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**Original Research**

# Clinical diagnosis of leptospirosis with negative serology: case series report

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## Abstract
Leptospirosis is a zoonotic disease with clinical manifestations that range from mild acute febrile illness that resolves on its own to severe, life-threatening conditions with multiple organ dysfunction. Clinical features and various diagnostic tests establish leptospirosis diagnosis, such as bacterial isolation and serological examination. Because the antibody response required for serological testing is often insufficient to detect until the second week of disease (when the immune phase begins), sensitivity, when symptoms begin, is limited. Often, patients present with obvious clinical symptoms of leptospirosis but have negative serological test results. This study aimed to determine how to establish the clinical diagnosis of leptospirosis in leptospirosis cases with negative serology test results. This study used a descriptive method with a case series research design that analyzed four series of leptospirosis cases. The result of this study is that some cases of leptospirosis have obvious symptoms, but serological examination shows negative results. This can happen because new antibodies can be detected on day 6 to day 10 of the illness and reach their peak in 3-4 weeks so that the establishment of a clinical diagnosis of leptospirosis can be established from clear clinical symptoms and other laboratory tests. Establishing the clinical diagnosis of leptospirosis with negative serology can be done with the Faine scoring system.

**Keywords**: clinical suspicion; leptospirosis; serology test

## 1. Introduction

Leptospirosis is a zoonotic disease that mostly affects the tropics, although it can sometimes occur in temperate climates. Leptospirosis can occur in mammals and humans after exposure to the causative organism. The most common cause of leptospirosis in humans is the genus Leptospira interrogans, which can be infected through broken skin/wounds (Alikhani et al., 2018).

Leptospirosis has clinical manifestations that range from mild acute febrile illness that resolves on its own to severe, life-threatening conditions with multiple organ dysfunction. The diagnosis of leptospirosis is made based on a clinical picture indicating a history of risk exposure. Leptospirosis should be suspected in any patient with a history of exposure to risk factors. There are several diagnostic tests used in establishing the diagnosis of leptospirosis. Diagnostic accuracy also varies, especially for serological tests. The threshold value limits for seropositivity in a single sample depend on regional seroprevalence (Rajapakse, 2022).

The diagnosis of leptospirosis is based on the presence of suggestive clinical symptoms and the presence of a history of risk exposure. Leptospirosis should be suspected in any patient who has had a risk exposure. Many diagnostic tests are available for leptospirosis. The effectiveness of diagnostic tests also varies. Antibodies can be detected on the 6-10th day of the disease and reach their peak within 3-4 weeks. Diagnostic tests are generally divided into tests that provide direct evidence of leptospira infection, such as by DNA or culture. Moreover, tests that provide indirect evidence of infection, such as antibody reactions to leptospira (Rajapakse, 2022).

According to research conducted by Chaudhry et al. (2013), there were no significant differences in clinical features and laboratory parameters in the two groups (seropositive and seronegative), except lymphadenopathy, serum AST/ALT levels, and serum creatinine, which were significantly higher in the seropositive group (Chaudhry et al., 2013). Often, patients present with obvious clinical symptoms of leptospirosis but have negative serology test results. In most cases, researchers cannot perform sequential serological examinations, so the diagnosis of leptospirosis is maintained from obvious clinical symptoms to avoid delays in diagnosis. Thus, this study aims to determine how to establish the clinical diagnosis of leptospirosis in leptospirosis cases with negative serology test results.

## 2. Research Methods

This study used a descriptive method with a case series research design to determine how to establish the clinical diagnosis of leptospirosis in leptospirosis cases with negative serology test results. The research data was obtained from the year with the results of a literature search that discusses establishing the diagnosis of leptospirosis with negative serology test results. The search for publication articles used in this literature review uses online databases 2018-20122 in the form of PubMed, Google Scholar, and Science Direct using keywords (Leptospirosis OR Leptospira) AND ("negative serology" OR "serology test OR "clinical suspicion").

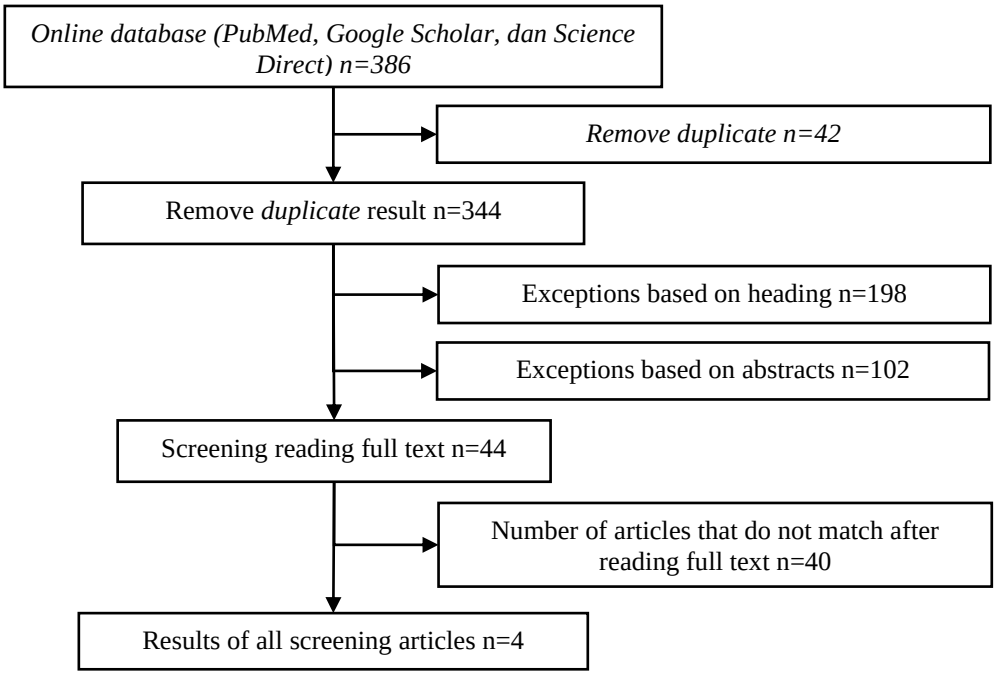


**Figure 1.** PRISMA Flowchart

## 3. Results and Discussion

### 3.1. Result

From searching case series data, 4 cases were obtained by this study, including:

#### 3.1.1. Case 1

A case report was reported by Tomacruz et al., 2019, with complaints of loss of consciousness. One week before the complaint appeared, the patient was passing through a flooded area when the patient had an open wound on his left leg. Four days later, he developed a fever with a temperature of 38.9 C, general weakness and mild behavioral changes, increased irritability, poor appetite, myalgia,

bilateral stiffness of the upper extremities, and an eyeball turning upwards lasting a few seconds. There is no cough, abdominal pain, nausea or vomiting, loose stools, dysuria, hematuria, or changes in urine output. Neurological manifestations include delirium, worsening disorientation, improper speech, and visual and auditory hallucinations. On physical examination, it was found that Conjunctival Suffusion, Anicteric Sclera, and abrasion of the left foot's big toe are slightly purulent. No rash, signs of bleeding, jaundice, flatulence, or tenderness. Lab results showed increased liver enzymes in AST and ALT, serum creatinine, and total creatinine kinase. Urine culture does not indicate the presence of organisms. MAT tests taken at admission (4th sick day), day 4 (8th sick day), and 7th day (11th sick day) showed initially negative seroconversion, then positive at 1:400 dilution, and quadrupled at 1:1600 dilution.

### 3.1.2. Case 2

Other cases of leptospirosis were reported by (Muthuppalaniappan et al., 2018), a 29-year-old male complaining of palpitations, a week's history of fever, myalgia, progressive general muscle weakness, and diarrhea. He loves fishing and claims to care for his mother's pet, a cat, in the past two weeks. On physical examination, they found jaundice. Temperature 38.4°C. hematuria 3+ and proteinuria 2+. ECG shows sinus tachycardia. Laboratory results showed thrombocytopenia, hyperuricemia, and increased serum creatinine of 534 mol/L. Liver function tests were also impaired with increased bilirubin by 281 mol/L, alkaline phosphatase (ALP), alanine aminotransferase (ALT), increased creatinine kinase, and CRP. Show a negative result at the beginning. A positive MAT test with increased titer at 1:320 and anti-leptospira IgM by ELISA at 1:2560 confirmed suspicion of leptospirosis on day 14 of illness.

### 3.1.3. Case 3

Cases reported by (Predescu et al., 2018), a 62-year-old male with complaints of fever of 38 C, chills, muscle aches, nausea, vomiting, abdominal pain since 14 days ago accompanied by jaundice, and shortness of breath. The results of the clinical examination of the patient seemed confused with jaundice, tachypnea, anuria, hepatomegaly accompanied by hepatic tenderness. Laboratory examination with leukocytosis with neutrophilia, thrombocytopenia, increase in serum creatinine, increase in ALT and AST, hyperbilirubinemia, rhabdomyolysis, proteinuria, and hematuria. ECG examination with atrial fibrillation results and Doppler echocardiography with LVEF 40-45%. Physical examination found a rhythmic rhythm, weak heart sounds, and hepatomegaly. Initial examination with a negative rapid agglutination test and re-examination with an ELISA test for leptospirosis showed positive results. Day 17 ascites (+) peritoneal cavity and cardiomegaly (+).

### 3.1.4. Case 4

Later cases were reported by (Khanal et al., 2022), a 56-year-old woman with complaints of fever with a temperature of 38.8 C for five days accompanied by chills, sciatica aches, back pain, and polyarthralgia. Other complaints include changes in the color of the eyeballs to icteric yellow (+/+), darker urine, and abdominal pain in the right upper quadrant. Her urine output decreased to oliguria three days after the onset of fever, which then progressed to anuria in the following days. The heart is hard and delicate, measuring 3 cm below the costal arch on the right mid-clavicle line. Liver function tests showed conjugated hyperbilirubinemia, increased serum creatinine, AST, ALT, thrombocytopenia, and leukocytosis. Initial test results showed leptospira negative IgM and were later retested due to strong clinical suspicion of leptospira. Serological examinations of both IgM ELISA and MAT tests did show negative results at the beginning of the examination with an onset of <1 week (Musso & La Scola, 2013). However, with a re-serological examination after day ten from the

onset of symptoms, the initial negative serology test result will change to positive (Haake & Levett, 2015).

## 3.2. Results

Leptospirosis is an acute infectious disease in humans and animals (zoonoses) caused by microorganisms Leptospira sp (Setiati et al., 2017). The most commonly found serovars are Icterohaemorrhagiae, Canicola, Pomona, and Grippotyphosa (Browne et al., 2023). According to research in Iran, leptospirosis has a considerable prevalence in humans and animals (Khalili et al., 2020). Some patients can show severe symptoms with multiorgan involvement called Weil's disease. Weil's disease is characterized by multiorgan dysfunction with symptoms of high fever, significant jaundice, renal failure, liver necrosis, pulmonary involvement (especially pulmonary hemorrhage), shock, and hemorrhagic diathesis with a mortality rate of 5-10% (Mannam, 2018). The most commonly involved organs were the kidneys (48.7%), liver (30%), and heart (14.2%) (Warnasekara et al., 2019).

The risk of humans becoming infected depends on exposure to risk factors. Some people risk developing leptospirosis due to occupation, living environment, or lifestyle (Husni et al., 2023). The main occupational groups at risk are farmers or plantation workers, pet store workers, cattle ranchers, cleaners, sewers, slaughterhouse workers, meat processors, and the military. Other groups with a high risk of contracting Leptospirosis are natural disasters such as floods and an increase in the number of people doing water recreational sports (Ministry of Health, 2017).

The incubation period of leptospira is 1 to 2 weeks, but it can be 1 to 30 days (Sembiring, 2018). Leptospirosis can manifest as a biphasic disease (De Brito et al., 2018):

**a. The first phase of the disease (septicaemia phase)**

Lasts for 3-10 days and coincides with leptospiremia (bacteria in the blood). Symptoms include fever, headache, body aches, mild cough, rash, nausea, and vomiting. Signs that can be found include conjunctival suffusion, lymphadenopathy, and anorexia. If present, the skin rash is often temporary, lasting <24 hours. This phase is followed by a short period without fever of varying duration, which in turn is followed by the immune phase of the disease (Lane et al., 2016).

**b. The second phase of the disease (immune phase)**

In this phase, there can be recurrent fever accompanied by jaundice and kidney failure. During this period, leptospira is excreted in the urine. It can affect liver and kidney function, but both organ disorders can be cured. The difference between the first and second phases is unclear (Dey et al., 2021).

In some places, routine laboratory tests are important in assisting diagnosis and management, especially when a specific diagnosis of leptospirosis is not available at a healthcare facility or near a healthcare facility with a quick turnaround time (Sykes et al., 2022). Laboratory findings associated with leptospirosis are generally nonspecific, but mild leukocytosis may be found in 2-3 patients with a shift to the left and thrombocytopenia (Daher et al., 2014).

Markers of inflammation, such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), may be subject to increased (Becirovic et al., 2020; Le Turnier et al., 2019). In cases with more severe renal manifestations, serum creatinine is often elevated, and hypokalemia and hyponatremia may occur (Chancharoenthana et al., 2022). Even when the clinical manifestations are mild, conjugated hyperbilirubinemia is often found and can reach levels of up to 40-80 mg/dL. A mild increase in serum transaminases can also be found (Venkatesan et al., 2022). Urinalysis found occasional proteinuria, pyuria, and microscopic hematuria (Silva et al., 2016; Srisawat & Sitprija, 2019). Creatinine kinase and

serum amylase may also be elevated (Ghasemian et al., 2016). The cerebrospinal fluid examination is performed for signs of aseptic meningitis with lymphocytic pleocytosis, moderate protein elevation, and normal glucose levels (WHO, 2003).

According to research by Carrillo et al. (2019), the mean estimated glomerular filtration rate (GFR) can help establish the diagnosis. In the general population, average estimates of GFR are lower in people with positive anti-leptospira antibodies than in negative controls. In a prospective analysis, patients with high antileptospira antibody titers at baseline and follow-up had poor GFR (Carrillo-Larco et al., 2019).

Several laboratory tests can be performed to establish the diagnosis, including blood cultures, polymerase-chain reaction (PCR), dark field microscopy, and serological methods (Rajapakse, 2022). Blood culture examination is used for serovar identification and to determine antibiotic sensitivity. In addition, blood cultures require samples during the first week of illness. Other drawbacks are that culture examination takes much time and requires a high level of safety with low diagnostic results (Girault et al., 2017). PCR works for Early diagnosis (first week of acute/early disease) and genomic classification (Mullan & Panwala, 2016). Its sensitivity and specificity are quite high. Darkfield microscopic examination has low sensitivity and relies on the examiner's ability to recognize it (Bhatia et al., 2015; Kumar Singh, 2018).

Laboratory confirmation on serological examinations can be problematic, especially in resource-poor areas. Antibodies can be detected from day 6 to day 10 of illness and peak in 3-4 weeks (CDC, 2018). Acute and convalescent comparison has high sensitivity and specificity. A serological examination can be used to identify genera or serogroups. Serological examinations that can be done, among others:

**a. Microscopic Agglutination Test (MAT)**

MAT is the gold standard for screening to establish the diagnosis of leptospirosis. MAT is an inexpensive detection method with high specificity (Rahayu et al., 2021). However, MAT requires experienced observers to reduce variation between observers. In addition, MAT is quite time-consuming and dangerous because live cultures are needed to provide antigens. MAT can detect animal serogroup antibodies (Motto et al., 2021). The gold standard for leptospirosis enforcement. However, it requires an experienced observer to reduce variation between observers. It is time-consuming and dangerous because live cultures are needed to provide antigens (Budihal & Perwez, 2014).

**b. IgM enzyme-linked immunosorbent assay (ELISA)**

ELISA is easy to get and do. Sensitivity and specificity depend on the seropositive pattern of antibodies (Rajapakse, 2022).

Serological methods are the most commonly used means of confirming the diagnosis of leptospirosis. The gold standard for diagnosing leptospirosis is MAT, in which acute serum and recovery from suspected cases are mixed with a panel of live antigens from various serogroups of Leptospira organisms and examined for agglutination. Although there is some variability among laboratories/references, most commonly, a single titer of 1:100 (range 1:100 to 1:800) or a fourfold increase in titer between acute and convalescent serum serologically confirms the diagnosis. Leptospirosis. A positive MAT result predicts a 10,667 higher mortality rate than a negative MAT result (Perdhana et al., 2018).

Although the overall test characteristics are superior to those of culture and microscopy (sensitivity 90%, specificity > 90%), this method has some limitations. These tests require panels of living organisms specific to the area suspected of being infected by the patient and specialized laboratory expertise, limiting their use to reference laboratories. In addition, there was significant cross-reactivity between different Leptospira serogroups and other spirochetes (Treponema and Borrelia species) (Lane et al., 2016).

Because the antibody response required for MAT testing is often insufficient to detect until the second week of disease (when the immune phase begins), sensitivity when symptoms begin is limited. Several serologically-based methods have been developed for detecting the host's initial response during the first week of disease. The most commonly used is enzyme-linked immunosorbent assay (ELISA) (Dreyfus et al., 2022; Rosa et al., 2017). This test uses common leptospira antigens to detect IgM for pathogenic and non-pathogenic Leptospira serogroups (Lane et al., 2016). According to Munoz et al. (2020), there was only one laboratory-confirmed case in 39.9% of all suspected and/or suspected cases outbreaks. Sixty-one reported outbreaks were diagnosed using serological ELISA and/or MAT (Munoz-Zanzi et al., 2020). Clemente et al. (2022) show the results of almost all test kits detecting IgM antibodies against Leptospira species, except devices that use IgG as a marker of acute diagnosis of leptospirosis (Clemente et al. 2022).

The diagnosis of leptospirosis can also be established according to WHO recommendations, where a diagnostic score, namely the Faine score, is obtained based on six clinical criteria, two laboratory-determined criteria, and one epidemiological criterion. Leptospirosis is suspected if the score is ±20, with a strong suspicion if the score is ±24 (WHO, 2003).

### 3.2.1. Kriteria klinis Modified Faine's

**Table 1.** The scoring system uses the 2012 modified Faine Criteria (with changes) for the diagnosis of leptospirosis

| **Part A: Clinical data** | **Score** |
|---|---|
| Dizzy | 2 |
| Fever | 2 |
| Fever > 39 C | 2 |
| Conjunctival suffusion | 4 |
| Meningism | 4 |
| Myalgia | 4 |
| Sufusi konjungtiva+meningitis+myalgia | 10 |
| Jaundice | 1 |
| Albuminuria/nitrogen retention | 2 |
| Hemoptysis/dyspnea | 2 |
| | |
| **Part B: Epidemiological Factors** | **Score** |
| Rain | 5 |
| Contact with contaminated environments | 4 |
| Contact with animals | 1 |
| **Section C: Bacteriological and laboratory findings** | **Score** |
| Leptospira isolation in culture – specific diagnosis | |
| PCR | 25 |
| Positive Serology | |
| ELISA Ig M positive | 15 |
| Positive SAT | 15 |
| Other rapid tests | 15 |
| MAT- single positive with high titer | 15 |
| MAT- increased titer/seroconversion (paired serum) | 25 |
| Persumptive diagnosis of leptospirosis is made from: | |
| Part A or Part A &; Part B: >26 | |
| Part A, B, C (Total): >25 | |
| A score between 20-25 is suspected as a possible diagnosis of leptospirosis | |

Source: (Bandara et al., 2016)

### a. Laboratory Criteria

**Confirmation of the diagnosis:**

1) Isolation of Leptospira from clinical specimens; or
2) Fourfold or more increase in Leptospira agglutination titers between acute phase and recovery phase serum specimens studied in the same laboratory or
3) The presence of Leptospira in tissues through direct immunofluorescence; or
4) Leptospira 800 agglutination titer with Microscopic Agglutination Test (MAT) on one or more serum specimens or
5) Detection of pathogenic Leptospira DNA (e.g., by PCR) from clinical specimens (Haake & Levett, 2015).

**Presumptive leptospirosis:**

1) Leptospira agglutination titer 200 but <800 with MAT assay on one or more serum specimens or
2) Presence of Leptospira in clinical specimens with darkfield microscopy or
3) Detection of IgM antibodies against Leptospira in acute serum specimens (Washington State Department of Health, 2019).

Because the clinical manifestations of leptospirosis are highly nonspecific and have significant overlap with various other febrile illnesses, a combination of exposure history and symptoms should be confirmed immediately. Because the majority of patients who come to the hospital show clinical symptoms of acute fever that cannot be distinguished (Forbes et al., 2012). In establishing the diagnosis, the doctor must first consider leptospirosis (Windpessl et al., 2014). However, clinical suspicion alone may be sufficient to warrant empirical antibiotic treatment in most cases because delays in antibiotic administration can increase mortality (Goswami et al., 2014).

## 4. Conclusion

Often, patients present with obvious clinical symptoms of leptospirosis but have negative serology test results. In most cases, researchers cannot perform sequential serological examinations, so the diagnosis of leptospirosis is maintained from obvious clinical symptoms to avoid delays in diagnosis. Clinical manifestations that are often experienced by leptospirosis sufferers when coming to the hospital are fever with a temperature of >38 C, general muscle weakness, myalgia, changes in urine output into oliguria, diarrhea, nausea and vomiting, and abdominal pain, physical examination, jaundice, scleral icterus, conjunctival suffusion, abdominal pain, hepatomegaly, on laboratory examination of thrombocytopenia, leukocytosis, elevated liver enzymes in AST and ALT, hyperbilirubinemia, serum creatinine and total creatinine kinase, hematuria, and proteinuria. Previous exposure to risk factors can help establish the diagnosis. Early suspicion of the first serology test results helps patients get early and fast treatment. Thus, it is hoped that this study can be the basis for further research on establishing the clinical diagnosis of leptospirosis with negative serology test results.

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