

# Leptospirosis from water sources

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Leptospirosis outbreaks have been associated with many common water events including water consumption, water sports, environmental disasters, and occupational exposure. The ability of leptospires to survive in moist environments makes them a high-risk agent for infection following contact with any contaminated water source. Water treatment processes reduce the likelihood of leptospirosis or other microbial agents causing infection provided that they do not malfunction and the distribution networks are maintained. Notably, there are many differences in water treatment systems around the world, particularly between developing and developed countries. Detection of leptospirosis in water samples is uncommonly performed by molecular methods.

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Leptospirosis is a worldwide zoonosis caused by spirochaetes from the genus *Leptospira*. The genus currently contains 20 species containing nine pathogenic, six saprophytic, and five intermediate species.<sup>1</sup> Leptospirosis infections in human beings vary from asymptomatic to severe. Two phases of infection, acute and immune, are routinely characterized by a range of non-specific symptoms including fever, chills, headaches, conjunctival suffusion, excruciating myalgia, and arthralgia, and sometimes rigors vomiting, photophobia, a mucosal rash haemoptysis, hypotension, bradycardia, hepatosplenomegaly, and jaundice are also common. Death can occur from kidney failure, pulmonary haemorrhage, or other serious organ dysfunction.<sup>2-4</sup> However, the extent of organ damage is dependent on the virulence of the organism and host susceptibility.<sup>5</sup> Laboratory findings show significant differences in haemoglobin concentrations, haematocrits, counts of erythrocytes, leucocytes, neutrophils, platelets, and concentrations of creatinine, urea, protein, and albumin when comparing those with mild to those with severe disease.<sup>6</sup> Transmission of leptospirosis was first

recognized as an occupational hazard in industries related to agriculture, sewer maintenance, and animal husbandry, and results from direct or indirect contact with the urine of infected animals.<sup>1,2</sup> Other common modes of transmission include exposure to urine-contaminated water through recreational activities, adventure travel, and ingestion of contaminated water supplies.<sup>7-10</sup> Leptospires enter the body via small cuts or abrasions, through mucous membranes such as the conjunctiva and through wet skin.<sup>2</sup> Indirect exposure, and/or contact with contaminated water and soil, has been a major factor in numerous outbreaks and plays a crucial role in endemic settings. This paper provides a review of leptospirosis cases with transmission linked to potentially contaminated water sources, the public health implications of leptospirosis, and the current methods of diagnosis.

Outbreaks of leptospirosis have been associated with common water events such as rural and urban flooding, swimming, and other water sports as well as occupational exposure involved predominantly with farming and drinking contaminated water.<sup>5,10-12</sup> Both pathogenic and saprophytic strains of leptospirosis have been isolated from water sources including rivers and lakes as they are able to survive in moist soil and fresh water for long periods of time. Leptospires require fresh water to remain viable in the environment<sup>9</sup> and can survive for several months

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in running water but only several weeks in stagnant water, while some halophilic strains may be recovered from brackish and salt water.<sup>5,13–15</sup> Recently, two strains of *Leptospira kmetyi* (MS432 and MS422) were shown to survive for 3 days in artificial seawater and natural seawater. When the seawater was mixed with soil the strains were able to survive for four days.<sup>16</sup> This finding warns of the possible risks of the leptospiral infections in areas prone to ocean storm surges or tsunamis. Areas with high rainfall and warm climatic conditions provide optimal environments for the survival of leptospires. Most urban communities collect water from natural water bodies such as rivers, streams, or underground aquifers, and then store this water for long periods of time in a reservoir. Hospital data and seroprevalence surveys in the United States indicate that more than 70% of leptospirosis infections can be attributed to physical contact with contaminated water supplies.<sup>17</sup> This shows that environmental detection is important in the development of adequate control measures. Currently, detecting pathogenic *Leptospira* in water samples is difficult due to filtering problems with the volume of the sample water and leptospiral concentration in the sample and the number of other potential bacteria present in water samples, which can contaminate culture media. Furthermore, there is currently no DNA based methodology universally accepted to test water samples for the presence of leptospires and the effect of inhibitors on these molecular techniques also requires investigation. A number of molecular methods have recently emerged that will allow microbial agents to be detected in water samples. A DNA microarray has been developed to detect leptospires and 10 other commonly occurring pathogens in drinking water.<sup>18</sup> Another study that has recently detected *Leptospira interrogans* in drinking water has used 454 pyrosequencing and Illumina sequencing to investigate bacterial virulence in drinking water.<sup>19</sup> The methodology this study used to collect bacterial cells for DNA extraction required the use of water purifiers to filter ~1000 l of water. Collection and processing of such large samples may be problematic for many laboratories. Other studies have used smaller volumes with centrifugation to concentrate samples prior to extraction and polymerase chain reaction (PCR) detection of pathogenic leptospires by targeting the *lipL32* gene.<sup>20</sup>

### Transmission

Transmission of leptospirosis is facilitated by the survival of pathogenic leptospires in moist environments outside of their mammalian hosts.<sup>13</sup> Seasonal weather patterns involving flooding have long been recognized as a potential source of leptospirosis outbreaks and more recently, the contamination of

drinking water and urban water supply has been implicated.<sup>21</sup> It is estimated that in the United States, water borne illness rates are ~16 million cases per year.<sup>22</sup> Worldwide, there have been many outbreaks specifically associated with water contamination, most commonly in areas where sanitation is poor. Leptospires can survive for up to 152 days in fresh water by means of cellular aggregation and therefore water sanitation and hygiene are important factors in preventing and controlling the transmission of leptospirosis.<sup>13</sup> One study found that the levels of leptospires in urban water sources (underground, streams and open gutters) were significantly higher than the levels of leptospires found in rural water sources in the Peruvian Amazon regions of Iquitos. Furthermore, the authors found that the incidence of leptospirosis infection (and the corresponding serovar) was a direct reflection of these results.<sup>23</sup> Similarly, it was found that transmission of leptospirosis in Iquitos most likely occurs as a combination of environmental factors and human behaviour.<sup>24</sup> The importance of controlling environmental factors is highlighted in a recent study investigating household and environmental water source contamination by pathogenic leptospires in Chile. This study revealed that nearly 20% of human drinking sources and puddle water samples tested were contaminated with pathogenic leptospires. Not surprisingly the study also reported that lower income, increased temperature, and the presence of dogs and rodents signs were associated with contamination of some samples.<sup>20</sup>

A number of studies have suggested that leptospirosis infection can be acquired from drinking contaminated water. Thirty-three confirmed cases of leptospirosis were attributed to contaminated drinking water in 1984 in a small town in Italy.<sup>10</sup> Two deaths were credited to this outbreak, which was believed to be caused by drinking water from a fountain contaminated with leptospires of the serogroup Australis. There was evidence that a hedgehog became stuck and drowned in a water reservoir leading to the fountain water system. Although the fountain was not connected to the municipal water supply, people often drank directly from the fountain, which is fed by rain water from the mountain where water reservoirs are located. There was no indication of water treatment post outbreak other than the removal of the dead animal from the water reservoir. Samples were not taken from the hedgehog as it was believed that it had been dead for quite some time.

A similar event occurred in a nurses' hostel in Chennai, South India when 69 residents tested positive serologically by microscopic agglutination testing (MAT) and their drinking water source tested positive by PCR for leptospires in 2002.<sup>25</sup> The drinking water was sourced from an underground storage tank that was filled from a water tanker weekly.

Collection was performed using a bucket on a rope and the tank was usually left open. Control measures were introduced to remove any further contamination of water sources including chlorination, boiling, education, and the removal of large numbers of rats and mice in the area. Following the implementation of water sanitation and control methods, no further cases were detected. A 48 year old man in Japan, who had a laboratory confirmed diagnosis of leptospirosis, (serovar *Leptospira autumnalis*) was also believed to be infected after drinking water from a well following an earthquake.<sup>26</sup> Although water testing was not performed, the well was slightly muddy and many rats had inhabited the area around the well following the earthquake. Natural disasters including earthquakes, floods, typhoons, landslides, and tsunamis have been linked to communicable disease outbreaks generally as a result of a lack of clean drinking water and sanitation facilities. Following typhoon Dina in 1987, an outbreak of leptospirosis occurred in two groups of US military personnel in Okinawa, Japan – those that were exposed during training exercises and those exposed whilst engaging in recreational swimming.<sup>27</sup> In 1998, an outbreak of leptospirosis (52 cases) occurred following a triathlon in Illinois, USA.<sup>12</sup> Investigations suggested that swallowing a mouthful of contaminated water was the only factor significantly associated with an increased risk of developing leptospirosis as seropositivity was demonstrated in the full cohort of racers. Although it is not known whether a heavy rainfall event contributed to this outbreak, it shows that leptospires are able to survive in fresh water and act as a transmission vehicle in this type of environment.

Early guinea pig studies from Japan showed that the animals could be infected with leptospires by intraperitoneal, subcutaneous, or oral injection routes, and death resulted in 5–10 days depending on the route of infection.<sup>28</sup> Given that oral infection has been reported, it is surprising that there is a paucity of research outlining more outbreaks of leptospirosis from drinking water. Whether this is due to a lack of reporting systems in developing countries, sub-clinical infections or protection/attenuation of infection from natural host defences, or a combination of these is difficult to determine. Recent research has shown that low passage, pathogenic leptospires rapidly agglutinate in saliva, and the mucosal surface of the mouth is an effective barrier as submucosal injection of leptospires caused death but infection by drinking contaminated water did not.<sup>29</sup> This research also revealed the utility of gastric acid in preventing infection as intragastrically infected animals displayed no sign of illness.

### Public Health Perspectives

Outbreaks of leptospirosis have been attributed to a number of factors with a large proportion of

infections resulting from contaminated water sources. *Leptospira* can survive in ponds, rivers, lakes, surface water, and moist soil when the environmental temperature is warm and are generally transmitted through direct or indirect contact with the urine of infected animal. Current prevention and control methods of leptospirosis consist of source/rodent reduction, environmental and water sanitation, hygienic work, and personal practices. There is no universal control method applicable to all epidemiological setting as the characteristics of the environments differ from place to place. Understanding the eco-epidemiological and cultural characteristics of communities where leptospirosis is a problem is an essential prerequisite for evolving effective and acceptable control measures. Global climate change is also considered a factor contributing to leptospirosis as an emerging disease as increased temperatures are able to lengthen the survival of leptospires in the environment and can result in the expansion of habitats into higher elevations and latitudes.<sup>30</sup>

The water treatment processes in developing and modernized countries differ significantly. Poor water quality and sanitation accounts for 1.7 million deaths in developing countries each year, mostly in children.<sup>31</sup> Most infectious agents in water in developing countries are controlled by economically feasible methods such as chlorine treatment; however, recontamination of the treated water is a major problem. In some countries, water is not treated by any methods – it is simply collected from a well and consumed. Factors including inadequate reservoir and storage design and construction, inadequate maintenance of storage facilities, and poor quality control checks have also lead to the contamination and recontamination of drinking water.<sup>32</sup> A study of drinking water sources in rural areas of Beijing found that well construction was a major factor in bacterial contamination of drinking water.<sup>33</sup> Shallow wells with open tops and no well housing were found to be most likely to have high bacterial contamination. Collection of water for consumption in many developing countries is performed by hand – using buckets or urns to carry water from the drinking water source to the community or households, which provides a means for contamination from environmental sources. Informal water distribution supplies, such as private systems or community run systems have also been linked to high levels of microbial contamination in drinking water. In the slums of Mumbai, people rely on community run drinking water systems. Levels of microbial contamination in the water sources were assessed and it was found that approximately 50% of water was contaminated.<sup>34</sup> However, they noted that this contamination occurred post-source. These are important considerations for the prevention of leptospiral

contamination of water systems that may be exposed to animals shedding leptospire in their urine at or near a water source or open storage area. Diligence should also be applied to feral animal, domestic animal, and rodent control around these areas.

### Diagnosis/Detection

The diagnosis of leptospirosis in blood samples from human beings and animals is challenging as the majority of infections are subclinical or mild and leptospirosis usually presents as a non-specific acute, febrile illness.<sup>1,2,5</sup> Diagnosis of leptospirosis can occur at two stages of infection. The acute phase, bacteraemia, generally occurs between days 3 and 10 post-infection and can most effectively be diagnosed with molecular diagnostic methods such as PCR and blood culture isolation. During this stage, leptospire are present in blood and remain, in decreasing numbers, until approximately day 15.<sup>1</sup> The immune phase begins at approximately day 4 and can last up to day 30. During this phase, an increase in antibody response is correlated to the elimination of *leptospire* in the blood. Serological diagnosis methods including the MAT and enzyme-linked immuno-sorbent assay (ELISA) can determine an infection in this phase.<sup>5</sup>

Localized environmental detection is an important process in the development of control measures for leptospirosis. The detection of leptospire in water is mostly performed by molecular methods with culture methods still being utilized in some laboratories. The main issue with using leptospiral culture methods when performing environmental testing is the potential bacterial contamination in general and contamination with non-pathogenic leptospire specifically. A quantitative real-time PCR and sequencing has been used to identify *Leptospira* species in human samples and water samples in Iquitos, Peru, to compare urban and rural environmental surface waters.<sup>23</sup> The authors found that the distribution of *Leptospira* in human samples mirrored that found in environmental water samples. A PCR has been designed, which can differentiate between pathogenic and saprophytic (non-pathogenic) leptospire.<sup>35</sup> As outlined previously, a DNA microarray has been developed to detect leptospire and 10 other commonly occurring pathogens in drinking water.<sup>18</sup> Recently *L. interrogans* in drinking water was detected using 454 pyrosequencing and Illumina sequencing.<sup>19</sup>

Currently, testing for leptospirosis in water samples is not common practice. Culture isolation is limited due to the presence of non-pathogenic leptospire in the environment. Whilst PCR's have been developed to differentiate pathogenic from non-pathogenic leptospire, validated protocols for testing leptospirosis in water samples have not yet been

developed to a point where they are universally accepted or routinely performed. Such tests will be required to be sensitive and specific as well as robust, non-labour intensive, and cheap to perform.

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