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Review article

DECIPHERING LEPTOSPIROSIS-A DIAGNOSTIC MYSTERY: AN INSIGHT

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ABSTRACT

Leptospirosis is an emerging infectious disease which has been recognized as the most common zoonotic infection in the world. It affects human beings and many other species of vertebrates . Most commonly, the infection is acquired by direct or indirect exposure to urine of reservoir animals through contaminated soil, mud & water entering via small abrasions or breaches in the skin & mucous membranes during occupational, recreational or vocational activities. The signs & symptoms resemble a wide range of bacterial & viral diseases & sometimes can present as food poisoning, chemical poisoning & snake bite also due to which the diagnosis is often missed. This review article aims to focus on the role of Dark Field Microscopy (DFM), culture, Enzyme Linked Immuno Sorbent Assay (ELISA), Macroscopic Slide Agglutination test (MSAT), Microscopic Agglutination Test (MAT) and Faine's criteria in the diagnosis of leptospirosis.

Keywords: Dark Field Microscopy, Enzyme Linked Immuno Sorbent Assay, Macroscopic Slide Agglutination test, Microscopic Agglutination Test, Faine's criteria

INTRODUCTION

Leptospirosis also known by various names like "Weil's disease", "Pretibial fever", "Fort Bragg fever", "Peapicker's fever" in different parts of the world is an acute bacterial infection caused by spirochetes belonging to the genus *Leptospira* that can lead to multiple organ involvement and fatal complications.[1] It has been recognized as the most common zoonotic infection in the world.[2] Leptospirosis has a wide geographical distribution and occurs in tropical, subtropical and temperate climatic zones. The incidence is higher in the tropics than in temperate regions.[3] Most countries in the South East Asia region are endemic to leptospirosis.[2]

Leptospirosis affects human beings and many other species of vertebrates .[2] Most commonly, the infection is acquired by direct or indirect exposure to urine of reservoir animals through contaminated soil, mud & water entering via small abrasions or breaches in the skin & mucous membranes during occupational, recreational or vocational activities.[4] A small number of organisms can cause infection. The incubation period usually ranges from 7-10 days. Leptospirosis may follow a biphasic course. During the first 10 days, there is a phase of leptospiraemia when the leptospires multiply in blood and spread to different organs. The chances of recovery of leptospires from blood or other tissues or body fluids is usually high during this stage. This phase is followed by immune phase or leptospirurea phase when the organisms are excreted in the urine. In this phase, the chance of recovery of organisms

from the blood is low. The ideal specimen for isolation or demonstration of leptosires during immune phase is urine.[5] The signs & symptoms resemble a wide range of bacterial & viral diseases & sometimes can present as food poisoning, chemical poisoning & snake bite also due to which the diagnosis is often missed.[6] The most common clinical syndrome is anicteric leptospirosis which is a self limiting illness.[4] Weil's disease or icteric leptospirosis, is generally the most severe illness, with symptoms caused by liver, kidney & vascular dysfunction (jaundice, oliguria/anuria, bleeding & lethal pulmonary haemorrages).[4] The case fatality rate of leptospirosis is upto 10%.[4]

The clinical diagnosis of leptospirosis may be difficult to arrive at. A high index of suspicion is required in patients with Pyrexia of Unknown Origin (PUO) with or without jaundice, especially in presence of history of animal contact, diagnosis may be established by laboratory investigations.[7] The commonly followed case definition, which is also recommended by the WHO and International Leptospirosis Society prescribes that any person presenting with acute onset of fever, headache and body aches associated with severe muscle tenderness particularly in calf muscles, haemorrhages including sub-conjunctival haemorrhage, jaundice, cough, breathlessness and haemoptysis, oliguria, signs of meningeal irritation should be suspected as a case of leptospirosis and investigated[8]. A suspect, who tests positive in any of the screening tests such as dipstick, lateral flow or latex agglutination test should be considered as a probable case. Successful isolation of Leptospires from clinical specimens, a four-fold or higher rise in titre or seroconversion in paired Microscopic Agglutination Test (MAT) or a positive Polymerase Chain Reaction (PCR) is considered as confirmatory evidence of current leptospiral infection [8]. Owing to shortcomings of laboratory tests in establishing early diagnosis of leptospirosis, the World Health Organization (WHO), introduced Faine's criteria which includes the scoring of clinical, epidemiological and laboratory parameters of patients (Parts A, B and C respectively) [9].This criteria has been simultaneously modified and validated by Brato et al and Shivkumar et al, who recommended addition of abdominal symptoms, local factors like rainfall and newer investigations in the total scoring respectively [10,11]. Microbiological diagnosis of leptospirosis aims at demonstrating the leptospires, by culturing them or by demonstrating an appreciable antibody response to them.[12]

Laboratory diagnostic tests are broadly divided into two categories viz., Direct evidence - isolation of organism or demonstration of leptospires by dark field microscopy or amplification of specific fragment of leptospiral DNA; and Indirect evidence- detection of antibodies to leptospires.[13]

This review article aims to focus on the role of Dark Field Microscopy (DFM), culture, Enzyme Linked Immuno Sorbent Assay (ELISA), Macroscopic Slide Agglutination test (MSAT), Microscopic Agglutination Test (MAT) and Faine's criteria in the diagnosis of leptospirosis.

REVIEW

Dark-Field Microscopy (DFM)

DFM has often been employed for examination of body fluids such as blood, urine, CSF and dialysate fluid.[14] The specimens should be taken aseptically and sent to laboratory without delay. They must not be frozen.[5] Oxalate, citrate, heparin or EDTA may be used as anticoagulant for blood or pleural fluid.[5,15] Approximately 10^4 leptospires/ml of sample are necessary for one organism per field to be visible by DFM.[14]

DFM is the procedure of choice for the demonstration of the organisms in tissue fluids.[16] It is particularly useful for observing leptospires in culture, particularly when they are present in large numbers. It can be used for observing agglutination in the MAT.[17] However, it is technically demanding. Recognizing leptospires is difficult, particularly when only small numbers are present.[17] Reading the results is always subjective as in the majority of the samples the number of organism per field ranges from 0-2 and there is always doubt about typical motility.[5] Double centrifugation of the sample at low speed to separate the cellular elements, and then at high speed, help concentrate the leptospires.[12] The usefulness of differential centrifugation is limited and the motility of the organism further reduces after centrifugation at higher g.

The advantage of this technique is that laboratories, where the facilities for the other tests are not available, can undertake this technique. But the results should be confirmed with other standard tests. The various limitations of this technique include the following: Low sensitivity (40.2%) and specificity (61.5%). Serum proteins and fibrin strands in blood resemble leptospires thereby making it extremely difficult to diagnose the disease. The concentration of organism is frequently too low in the specimens. This technique requires expertise. [5]

Culture: Leptospires are obligate aerobes with an optimum growth temperature of 28° C to 30°C.[14] They can be grown in liquid, semi-solid (containing 0.2-0.5% agar) or solid media.[18] The only organic compounds required for their growth are Vitamins B1 and B12, and long chain fatty acids . Fatty acids are the main source of carbon and energy for leptospires. These are also required as a source of cellular lipids, as leptospires cannot synthesize fatty acids de novo. Liquid medium is used for the cultivation of leptospires which can later be used for harvesting antigens to be used in various serological tests. Liquid medium can be converted into semisolid and solid by the addition of agar or agarose. Semisolid media contain 0.2-0.5% agar whereas solid medium contain 0.8-1% agar.[18,19] Semisolid medium is commonly used for the isolation of leptospires and for maintaining the cultures. In semisolid media, growth reaches a maximum density in the form of a discrete zone under the surface of the medium usually within 7-21 days. This growth is known as Dinger's ring and is related to the optimum oxygen tension.[14] Solid medium is not ideal for isolation or maintenance of leptospires and mainly used for the research purpose to clone the leptospires from mixed letospira cultures.[19]

A wide variety of culture media can be used for the cultivation of leptospires.[19] The routinely used culture media are described briefly here:

a) Media that contain rabbit serum: These include Korthof's medium, Fletcher's medium and Stuart's medium. Rabbit serum contains nutrients including high concentrations of bound vitamin B12 which helps in the growth of leptospires. All these media can be used for the isolation of leptospires from the clinical specimens and for the maintenance of leptospires but not for the preparation of antigens for MAT.[19]

b) Fatty acid albumin medium: In this medium long chain fatty acid is used as a nutritional source and serum albumin as detoxicant. This medium is popularly known as Ellinghausen-McCullough-Johnson-Harris (EMJH) medium and widely used for isolation, maintenance and preparation of antigens for MAT and for growing leptospires in bulk.[19]

c) Protein-free medium: In this medium long chain fatty acids are treated with charcoal to detoxify the free fatty acids which are highly toxic to leptospires.[19]

Culture media can be enriched by addition of 1% fetal calf serum or rabbit serum to cultivate the fastidious leptospiral serovars.[19] Due to the ability of leptospires of incorporating purine and not pyrimidine bases into their nucleic acids, they are resistant to the antibacterial activity of the pyrimidine analogue,5-Fluorouracil.[12] Selective culture media, containing 5-FU 50-1000 µg/ml or a combination of nalidixic acid 50 µg/ml, vancomycin 10 µg/ml and polymixin B sulphate 5 units/ml or a combination of actidione 100 µg/ml, bacitracin 40 µg/ml, 5 FU 250 µg/ ml, neomycin sulphate 2 µg/ml, polymixin B sulphate 0.2 µg/ml and rifampicin 10 µg/ml can be used to avoid the contamination.[19] Contaminated cultures may be passed through a 0.2µm or 0.45µm filter before subculture into fresh medium.[20]

The infecting strains can often be isolated in culture, provided that suitable material is obtained before antibiotics have been administered. Early in the course of illness – during the leptospiraemic phase – the samples of preference are blood or cerebrospinal fluid; later during the phase of leptospiruria – it is urine.[12]

***Blood*:** Leptospires have rarely been isolated from blood weeks after the onset of symptoms.[14] Blood should be cultured in the first ten days of the illness and before antibiotics are given. Venous blood is collected with aseptic precautions and ideally inoculated at the bedside into blood culture bottles containing culture medium for Leptospira. Only few drops of blood are inoculated into several tubes, each containing 5 ml of a suitable medium as large inocula will inhibit the growth of leptospires.[17] Cultures are incubated

between 28 to 30°C for 4 to 6 weeks or longer.[5,14] Culture are examined using dark field illumination initially on first, third and fifth days followed by 7-10 days interval up to 6 weeks.[5] Blood culture is particularly valuable in man, as the serological response can be slow and may be absent altogether if antibiotics are given early.

***Urine*:** During the leptospiruria phase which is characterized by increasing concentrations of antibodies after about 1 week from onset of clinical illness, the urine and the renal cortex post mortem are the most suitable inocula for the isolation of leptospires from humans. Fresh mid-stream urine is collected and inoculated immediately. One drop of undiluted urine is inoculated into the first tube containing 5 ml of culture medium. Alternatively, urine samples may be centrifuged for 30 minutes at 1600 g or 1 min at 10,000 g and the pellet re-suspended in medium, after which ten-fold serial dilutions are made immediately in 1 or 2 additional tubes. Since urine is acidic and decreases the viability of leptospires, it should be inoculated into the medium within 2 hours after voiding. Viability is reported to be increased in urine samples neutralized with sodium bicarbonate and by using phosphate-buffered bovine serum albumin solution. Media containing 5-fluorouracil or appropriate antibiotics that suppress the growth of bacterial contaminants and leave leptospires unaffected may be beneficial in reducing the contamination of media inoculated with urine samples obtained from various patients clinically suspected to be suffering from leptospirosis.[17]

***Cerebrospinal Fluid* (CSF):** Leptospires may be observed in CSF by DFM and can be isolated by inoculating 0.5ml CSF into 5 ml semi-solid culture medium during the first week of illness (usually upto 10 days of onset of clinical signs & symptoms).[17,21]

The various advantages of this technique include the following: Isolation in culture is a definite proof of infection. Circulating serovars can be identified. Local isolates can be used as antigens in MAT and vaccine development. [5]

The various disadvantages of this technique include the following: Leptospires are fastidious organisms which require special medium for isolation. Leptospires grow slowly and therefore, isolation of leptospires from clinical specimens takes several days to several weeks. The technique is laborious, time consuming and is not possible in small laboratories. Contamination of culture media by other micro-organisms or by saprophytic leptospires is common in routine practice. The successful isolation rate is less due to prior use of antibiotics, imperfectly cleaned glass ware or wrong incubation temperature and pH. [5]

The various serological tests used for the diagnosis of leptospirosis can be divided into two groups, those, which are Genus specific, and those, which are Serogroup specific.(13) The various Genus specific tests are Macroscopic Slide Agglutination test (MSAT), Indirect Fluorescent Antibody Test (IFAT), Indirect Hemagglutination Test (IHA), Counter Immuno Electrophoresis (CIEP), Complement Fixation Test (CFT), Enzyme Linked Immuno Sorbent Assay (ELISA), Microcapsule Agglutination Test (MCAT) and Lepto-Dipstick. The Serogroup/serovar specific tests are Microscopic Agglutination Test (MAT) and Serovar specific ELISA.[12]

Leptospires have a complex antigenic structure.[12] Within the two species *L.interrogans* and *L.biflexa*, there are many serotypes (now referred to as sero-varieties or serovars) that are distinguished by cross-agglutinin-absorption tests or by antigenic factor analysis. Some of the serovars are closely related because of common antigens and form clearly defined serogroups.[18] The somatic antigen is genus specific. The surface antigen which is a polysaccharide is serovar specific.[12] The outer membrane is lipopolysaccharide (LPS) in nature and is a potent immunogen. It is the major antigen and the target of antibody and complement-mediated bactericidal activity. Antibodies directed against this antigen are protective in nature. Flagellar antigen is composed of both genus and serotype specific antigens. Some serovars, e.g. *L. icterohaemorrhagie*, have an additional Vi antigen associated with virulence.[12] The leptospiral outer membrane contains both transmembrane proteins, such as the porin OmpL1 and lipoproteins such as LipL41 and LipL36 .[22] Outer membrane proteins (OMPs) that are exposed on the leptospiral surface are potentially relevant in pathogenesis because of their location at the interface between leptospires and the mammalian host. Results of surface immunoprecipitation studies, immunoelectron microscopy & topological

considerations suggest that OmpL1 and LipL41 are present on the surface. Unlike leptospiral LPS, OmpL1 and LipL41 are antigenically conserved among pathogenic *Leptospira* species. OmpL1 and LipL41 are expressed during infection of the mammalian host.[22] LipL32, the 32-kDa lipoprotein is also a prominent immunogen during human leptospirosis. The sequence and expression of LipL32 is highly conserved among pathogenic *Leptospira* species. LipL32 may be important in the pathogenesis, diagnosis, and prevention of leptospirosis.[23]

Enzyme linked Immuno Sorbent Assay (ELISA)

This is now widely used as a genus specific screening test in man.[12] It is a very sensitive and specific test for the biological diagnosis of leptospirosis.[17] It is of particular value as a serological screening test because of its relative simplicity in comparison with the MAT.[17] Both peroxidase and urease labelled conjugates have satisfactorily been used. Stable reagents are available and form the basis of bedside tests, which are read visually. The use of computer assisted automated readers and the appropriate controls has improved the reproducibility and predictive value of this test. They can be performed with commercial kits or with antigen produced "in house".[12] Antigen preparation for ELISA is done cultivating *Leptospira interrogans* serovar *Copenhageni* strain Wijnberg or *L. biflexa* serovar *Patoc* strain Patoc I in EMJH medium for 10-12 days at 30ºC in shaking incubator. Abundant growth is necessary to produce a good antigen.[5] Several attempts have been made to develop serotype specific ELISA tests with a variety of extracted antigens. Tests based on boiled whole cell antigens tend to be genus specific but those based on ultrasound-disintegrated or phenol-extracted preparations show considerable serotype specificity.[12]

The ELISA test gives a positive response in the diagnostic evaluation of leptospirosis a little earlier than the MAT because it is more sensitive to IgM antibodies. A response 6–8 days following the appearance of the first clinical signs is generally observed. On the other hand, the test may become negative more quickly than MAT, although low levels of specific IgM may persist. A potential advantage of the ELISA test is that it may help to differentiate between current leptospirosis and previous leptospirosis since antibodies from past infection or immunization may not be detectable. However, if a total human anti-Ig or IgG conjugate is used instead, the positivity of the test may be extended, allowing the detection of residual antibodies in recovered or immunized patients. The level of positivity observed with a total anti-Ig conjugate is then always equal to or higher than the maximum observed with anti-IgG or anti-IgM antibodies.[17] Other advantages include the following: Single antigenic preparation can be used for ELISA; Heat stable antigens which are stable at room temperature for long periods are generally used; ELISA allows rapid processing of large number of samples.

A limitation to the use of single serum samples for serodiagnosisis the persistence of antibodies. Anti-leptospiral IgM antibodies are also persistent, but the rate of decline shows marked variation. Thus, a single IgM positive sample taken during an acute febrile illness with symptoms suggestive of leptospirosis is presumptive evidence of infection, but this finding requires confirmation by testing a convalescent sample, preferably by the use of an alternative method.[24] Infecting serovar cannot be assessed which makes it comparatively less specific.[12] Another disadvantage of this procedure is that it usually requires calibration of cut-off values and significant titres.[5]

Macroscopic Slide Agglutination Test (MSAT)

A rapid macroscopic slide agglutination test can be used to screen human and animal serum samples. This test is carried out with a dense suspension of leptospires, which agglutinate into clumps visible to the naked eye. It can be performed on slides or plates. There can be different ways to prepare the antigens. One may either use a single serovar or multiple serovars to prepare the antigens. When multiple serovars are used as antigens, antigens can be pooled. Since the technique will require lot of laboratory work, generally a single non-pathogenic serovar patoc (strain Patoc 1) is used.[12] The basic principle of the test is similar to other slide agglutination tests used in other infectious diseases such as enteric fever or brucellosis. The reaction is recorded as ++ when the clumps are large and definite, + when the smaller clumps are well

defined but the suspension is not clear, +/- when fine clumps are visible but the suspension is not clear and negative when the mixture in the drop is unchanged. Agglutination of + and ++ is considered as positive.[5] It is a simple, rapid, and sensitive diagnostic test for active leptospirosis and allows a provisional diagnosis of acute leptospirosis to be made within a few minutes.[12] The antigen is broadly reactive and stable for six months at 4ºC to 8ºC.[5] It is more sensitive than Microscopic Agglutination Test (MAT) in the early stage of the disease. The sensitivity of this test can be enhanced by adding the locally-prevalent serovars. MSAT has shown good correlation with both IgM ELISA and MAT in various studies, and therefore can be used as a valuable and simple screening test. It is not however suitable for retrospective or survey work.[12] A high percentage of false positive reactions are observed, probably due to lack of standardization and quality control of the antigen preparation. The number of false negative reactions are comparatively low.[5] Positive reactions should therefore be confirmed by complement fixation or microscopic agglutination tests.[12]

Microscopic Agglutination Test (MAT) : This test is considered the gold standard for serodiagnosis of Leptospirosis.[25] MAT is carried out with suspensions of live cultures or with cultures killed by the addition of neutralized formaldehyde.[12] There is a difference in appearance between the clumps of agglutinated living leptospires and those of killed cultures. Living leptospires are agglutinated into highly refractile spheroids of various sizes which may join to produce elongated masses of confluent spheroids. By contrast, the agglutinated killed leptospires form looser masses with an irregular often angular, outline; these appear flattened, resembling small piles of threads, or snowflakes, or pieces of cotton wool.[12]

Agglutinating antibodies can be of both IgM and IgG classes.[17] The degree of agglutination can only be assessed in terms of the proportion of free leptospires. The accepted endpoint of an agglutination reaction is the final dilution of serum at which 50% or more of the leptospires are agglutinated. Preparations for MAT require meticulous culture of a collection of the live strains used as antigen suspensions, their regular subculture and quality control for authenticity and purity and also skilled educated personnel.[11]

A recent advance is the use of standardized preparations of dried leptospires available to accredited diagnostic laboratories from a central reference laboratory. Multi-antigen MAT uses a battery of strains giving comprehensive coverage of all serogroups and provides an alternative to the so-called 'genus-specific' tests as a means of diagnosing leptospirosis. However, the necessity to maintain large number of live strains of *L. interrogans* limits its use to reference laboratories. Wherever possible, local isolates of known identity should be included in the battery of strains in order to increase both the sensitivity and the specificity of this widely accepted gold standard test.[12]

Interpretation of diagnostic MAT: The MAT is usually positive 10–12 days after the appearance of the first clinical symptoms and signs but seroconversion may sometimes occur as early as 5–7 days after the onset of the disease.[17] The antibody response may be delayed if antibiotic therapy was given before the test. A patient with a compatible history may be considered to have current leptospirosis if he has IgM antibodies to leptospira and a MAT titre of >/=80.[26]

The optimal cut-off titre is assessed by carrying out a baseline study on distribution of titres in the community as well as among confirmed patients. Using this data it is possible to estimate the sensitivities and false positivity rates at different cut-off titres. The titre that gives the lowest number of false results is then chosen as the optimal cut-off titre.[5]

In a non-endemic area, even a low level of antibodies may signify leptospirosis in the first week of a clinically compatible illness. The titre will rise in a second specimen taken after 3 to 7 days. If the titre remains below 100, even on repeated testing then it could probably be due to previous leptospirosis, and not current illness. In endemic areas, the diagnosis will be confirmed if the titre rises on retesting, but will be negated if it is unchanged, assuming that the infecting serovar was included among the antigens used for MAT.[12]

MAT is the serological test used in reference laboratories, because of its high degree of sensitivity and specificity.[27] MAT remains very

useful for epidemiologic studies, identification of strains, assessment of the probable infecting serogroup and confirmation of illness for public health surveillance.[28]

The disadvantages of MAT are that 14–21 strains have to be maintained in culture, which is often very difficult. Procedure is complex and time consuming. Reading results requires experienced personnel.[5] It cannot be standardized as live antigens are often used and various factors, such as the age and density of the antigen culture, can influence the agglutination titre.[17] Co-agglutinins (cross-reactions) are frequently present in the sera of patients with leptospirosis. Antibodies which cause cross-reactions are often the first to appear but they disappear rapidly. Homologous antibodies, although they appear slightly later, persist much longer, thus allowing the presumptive identification of the serogroup responsible for the infection and also the detection of traces indicating previous infections.[17] Some patients have serological evidence of previous infection with a different leptospiral serogroup. In these patients, serological diagnosis is complicated further by the "anamnestic response," in which the first rise in antibody titre is usually directed against the infecting serovar from the previous exposure. Only later does it become possible to identify the serovar or serogroup responsible for the current infection, as the titre of specific antibody rises. Paradoxical reactions also occur in patients who have such infections, and interpretation of serology is further complicated.[14] The MAT cannot differentiate between agglutinating antibodies due to current, recent or past infections.[17] Therefore, the use of MAT is only restricted to some of the specialised laboratories.[29]

Faine's criteria for diagnosis of leptospirosis

Faine had formulated a criteria for diagnosis of leptospirosis on the basis of clinical, epidemiological and laboratory data (Parts A, B and C respectively). A presumptive diagnosis of leptospirosis may be made if:

(i) Parts A and B score = 26 or more (Part C laboratory report is usually not available before fifth day of illness; thus it is mainly a clinical and epidemiologic diagnosis during early part of disease).

(ii) Part A+B+C = 25 or more A score between 20 and 25: Suggests a possible but unconfirmed diagnosis of leptospirosis.[9]

Shivakumar et al from Chennai have suggested modification on Faine's criteria to include local factors like rainfall and newer investigations in the total scoring.[11] As per this, epidemiological and laboratory criteria (Parts B and C) are modified only; no modification is made in the clinical criteria (Part A).

The reasons for modification suggested are as follows:

(i) Most of the leptospirosis is reported in monsoon and post-monsoon period. Therefore they have suggested rainfall separately to be adjusted in the scoring criteria of Part B.

(ii) Microscopic agglutination test (MAT) is the Gold Standard test, but is complicated and less sensitive compared to newer tests like IgM ELISA and Slide Agglutination Test (SAT). IgM ELISA and SAT are simple, sensitive tests and can be used to diagnose current leptospirosis. Thus, they have been included with appropriate score being assigned to each one of these.

The difficulties in utilizing MAT are due to the following reasons:[30]

(a) The antibody titres rise and peak only in second or third week. Thus, paired sera are required to demonstrate four-fold rise of titre.

(b) The test is complicated requiring dark-field microscopy and cultures of various serovars, which may not be available in smaller laboratories.

The advantage of including simple diagnostic tests (IgM ELISA or SAT) in modified Faine's criteria is that it helps in diagnosing milder forms of leptospirosis which are associated with low clinical score (Part A). Suggestion of modification of existing Faine's criteria appears justified; however further evaluation is required.[30] Table 1shows comparison between Faine's & Modified Faine's criteria.

Table: 1. Table showing comparison between Faine's & modified Faine's criteria[9,11]

Faine's criteria		Modified Faine's criteria	
PART A: Clinical data	Score	PART A: Clinical data	Score
Headache	2	Headache	2
Fever	2	Fever	2
If fever, temperature 39°C or more	2	If fever, temperature 39°C or more	2
Conjunctival suffusion (bilateral)	4	Conjunctival suffusion (bilateral)	4
Meningism	4	Meningism	4
Muscle pain (especially calf muscle)	4	Muscle pain (especially calf muscle)	4
Conjunctival suffusion + meningism + muscle pain	10	Conjunctival suffusion + meningism + muscle pain	10
Jaundice	1	Jaundice	1
Albuminuria or nitrogen retention	2	Albuminuria or nitrogen retention	2
PART B: Epidemiological factors		PART B: Epidemiological factors	
Contact with animals or contact with known contaminated water	10	Rainfall Contact with contaminated environment Animal contact	5 4 1
PART C: Bacteriological and laboratory findings		PART C: Bacteriological and laboratory findings	
Isolation of leptospires in culture:	Diagnosis certain	Isolation of leptospires in culture:	Diagnosis certain
Positive serology (MAT)		Positive serology	
Leptospirosis (endemic)		ELISA IgM positive*	15
Single positive low titre	2	MSAT positive*	15
Single positive high titre	10	MAT single high titre*	15
		MAT rising titres (paired sera)*	25
Leptospirosis (non-endemic)		*Only one of these tests to be scored	
Single positive low titres	5		
Single positive high titres	15		
Rising titres (paired sera)	25		

CONCLUSION

Leptospirosis is probably an under diagnosed infectious disease which mysteriously mimics several clinical conditions. It is an emerging infectious disease, the current incidence of which only represents tip of an iceberg. Although, several modalities are currently available for diagnosing this deadly infectious disease, but each one of these has certain limitations. Newer diagnostic tests for leptospirosis are need of the hour which would aid clinical diagnosis during the initial phase of the disease and rapid case confirmation during outbreak surveillance.

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