliqués dans le développement de Legionella ne sont pas bien caractérisés.

Objectifs: identifier les facteurs environnementaux impliqués dans le développement de légionellose.

Méthodes: les paramètres physico-chimiques et les résultats d'analyse de légionelle ont été obtenus à partir de la base de données du LERES, Rennes, France. Certains paramètres tels que le fer, le chlore libre, température de l'eau, pH, le titre hydrométrique, les nitrates et la conductivité ont été sélectionnés sur la base de la littérature et les valeurs manquantes. La régression logistique a été réalisée pour corréler les légionelles et les paramètres physico-chimiques.

Résultats: La valeur moyenne de chlore libre, pH, de fer, température de l'eau, le titre hydrométrique et la conductivité ont été trouvés plus élevés dans les échantillons positifs en légionelle à l'exception des nitrates. Selon la régression logistique univariée, le chlore libre [OR 3,38, IC (de 1,93 à 5,91), $p < 0{,}0000$], pH [OR 5,05, IC (2,55 à 10,03), $p<0{,}0000$], la conductivité [OR 1,001, CI (1.001 - 1,002), $p<0{,}0000$] et température de l'eau [OR 1,06, IC (01/03 au 01/10), $p < 0{,}0001$] étaient significativement associés à la présence de légionelles au niveau de signification de 5%. Toutefois, titre hydrométrique de l'eau ($p<0{,}417$), le nitrate ($p<0{,}449$) et le fer ($p<0{,}645$) n'étaient pas corrélés avec la présence de légionelles. L'ajustement a été fait pour le nitrate, le titre hydrométrique et le fer en utilisant la régression logistique multivariée, mais il n'y avait pas de corrélation avec la présence de légionelles.

Conclusion: les facteurs physico-chimiques comme le chlore libre, pH, température de l'eau et la conductivité sont associés à la présence de légionelles. Ainsi, divers facteurs environnementaux sont impliqués dans le développement de légionelles.

Mots clés: Legionella, Legionella pneumophila, des échantillons d'eau, Rennes, France, les facteurs environnementaux, Fer, le titre hydrometrique, température de l'eau, le chlore libre, nitrate, pH, conductivité, corrélation et régression, GIS