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**Serologic Reactivity to *Leptospira* and Dengue virus of Febrile Patients
from Guayaquil Slums.**

Andrés Abril,

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**Burden of Leptospirosis in the Slums of Guayaquil during a rainy season 2008:
Serologic Reactivity to *Leptospira* and Dengue virus of Febrile Patients from
Guayaquil Slums.**

Gabriel Trueba Ph.D.

Director de la Maestría de Microbiología,
Director de Tesis y Miembro del Comité de
Tesis

Mauricio Espinel, Ph.D.

Miembro del Comité de Tesis

Renato Leon, Ph.D.

Miembro del Comité de Tesis

Stella de la Torre, Ph.D.

Decana del Colegio de Ciencias Biológicas

Víctor Viteri Breedy, Ph.D.

Decano del Colegio de Postgrados

Quito, mayo de 2012

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PART I

GENERAL INTRODUCTION

1. URBAN LEPTOSPIROSIS

Leptospirosis is a zoonosis that occurs throughout the world but is most commonly seen in tropical climates (1). It has risen as a globally important infectious disease as shown in some reports and studies from outbreaks like the 1995 Nicaragua epidemic of severe pulmonary haemorrhage syndrome (SPHS)(2,3), identification of disease among US inner-city homeless population (1,2), the 1998 Lake Springfield Triathlon (2) and 2000 Borneo Eco-Challenge (2,4). Moreover, it was traditionally thought that leptospirosis is only a major health problem in developing, tropical countries (1,4); however, the disease has been under recognized in urban populations of developed countries as well as in temperate rural regions of the world especially during rainy seasons (1,4, 5). But, the real importance of the disease is the incidence related to the lack of sanitary infrastructure. Additionally poorly understood risk factors, pathogenicity, immunopathology are important factors for mortality associated to the disease (1,5,6). Challenges related to prevention are largely dependent on sanitation measures which may be difficult to implement, especially in developing countries, as our country Ecuador (1,6).

1.1. Epidemiology

Leptospirosis has a changing epidemiology especially in developing countries, as our country where it is a significant health burden for poor rural populations (1-4,5). Recently, leptospirosis has been recognized as an urban problem. Rural populations have moved to cities (5,7) and live in urban slums where the lack of basic sanitation is evident and produces ecological conditions for rodent-borne transmission (5-7). In South American countries such as Ecuador, outbreaks occur yearly in poor urban communities during periods of heavy rainfall (8). Pathogenic spirochetes of the genus *Leptospira* colonize the kidneys of animal carriers such as dogs and rats which eliminate the spirochetes in their urine. (6,7) The cycle of infection continues when rainwater washes off leptospires from contaminated soil and carries them into puddles, rivers and flooding water where pathogenic *Leptospira* can survive for long periods of time and can infect people through the contact of this water with their mucosa or lacerated skin (6,8-10).

Leptospirosis, traditionally considered an occupational disease (3,7), is now identified among people practicing recreational activities (10,11), water sports (1,5), travel and adventure tourism (4,5-9,12). However; it continues being under recognized or under reported despite the efforts of Leptonet, a surveillance system developed by the International Leptospirosis Society. (13)

Syndrome-based or febrile cases analysis have been effectively used in outbreaks (6,14,15) and found that a large proportion of leptospirosis cases had been unrecognized or attributed to other tropical diseases, because clinical symptoms are very similar to other illnesses (2,11,16). Also, in our countries, surveillance underestimates the impact of leptospirosis, because clinical diagnosis is difficult, in fact, classical severe manifestations do not differ from other febrile syndromes; for example, in some studies leptospirosis was found to be the cause of disease in many of the patients with non-viral hepatitis jaundice and non-malarial fever (9,16). Also, only a few laboratories carry out paired serum analysis and perform the standard diagnostic test, microagglutination test (MAT) (8,16).

There is a wide range of clinical manifestations, some people can develop mild symptomatic infections while others develop severe hemorrhagic disease forms such as Weil's disease (jaundice, acute renal failure and bleeding) (6,9) and SPHS (severe pulmonary hemorrhagic syndrome) (3). In some studies it was found that asymptomatic infections occurred in 60–70% of all serologically identified infections (11).

Adult males suppose to have increased risk for acquiring leptospirosis due to their habitual jobs that are related with risk factors; however, recently it was noticed that young urban slum residents had serologic evidence of infection, especially after exposure to floods (14,17). The disease is commonly seen during rainy seasons in some populations inhabiting slums such as Guayaquil lacking sanitary infrastructure. In such a conditions women and men had similar risk for acquiring infection (14,18). Epidemiological studies are useful for identifying risk factors and guided efforts (interventions) to control the disease. (10,11,19).

In urban slums in developing cities and countries, there are infrastructural deficiencies, such as open sewers, exposed garbage and numerous rodents that contribute to the transmission of leptospirosis during epidemics, especially during rainy seasons (7,8,13,16). Chemoprophylaxis as a prevention method may be useful in very small groups; however, it

won't be of much use in large risk populations. Vaccination of domestic animals is also very important but wild reservoirs are difficult to handle for vaccination purposes (12).

1.2. Clinical and laboratory diagnosis

Antibiotic therapy provides the greatest benefit when initiated early, this emphasized the importance of early diagnosis (12). However, it is difficult to assess because severe late-phase disease shows the classic manifestations of the infection but early-phase leptospirosis has non-specific presentation which can be misdiagnosed and confounded with other febrile illness (20,21). Moreover, misdiagnosis has become critical, where dengue and other infectious diseases with similar clinical presentations are endemic (17,22). Co-infection with diseases such as scrub typhus and malaria have been reported and presents another challenge in the diagnosis due to their unspecific syndromes similar to the ones produced by leptospirosis. (23). Most cases of leptospirosis probably remain unrecognized unless the diagnosis is suggested by a specific exposure or when the disease occurs in the context of an outbreak (7-10,15,23)

There is also lack of adequate laboratory tests for confirmatory diagnosis (8). MAT is the best tool so far, however it is necessary to have paired serum samples, as well, it is necessary to culture leptospiral strains which is difficult and risky (24). Efforts have focused on developing serologic tests that use whole *Leptospira* antigen preparations to get an easier diagnostic test. (25-28).

ELISA tests has shown to be a useful tool for the diagnosis of leptospirosis; however, some reports show low sensitivities during acute-phase illness, in fact it may detect fewer than 25% cases during the first week of illness (30-32). Screening for immunoglobulin M antibodies was evaluated for its ability to detect human leptospirosis (31,32), but these tests exhibited less sensitivity compared with *Leptospira* MAT (24,31). Sensitivity appears increased during the second week of illness, and testing of a late acute-phase sample (after ten days of illness) is therefore recommended; however, antibodies may persist for years after exposure. PCR based diagnosis of leptospirosis cannot identify the infecting serovar, which reduces its value in terms of epidemiologic research and public health. (25,31,33-35).

The currently gold standard is microscopic agglutination test (MAT) due to its high sensitivity and specificity but it does not permit early diagnosis because it cannot detect infection until 5–7 days after exposure. (24,25,31,36). Development of several other diagnostic methods, including serologic testing as immunofluorescence, enzyme-linked immunosorbent assay, and Western blot analysis has been reported. (37-43)

Several assays based on targeting genes such as those encoding OmpL1, DNA gyrase, RpoB, Lig, LipL32/Hap1, putative transcriptional regulator, and repetitive DNA elements have therefore been developed (40). Real-time, quantitative TaqMan PCR has also been developed. Several assays have been developed to overcome the typing of *Leptospira spp.*, such as single-strand conformation analysis, restriction enzyme analysis of PCR products, direct sequencing of amplicons, low-stringency single specific PCR, and multilocus variable number tandem-repeat analysis (43-48). A multiplex PCR assay to differentiate pathogenic and saprophytic *Leptospira* has also been developed. (47)

Epidemiologic tools such as serologic and molecular typing can be useful because knowing the prevalent serogroup could be the first step to identify reservoirs and generate control strategies; however, reliable molecular strategies are not available yet, so results from MAT testing of patient sera would be used as a surrogate to infer the infecting serogroup. Pulsed-field gel electrophoresis, 16S rRNA sequencing or PCR-based typing methods have not gained wide acceptance because of their limited discriminatory power, lack of adequate electronic databases of typing and sequence patterns or low reproducibility. (27,29,34,49)

1.3. Pathophysiology and clinical management

As shown in figure 1, clinical manifestations range from an influenza-like illness to fulminant disease with jaundice, acute renal failure, aseptic meningitis, and hemorrhagic diathesis like Weil's disease and SPHS (50) Early diagnosis is important since complications depend on the age population, because older adults have an increased risk for death, require aggressive treatment and monitoring (50). Prognostic factors for mortality are hypokalemic acute renal failure, respiratory insufficiency, hypotension, arrhythmias and altered mental status. (51,52).

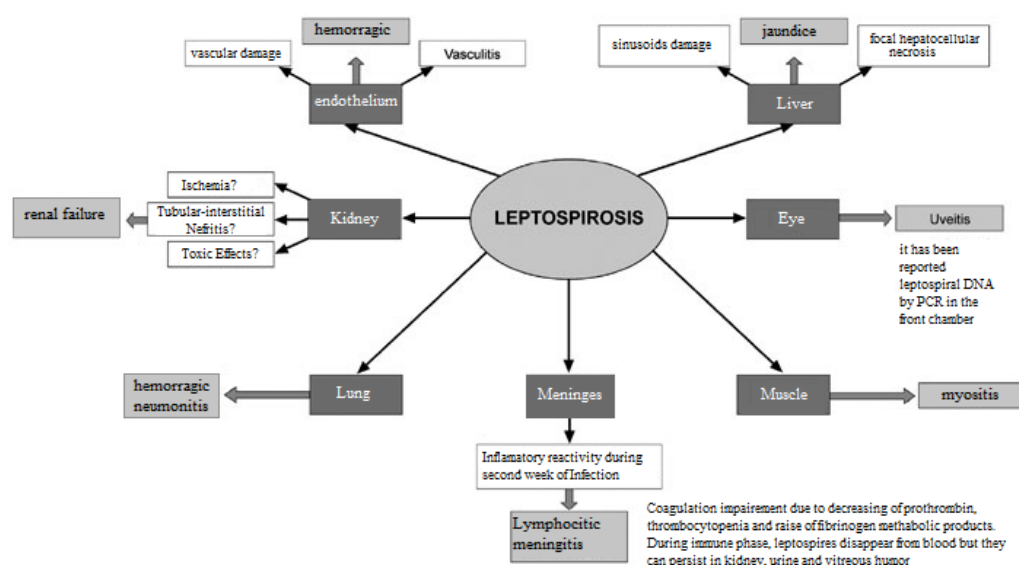


Figure 1. Clinical Syndromes caused by *Leptospira*. Adapted From: Zunino M, Pizarro P. Leptospirosis: Puesta al día. Rev. chil. infectol.2007, vol.24, n.3,

Leptospirosis-associated SPHS is now recognized as a widespread public health problem. It should be suspected when the patients show signs of respiratory distress, without haemoptysis (3,51). SPHS patients seem to have a high (approximately 1000000 bacteria/mg) leptospiral load in the lungs (52). However, few intact leptospirae are observed in autopsy, suggesting an immune-mediated process with immunoglobulin and C3 deposited along the alveolar basement membrane. (51-54).

Acute and fulminant pulmonary edema (PO) or acute lung injury (ALI) has been reported (52). Lung parenchyma showed a marked increase in inducible nitric oxide synthase (iNOS) activity, suggesting that iNOS and nitric oxide (NO) may also play a role in the pathogenesis of PO, but the mechanisms remain unclear (53).

The renal failure causes impaired proximal sodium reabsorption, increased distal sodium delivery and potassium wasting (55,56). The target may be the sodium–potassium–chloride co-transporter since leptospiral outer membrane extracts inhibit transporter activity and leptospiral derived unsaturated fatty acids act as toxins that inhibit kidney sodium–potassium ATPase (57,58).

Weil made the first description of the disease in 1886; therefore, it is called Weil's disease, the most severe form of leptospirosis (59). This presentation, with high fever, intense jaundice related with elevations of liver transaminases, hemorrhagic diathesis requiring

vitamin K, hepatic and renal dysfunction, mental status changes, cardiovascular collapse, and significant mortality, is not always present (52,59).

1.4. Genome and microbiology

The genomes of serovar *L. interrogans* serovars Icterohaemorrhagiae and Copenhageni were published in 2003 and 2004, respectively (9,61). A database, is available online for comparison of the two genomes (13,60). Leptospiral genome is formed by two circular chromosomes (figure 2), the chromosome I with approximately 4.3 Mb and the chromosome II with approximately 350 kb which is highly conserved among the serovars that are members of the same serogroup. (9)

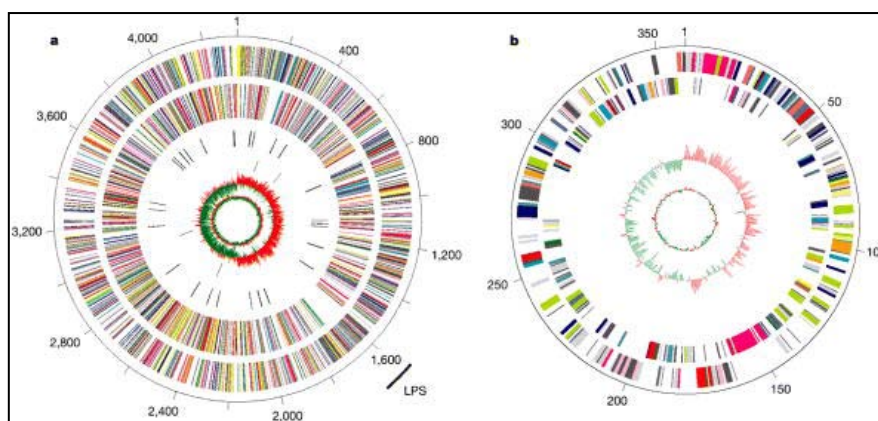


Figure2. *L. interrogans* Lai genome. **a.** Large chromosome (CI); circles range from 1 (outer) to 6 (inner) kilobases; **b.** small chromosome (CII); circles from I (outer) to IV (inner) kilobases. Genes on forward and reverse strand; tRNA genes; rRNA genes; GC bias ((G-C)/(G + C); G + C content (from circle 1 to 6 respectively). They are represented as follows: amino acid biosynthesis(orange), purines, pyrimidines, nucleosides and nucleotides(green), fatty acid and phospholipid metabolism(blue), biosynthesis of cofactors, prosthetic groups and carriers(magenta), central

intermediary metabolism(khaki), energy metabolism(cyan), transport and binding proteins(orchid), DNA metabolism(yellow), transcription(dark green), protein synthesis (brown), protein fate (red), regulatory functions(green-yellow), cell envelope(pink), cellular processes(salmon), other categories(navy), conserved(light grey), hypothetical(dim grey), unknown function protein(slate grey), tRNA and rRNA(black) FROM Ren SX, Fu G, Jiang XG, et al. Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. Nature 2003; 422:888–893.

These genomes contained 4,768 and 3,728 predicted open reading frames (ORFs), and 118 and 64 unique ORFs respectively (6,9,61). Lipopolysaccharide (LPS) is believed to influence reservoir specificity Icterohaemorrhagiae and Copenhageni are the striped field mouse (*Apodemus agrarius*) and the domestic rat (*Rattus norvegicus*), respectively (9).

Unlike other spirochaetes, *Leptospira* have a haem biosynthesis and uptake pathway, and an alternative pyruvate pathway which is used to synthesize isoleucine (62,63). *Leptospira* also has three toxin – antitoxin systems that may mediate global gene regulation during nutritional stress. Moreover, *Leptospira* have over 70 genes with putative regulatory roles; this repertoire is more than twice the number seen in other spirochetes (61,64). It has also genes encoding flagellin and recA (65). Importance of host-induced genes infection processes have been determined to be coordinately regulated or stimulated by host factors.

The genomic sequences of *L. interrogans* serovar Icterohaemorrhagiae (*Lai* strain) and *L. interrogans* serovar Copenhageni strain Fiocruz L1–130 indicate that there is no plasmid in *Leptospira* spp. (9,61).

1.5. Pathogenesis

Disease determinants are inoculum size, host factors and the pathogen's virulence characteristics. The inoculum size could be influenced by repeated exposure (risk associations) like the relation of urban slum resident and open sewer or flooding water (8, 18).

Identification of host genetic susceptibility factors for leptospirosis in outbreak investigations can provide clues in disease pathogenesis, as host factors involvement (67). It was found an association between HLA-DQ6 genotype and the risk of acquiring leptospirosis by swallowing water; however It could be related with the inoculum's size effect (61).

The pathology of leptospirosis seems to have an underlying immunopathogenic process. Poor outcomes in patients have been proved to be related with differences in plasma tumor necrosis factor (TNF)- α levels. Whole *Leptospira* induces type 1 cytokines from whole blood of naive individuals (68). *Leptospira* led to expansion of γ - δ cells at high concentration of bacteria and α - β T at low numbers of *Leptospira*. Stimulated γ - δ T cells release IFN- γ without antigen processing. *Leptospira* LPS activates cells through Toll-like receptor-2 which is associated to a unique structure of *Leptospira* lipid A. Also, leptospiral glycolipoprotein induce naive PBMCs to secrete TNF- α and IL-10 and induce cell activation (69-72).

1.6. Virulence

The ability of the pathogen to rapidly penetrate and disseminate through intercellular junctions, and establish persistent colonization in the renal tubules is a characteristic of leptospirosis (73,74). It has been proposed that O - antigen regulation may determine whether *Leptospira* cause acute disease or persistent infection. (2,73). Nally and colleagues noted that features of the lipopolysaccharide O antigen (Oag) content of leptospires in guinea pig liver was markedly reduced compared to that of organisms found in rat renal

tubules or cultivated in vitro. Their findings show an association of Oag loss with disseminated lethal infection and an association of Oag with renal tubular colonization (73), but these results aren't well accepted and need to be reproduced in Vivo. Nevertheless, the lipopolysaccharide O antigen is well known as virulence factor, and it is probably that its modifications let the bacteria to escape from antibody elimination (13)

Based on genomic data, there are over 260 membrane-associated surface proteins (75). Studies have tried to evaluate differential expression of target genes when cultured under conditions that mimic the host environment (61,76) and to confirmed surface expression with immunofluorescence and electron microscopy, immunochemical analysis of outer membrane vesicles and ELISA with intact *Leptospira* (59,76,77).

There are reported six surface proteins, these include porin OmpL1; peripheral protein P31LipL45; lipoproteins LipL41, LipL32, LipL21 and LipL48 and over 10 candidate proteins related con leptospiral pathogenesis (75-77). LipL32 and LipL21 are very interesting because they are related to all pathogenic *Leptospira* (75,76). A very important finding has been the identification of a set of proteins that are called Lig proteins that are 90-amino acid bacterial immunoglobulin-like proteins (Big) which are related to virulence factors such as intimin, invasin and BipA proteins (41,75). Another interesting discovery is that lig genes, that include ligA and ligB and a pseudogene ligC, are present only in pathogenic *Leptospira* species. These genes encode two large lipoproteins surface expressed that are reduced when virulent strains are attenuated during culture passage (76). Furthermore, these proteins have been shown to confer protective immunity and they could serve as serodiagnostic markers for infection (79)

1.7. Vaccines and immunity

Vaccines are supposed to be used as prevention against the disease in humans or limiting the spread of transmission to humans in reservoirs. Bacterin vaccines have been used for years in the veterinary field all over the world; however, Cuba and China use them in humans. (80-82).

Some authors identified surface-exposed proteins conserved across pathogenic serovars and may elicit cross-protective immunity (83,84). LipL41 and OmpL1 recombinant immunization induced protective responses in hamsters (77,84). It was found also that

immunization with a viral construct which encodes Hap1 (LipL32) can confer protection (75, 83). Lig proteins are also candidates to develop effective vaccines (76,78).

Immunity has been believed to be antibody-mediated because it was found that immunization can be passively transferred in hamsters (85). However, most of responses were found to be correlated with Th1 responses, characterized by CD4 and γ - δ T cell production of IFN- γ . So the efforts will be focus on developing strategies to enhance these types of immune responses. (80,86,87)

1.8. Treatment

Despite the fact that we are currently 60 years into the antibiotic era, the optimal treatment approach for leptospirosis is not clearly defined (88). As is resumed in table 1, recent human studies suggest that the later generation cephalosporins, namely ceftriaxone and cefotaxime, are likely the treatment of choice in cases of suspected or confirmed leptospirosis (80). The once-daily dosing and broad spectrum of activity of ceftriaxone make it a particularly attractive option. If these medications are not available, the classic treatments of penicillin and doxycycline remain viable options. The macrolides appear effective in the therapy of at least mild disease. The aminoglycosides are often used as first-line therapy, but we are hesitant to recommend their use due to nephrotoxicity. The fluoroquinolones hold promise as potential therapies, but there are not currently enough human data to support their use. Further research into this important but often overlooked disease is clearly needed. (90,91)

TABLE 1 Treatment options for leptospirosis. Taken from Griffith M, Hospenthal D, Murray C. Antimicrobial therapy of leptospirosis. *Curr Opin Infect Dis* 2006 19:533–537

	Primary agents	Alternative agents	Other potential agents
Severe disease	Ceftriaxone, cefotaxime, or penicillin G	Doxycycline or aminoglycosides	Cefepime or extended spectrum penicillins or carbapenems
Mild to moderate Disease	Doxycycline	Azithromycin or amoxicillin or aminoglycosides	Fluoroquinolones

2. Dengue

Dengue is known as the most important human arboviral disease worldwide (92,93). In some regions it is endemic and has a potential of generating epidemics usually associated with the rainy season, despite the control strategies of health authorities which are for the most cases insufficient to avoid transmission (94). Incidence of dengue hemorrhagic fever is increasing due to the circulation of more than one viral serotype, DEN-1, DEN-2, DEN-3, DEN-4 (94-96). This and other epidemiological factors add up so that dengue is currently considered one of the most serious public health concerns (94,97). Identification of recurrences and isolation of severe cases reflect the lack of efficient control strategies and adequate epidemiological surveillance systems (146). Among the many aspects on dengue epidemiology to better develop a control to annual outbreaks, one of the main problems is the difficulty in the correct identification of dengue cases (92-94,98).

2.1. Epidemiology

Dengue is now endemic in more than 100 countries in Africa, America, Eastern Mediterranean, Western Pacific, and particularly in South East Asia and it is of deep concern that its global prevalence has grown dramatically in recent years (99).

Dengue virus belong to the *Flavivirus* genus within the Flaviviridae family (single stranded positive sense RNA viruses) and it is found in tropical and subtropical areas where environmental conditions favor the presence and breeding of its vector, the *Aedes aegypti* mosquitoes. (94,100). In the Americas, dengue was first recognized in the 18th century, and largely eradicated during the 1950-60's due to intense vector control strategies. Re-introduction occurred after intensive top-down approaches to eradicate vectors ceased in the 1970's and soon dengue spread in the Caribbean and Central America and re-emerged in Ecuador in 1988 (101). The World Health Organization (WHO) indicates that South-East Asian and Western Pacific Regions bear nearly 75% of the current global disease burden (94,100).

Dengue haemorrhagic fever (DHF) was first recognized in Manila in 1954 especially infecting children and was characterized by the acute onset of high fever, petechial haemorrhage and shock.(102,103) In 1958, an outbreak of DHF occurred in Bangkok

becoming a serious public health problem, causing morbidity and mortality among children in many regions of world (104,105)

Mosquitoes of the genus *Aedes*, subgenus *Stegomyia*, like *Aedes aegypti* and *A. albopictus* are the main epidemic vectors which are well established in tropical and subtropical regions as peridomestic mosquitoes (100,106). *Aedes aegypti*, the main vector, is well adapted to urban life and typically breeds in clean, stagnant water like rain water, thus the illness usually increases during rainy seasons where mosquitoes are more abundant (93-96,106).

WHO has classified dengue as a significant health, economic, and social problem on endemic and hyperendemic areas where usually the four serotypes circulate (94,95). The incidence and prevalence vary from year to year, with epidemics every 3 to 5 years. Multiple factors are responsible for the dynamic patterns of transmission of the disease: population growth, rapid uncontrolled urbanization, increased movement of viruses via international travel, demographic changes, poor vector control, genetic changes in the virus, immunological profile and modulating climatic factors. (98,107-109)

El Niño Southern Oscillation (ENSO) and global warming are also known as other factors contributing to the cyclical pattern of dengue activity (97,98). Furthermore, if it is seasonal within a year in most endemic countries it is related to seasonal rainfall and temperature changes(107,109), its greatest burden in endemic countries is concentrated in children (110,111). The spread of infection is also enhanced by traveling which facilitates the transmission of infected individuals and mosquito larvae to non-infected areas (112,113).

2.2. Clinical and laboratory diagnosis

Dengue virus infection is clinically similar to many other acute tropical diseases where fever is the main symptom, thus laboratory testing and confirmation of clinical diagnosis is very important in early diagnosis and patient management (20,59,114).

Dengue symptoms range from a self-limiting infection to the life-threatening dengue complications such as hemorrhagic fever and dengue shock syndrome (DHF/DSS)(113). Classical dengue causes headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, leucopenia; whereas dengue haemorrhagic fever include in

addition to fever (or history of acute fever) lasting two to seven days, haemorrhagic tendencies (positive tourniquet test; petechiae; ecchymoses or purpura; bleeding from the mucosa, gastrointestinal tract, injection sites, or other locations; haematemesis or melena; and thrombocytopenia ($\leq 100\,000 \times 10^6$ cells/l). (115). Nevertheless, final diagnosis should be based only on laboratory confirmation of dengue especially in areas where other diseases causing similar symptoms are also present (115,116)

The laboratory diagnostics of dengue include methods for detection of the virus (by cell culture, immunofluorescence), detection of virus antigen (ELISA), detection of anti-dengue virus antibody by hemagglutination inhibition-HI or complement fixation test (CF), neutralization tests and detection of virus nucleic acid (RT-PCR) (116, 117) .

However, for a confirmatory molecular diagnosis viruses should be identified by isolation or nucleic acid detection. Commercially kits have been developed for the rapid detection of dengue infections (118,119). These kits are designed to detect the presence of NS1 antigen and/or anti-dengue antibodies (99,119). The detection for clinical application has been described in ELISA and RDTs. (118,121,122).

Rapid diagnostic tests (RDTs) for IgM and IgG antibodies detection have shown to be efficient and use low-technology (120). The tests are based on detecting IgM antibodies and/or NS1 antigens. NS1 is detectable in the first 5 to 6 days of infection in contrast to IgMs that only develop after 4 to 5 days of infection with the virus. IgM antibodies detection by RDTs generally has a higher sensitivity (118,123,124), however, RDTs that detect NS1 antigens are highly sensitive and specific for the early stages of the infection. (125).

The use of anti-dengue IgM and IgG antibody detections allow the classification of primary and secondary (or later) dengue infections respectively (120,126). This improves patient management because dengue patients are commonly identified during secondary infections which is associated to severe clinical complications.(117)

Instrumental methods such as quartz crystal microbalance, surface plasmon resonance, photonic crystal and electrochemical impedance spectroscopy have shown promising results. Nanosized materials including liposomes, nanowires and nanopores, coupled to

conventional fluorescence, potentiometry and voltammetry methods are also described and could be valuable diagnostic tools. (127)

2.2.1. Serologic Diagnosis

These methods include hemagglutination-inhibition (HI), complement fixation (CF), neutralization test (NT), immunoglobulin M (IgM) capture enzyme linked immunosorbent assay (MAC-ELISA) and indirect immunoglobulin G ELISA. Despite these are useful, they show high cross-reactivity, among serotypes and with other flaviviruses (yellow fever virus, Japanese encephalitis virus, or St. Louis encephalitis virus), and others like Oropouche, Mayaro or Chikungunya viruses. (128)

Hemagglutination-inhibition test (HI) was the standard method used before due to its high sensitivity and relatively easy execution. However, it lacks specificity, require paired samples, and can't identify serotypes. Complement fixation test (CF) is not used for routine dengue diagnosis; it is based on the fact that the complement will be consumed during the antigen-antibody reaction. (129)

Neutralization test (NT) is the most sensitive and specific serological test for diagnosis and serotyping. The disadvantages are its high cost, the long time necessary to perform the assay and the associated technical difficulties (128). Finally, ELISA is considered the most useful test for diagnosis to detect acute phase (IgM) and convalescent phase (IgG) antibodies. (128-132)

Immunoglobulin M (IgM) capture enzyme linked immunosorbent assay (MAC-ELISA) is less sensitive but its specificity is similar to that of HI in primary and secondary infections (129,133). Other ELISA's techniques for the detection of total immunoglobulin of flavivirus have been described and used only in seroepidemiological studies(131,132-136). Hemolysis in gel, the hemoadsorption immunosorbent technique and the staphylococcal agglutination-inhibition reaction were techniques that have also used in the past.(137-139)

2.2.2. Viral Isolation

Four methods are routinely used; intracerebral inoculation of newborn mice, inoculation on mammalian cell cultures, intrathoracic inoculation of adult mosquitoes, and inoculation on

mosquito cell cultures. (116, 128). Intracerebral inoculation can detect the four serotypes in mice; however, it has high cost, long time for isolation, and low sensitivity (140,141). Mammalian cell culture presents the same disadvantages, although this method seems to be very commonly used. (128)

Mosquito inoculation is the most sensitive but least used method. *Aedes aegypti*, *A. albopictus*, *Toxorhynchites amboinensis* and *T. splendens* are used to perform the test. The viral detection is made through indirect immunofluorescence assay (IFA) on mosquito's brain or salivary glands (135). Mosquito cell culture is the most recent methodology developed, it is a quick, sensitive and relatively inexpensive. The mosquito cells are relatively easy to maintain and grow at room temperature and it is possible to keep cultures for up to 14 days without replacing the medium (116,142,143).

2.2.3. Molecular Detection

Nucleic acid hybridization using RNA either with biotinylated probes or ³²P-labelled probes is used primarily in epidemiological studies as a research tool rather than a routine diagnostic method (144,145). Reverse transcription - Polymerase Chain Reaction (RT-PCR) has been developed but it depends of many variables. Furthermore, according to the World Health Organization (WHO), despite PCR is a powerful method to be used for dengue diagnosis, it still needs to be better standardized. (146-149)

New immunohistochemical methods consisting of monoclonal or polyclonal antibodies labeled with peroxidase/ alkaline-phosphatase enzymes that detect virus on a great variety of fixed samples have been also used and evaluated(150). In addition to specific amplification and restriction enzyme analyses, other studies have demonstrated that nucleotide sequencing of gene fragments amplified by RT-PCR can be used as a fast method for classification of dengue virus serotypes and phylogenetic studies (146,151).

2.3. Pathophysiology and clinical management

Early diagnostic of dengue infection allows for timely clinical intervention and etiological investigation (99). Diagnosis of disease during the acute phase should be a priority for public health programs because dengue virus could cause significant pathologies including

death of the patient (152,153). In the last century dengue fever became one of the leading causes of morbidity and mortality throughout the tropics (94,113).

The main explanation for the progression of dengue symptoms and complications has been associated to "antibody-dependent enhancement" (ADE) which means that enhancing antibodies acquired at primary infection increase the number of infected cells, and thus the levels of viremia, during secondary infection (114,154).

The World Health Organization (WHO) classified Dengue due to clinical manifestations in Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF), and Dengue Shock Syndrome (DSS) (155). The definition for DF must meet four criteria: fever, minor hemorrhagic manifestations, and thrombocytopenia. In turn, DHF is divided into four grades (DHF I–IV), where Grades III and IV are DSS, with hypotensive shock or narrow pulse pressure plus clinical signs of shock. However, limitations have been noted regarding its complexity and applicability, particularly in patients with severe symptoms.(156-159). A new classification has been propose, assigning clinical symptoms into Dengue without Warning Signs, Dengue with Warning Signs, and Severe Dengue (see treatment) (113,155,159).

The immunopathogenesis remains poorly understood but the target cells for dengue virus include dendritic reticulum cells, monocytes, lymphocytes, hepatocytes, and vascular endothelial cells (154). The virus binds to Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) which has a high affinity for ICAM3, expressed in activating T-cells (154,160,161). In mature dendritic cells, antibodies can enhance dengue infection via Fc receptors and then T-cells become activated and generate abundant cytokines, including tumor necrosis factor-alpha, interleukins (IL-2, IL-6, and IL-8), implicated in vascular leak and shock in addition to activating effector cells (154,162).

The virus manipulates many different cellular pathways, including autophagy. For example, DENV2 infection of a hepatocyte cell line induce autophagy due to virus induced autophagosomes co-localized with a marker of lysosomal fusion (LAMP1) (163). Also, NS1 is co-localized with autophagosomes as well as LAMP1 and the ribosomal protein L28 (163-165). An endosomal marker (M6P-R) co-localized with autophagosomes indicates that some autophagosomes may fuse with endosomes to form organelles called amphisomes. (163,166) Virus replicates on virally induced characteristic double membrane

vesicles (DMVs), and autophagosomes are DMVs, it has been hypothesized that it might replicate on amphisomes and thus link virus entry and replication. (163-166)

T-Cell Activation and Apoptosis may lead to the sudden onset of vascular permeability and hemorrhage that characterizes severe forms of dengue disease (168). T-cell response may cause suboptimal killing of the infected monocytes and serve to augment the severity of the second infection due to higher viral loads (168,169). Dengue virus can also attach to the Fc receptors on macrophages, monocytes, and other cells when the virus is coated with antibody. The antibody actually enhances the infectivity of these viruses by providing new receptors for the virus and promoting viral uptake into target cells and it is mostly related with the severity of secondary infection with a different virus serotype (169-171). Most of the flaviviruses are serologically related, and antibodies may neutralize or enhance another virus. (128)

Most infections produce, in decreasing order of frequency, an asymptomatic infection, mild nonspecific symptoms, or classic dengue (94, 169). The more severe manifestations of shock and hemorrhage occur only in 5% of infections (169). The greatest risk factor for the development of severe dengue is secondary infection with a different dengue serotype from the original infecting virus (168,172). Severe illness during secondary dengue infections was associated with higher peak plasma virus titers (172).

2.4. Genome and microbiology

Studies of dengue virus evolution are possible thanks to increasing availability of viral gene sequence data (173-175). DENV sequences have shown remarkable stability over the entire history as evidenced in the NCBI Entrez protein database. Hence, these conserved sequences are unlikely to significantly diverge in newly emerging DENV isolates in the future (174). There is high rate of clade extinction with fluctuations in genetic diversity. It is possible that genetic variations are synonymous and moreover deleterious acting as a constraint to Dengue evolution, apparently, recombination is the most debated mechanism of viral variation; however, the variations tend to be deleterious which reduce fitness and therefore is cleared by purifying selection (173,174). Dengue virus has a relatively recent evolutionary history, with the four serotypes establishing endemic transmission in humans in the last few hundred years. There is evidence that viral strains differ in key phenotypic

features such as virulence, and for positive selection at immunologically important sites. (175)

Dengue as other flaviruses has a positive-stranded RNA genome, an icosahedral capsid, an envelope and varies from 40 to 65 nm in diameter (173). The E viral glycoprotein folds over, pairs up with another E glycoprotein, and lies flat across the surface of the virion to form an outer protein layer. (Figure 3).(163,176)

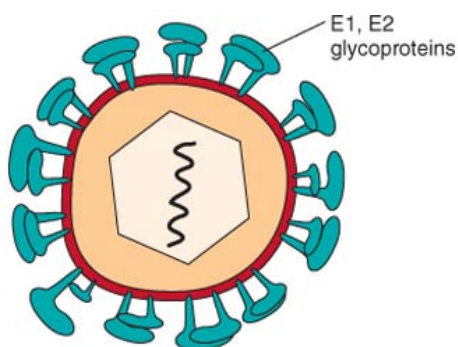


Figure 3. Structure of Flavivirus Dengue. Taken from Murray et al: Medical Microbiology; 6th edition.

The virus initiates infection of a permissive cell via clathrin-mediated endocytosis and then releases its genomic RNA into the cytosol after fusing with the late endosome (166,163,176). The entire viral RNA is translated as one open reading frame into a single polyprotein, and is subsequently cleaved by cellular and viral proteases to release three structural proteins and seven non-structural proteins (163).

The non-structural proteins, including four nonstructural early proteins called NS 1 through 4, a protease and an RNA-dependent RNA polymerase act to replicate the viral genome and assemble the new virions (177,178). In fact, a full-length, 42S, negative-sense RNA is synthesized as a template to produce more 42S positive-sense mRNA (176,178). The NS1 is a highly conserved glycoprotein that is present in all serotypes and during the early clinical phase of disease (128).

In addition, a 26S late mRNA is transcribed from the template which encodes the capsid (C) and envelope (E1 through E3) proteins (99,123,176,178). The structural genes are at the 5'-end of the genome so they are synthesized first and with the greatest efficiency. This feature may contribute to the lag before detection of their replication. (178) (Figure 4)

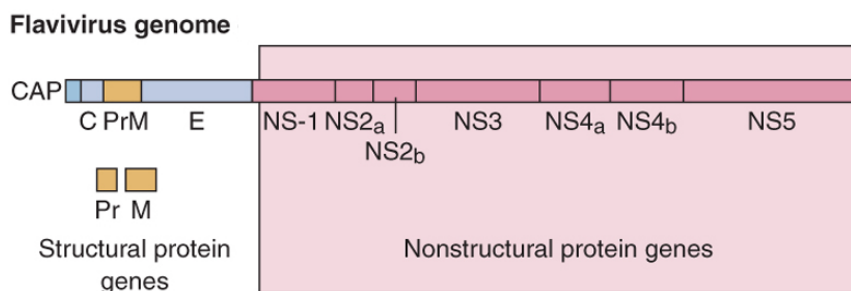


Figure 4. Dengue Genome map. Taken from Murray et al: Medical Microbiology; 6th edition.

2.5. Pathogenesis

Dengue hemorrhagic fever is primarily a disease of infants and children (94, 168), although adults may also be afflicted with severe disease (171). Unlike other infectious diseases, severe forms of dengue are more common in well-nourished children, and malnutrition protects against severe dengue vasculopathy. This negative association may be related to suppression of cellular immunity in malnutrition (168,179).

The viruses attach to specific receptors expressed on many different cell types from many different species. Then they enter the cell by endocytosis fusing its membrane with the membrane of the endosome on acidification of the vesicle to deliver the capsid and genome into the cytoplasm (163,176,178,180). Once in the cytoplasm, genomes bind to ribosomes as mRNA which is translated into a polyprotein, then cleaved into the four nonstructural early proteins described in last section (128,177,178). The structural proteins are produced by protease cleavage of the late polyprotein that was produced from the 26S mRNA (176,178). Thereafter, envelope glycoproteins are translated, glycosylated, and cleaved from the remaining portion of the polyprotein to produce the E1, E2, and E3 glycoprotein spikes. The glycoproteins are processed by the normal cellular machinery in the endoplasmic reticulum and Golgi apparatus and are acetylated and acylated with long-chain fatty acids (163,178,180, 181)

The C proteins associate with the genomic RNA soon after their synthesis and form an icosahedral capsid. Once this step is completed, the capsid associates with portions of the membrane expressing the viral glycoproteins. The virus is then released by exocytosis or cell lysis mechanisms (181). (Figure 5)

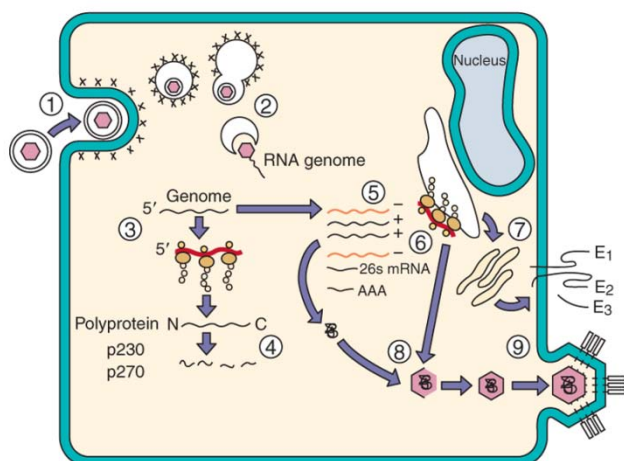


Figure 5. Replication and pathogenesis. Taken from Murray et al: Medical Microbiology; 6th edition.

2.6. Virulence

Due to the lack of specificity of clinical presentation and specialized laboratories, the incidence of dengue and its economic costs are certainly underestimated (182). According to the World Health Organization of the 100 million cases of dengue fever per year, up to 500,000 develop dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) as the result of a secondary infection with other serotype (94,168-170) .

Dengue virus infect both vertebrates and invertebrates. A full cycle occurs when the virus is transmitted by the invertebrate arthropod vector and amplified in a susceptible, immunologically naïve host (reservoir) that allows the reinfection of other arthropods (182,183). Birds and small mammals are the usual reservoir hosts but reptiles and amphibians can be too. (94,98,110,180)

Increasing numbers of dengue cases occur coinciding with monsoon season and period of high vector prevalence. Surveillance of mosquitoes can help monitor the infection rates and the specific serotype also can predict an epidemia (100, 107). The disease occur during the summer months and rainy seasons, when the arthropods breed and the arboviruses are cycled among a host reservoir, an arthropod, and human hosts (107, 158). This cycle maintains and increases the amount of virus in the environment. In the winter, the vector is not present to maintain the virus but it can persist in arthropod larvae or eggs or in reptiles or amphibians that remain in the locale or migrate with the birds and then return during the summer.(111,112,158)

When humans travel into areas where the *Aedes aegypti* mosquito is present and the virus circulating, they are at risk of being infected. Pools of standing water, drainage ditches, and trash dumps in cities can also provide breeding grounds for the vector (98,110,111). An increase in the population of these mosquitoes also increases the risk for infection. These viruses include at least two forms of transmission, can be maintained in a sylvatic or jungle cycle, in which monkeys are the natural hosts, and human can be accidentally infected if it is bitten by infected mosquitoes in the forest in a sylvatic transmission cycle that has not been extensively studied and also in an urban cycle which is the most common, in which humans are the hosts. (98,107,110)

2.7. Vaccines and immunity

Despite some cross reactivity shown in dengue pathogenesis, there is no cross protection between the four serotypes and there is no specific treatment but the high human and economic costs as well as the absence of specific preventive measures show the need to develop a vaccine. (182)

The challenges to develop a vaccine are; 1. absence of an animal model which is important for the preclinical analysis; 2. need to develop a live attenuated vaccine; 3. existence of 4 antigenically distinct serotypes (173,178,181); 4. immunologic risks related to antibody-dependent enhancement (170); 5. absence of a well defined correlation between protection and preexisting vaccines; 6. complexity associated to industrial production of a tetravalent vaccine. (154,180)

Introduction of dengue vaccination in the national immunization programs must be directed to regional epidemiological specificities and take into account the special features of each country.(172) Clinical studies with the most promising tetravalent vaccine were already started. In fact, Phase II clinical trials are now under way in children and adults in Mexico, Peru, and the Philippines. (111,140,184,185)

Mice are often used as a small-animal model; however, the results are not always predictive of what will happen in humans.(94) The second animal model is the nonhuman primate, which demonstrate viremia but do not present clinical evidence so it is difficult to predict attenuation in humans. Despite all limitations, four types of dengue vaccines are in development including live attenuated vaccines, chimeric live attenuated vaccines, whole

virus inactivated or subunit vaccines, and nucleic acid-based vaccines (182) . Live attenuated vaccines (LAVs) can induce important humoral and cellular immune mimicking a natural infection but the viruses must be sufficiently attenuated, have low viremia, low reactogenicity, and high immunogenicity for prevent transmission by mosquitoes (186) .

Chimeric Live Attenuated Vaccines are the most advanced developed for dengue/yellow fever. This vaccine uses the 17D yellow fever vaccine virus as its genetic backbone and replaces the yellow fever envelope (E) and *prM* genes with those from each of the four dengue viruses. This vaccine was shown to be attenuated, efficacious, safe, and highly unlikely to be transmitted by arthropod vectors (187) . Another chimeric live vaccine uses the PDK-53 DEN-2 vaccine candidate as a backbone. Similar to the concept of the yellow fever/dengue chimeric vaccine, the *prM* and *E* genes of DENV-2 are replaced with those of DENV-1, DENV-3, and DENV-4 (188) . A third chimeric live vaccine uses a DENV-4 attenuated by a $\Delta 30$ deletion of the 3'-untranslated region as the backbone (189,190) .

Whole-virus inactivated vaccines have two major advantages over live attenuated virus vaccines. First, it is not possible for inactivated vaccines to revert to a more pathogenic phenotype; second, induction of a balanced antibody response is easier to attain (190).

DNA shuffling and screening technologies have been used to construct DNA expression vectors encoding the epitopes of the four dengue serotypes and show immunogenicity (191). DNA vaccines afford advantages in terms of ease of production, stability, and transport at room temperature, decreased likelihood of replication interference, and the possibility to vaccinate against multiple pathogens in a single vaccination. (190-192)

2.8. Prevention and treatment

Prevention requires control of vector mosquitoes; however it is difficult to implement and maintain (94,115). Management; otherwise, depends on symptomatic treatment of hemorrhagic complications and hypovolemic shock, especially in severe forms. (194)

Despite of a lack of an specific antiviral therapy for dengue, antiviral therapy that blocks viral replication can clear viremia in a dose dependent manner, and reduce the spread of virus and the transmission. Several small-molecular inhibitors have been shown to target viral entry (195,196). Currently, the most advanced targets for specific anti-dengue virus

therapy are the NS2B/NS3 protease and the NS5 RNAdependent RNA polymerase, E protein, NS3 helicase, and NS5 methyltransferase (196,198).

Fluid replacement is the only recognized form of intervention for most patients with dengue haemorrhagic fever and dengue shock syndrome according to the individual dynamic evolution of the disease (117,129). Therapeutic regimens have been proposed according to World Health Organization's guidelines. Table 2 shows a summary of these guidelines. (94,199,200)

Treatment schemes are organized in five levels according to dengue severity (115). Treatment is focused on adequate oral hydration and control of fever with antipyretics. Admission to hospital is required to enable fast and monitored hydration in the event of haemoconcentration or thrombocytopenia (level C), third space plasma leakage (level D), dengue shock syndrome with pulse pressure <10 mm Hg (level E). (94,199,200)

Table 2. Treatment guidelines according to level of dengue severity. **FROM:** Teixeira MG, Barreto ML. Diagnosis and management of dengue BMJ. 2009 Nov 18;339:b4338

Features	Treatment
Level A	
Patient with signs and symptoms of dengue fever	Oral hydration
No warning signs	Antipyretics and analgesics*
Level B	
Patient with signs and symptoms of dengue fever	Vigorous oral hydration
Spontaneous haemorrhagic manifestations or positive tourniquet test; absence of other warning signs	Antipyretics and analgesics*
Level C	
Patient with signs and symptoms of dengue fever	Keep the patient under strict observation at a health care unit with enhanced oral hydration
Raised packed cell volume (until 10% above baseline value) with or without thrombocytopenia ($50 \times 10^6/l$ to $100\,000 \times 10^6/l$); with or without other warning signs	Antipyretics and analgesics*
Level D	
Haemoconcentration: raised packed cell volume (>10% above baseline value). In the absence of this information consider the following values for increased haemoconcentrations—children, >42%; women, >44%; men, >50%; with or without other warning signs	Intravenous hydration with crystalloid solution and plasma expander in a healthcare unit under medical supervision for at least 24 hours; provide sufficient fluids to prevent shock; antipyretics and analgesics*; clinical re-evaluation and measurement of packed cell volume after hydration
Level E	
Shock (pulse pressure <10 mm Hg)	Ideally in an intensive healthcare unit; intravenous colloid solution (hydroxy ethyl starch 6%, molecular weight 200,000; Dextran 70) is mandatory
Haemorrhagic manifestations present or absent	Transfusion of fresh whole blood or fresh frozen plasma in case of severe gastrointestinal bleeding
Essential laboratory tests: complete blood count, determination of serum albumin, chest radiography. Other tests might be needed: glucose, urea, creatinine, electrolytes, serum aspartate aminotransferase and serum alanine aminotransferase, blood gas analysis, and ultrasonography of abdomen and chest.	
*Do not use aspirin or anti-inflammatory drugs.	

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PART II

Serologic Reactivity to *Leptospira* and Dengue virus of Febrile Patients from Guayaquil Slums.

*Andrés Abril Gamboa,¹ Luis Vasco¹, Mauricio Espinel¹ Josefina Coloma², and
Gabriel Trueba¹.*

1. Microbiology Institute, Universidad San Francisco de Quito, Quito, Ecuador.
gtrueba@usfq.edu.ec
2. Department Public Health-Infectious Diseases, University of California, Berkeley
USA. colomaj@berkeley.edu

RESUMEN

Antecedentes del estudio: Durante los periodos lluviosos los barrios marginales de Guayaquil-Ecuador han sido constantemente afectados por leptospirosis y dengue, dos enfermedades que presentan una sintomatología muy similar. El propósito del presente trabajo fue investigar la frecuencia de leptospirosis en la población de los barrios marginales de Guayaquil y el grado de dificultad que existe para distinguir clínicamente leptospirosis de dengue.

Materiales y Métodos: Muestras de sangre de pacientes febriles provenientes de los barrios pobres de Guayaquil fueron colectadas durante la estación lluviosa del año 2008. Las muestras se sometieron a análisis de ELISA IgM para dengue y leptospirosis. Adicionalmente se obtuvieron datos retrospectivos (2003-2007) de historias clínicas de pacientes que acudieron a uno de los hospitales públicos más grandes de Guayaquil.

Resultados: De un total de 135 pacientes febriles, 15 (11.1%) fueron positivos a leptospirosis por ELISA, 36 (26.7%) fueron positivos a dengue, 3 (2.2 %) fueron positivos a ambos patógenos y 81 (60%) fueron negativas para los dos. Según el diagnóstico clínico los casos febriles se clasificaron 68.1% como dengue, 20.7% como leptospirosis, y 9.6 % como malaria y 1.5% como otro. Sin embargo, 60,0% de los pacientes diagnosticados clínicamente como dengue tuvieron anticuerpos contra *Leptospira* y no para dengue y 25.0% pacientes diagnosticados como leptospirosis tuvieron anticuerpos contra el virus de dengue y no para *Leptospira*. Adicionalmente, los archivos hospitalarios (2003-2007) indicaron que 72.8% de los pacientes diagnosticados clínicamente como dengue tuvieron anticuerpos contra *Leptospira* y carecían de anticuerpos contra virus de dengue.

Conclusiones: Los resultados sugieren que dengue y leptospirosis son dos enfermedades que ocurren con frecuencia y simultáneamente en los meses lluviosos en la ciudad de

Guayaquil. También sugiere que ambas enfermedades son frecuentemente identificadas erróneamente lo que representa un problema grave de salud pública pues ambas enfermedades requieren un tratamiento médico completamente distinto.

ABSTRACT

Background: Leptospirosis and dengue fever are two diseases causing very similar symptoms in Guayaquil during rainy seasons. The purpose of this study was to assess the frequency of leptospirosis and dengue during a rainy season and the difficulty to distinguish clinically both diseases.

Methods: Blood samples from febrile patients from poor neighborhoods in Guayaquil were obtained during the rainy season of 2008 and analyzed by IgM ELISA for dengue and leptospirosis. Additionally, retrospective data (2003-2007) from febrile patients who attended one of largest public hospitals in Guayaquil were obtained.

Results: From 135 febrile patients samples, 15 (11.1%) were positive to leptospiral ELISA, 36 (26,7%) were positive for dengue, 3 (2.2%) were positive for both pathogens and 81 (60%) were negative to both. Based on clinical diagnosis, febrile cases were classified 68.1% as Dengue, 20.7% as leptospirosis, 9.6% as malaria, and 1.5% as other. However 60.0% of patients clinically diagnosed as dengue had antibodies against *Leptospira* but not for dengue virus and 25.0% patients diagnosed as leptospirosis had antibodies for dengue but not for *Leptospira*. In addition the hospital archives indicated that 72.8% of the patients diagnosed clinically as dengue had antibodies to *Leptospira* and lacked of antibodies to dengue.

Conclusions: The results suggest that dengue fever and leptospirosis are two diseases that occur frequently and simultaneously during the rainy months in Guayaquil. This study also suggests that both diseases are often misidentified which is a public health problem because both diseases require different medical treatment.

INTRODUCTION

Leptospirosis, a zoonosis that occurs throughout the world, especially in tropical climates (1), is caused by any of the eleven pathogenic species of the spirochete *Leptospira* (2) which causes a spectrum of disease ranging from flu-like to life threatening hemorrhagic syndromes (1,3-8). In developing countries the disease causes a significant health burden in slums and rural populations during rainy seasons (3-6,9,10). Although leptospirosis is classically associated to individuals working in close proximity to domestic and wild animals (11,12) recent reports show increasing numbers of cases in people exposed to fresh water in urban settings or during recreational activities (13-16). Urine from animal reservoirs (dogs, pigs, rats, etc) is removed from soil by rainwater and carried by flooding water to rivers and puddles where humans and other animals get infected by skin or mucosal exposure (3,4, 5,16,17,18). Urban slums are especially vulnerable to this infection due to deficient sewage systems (1,3-5,12), poor drainage and large number of animal carriers (especially rats and dogs) (18-24). A recent report suggests that leptospirosis is the most frequent infection in febrile patients inhabiting tropical towns in Ecuador (42). Outbreaks of leptospirosis have been reported in Guayaquil since the beginning of the twentieth century and the most important recorded occurred during the winter and spring 1997-1998 which was associated with 11.8% mortality, high hospitalization rates and 12% seroprevalence after the outbreak (25).

In the Americas, dengue was first recognized in the 18th century, and largely eradicated from the continent in the 1950-60's. Re-introduction occurred after intensive top-down approaches to eradicate vectors ceased in the 1970's and soon dengue spread in the Caribbean and Central America and detected in Ecuador in 1988 (39). Dengue viruses are

transmitted by mosquitoes of the genus *Aedes*, which are widespread in tropical and subtropical climates (26,27). This illness tends to increase during rainy seasons due to the presence of clean water collections favoring the multiplication of mosquitoes (28-30). There are four distinct serotypes of dengue arbovirus: DENV1, DENV2, DENV3, DENV4 (31,32). Similar to leptospirosis, dengue viruses can cause symptoms ranging from the classical self-limiting flu-like disease to a severe, potentially fatal hemorrhagic syndrome known as dengue shock syndrome (29, 32, 33). Dengue is also an emerging disease because of the recent geographic expansion of the vector especially in Western Pacific Regions (32,34). The combination of multiple factors have contributed to the dissemination of the disease including population growth associated with rapid uncontrolled urbanization, increased population mobility, demographic changes, poor vector control, genetic changes in circulating or introduced viruses, and modulating climatic factors (5,6,35-37).

Leptospirosis and dengue fever are two diseases that co-occur in rainy seasons in tropical cities such as Guayaquil and share many symptomatic features (40,41). In fact, both diseases are part of what is called “acute undifferentiated febrile illness” (AUI) which is common in tropical regions of the developing world (42). The purpose of this study was to assess the burden of leptospirosis in the slums of Guayaquil and its possible confusion with other tropical diseases such as dengue (38, 40-45). For this purpose we analyzed the presence of anti-leptospira and anti-dengue virus IgM antibodies in blood from febrile patients inhabiting a slum of Guayaquil during rainy season 2008, additionally we did a retrospective review of the clinical archives of the main infectious diseases public hospital of Guayaquil.

MATERIALS AND METHODS

Study site and population

The study was conducted in the Bastion Popular community (Figure 1A) which is situated in the north part of Guayaquil, in a community with over 90,000 inhabitants . It is a region with several valleys and it is a densely-populated slum (Figure 1B). In total, 3,70% of the population of Guayaquil reside in this area with significant levels of poverty. Subjects were enrolled into the study during the rainy season between December 2007 and May 2008

Household survey

The study team (brigades) of community health workers, nurses and physicians conducted interviews during house visits and administered a standardized questionnaire (Appendix 2) to obtain information on demographic and socioeconomic indicators, employment and occupation, and exposures to sources of environmental contamination and potential reservoirs in the household and workplace. Subjects were asked to report the highest number of rats sighted within the household property and the site of work-related activities. The study team surveyed the area within the household to determine the presence of dogs, cats and chickens. The survey was provided by Albert Ko as far it was previously used in Brazil (61) An informed consent (Appendix 4) was developed and all patients enrolled voluntarily and were provided with written consent forms. All protocols were approved by Universidad San Francisco de Quito bioethics committee. All data collected was confidential and exclusively intended for scientific research purposes

Dried blood spots

Febrile patients, residing at the Bastión Popular areas of Guayaquil, were identified by the health brigades of the Ecuadorian Ministry of Health and asked to donate a drop of blood following standardized protocols (47) after voluntarily accepting to be part of the study and filling out the consent form and the household survey.

Blood extraction, transportation and sample storage is indicated in Appendix 6. To assure the correct application of protocols, every week the process was evaluated in a meeting with the health personnel during the time of the study.

Sample exclusion criteria were patients showing diarrheic symptoms, common cold symptoms, and patients younger than 4 years of age. The first were defined based on characteristics of other well defined syndromes and the last considering that children less than 4 years do not work or study. The main inclusion criteria was the clinical evidence of fever of unknown origin, also secondary inclusion criteria included living or working at Bastion Popular, and symptoms such as chills, myalgia, cephalgia or Icteric-febrile syndrome.

Blood drops were extracted by the health personnel, subsequently samples were allowed to dry for at least 4 hours, wrapped with waxed paper and stored with silica gel for transportation until placed inside plastic bags that were sealed and stored for up to two weeks at room temperature. Finally samples were stored at -20° at the Microbiology lab at San Francisco de Quito University until processing (48-51). The samples were labeled with an arbitrary code corresponding to the same code assigned at the survey avoiding the use

the patient's names for ethical reasons. All samples that were not treated following the established standardized operating procedures were discarded and a new sample obtained.

Serological Tests

A modified procedure for an ELISA kit for both leptospirosis and dengue (PanBio Pty. Ltd, Australia) was used. A 6 mm punch of the blood spot in filter paper was eluted in 150 µl of PBS and 29 µl of the eluted serum was placed in a well containing 71µl of serum diluent reagent (49,50). Plates were covered and incubated at 37 °C for 30 minutes, then washed with washing buffer 6 times, and allowed to dry. The rest of the procedure was performed following the instructions provided by the manufacturer.

A microagglutination test was performed on leptospira positive sera as described (54) however, we used the corresponding sera elution correction in order to reach 1:100 sera dilution (54,55).

Retrospective Hospital Data

Partial data from leptospirosis suspected patients (LSP), that attended the Hospital de Infectología José Daniel Rodríguez Maridueña in Guayaquil during the last 5 years (2003-2008) was obtained. Data was analyzed based on a modification of the interview field form to obtain information on the following. Demographic and socioeconomic indicators, employment and occupation, exposures to sources of environmental contamination and potential reservoirs in the household, workplace, evidence in the clinical record listed prepared by the physician who reported the case of any of these risk factors listed above.

Names of patients were omitted and were only recopilated from cases reported with one of the suspected diagnosis of leptospirosis.

The data collection was carried out separately and independently of the field study.

Statistical and Epidemiological Analysis

Analysis was performed using EpiInfo version 3.4.3 and StatView packages.

RESULTS

All inhabitants at Bastion Popular were eligible residents at the slum community, 135 were enrolled in the study. In this group we had a higher proportion of females (64.4%) than males (34.8%) and a mean age of 25,4 years (SD: 16,63; 4-78 years old). Among the subjects, 51(37,8)% had their houses constructed over water, and the frequency of work and residence in the same place was 36,3%, observing a high frequency of subjects that reported the activity of housewife or householder as principal work. Frequencies of household risk factors, work places association are listed on **Table 1**.

From 135 blood samples obtained from febrile patients at Bastión popular and close to Pascuales, the calculated prevalence was 11.1% (95% CI 6,4–17,7) and 26,7% (95% CI 19,4–35). for *Leptospira* and dengue antibodies respectively. Three samples (2.2%; 95% CI 0,5-6,4) were positive for both antigens and 81 (60%; 95% CI 51,2 – 68,3) were negative to both by IgM ELISA procedure. Dengue fever was clinically diagnosed in 68.1% of the febrile patients, leptospirosis in 20.7%, malaria 9.6%, and other 1.5% (**Table 2**). Clinical diagnosis of these patients showed little correspondence to their serologic

reactivity; 9 patients (60%) clinically diagnosed as having dengue fever showed positive IgM titers to *Leptospira* and no reactivity to dengue. Conversely 25% of patients clinically diagnosed as leptospirosis cases had positive IgM titers to dengue virus and did not show any reactivity to the leptospiral test. *Leptospira* ELISA positive sera showed highest MAT titers to serovar Patoc in 7 sera, serovar Panama in 3 sera, serovar Pomona in 3 sera, serovar Icterohaemorrhagiae in 2 sera, serovar Tarassovi in 2 sera and serovar Autumnalis in 1 serum.

Prevalence was adjusted for the age and gender distribution of enrolled subjects in the study. Prevalence was highest among children and adults; 33,3% for 5–14 years of age and 27.8% for 25-34 years of age (95% CI 13,3– 59 and 95% CI 9,7– 53,5, respectively) (**Table 1**). However, 16,7% (95% CI 3,6–41,4) of 15-24 and 35 – 44 years of age had evidence for leptospirosis. The prevalence of dengue was highest among adolescents and adults; in fact, 25,6% for 15–24 years of age and 30,8% for 35-44 years of age (95% CI 13– 42,1 and 95% CI 17– 47,6, respectively).

The prevalence of *Leptospira* was higher in females than males (7.6 versus 3.8%, respectively; OR 2,6 95%; CI 0,65–10.38 p= 0,09); and the prevalence of dengue was higher in females than males (20,5 versus 6.8%, respectively; OR 3,16; 95% CI 1.19–8,38 p=0,01).

High frequencies of related jobs are reported on cases. In fact, on leptospirosis cases, housewives and students are more frequent (33,3% and 38,9%, respectively) and on Dengue cases, housewives (53,8%) are the most common affected (**Figure 1 and 2**). There isn't significant risk association with the disease.

The main risk factors associated to patients with sera positive to leptospirosis were having their home over water, presence of garbage, contaminated drain water at home, sewer contact and evidence of rats at home; moreover, two relevant and statistic significant risk factors are the evidence of rats walking at daylight, and home flooding (**Table 3**), whereas risk factor associated to sera positive to dengue ELISA were garbage close to home, proximity to drain water and sewer, home flooding and street flooding; however, none of these show statistic relevance.

Subjects who resided in houses with flooding water had a 4,085 times (95% CI 1,006-16,57) increased risk for having anti-*Leptospira* antibodies than those who resided in houses over dry properties. People living in houses with rats walking during the day had 5 times (95% CI 1,06-23,46) more risk for leptospirosis. Sighting of two or more rats was a sign for rat infestation, and is a significant reservoir-associated risk factor for the household.

Having pets and reporting sighting rats at the workplace environment was not associated to risk according to the analyzed data. Open rainwater drainage structures and garbage deposits were distributed throughout the study area; yet sewers were very infrequent at the slum. (**Figure 3**).

The main symptoms associated to leptospirosis ELISA positive patients at community were fever, headache, joint and muscular pain, respiratory difficulties, jaundice, oliguria, and conjunctiva suffusion (**Table 3**). Most frequent symptoms associated to dengue ELISA positive sera were fever, headache, joint and muscular pain, respiratory difficulties, jaundice, conjunctiva suffusion. Hospital records (2003-2007) showed similar symptoms for leptospirosis and dengue: fever, headache, generalized muscle pain, jaundice and conjunctiva suffusion (Data not Shown)

Archival clinical chart data from the hospital during the period of 2003 to 2007 showed that from a total of 107 records recovered, the number of suspected dengue cases was 59 and the number of suspected leptospirosis cases was 29. Inconsistencies between clinical presumptive diagnosis and laboratory results attached to clinical records was also evident in these records; 72.88% of patients clinically diagnosed as dengue showed positive serology to *Leptospira* and no reactivity to dengue virus and 17.2% of patients clinically diagnosed as leptospirosis had positive serology to dengue virus and no reactivity to *Leptospira*, 25.4% of patients clinically diagnosed as dengue had positive sera for both, *Leptospira* and dengue virus, 1 case reported as dengue fever, and 5 reported as leptospirosis were negative for both ELISA tests.

DISCUSSION

The data presented here suggests that dengue and leptospirosis are two very common infectious diseases that co-occur during rainy seasons in the slums of Guayaquil, and dengue fever was the most frequent clinical diagnosis of febrile presentations during the rainy season of 2008. Archival data obtained from one of the largest public hospitals also suggests that dengue and leptospirosis were the most frequent causes of febrile disease (including malaria and hepatitis) from 2003 to 2007 (data not shown).

Our results indicate that 68.5% of the febrile cases were misdiagnosed as either leptospirosis or dengue fever. The discrepancy between the clinical diagnosis and the serology may reflect the similarity of the clinical manifestations of these two diseases (**Table 2**), as shown in other studies (61-63).

Misdiagnosis of these two diseases is an important public health concern because the two diseases require different therapeutic approaches. Unlike with dengue fever, clinical

complications of leptospirosis can be easily avoided using antibiotics (3,17,23,38,56,57). Complications due to dengue can be avoided by early support intervention and hospital referral of cases with alarming signs and symptoms (58). Both diseases share some risk factors which are associated to low income communities in tropical cities and rainy seasons (accumulation of water and deficient drainage) (60-63). These conditions are likely to worsen due to global warming (24,25,27,36,58) and rapidly expanding slums (30-33). A previous study on slum community performed in Salvador showed a 12% prevalence rate of anti *Leptospira* antibodies in general population, and other study from Brazil slums showed a 15% (61,65) in general population too using MAT as diagnostic tool(61). In this community-based survey at slums of Guayaquil, we found that 11,1% of all febrile cases had IgM against *Leptospira* ..

In conclusion, our findings, suggest that differences between dengue and leptospirosis are not so evident for clinicians, and dengue was the most frequent clinical diagnosis among the population enrolled.

Our study has limitations. Anti-*Leptospira* IgM antibodies can persist for years after initial exposure (19) and IgM antibodies may remain detectable for at least as long as 3 months after symptom onset (57). It is therefore difficult to differentiate between current and past infections. In fact, it is difficult to attribute the acute symptoms reported to an acute leptospirosis infection or to a more recent infection of different etiology characterized as an AUI (42). Therefore, it should have done a seroconversion test to corroborate that the leptospirosis infections were recent (63).

A second limitation is the reported low sensitivity of ELISA test (<25%) when applied to acute-phase specimens (64,67). It is therefore likely that the rates of leptospiral infection in our study population are higher than reported. Also, the study was performed through

home visits so the population studied reflected household characteristics; however, people that were working far from their homes at the same time of interview weren't included.

A third potential limitation is that we weren't able to calculate predictive values for the modification of the ELISA protocol using, however, dried blood spots have been used with high sensitivity and specificity for prevalence studies, outbreak surveillance and population screening for other diseases (47,49).

Since the study was performed in a single community our findings may not be generalizable to other slum settings. However, a large proportion of the world's slum population resides in tropical climates similar to this slum and reported similar conditions (Figure 3).(61,62,63,65) Then our findings may therefore be relevant to other slum communities where leptospirosis and Dengue are endemic.

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TABLES AND FIGURES

TABLE 1. Demographic characteristics. Numbers correspond to febrile patients from slums in Guayaquil

Demographic	Total (n=135)		Positives IgM ELISA				Negatives	
	Population enrolled		Leptospira (n=18)		Dengue (n=39)		For both antigens (n= 81)	
	Yes		Yes		Yes		Yes	
Age	N°	%	N°	%	N°	%	N°	%
05 to 14	44	32,6	6	33,3	4	10,3	34	42,0
15 to 24	29	21,5	3	16,7	10	25,6	16	19,8
25 to 34	23	17,0	5	27,8	7	17,9	12	14,8
35 to 44	21	15,6	3	16,7	12	30,8	8	9,9
45 and over	18	13,3	1	5,6	6	15,4	11	13,6
Gender								
Male	48	35,6	5	27,8	9	23,1	34	41,9
Household factors								
Home over water	51	37,8	5	27,8	14	35,9	34	42
Garbage at home	95	70,4	12	66,7	26	66,7	60	74,1
Inundation	67	49,6	5	27,8	14	35,9	49	60,5
Rats proximity	104	77,0	11	61,1	32	82,1	63	77,8
Dogs	86	63,7	9	50,0	27	69,2	52	64,2
Cats	57	42,2	10	55,6	13	33,3	34	42,0
Chicken	58	43	10	55,6	13	33,3	36	44,4
Work related exposures								
Contact with contaminated environment ^a	55	40,7	8	44,4	19	48,7	30	22,2
Rats	16	11,8	1	5,6	4	10,3	11	13,6

a Reported exposure to mud, garbage, flooding water or sewage water in the workplace.

FIGURE 1. Frequency of distribution of work in leptospirosis cases.

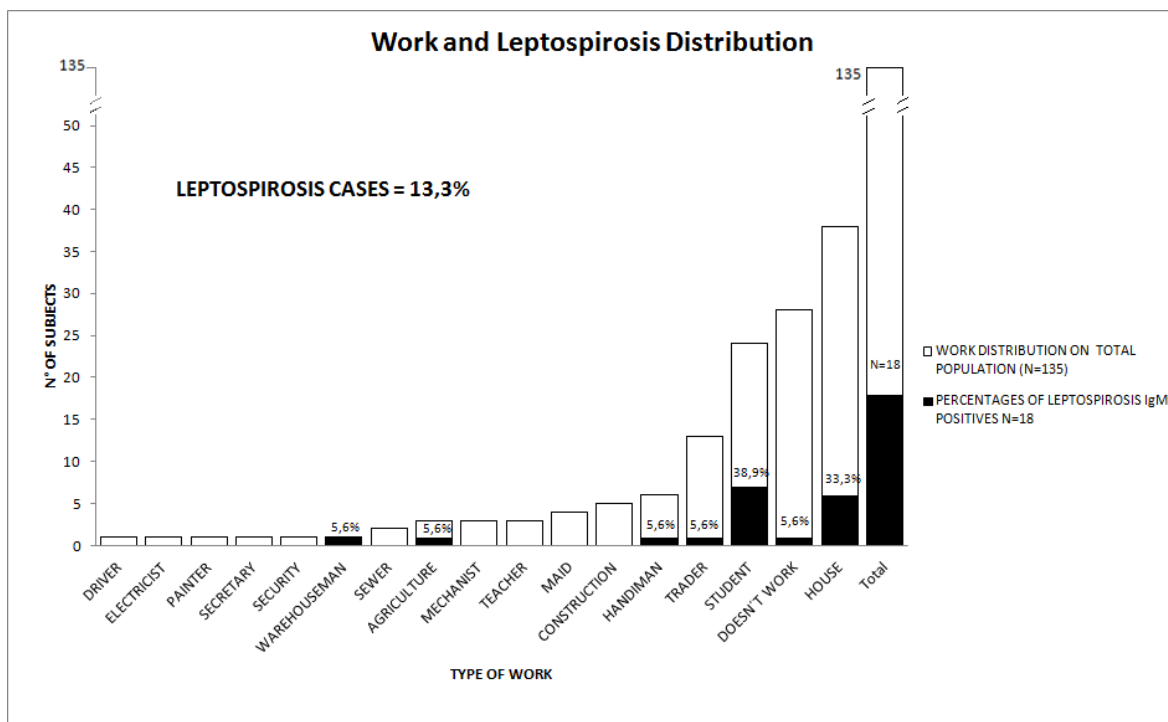


FIGURE 2. Frequency of distribution of work in Dengue cases

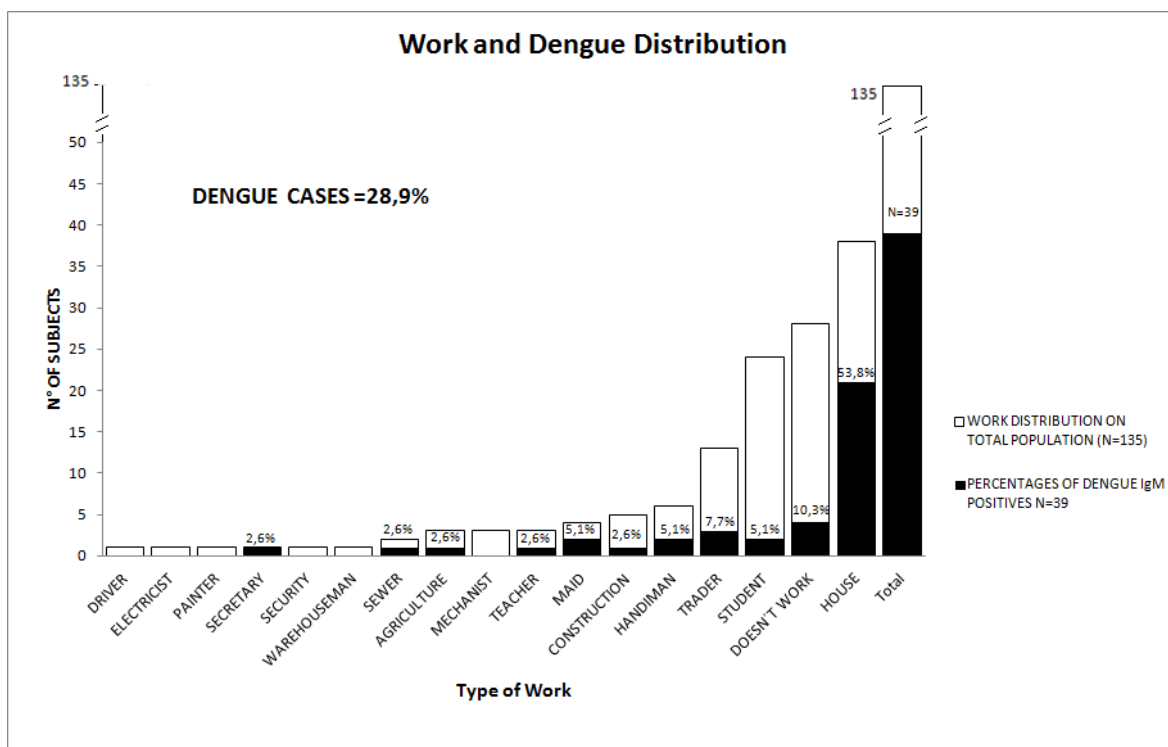
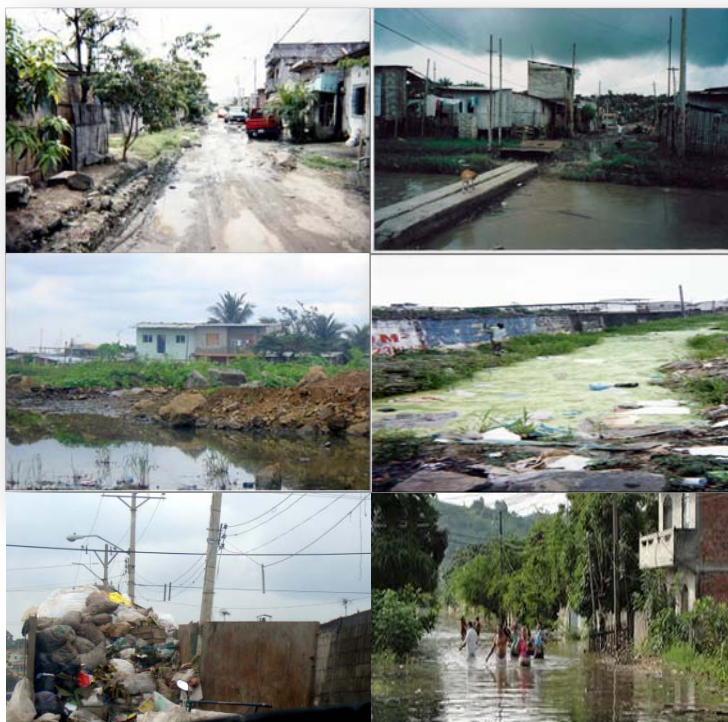


FIGURE 3. Slum community site in Bastión Popular, Guayaquil.



Photographs of the typical environment at the community study site, which shows the proximity of households to flooding water and refuse.

TABLE 2. Clinical diagnosis compared to serologic results. Numbers correspond to febrile patients from slums in Guayaquil diagnosed clinically and by ELISA.

Clinical Diagnosis	Number of ELISA Positive Sera			
	Dengue	<i>Leptospira</i>	Both	None
Leptospirosis	9	4	1	14
Dengue Fever	23	9	2	58
Malaria	4	2	0	7
Other	0	0	0	2
Total	36	15	3	81

TABLE 3. Clinical manifestations in patients with positive serology leptospirosis and dengue. Data obtained from interview forms collected from febrile patients in the slums of Guayaquil. Numbers indicate percentages.

SYMPTOMS	Positive IgM ELISA	
	<i>Leptospira</i>	Dengue virus
Fever	100.0	100
Headache	97.4	97,4
Join and muscular pain	94.5	92,3
Respiratory difficulties	61.5	61,5
Jaundice	56.4	53,8
Oliguria (patient's perception)	38.5	28,2
Conjunctiva suffusion	33.6	46,1
Haemoptysis	10.3	10,2
Calf pain	5.1	-----
Limb pain	3.7	10,2

TABLE 4. Risk factors. Data obtained from interview forms collected from febrile patients which report positive antibodies against *Leptospira* and/or dengue virus in the slums of Guayaquil. Values correspond to OR

Risk factor	Positive IgM ELISA			
	Leptospirosis	95% CI (min-max)	Dengue fever	95% CI (min-max)
Home over water	2,08	(0,51-8,33)	1,69	(0,68-4,2)
Garbage	1,75	(0,39-7,7)	1,27	(0,48-3,3)
Drain Water at home	3,5	(0,8-14,8)	1,67	(0,68-4,11)
Drain Water at work	1	(0,05-17,32)	0,24	(0,04-1,27)
Sewer contact	2,12	(0,17-25,77)	1,76	(0,39-7,9)
Home inundation	4,085	(1,006-16,57)	2,31	(0,9-5,7)
Street inundation	0,43	(0,06-2,76)	1,37	(0,45-4,14)
Rats at home	5,09	(0,88-29,2)	0,84	(0,27-2,61)
Rats walking at light day home	5	(1,06-23,46)	1,77	(0,6-4,55)

5. ADENDUM

5.1 LEPTOSPIRAL CULTURES.

The bacteria were cultivated at 30°C and the serovars used as antigens were the recommended on standard methods of the Royal Tropical Institute. The *Leptospira*'s culture had a cell count of 10^7 or 10^8 , determined on a Petroff - Hausser chamber count in a home prepared supplement for EMJH liquid medium. (201)

5.2 WATER SAMPLES COLLECTION

The water samples that were positive at PCR, were collected at Bastión Popular, Block 1B and at "Las Lojas" which is the place with more confirmed cases of leptospirosis.

5.3 DNA ISOLATION.

Extraction of DNA from water samples were performed and compared with a DNA extract from a strain cultured (*L. autumnalis*), approximately 10^6 - 10^8 cells from 1 ml of a 7-day *Leptospira* culture in EMJH was used as positive control. Subsequently, it was performed CTAB DNA extraction Protocol. The samples were added 700 ul of CTAB (2% (w/v) CTAB, 1.4M NaCl, 20mM EDTA, pH 8.0) for 2 hours at 65° C°, then added 700 ul of chloroform-isoamyl alcohol (24:1) and centrifuged at 12.000 RPM for 5 minutes to separate DNA from other particles in solution. DNA isolated was precipitated overnight using 3M Sodium Acetate (pH 5.0), and 100% ethanol. Finally, after 24 hours the solution was centrifuged at 14000 RPM for 10 minutes to obtain a DNA pellet which was washed with 70% ethanol and suspended in TE buffer (10mM TRIS – HCl, pH 8.0; 0.1mM EDTA). DNA was stored at -20°C.

5.4 PCR PROTOCOL FOR DETECTION OF LEPTOSPIRAL DNA IN FLOODING WATER

AB/CD primers were used to amplify standard *Leptospira*'s sequence. Primer A, 5'-GGCGGCGCGTCTITAAACATG-3' and B, 5'-TTCCCCCATTGAGCAAGATT-3', which correspond to nucleotides 38 to 57 and 348 to 368 in the primary structure of the *L. interrogans* rrs (16S) gene, respectively and primers C, 5'-

CAAGTCAAGCGGAGTAGCAA-3' and D, 5'-CTTAACCTGCTGCCTCCCGTA-3', which correspond to nucleotides 58 to 77 and 328 to 347, respectively. PCR reactants and protocol was performed as described at Merien et al. 1992. (49,202)

In order to probe if this isolated DNA from water samples was from a pathogenic leptospiral serovar, we performed a more specific PCR, with primers G1 and G2; G1, 5'-CTGAATCGCTGTATAAAAGT-3' and G2, 5'-GGAAAACAAATGGTCGGAAG-3', derived from sequences obtained from a genomic library of *L. interrogans* serovar *icterohaemorrhagiae* were used to differentiate pathogens from environmental strains. (203)

We performed a PCR with DNA from cultured *Leptospira* to probe that the protocol was correct

6. RESULTS OF DETECTION OF LEPTOSPIRAL DNA IN FLOODING WATER

A PCR with primers A, 5'-GGCGGCGCGTCTITAAACATG-3'; B, 5'-TTCCCCCATTTGAGCAAGATT-3'; C, 5'-CAAGTCAAGCGGAGTAGCAA-3'; and D, 5'-CTTAACCTGCTGCCTCCCGTA-3' was performed demonstrating evidence of leptospiral DNA in environment. However, only two water samples amplified the DNA (Figure 6, arrows), none amplified with C-D primers (not shown).

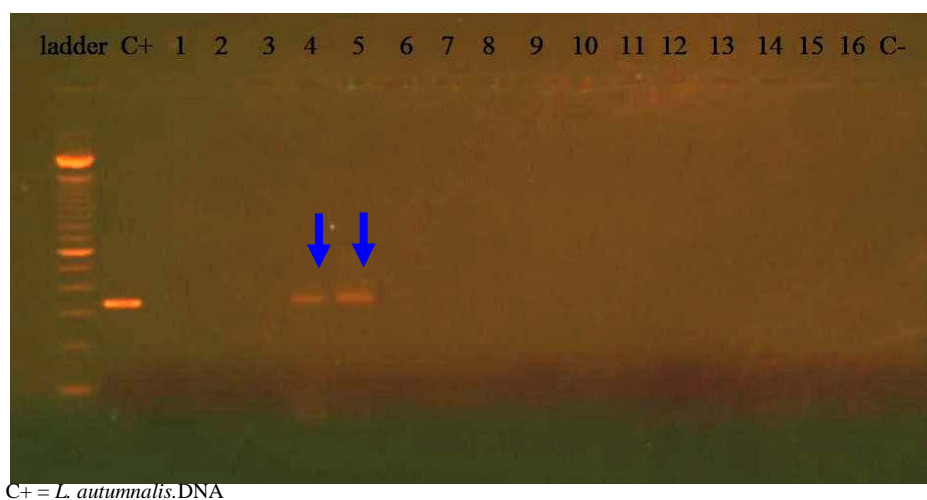


Figure 4. PCR. Presence of leptospiral DNA on residual flooding water. PCR performed with AB/CD, primers

Primers G1 and G2 show no amplification of any sample. Neither those that were positive to A-B primers (Figure 7). The PCR with DNA from cultured *leptospira* probed that the protocol was well done (Figure 8). Then, none water sample seems to be positive for pathogenic strain.

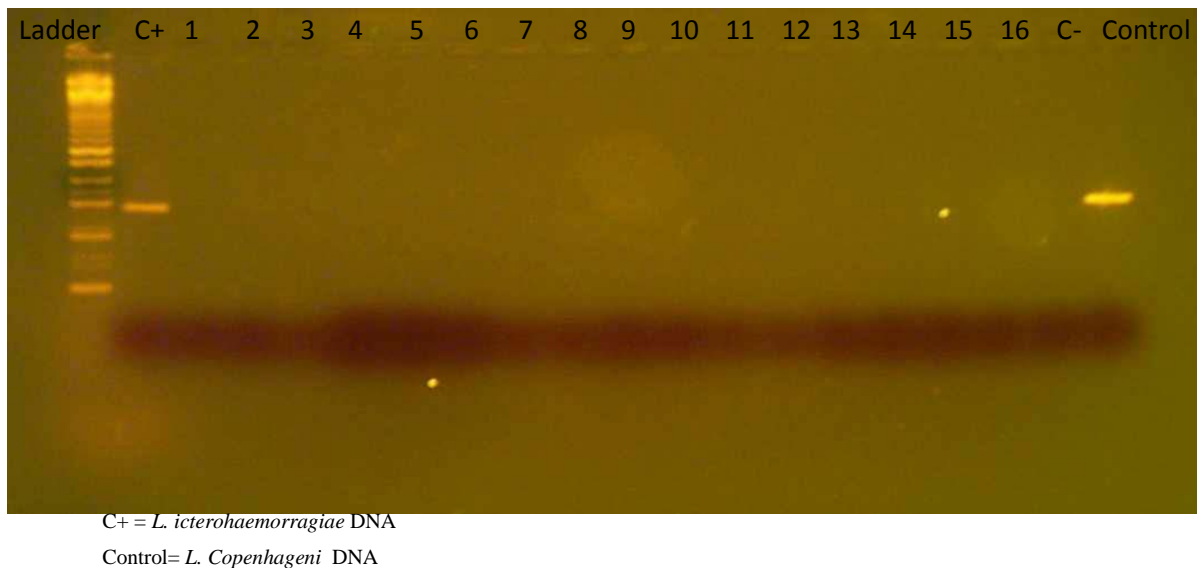


Figure 5. PCR. . PCR performed with primers G1 and G2

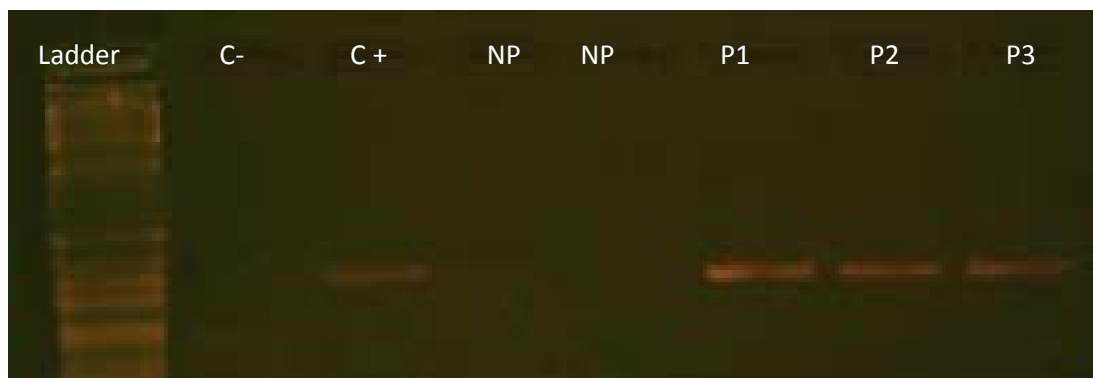


Figure 6. PCR. . PCR performed with primers G1 and G2. C- (No-DNA), C+ (DNA from *L. icterohaemorrhagiae* previously probed). NP (non pathogenic *leptospira*). P (pathogenic *Leptospira* P1. *L. interrogans*, P2 *Leptospira autumnalis*, P3 *Leptospira Copenhageni*)

7. SUPPLEMENTARY INFORMATION

7.1 ELISA Absorbances

This table shows the distribution of the analysis of the samples, the localization in the ELISA's plate of the each valid sample

SAMPLES																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A		102	156	14	162	115	56	63	201	6	167	24	165	353	22	A9	A11
B		351	157	16	163	116	57	64	103	7	171	25	166	352	138	A5	A13
C		251	158	15	118	51	58	151	105	8	170	65	A12	354	33	A3	A14
D		252	159	17	119	52	53R	152	107	9	169	66	A1	355	34	A2	A15
E		253	10	18	120	53	59	153	108	126	20	67	130	25	35	A7	A16
F	1	254	11	19	123	A	60	154	3	127	21	252	134	23	36	A10	A17
G	2	111	12	160	113	54	61	155	4	128	22	253	133	21	37	A4	A18
H	101	112	13	161	114	55	62	156F11	5	168	23	164	136	24	40	A8	A19

This table shows the index value that each sample get in the analysis of the absorbance.

INDEX VALUES																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A		0,801	0,356	0,150	0,100	0,325	1,576	0,519	1,504	0,291	0,797	0,319	0,331	0,328	0,772	0,394	0,366
B		0,457	0,910	1,007	0,172	0,206	2,436	0,425	0,066	1,811	0,131	0,310	0,141	0,275	0,088	0,413	0,231
C		0,153	0,131	0,632	0,457	0,078	2,821	0,272	0,150	0,194	0,106	0,450	1,373	0,350	0,150	2,145	0,197
D		0,453	1,073	0,156	0,103	0,119	0,632	0,266	0,066	0,866	0,178	0,169	0,544	0,131	0,097	0,782	3,399
E		0,122	0,088	0,184	0,303	0,469	4,046	0,391	0,485	0,103	0,184	0,538	0,300	0,184	0,210	0,444	0,172
F	0,747	0,225	0,319	0,535	0,119	1,041	0,463	0,341	0,222	0,084	0,194	0,331	0,241	0,381	0,591	0,281	1,295
G	5,203	0,113	0,206	0,144	0,169	0,260	0,347	0,188	0,116	0,084	0,194	0,353	0,210	0,725	0,191	0,194	1,123
H	0,344	0,172	0,247	0,303	0,288	1,735	0,913	0,510	0,197	0,103	0,322	0,144	0,188	0,569	0,053	0,128	1,207

This table shows the Panbio units of each sample.

PANBIO UNITS																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A		8,01	3,56	1,50	1,00	3,25	15,76	5,19	15,04	2,91	7,97	3,19	3,31	3,28	7,72	3,94	3,66
B		4,57	9,10	10,07	1,72	2,06	24,36	4,25	0,66	18,11	1,31	3,10	1,41	2,75	0,88	4,13	2,31
C		1,53	1,31	6,32	4,57	0,78	28,21	2,72	1,50	1,94	1,06	4,50	13,73	3,50	1,50	21,45	1,97
D		4,53	10,73	1,56	1,03	1,19	6,32	2,66	0,66	8,66	1,78	1,69	5,44	1,31	0,97	7,82	33,99
E		1,22	0,88	1,84	3,03	4,69	40,46	3,91	4,85	1,03	1,84	5,38	3,00	1,84	2,10	4,44	1,72
F	7,47	2,25	3,19	5,35	1,19	10,41	4,63	3,41	2,22	0,84	1,94	3,31	2,41	3,81	5,91	2,81	12,95
G	52,03	1,13	2,06	1,44	1,69	2,60	3,47	1,88	1,16	0,84	1,94	3,53	2,10	7,25	1,91	1,94	11,23
H	3,44	1,72	2,47	3,03	2,88	17,35	9,13	5,10	1,97	1,03	3,22	1,44	1,88	5,69	0,53	1,28	12,07

7.2 Appendix 1

HOJA INFORMATIVA

Burden of Leptospirosis in the Slums of Guayaquil,

Presencia de Leptospirosis en Barrios marginales de Guayaquil.

1) Introducción:

Leptospirosis, es una de las enfermedades adquiridas desde animales más comunes del mundo. Se puede presentar como una gripe simple o también como un problema hemorrágico que puede llevar a la muerte. El microbio vive en los riñones de los animales que lo portan como perros y ratas, sin que estos presenten enfermedad. Lo eliminan en la orina, la cual tras la lluvia, es llevada hacia ríos o reservorios de agua donde el microbio puede seguir viviendo por mucho tiempo entonces las personas que tienen contacto con el agua de estos sitios pueden contagiarse (NO SIEMPRE), es posible que exista este microbio en estas zonas de Guayaquil por estar sus casas construidas sobre sitios donde se encuentra naturalmente agua.

Existen casos confirmados de hemorragia por leptospirosis en Guayaquil que puede ser confundida con fiebre amarilla, dengue o malaria. Sin embargo no se le ha puesto mucha importancia, a pesar de existir posibilidades de contagio, como inundaciones, o falta de buenos drenajes cuando hay épocas de lluvia, aumentando la cantidad de agua donde podría estar viviendo el microbio, ya que existen animales callejeros cuyas orinas pueden estar infectadas.

2) Justificación y Objetivos de la Investigación:

Existe un gran número de cuadros clínicos asociados a leptospirosis en Guayaquil que es ampliamente mal reconocida, o mal diagnosticada, debido a que no es un problema de reporte obligatorio a nivel Ministerial, no hay un diagnóstico relevante y la presentación clínica puede no ser reconocida y confundida por los clínicos.

3) Metodología empleada

a) Pacientes que se considere pueden presentar Leptospirosis de los barrios marginales de Guayaquil, así como del Hospital de Infectología.

b) El estudio dura aproximadamente 5 meses, a partir de Diciembre del 2007 hasta Abril del 2008

c) Se recolectarán muestras de gotas de sangre mediante una lanceta, haciendo punción (pinchazo) sobre el pulpejo de un dedo de la mano, y se coloca esta muestra sobre un papel filtro.

d) Es un estudio, descriptivo, epidemiológico, que busca encontrar la prevalencia, e incidencia de la patología.

4) Procedimientos y su propósito:

a) Entrevistas

b) Cuestionarios

c) Muestras para el laboratorio, extracción de muestras de sangre por punción en el dedo de la mano, para el análisis de la presencia del patógeno.

d) Muestras complementarias en caso de ser necesarias con autorización y consentimiento informado

5) Incomodidades y riesgos derivados del estudio:

a) Es un procedimiento inocuo, solo conlleva la punción del pulpejo del dedo, sin mayor relevancia o posibles complicaciones del procedimiento

b) No existe tampoco, la posibilidad del uso de los datos personales de cada paciente para otras circunstancias, excepto que para control epidemiológico.

6) Beneficios derivados del estudio:

a) No existen beneficios individuales, el beneficio sería colectivo, pues si la sospecha de que existe mayor cantidad de casos de leptospira que lo que se diagnostica, se podría implementar un sistema de vigilancia epidemiológica con el fin de reducir los casos.

7) Privacidad y Confidencialidad

a) Todos los datos proporcionados serán manejados con total confidencialidad por las personas implicadas en el estudio.

8) Participación voluntaria y Retiro del Estudio

a). Es voluntaria su participación, así como la posibilidad de retirarse del estudio en cualquier momento, sin que ello acarree una sanción o la pérdida de cualquier beneficio que exista.

b). Todos los hallazgos nuevos que se descubran durante el transcurso del estudio, que pudieran afectar su deseo de seguir participando, deberán ser comunicados al sujeto en investigación.

c). El paciente tiene todo el derecho de negarse a la participación si el encuestador o entrevistador intenta usar métodos de persuasión que involucren falta al respeto a la moral o ética

7.3 Appendix 2

Impacto de la Leptospirosis en Barrios Marginales de Guayaquil

Fecha de entrevista	mes	dia	año
Lugar de la Entrevista:			
Entrevistador:			

I. IDENTIFICACIÓN:

1.1	Nombre del paciente		
1.2	Edad:		
1.3	Fecha de nacimiento:	mes	dia
			año
1.4	Sexo: Masculino <input type="checkbox"/> Femenino <input type="checkbox"/>		
1.5	Dirección del domicilio:		

II. PRESENTACION CLINICA

2.1	Cuántos días ha estado enfermo (a)?		
2.2	Febre:	Temperatura	
	Si <input type="checkbox"/>	No <input type="checkbox"/>	
2.3	Tomó antibiótico?		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No sabe <input type="checkbox"/>
2.4	Ictericia:		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	
2.5	Sufusión Conjuntival:		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	
2.6	Dificultad respiratoria:		

	Si <input type="checkbox"/>	No <input type="checkbox"/>	
2.7	Hemoptisis:		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	
2.8	Frecuencia Respiratoria:		
2.9	Oliguria		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No sabe <input type="checkbox"/>
2.10	Dolor muscular/articular		Localización del dolor
	Si <input type="checkbox"/>	No <input type="checkbox"/>	
2.11	Dolor de cabeza		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	
2.12	De acuerdo a los síntomas usted sospecha de:		
	Dengue <input type="checkbox"/>	Leptospirosis <input type="checkbox"/>	Malaria

III. Factores de riesgo:

3.1	Su casa esta construída sobre agua?:
	Si <input type="checkbox"/> No <input type="checkbox"/>

3.2	<p>En que trabaja usted?:</p> <p>a. Alcantarilla desagües Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. Construcción Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>c. Vendedor ambulante Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>Otro trabajo:</p> <p>d. Agricultura/ganadería Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>e. Mecánico Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>f. estudia Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.3	<p>Trabaja en el barrio que vive?</p> <p>Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.4	<p>DURANTE LAS ÚLTIMAS TRES SEMANAS USTED ESTUVO :</p> <p>EN CONTACTO CON LODO</p> <p>a. cerca de casa? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. en el trabajo? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>En caso afirmativo</p> <p>Usó guantes o botas? Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.5	<p>EN CONTACTO CON BASURA</p> <p>a. cerca de casa? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. en el trabajo? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>En caso afirmativo</p> <p>Usó guantes o botas? Si <input type="checkbox"/> No <input type="checkbox"/></p>

3.6	<p>EN CONTACTO CON AGUA DE INUNDACION</p> <p>a. cerca de casa? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. en el trabajo? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>En caso afirmativo</p> <p>Usó guantes o botas? Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.7	<p>EN CONTACTO CON AGUA DE ALCANTARILLA</p> <p>a. cerca de casa? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. en el trabajo? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>En caso afirmativo</p> <p>Usó guantes o botas? Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.8	<p>Entró a una alcantarilla?</p> <p>a. cerca de casa? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. en el trabajo? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>En caso afirmativo</p> <p>Usó guantes o botas? Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.9	<p>Se inundó su casa durante la lluvia?</p> <p>Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.10	<p>Se inundó la calle cercana a su casa por la lluvia?</p> <p>Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.11	<p>Se inundó su lugar de trabajo</p> <p>Si <input type="checkbox"/> No <input type="checkbox"/></p>

7.4 Appendix 3

INSTRUCTIVO PARA LLENAR LA ENCUESTA

I. CAPACITACIÓN DE ENCUESTADORES.

El personal destinado para las encuestas, será escogido mediante un proceso previo de selección. El proceso de capacitación será realizado mediante presentaciones y demostraciones prácticas de la encuesta. Preferentemente sería llenado en el momento de la Anamnesis.

La guía será entregada a los encuestadores con el fin de facilitar el proceso de llenado del cuestionario adjunto.

RESPONSABILIDAD

Encuestadores:

Su función será la de llenar el cuestionario adjunto, además de recolectar las muestras de gota seca de sangre utilizando el procedimiento detallado.

Se debe recordar que las encuestas están encaminadas a recolectar información sobre los factores de riesgo que nos inviten a sospechar en el diagnóstico de Leptospirosis, por tal razón es de gran importancia que se llene correctamente el cuestionario.

GUIA

NO olvidar poner el número correspondiente a la encuesta y que corresponde al número de muestra.

CUESTIONARIO N#

los pacientes no manejan muchas veces los términos médicos, por tal razón debe explicárselos de manera concisa y en lenguaje común, sin embargo es el personal de salud que está recogiendo la encuesta quien llene los espacios en este apartado, pues involucra criterio médico y diagnóstico presuntivo. La temperatura y frecuencia respiratoria son tomados en el momento de la encuesta.

2.1	Cuántos días ha estado enfermo (a)?	
2.2	Fiebre: Si <input type="checkbox"/> No <input type="checkbox"/>	Temperatura
2.3	Tomó antibiótico? Si <input type="checkbox"/> No <input type="checkbox"/> No sabe <input type="checkbox"/>	
2.4	Ictericia: Si <input type="checkbox"/> No <input type="checkbox"/>	
2.5	Sufusión Conjuntival: Si <input type="checkbox"/> No <input type="checkbox"/>	
2.6	Dificultad respiratoria: Si <input type="checkbox"/> No <input type="checkbox"/>	
2.7	Hemoptisis: Si <input type="checkbox"/> No <input type="checkbox"/>	
2.8	Frecuencia Respiratoria :	
2.9	Oliguria Si <input type="checkbox"/> No <input type="checkbox"/> No sabe <input type="checkbox"/>	
2.10	Dolor muscular/articular Si <input type="checkbox"/> No <input type="checkbox"/>	Localización del dolor
2.11	Dolor de cabeza Si <input type="checkbox"/> No <input type="checkbox"/>	
2.12	De acuerdo a los síntomas usted sospecha de:	

	Dengue		Leptospirosis		Malaria
--	--------	--	---------------	--	---------

III. Factores de riesgo:

El siguiente apartado, nos ayudará a investigar cuales son las condiciones de vida más prevalentes que involucran el riesgo de la enfermedad, son realmente importantes que se llene por completo este apartado, sin embargo, las respuestas pueden ser variables, o pueden existir casilleros que queden vacíos, por ejemplo, el apartado 3.12 averigua sobre la presencia de ratas, si la respuesta es negativa, los apartados, 3.13 y 3.14 se anulan automáticamente.

Las preguntas que tengan como respuesta si o no, no deben contener aclaraciones extras, cuando existe la posibilidad de añadir algún dato, realizarlo de forma clara y consisa. No poner más datos de los requeridos.

En el apartado 3.2, acerca del trabajo del paciente encuestado, puede existir la posibilidad de anotar otra ocupación, sin embargo debe relacionarse con la patología a investigar, y que preferentemente tenga factores de riesgo de desarrollo de leptospirosis y/o dengue, (que pueda tener contacto con agua contaminada) Ej. Recolector de Basura, Bombero.

Los demás acápite se llenarán de acuerdo a su contenido.

3.1	<p>Su casa esta construída sobre agua?:</p> <p style="text-align: center;">Si <input type="checkbox"/> No <input type="checkbox"/></p>
-----	---

3.2	<p>En que trabaja usted?:</p> <p>a. Alcantarilla desagües Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. Construcción Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>c. Vendedor ambulante Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>Otro trabajo:</p> <p>d. Agricultura/ganaderia Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>e. Mecanico Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>f. estudia Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.3	<p>Trabaja en el barrio que vive?</p> <p>Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.4	<p>DURANTE LAS ULTIMAS TRES SEMANAS USTED ESTUVO :</p> <p>EN CONTACTO CON LODO</p> <p>a. cerca de casa? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. en el trabajo? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>En caso afirmativo</p> <p>Usó guantes o botas? Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.5	<p>EN CONTACTO CON BASURA</p> <p>a. cerca de casa? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. en el trabajo? Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>En caso afirmativo</p> <p>Usó guantes o botas? Si <input type="checkbox"/> No <input type="checkbox"/></p>

3.6	EN CONTACTO CON AGUA DE INUNDACION			
	a. cerca de casa?	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	b. en el trabajo?	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	En caso afirmativo			
3.7	EN CONTACTO CON AGUA DE ALCANTARILLA			
	a. cerca de casa?	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	b. en el trabajo?	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	En caso afirmativo			
	Usó guantes o botas?			
		Si	<input type="checkbox"/>	No <input type="checkbox"/>

DURANTE LAS ULTIMAS TRES SEMANAS

3.8	Entró a una alcantarilla?			
	a. cerca de casa?	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	b. en el trabajo?	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	En caso afirmativo			
3.9	Se inundó su casa durante la lluvia?			
		Si	<input type="checkbox"/>	No <input type="checkbox"/>
3.10	Se inundó la calle cercana a su casa por la lluvia?			
		Si	<input type="checkbox"/>	No <input type="checkbox"/>
3.11	Se inundó su lugar de trabajo			
		Si	<input type="checkbox"/>	No <input type="checkbox"/>

3.12	<p>Ha visto ratas ultimamente?</p> <p>a. Cerca de la casa Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. Cerca del trabajo Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.13	<p>Cuántas ratas ha visto juntas?</p> <p>a. Cerca de la casa? _____</p> <p>b. En el trabajo?</p>
3.14	<p>Ha visto ratas caminando durante el día?</p> <p>a. Cerca de la casa Si <input type="checkbox"/> No <input type="checkbox"/></p> <p>b. En el trabajo Si <input type="checkbox"/> No <input type="checkbox"/></p>
3.15	<p>Tienen perro en la casa o los perros de su vecino visitan su casa?</p> <p>No <input type="checkbox"/> Si <input type="checkbox"/></p>
3.16	<p>Tienen gato en casa?</p> <p>No <input type="checkbox"/> Si <input type="checkbox"/></p>
3.17	<p>Tiene gallinas en casa?</p> <p>No <input type="checkbox"/> Si <input type="checkbox"/></p>
3.18	<p>Tiene otro animal en la casa?</p>

7.5 Appendix 4

DECLARACIÓN DE CONSENTIMIENTO INFORMADO

Yo, _____, de ____ años de edad, con CI N° _____, manifiesto que me han explicado y entiendo la importancia que tiene mi contribución a este estudio, para investigar la presencia de Leptospirosis (enfermedad grave transmitida por ratas y perros) y Dengue (enfermedad transmitida por mosquitos).

He sido informado/a de que mis datos personales no serán entregados a nadie.

También se me ha indicado que mi colaboración es voluntaria y que puedo decidir no participar.

ACEPTO donar tres gotas de mi sangre para este estudio y llenar la encuesta solicitada.

Firma Paciente

CI:

Firma Investigador

CI:

Fecha:

Cualquier pregunta favor dirigirse a:

Dr. Andrés Abril G, e-mail: aabril@usfq.edu.ec,

Dirección: Diego de Robles y Pampite, Círculo de Cumbayá(Universidad San Francisco de Quito)

Teléfono: (593-2) 297-1700, 297-1701, 297-1702, 297-1703, 297-1704, 297-1705, 297-1706, 297-1707, 297-1708

Ext. 1234

7.6 Appendix 5

Indicaciones y responsabilidades para el Investigador al momento de obtener el consentimiento informado

- La Obtención del consentimiento informado para la participación de sujetos humanos en investigación biomédica es el aspecto más importante y crítico a la vez, en el reclutamiento de voluntarios, y se debe tener presente que constituye un proceso interactivo y dinámico que no termina con la firma del consentimiento informado si no que se prolonga durante todo el estudio.
- Al elaborar el consentimiento informado, el investigador debe usar un lenguaje claro y sencillo, de preferencia con expresiones usadas comúnmente en nuestro país para mencionar enfermedades o situaciones especiales, que aclaren o faciliten el entendimiento del estudio.
- Obtener el consentimiento informado del candidato voluntario que participará en la investigación o, en caso de que la persona carezca de capacidad de dar su consentimiento informado, el consentimiento podrá obtenerse por poder de un representante debidamente autorizado.
- Proporcionar al candidato voluntario, toda la información relevante respecto al estudio que se va a realizar para que pueda dar su consentimiento sin dudas ni temores.
- Ofrecer al candidato voluntario, amplias oportunidades de hacer preguntas con respecto a sus dudas y temores, estar dispuesto y seguro a contestarlas, de manera clara y amable.
- Asegurarse que el posible voluntario comprenda esta información. Los investigadores pueden solicitar a los participantes que discutan la información que han recibido, se pueden hacer las siguientes preguntas: ¿Me puede decir cuál es el propósito de nuestro estudio? ¿Cuáles son los riesgos del estudio?
- Darle el tiempo suficiente para discutir su participación si él lo desea, con familiares o médico de cabecera y poder tomar su propia decisión.

- Excluir toda posibilidad de persuasión encubierta injustificada, influencia indebida o intimidación, no ofrecer estímulos que posteriormente no se los podrá cumplir.
- El presunto voluntario o su representante autorizado debe firmar el documento que acredite su consentimiento y debe entregársele una copia del mismo.
- Renovar el consentimiento informado de cada participante si las condiciones o procedimientos de la investigación sufren modificaciones importantes.
- Se debe tener especial atención para cubrir las necesidades de poblaciones vulnerables como los niños, los enfermos en estado crítico, mentalmente incapacitados o las comunidades nativas, para modificación del lenguaje y procedimientos a realizar.

7.7 Appendix 6

Impacto de la Leptospirosis en Barrios Marginales de Guayaquil

Fecha	mes	día	año
Historia Clínica :			
Investigador:			

I. IDENTIFICACIÓN:

1.1	Nombre del paciente		
1.2	Edad:		
1.3	Fecha de nacimiento:	mes	día
1.4	Sexo: Masculino <input type="checkbox"/> Femenino <input type="checkbox"/>		
1.5	Dirección del domicilio(si es recuperable)		

II. PRESENTACION CLINICA

2.1	Cuántos días ha estado enfermo (a)?		
2.2	Fiebre:	Temperatura	
	Si <input type="checkbox"/>	No <input type="checkbox"/>	
2.3	Tomó antibiótico?		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
2.4	Ictericia:		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
2.5	Sufusión Conjuntival:		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>

2.6	Dificultad respiratoria: Si <input type="checkbox"/> No <input type="checkbox"/> No Hay datos <input type="checkbox"/>
2.7	Hemoptisis: Si <input type="checkbox"/> No <input type="checkbox"/> No Hay datos <input type="checkbox"/>
2.8	Frecuencia Respiratoria:
2.9	Oliguria Si <input type="checkbox"/> No <input type="checkbox"/> No Hay datos <input type="checkbox"/>
2.10	Dolor muscular/articular <input type="checkbox"/> Si <input type="checkbox"/> No <input type="checkbox"/> Localización del dolor
2.11	Dolor de cabeza Si <input type="checkbox"/> No <input type="checkbox"/> No Hay datos <input type="checkbox"/>
2.12	Diagnóstico inicial: Dengue <input type="checkbox"/> Leptospirosis <input type="checkbox"/> Malaria

III. DIAGNÓSTICO Y TRATAMIENTO

3.1	Métodos Diagnósticos
3.2	Presuntivo: Si <input type="checkbox"/> No <input type="checkbox"/> Criterios
3.3	Confirmatorio por laboratorio? Si <input type="checkbox"/> No <input type="checkbox"/> No hay datos <input type="checkbox"/>

3.4	Seguimiento:	
	Si <input type="checkbox"/>	No <input type="checkbox"/>
3.5	MEDIDAS FARMACOLÓGICAS?	
3.6	Hospitalización?	
	Si <input type="checkbox"/>	No <input type="checkbox"/> <input type="checkbox"/>
3.7	Farmacoterapia?	Medicamento.
	Si <input type="checkbox"/>	No <input type="checkbox"/>
3.8	Terapia Múltiple	
	Si <input type="checkbox"/>	No <input type="checkbox"/>
3.9	Diagnóstico confirmado	
	Dengue <input type="checkbox"/>	Leptospirosis <input type="checkbox"/> Malaria

IV. EVOLUCIÓN CLINICA

4.1	Cuántos días de seguimiento	
4.2	Fracaso terapéutico	Conducta
	Si <input type="checkbox"/>	No <input type="checkbox"/>
4.3	Complicaciones	
	Si <input type="checkbox"/>	No <input type="checkbox"/> No Hay datos <input type="checkbox"/>
4.4	Nueva Hospitalización	

	Si <input type="checkbox"/>	No <input type="checkbox"/>
4.5	Transferencia:	
	Si <input type="checkbox"/>	No <input type="checkbox"/>
4.6	Muerte:	
	Si <input type="checkbox"/>	No <input type="checkbox"/>

V. Factores de riesgo: "Solo si existen"

5.1	Casa esta construída sobre agua?:			
	Si	<input type="checkbox"/>	No	<input type="checkbox"/>
5.2	Trabajo:			
	a. Alcantarilla desagües	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	b. Construcción	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	c. Vendedor ambulante	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	Otro trabajo:			
	d. Agricultura/ganadería	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	e. Mecánico	Si	<input type="checkbox"/>	No <input type="checkbox"/>
	f. estudia	Si	<input type="checkbox"/>	No <input type="checkbox"/>

5.4	EN CONTACTO CON LODO		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
5.5	EN CONTACTO CON BASURA		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
5.6	EN CONTACTO CON AGUA DE INUNDACION		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
5.7	EN CONTACTO CON AGUA DE ALCANTARILLA		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
5.8	Tienen perro en la casa?		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
5.9	Tienen gato en casa?		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
5.10	Tiene gallinas en casa?		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>
5.11	Tiene otro animal en la casa?		
	Si <input type="checkbox"/>	No <input type="checkbox"/>	No Hay datos <input type="checkbox"/>

7.8 Appendix 7

MANUAL DE PROCEDIMIENTOS

I. CAPACITACIÓN DE TOMA DE MUESTRAS SEROLÓGICAS

El personal destinado para la toma de muestras serológicas será escogido mediante un proceso previo de selección. El proceso de capacitación será realizado mediante presentaciones y demostraciones prácticas de las guías de procedimientos. El entrenamiento para la recolección, cuidado y almacenamiento de los especímenes de colectados en conjunto con las encuestas escritas será dirigido a supervisores de área y entrevistadores. Mientras que el entrenamiento para el proceso utilizado en la recolección, cuidado y almacenamiento de muestras de laboratorios será dirigido a técnicos de laboratorio y a los supervisores de área.

Posterior al entrenamiento, se realizarán entrevistas prácticas en grupos pequeños con el objetivo de escoger al personal idóneo. La correspondiente guía de procedimientos será entregada a cada uno de los supervisores y entrevistadores elegidos.

RESPONSABILIDAD DE CADA UNA DE LAS PERSONAS INVOLUCRADAS DURANTE EL PROCESO DE RECOLECCION DE LAS MUESTRAS

Recolectores de muestras de laboratorio:

Deberán recorrer cada uno de los laboratorios asignados recolectando y preparando las muestras de sangre. Para el proceso de recolección deberán seguir el protocolo descrito en la guía de procedimientos detallada a continuación. Durante el proceso de recolección asignarán un código a cada muestra. Finalmente deberán entregar las muestras al supervisor de área.

Encuestadores:

Su función será la de recolectar las muestras de gota seca de sangre utilizando el procedimiento detallado a continuación, el tiempo necesario para recolectar cada muestra se estima en 5 minutos. En este tiempo, deben cambiar de guantes a un par nuevo, tomar la muestra, rotularla con el código asignado y colocarla cuidadosamente en la caja destinada para este fin (detallado a continuación en la guía de procedimientos).

Supervisores de área:

Dependiendo de la cantidad de muestras, pueden existir supervisores de área, caso contrario, solo existirán encuestadores. Los supervisores estarán capacitados para realizar un control de calidad (detallado a continuación en la sección de control de calidad) y almacenamiento (detallado a continuación en la guía de procedimientos) tanto de las muestras de laboratorio como las muestras recolectadas durante las entrevistas. Las muestras de gota de sangre seca serán mantenidas en un lugar seco y enviadas en un sobre al laboratorio en Quito. El supervisor deberá llevar un control de las muestras recibidas con la identificación respectiva de los encuestadores y recolectores de muestras de laboratorio.

GUIAS DE PROCEDIMIENTOS

Incluyen todo el proceso desde la toma de muestra, cuidados y precauciones, hasta su almacenamiento y envío al laboratorio de la USFQ en donde serán procesadas. Se llenará un formulario durante la toma de muestras, esto permitirá llevar un orden además de ser parte del control de calidad de las muestras.

MUESTRAS DE SANGRE DE LABORATORIOS

Recolección y preparación de muestra

Las muestras de sangre deben estar en tubos con anticoagulante (como la heparina, citrato o EDTA). Generalmente estos tubos tienen tapa morada o tapa verde. La muestra de sangre no debe ser centrifugada.

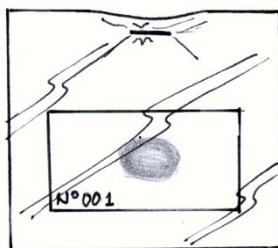
1. Ponerse guantes antes de manipular los tubos
2. Antes de iniciar colocar el código de la muestra en el extremo derecho del papel filtro.
3. Agitar el tubo en donde se encuentra la sangre de tal manera que la sangre se mezcle bien.
4. Con la pipeta plástica nueva, se debe tomar una pequeña cantidad de sangre y se deja caer la sangre (gota por gota) sobre el círculo marcado en el papel filtro. De tres a cinco gotas serán depositadas hasta llenar el círculo que se encuentra dibujado en el papel filtro.
5. PARA CADA MUESTRA DE SANGRE SE DEBE USAR UNA PIPETA PLASTICA DIFERENTE. LUEGO QUE SE HA USADO UNA PIPETA PLASTICA, ESTA DEBE SER COLOCADA EN EL RECIPIENTE DE BASURA CONTAMINADA.

6. Tener mucho cuidado en que no salpique sangre de un tubo en el papel filtro porque se pueden mezclar sangres diferentes. Si esto llegara a ocurrir, desechar el papel filtro y repetir el proceso utilizando un papel filtro nuevo.
7. Los papeles filtro con la gota de sangre deben ser luego secados a temperatura ambiente por 4 horas (24 horas en la costa) en posición horizontal. Utilizando un alfiler, sujetar cada muestra sobre la lámina de corcho que se encuentra en la caja.

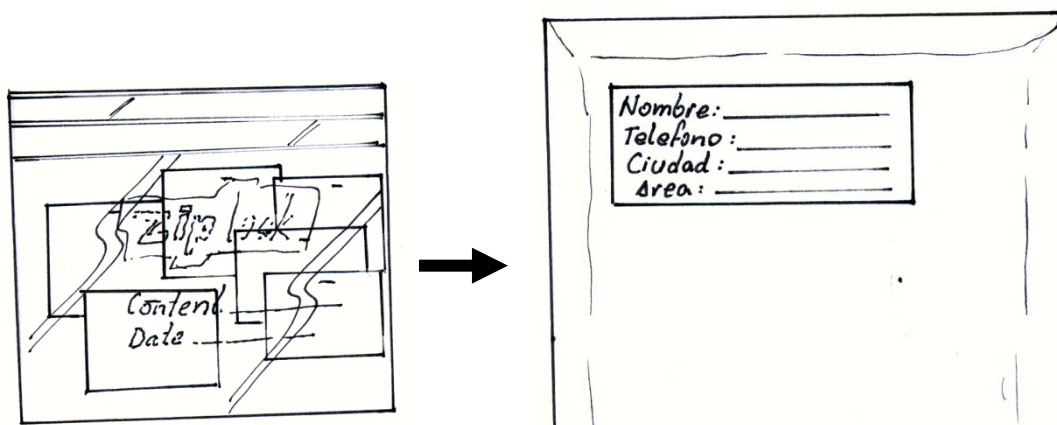
Como se guardan las muestras

1. Una vez secas las muestras se coloca cada muestra en una funda de papel encerado, de tal manera que cada fundita contenga un solo papel filtro. Grapar el extremo superior de la fundita de tal manera que el papel filtro no salga de ella en ningún caso. Las muestras deben estar completamente secas antes de ser guardadas, de lo contrario hay el riesgo de que se contaminen.

CUIDADO: Colocar el papel filtro con la muestra en el lugar designado para este propósito y no permitir que éstos toquen otras superficies ni durante ni después de que se sequen.



2. Finalmente, las muestras serán colocadas en una funda plástica junto con una fundita de silica. Cada funda plástica “zip lock” contendrá entre 15 a 20 muestras y será colocada dentro de un sobre de Manila. El sobre será entregado a cada uno de sus supervisores de área quienes son los encargados de revisar y enviar las muestras a al laboratorio de Quito.



3. La siguiente información debe ser enviada junto a las muestras.

Nombre del laboratorio: _____

Provincia: _____

Ciudad: _____

Nombre del supervisor : _____ tlf: _____

Nombre de la persona que colecta la muestra _____ tlf _____

Código de muestra	Observación

Precauciones a tener durante la recolección y el manejo de la muestra

1. La toma de muestras solo debe llevarse a cabo por personal entrenado.
2. Usar un par de guantes durante todo el procedimiento. El uso de guantes disminuye la incidencia de contaminación con sangre.
3. No lavar los guantes con agentes desinfectantes, esto puede causar la penetración de líquidos por agujeros imperceptibles.
4. No manipular las muestras sin guantes.
5. Todos los materiales contaminados con sangre deben ser eliminados previa neutralización por medios térmicos o con una solución de hipoclorito de sodio al 0.5%.

Desechar el material corto punzante en el sitio destinado con este propósito, no ponga en el tarro de basura.

.MUESTRAS RECOLECTADAS DURANTE LAS ENCUESTAS ESCRITAS: GOTA DE SANGRE SECA

Para tomar la muestra de sangre se deben seguir los pasos que se describen a continuación.

Preparación del Accu Check Softclix

El Accu Check Softclix es un punzador, que sirve para pinchar el dedo y obtener la muestra de sangre de cada una de las muestras. Cada vez que se desee obtener una muestra, se debe cambiar de aguja, por ningún motivo se utilizará la misma aguja para obtener una muestra de sangre de dos personas diferentes.

1. Ajustar la profundidad del pinchazo: Con la parte delantera de la tapa del Accu-Chek Softclix puede ajustar la profundidad del pinchazo a su propio espesor de piel.



2. Sacar la tapa: A continuación, saque la tapa del Accu-Chek Softclix para poder colocar la lanceta Accu-Chek Softclix II.



3. Colocar la lanceta: Coloque la lanceta hasta escuchar un claro clic. Quite luego la cobertura de protección de la lanceta y vuelva a colocar la tapa del Accu-Chek Softclix. Fíjese en la pequeña ranura ubicada en el borde blanco de la tapa y asegúrese que esté orientada hacia el centro del agujero en forma U en el punzador.



4. Tensar el Accu-Chek Softclix: Presione el botón en el lado posterior del Accu-Chek Softclix para tensar el punzador. Puede controlar que esté tensado comprobando si el botón destensador tiene un color amarillo.



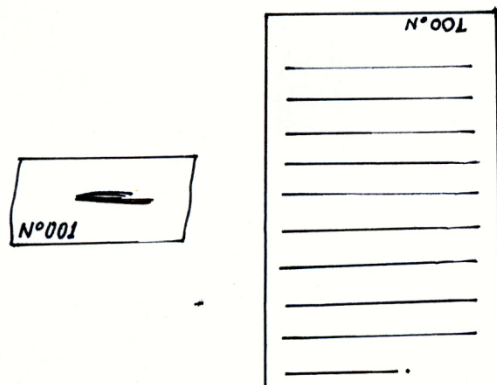
5. Sacar sangre: Antes de pinchar es importante lavarse las manos con agua caliente y luego secarlas bien. Para pinchar, elegir de preferencia el costado de la yema del dedo índice o anular. Coloque el Accu-Chek Softclix contra su dedo y presione el botón destensador amarillo.



6. Expulsar la lanceta: Para evitar infecciones y para seguir pinchando con el menor dolor posible, le recomendamos usar la lanceta sólo una vez. Después de utilizarla puede expulsar la lanceta en un contenedor de basura. Para eso, presione la parte posterior del Accu-Chek Softclix y al mover el mecanismo de expulsión hacia adelante, la lanceta usada caerá.

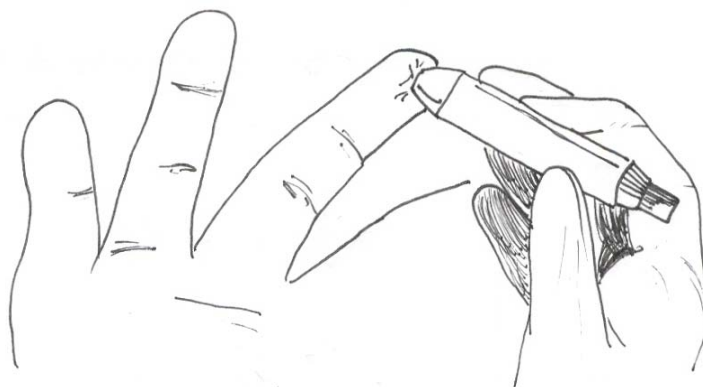


Recolección de muestra



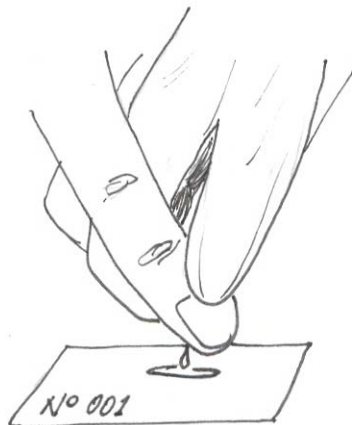
Antes de recolectar la muestra, el papel filtro debe ser rotulado con el mismo código que se encuentra en la esquina superior de la encuesta que el paciente está llenando.

Las muestras deben ser tomadas del pulpejo digital anular. Para obtenerla se debe masajear hacia arriba de tal manera que la sangre se acumule en la yema del dedo.



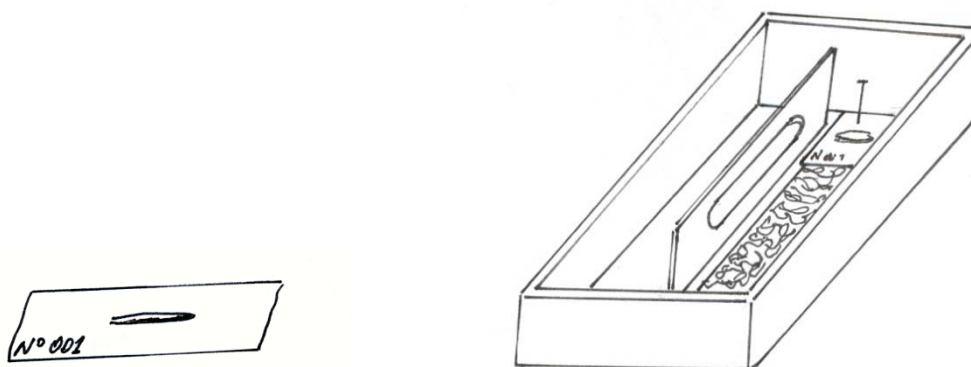
A continuación se limpia el pulpejo del dedo con alcohol isopropílico o metanol al 70% y se procede a punzar con la lanceta estéril. La primera gota debe ser eliminada limpiándola con una torunda. Si el pinchazo inicial es inadecuado, no pinchar nuevamente en el mismo sitio, otro dedo debe ser utilizado y se debe cambiar a una nueva aguja.

Nota: descartar la aguja utilizada en el recipiente marcado para “desechos cortopunzantes”. Por ningún motivo se debe reutilizar esta aguja para realizar un nuevo pinchazo.



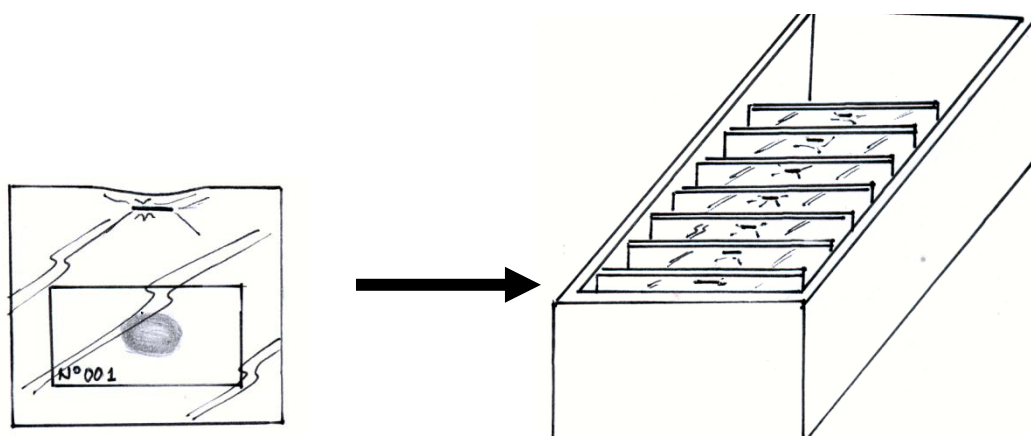
Las siguientes tres a cinco gotas serán depositadas hasta llenar el círculo que se encuentra dibujado en el papel filtro. Una buena muestra debe dar como resultado una circunferencia saturada de sangre, de 1.5 cm de diámetro. **CUIDADO:** No intentar esparcir la gota ni topar el papel filtro con el dedo. No utilizar los dos lados del papel filtro para colectar la muestra.

Almacenamiento y envío de muestra

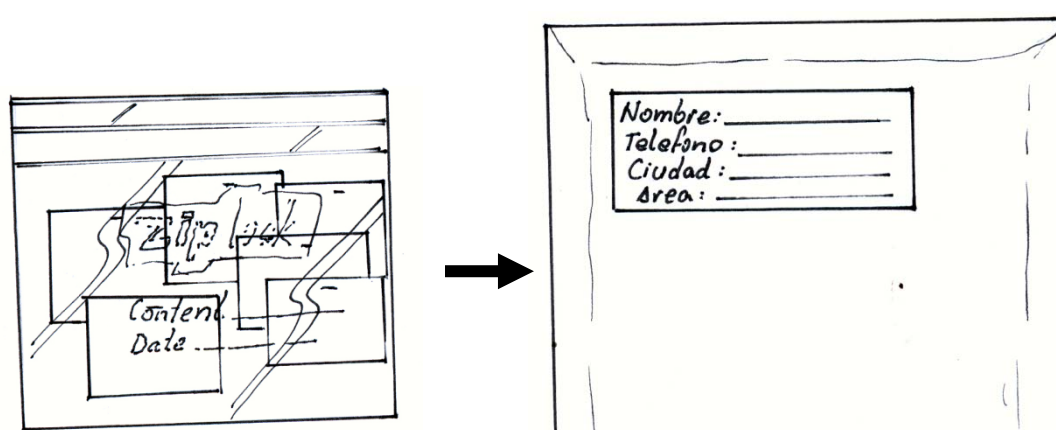


Los papeles filtro con la gota de sangre deben ser luego secadas a temperatura ambiente por 4 horas (24 horas en zonas húmedas) en posición horizontal. Utilizando un alfiler, sujetar cada muestra sobre la tira de corcho ubicada en la caja de trabajo que se les proveerá. **CUIDADO:** Colocar el papel filtro con la muestra en el lugar designado para

este propósito y no permitir que éstos toquen otras superficies ni durante ni después de que se sequen.



Una vez transcurrido el tiempo de secado, cada tira de papel filtro con la gota de sangre completamente seca, será introducida en una funda de papel encerado. La funda plástica debe ser grapada en la parte superior para que no se salga la muestra, de tal manera que no queden herméticamente cerradas. Solo se podrá colocar una muestra en cada fundita. Todo esto con el objetivo de que la muestra no se contamine ni tenga contacto con las demás muestras. Las muestras deben estar completamente secas antes de ser guardadas, de lo contrario hay el riesgo de que se contaminen.



Luego, las muestras serán colocadas en una funda plástica junto con una fundita de silica (la cual deberá ser perforada con un alfiler antes de ser introducida en la funda plástica). Cada funda plástica "zip lock" contendrá entre 15 a 20 muestras (cada una previamente

colocada en una funda pequeña) y será colocada dentro de un sobre de Manila. El sobre será entregado a cada uno de sus supervisores de área quienes son los encargados de revisar y enviar las muestras a al laboratorio de la USFQ en Cumbayá - Quito.

Precauciones y cuidados a tener durante la recolección de la muestra

1. La toma de muestras solo debe llevarse a cabo por personal entrenado.
2. Usar un par de guantes por cada paciente. El uso de guantes disminuye la incidencia de contaminación con sangre.
3. No lavar los guantes con agentes desinfectantes, esto puede causar la penetración de líquidos por agujeros imperceptibles.
4. No manipular las muestras secas sin guantes.
5. Desechar el material corto punzante en el sitio destinado con este propósito, no en el tarro de basura cotidiana.
6. Todos los materiales contaminados con sangre deben ser eliminados previa neutralización por medios térmicos o con una solución de hipoclorito de sodio al 0.5%, por esta razón los desechos deben ser llevados al laboratorio indicado para que allí sean decontaminados.

II. ANÁLISIS DE LABORATORIO

Para el análisis de laboratorio se utilizará el kit comercial IGM Elisa **Leptospirosis y/o Dengue** (PanBio, Australia). Según las especificaciones del fabricante.

III. CONTROL DE CALIDAD

Control de calidad del trabajo de campo

1. Los datos del entrevistador (Nombre, teléfono, ciudad, provincia y área) serán enviados con cada sobre para poder identificar el origen de las muestras.
2. En el caso de las muestras de suero o plasma provenientes de laboratorios, se deberá llenar un formulario que provea de el nombre del laboratorio, los códigos de las muestras y el nombre del responsable.
3. Cada encuestador, revisará la calidad y cantidad de las muestras, así como la rotulación de cada una.

4. El responsable deberá tener un registro con los códigos de todas las muestras a su cargo.
5. Una vez chequeadas las muestras, el responsable, las empacará y las enviará al laboratorio para su procesamiento.

IV. ANALISIS Y ENTREGA DE DATOS

Los datos serán analizados y entregados en una tabla en donde se especifique el código de cada muestra con su respectivo resultado.

7.9 BIBLIOGRAFÍA

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