



COMPARATIVE STUDY ON SERO PREVALENCE OF LEPTOSPIRA –IGM AMONG CRITICALLY ILL PATIENTS AND A COMMUNITY WHERE IDEAL ENVIRONMENT FOR LEPTOSPIROSIS OCCUR : A STUDY IN A MEDICAL COLLEGE & HOSPITAL

Swagnik Roy *	Associate Professor , Department of Microbiology , Zoram Medical College , Mizoram. *Corresponding Author
Bibhas SahaDalal	Assistant Professor , Department of Pathology , ESI-PGIMSR Medical College and Hospital , Joka , Kolkata.
Rajat Dasgupta	Tutor , Assistant Professor and Professor ,Department of Microbiology , KPC Medical College and Hospital , Kolkata.
Sourabh Mitra	Tutor , Assistant Professor and Professor ,Department of Microbiology , KPC Medical College and Hospital , Kolkata.
Amrita Roy	Assistant Professor , Department of Microbiology , KPC Medical College and Hospital , Kolkata.
Barun SahaDalal	Professor , Department of Microbiology , KPC Medical College and Hospital , Kolkata.

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INTRODUCTION

In 1886 leptospirosis was first described by Adolf Weil & leptospirosis causative agent *Leptospira* was first observed in 1907 from a post mortem renal tissue slice[1]. Leptospirosis is an emerging widespread zoonosis, caused by leptospira species. This type of diseases mostly occur in tropical , subtropical region and is a major public health problem with outcomes ranging from subclinical infections to fatal pulmonary haemorrhage and flu-like illness to a severe disease form known as Weil's syndrome. The causative agent of weill's disease is named as *L. icterohaemorrhagiae* was isolated in 1915 by Inada. Severe disease of leptospirosis includes jaundice, acute renal , intravascular disease and hepatic failure, pulmonary distress, which may result in death[2]. Leptospire are divided into pathogenic, non-pathogenic, and intermediate/opportunistic species based on DNA hybridization studies. The current study in genomic based classification indicates that there are at least 19 species which are divided into 13 pathogenic species and 6 saprophytic species[3,4], identified through DNA hybridization analysis [5,6] . Among these 19 species 7 species are the main agents of leptospirosis , those are : *L. interrogans*, *L. borgpetersenii*, *L. santarosai*, *L. noguchii*, *L. weilli*, *L. kirschneri* and *L. alexanderi*[7]. Under the new classification all recognized species are further subdivided into 24 serogroups and more than 200 serovars based on the surface lipopolysaccharide (LPS)[3,8]. *Leptospira interrogans* is very thin, flexible, tightly coiled, obligate aerobic spirochaete characterized by a unique flexuous type of motility with a single axial filament and hooked ends. . An important feature of the spirochetes is the location of the flagella, two endoflagella with their free ends towards the middle of the bacteria lie in the periplasmic space between the cell wall and the outer membrane. The motility of bacteria with external flagella is hindered in viscous solutions, but that of spirochetes is enhanced and it is theorized that this kind of flagella is responsible for the ability of spirochaetes to penetrate and invade host tissue. They do not stain well with conventional dyes, and resembles gram negative bacteria because of the Lipopolysaccharide membrane. They can only utilize Long Chain Fatty Acids as their sole carbon and energy source. Optimal growth temperature of pathogenic species in culture is 28°-30°C . They grow very slowly with a generation time of about 20 hours, colonies are visible after 3-4 weeks on solid medium.

occurs in heavy rainfall, flooded and poor sanitation areas . In west Bengal specially in Kolkata, there is high average rainfall with water holding capacity of soil [9]. As rodents like rats ,livestock and pets are reservoir for leptospire, their urine containing leptospire can contaminate the water by which humans get infected either by direct or indirect exposure to this contaminated water. Rats are main hosts of serovar *Icterohaemorrhagiae*, cattle of *Hardjo* and *Pomona*, pigs of *Pomona* or *Tarrossovi*, and dogs of *Canicola*[10]. On the other hand people living in urban slum encirclement with inadequate sanitation are at high risk of rat exposure and leptospirosis. Farmers, Sewage workers, Miners, Veterinarians and individuals who are involved in Water sports, Gardening, Ecotourism are at high risk for leptospirosis [11,33,61]. These occupations involve activities likely to result in exposure of wounds, cuts and to soil and water contaminated with the urine containing leptospire of rodents and animals from which workers get infected .All these above points are crucial reasons for the cases of leptospirosis in Kolkata.

The pathogenesis of Leptospirosis is not clearly known till now. Leptospire generally gain entry through small areas of damage on the skin, the conjunctiva or via mucous membranes and abrasion. They enter and spread to the whole body system and infect kidney, liver, heart and even Central Nervous System and may result rapidly from an apparently mild illness to severe condition such as pulmonary haemorrhage, jaundice, acute kidney injury and meningo encephalitis. After reaching the number of leptospire in the blood and tissues at critical level, lesions develop due to the action of undefined leptospiral toxin(s) may known as endotoxin or toxic cellular components and consequent symptoms appear. Endotoxin activity has been observed in several serovars of leptospire[12,13] Severe cases of leptospirosis should be treated with high doses of intravenous penicillin, less severe cases can be treated with oral antibiotics such as amoxicillin ,ampicillin[14]. Third-generation cephalosporins antibiotics also appear to be effective against leptospirosis[15]. The clinical diagnosis of leptospirosis are often nonspecific, that's why a timely and accurate laboratory diagnosis is essential to diagnose leptospirosis cases. Several types of method are used to diagnose leptospirosis such as: polymerase chain reaction (PCR)[16], Microscopic agglutination test (MAT), ELISA.

Leptospirosis is a potentially fatal zoonosis that is mostly

MAT, the reference serological testis considered as the 'gold

standard of serodiagnosis although with some limitations[17]. MAT requires technical expertise, can be done only in reference laboratories that maintain live *Leptospira* strains, and is the best interpreted with both acute and convalescent sera. Additionally, a live panel of leptospires require in MAT, is a laboratory biohazard[18]. Other limitations include a limited sensitivity during the early phases of illness(19), inter-laboratory variation due to subjective interpretation of agglutination and difficulty in standardisation[19,20]. **ELISA** is the simplest tool for the diagnose leptospirosis. *Leptospira* specific IgM antibody may be observed after 4 to 5 days of the onset of symptoms[21]. Samples can be screened for anti-leptospira IgM by ELISA. Positive ELISA is confirmed by using MAT. One study (Roy Sagnik et al) has been showed that the ELISA tests are the most readily applicable for the rapid detection and diagnosis of leptospirosis[9].

Mass immunization applying in people to prevent of this disease. Awareness of Leptospirosis through the advice of doctor, employers and general public will help to develop safer practices during recreational pursuits. Vaccination of human is beneficial method, where they are usually associated with animal sources though not universally accepted vaccine is available for human. Personal hygiene, Personal protective equipment, proper water treatment and most importantly control of rodents are some excellent way to prevent leptospirosis. The number of human cases over leptospirosis in worldwide is not known briefly. In October, 1995, it was reported that in rural Nicaragua, epidemic hemorrhagic fever, caused by leptospira not by jaundice or renal manifestation[22]. According to the current study it has been seen that incidents range from approximately 0.1-1/100000/year in temperate regions to 10-1000/100000 in the humid tropic region. Unlike leptospirosis mild cases can not be diagnosed. The mortality rate of leptospirosis is high, ranging from 2.5% to 16.45%. The mortality rate can be up to 56% at the age over 50 [9]. In 2011 reports from the Southern part of Gujarat revealed that 130 people were died within a span of two months due to only leptospirosis. In October 2012 one study reported 16 deaths were observed in Surat and Valsad districts of Gujarat due to leptospirosis[23]. The true statement of human leptospirosis in West Bengal state is not clearly known because lack of proper diagnostic techniques. In the year of 2013 -2016 one report from Kolkata showed that out of total 1527 patients 562 (36.8%) were diagnosed for leptospirosis. Out of these 562 diagnosed patients male patient was 410 (72.9%) and female was 152 (27.1%) and all the samples gave positive result in ELISA test [24]. Most of the people of this part of the planet suffering from infectious jaundice are sometimes confused with a viral hepatitis, but, many of these cases might be due to *Leptospira* infection. The aim of this study is to compare among the rate of the infected patients those who were suspected to leptospirosis, already admitted into hospital and, citizens (as a control) from a community where an ideal environmental condition for leptospirosis occur. We were focused mainly in the monsoon time (June-August) to get better results from the surrounding area.

MATERIALS AND METHODS

The study was conducted during five years 2014 to 2019 in a medical college and Hospital Kolkata.

The institutional Ethical Committee permission was taken.

Sample selection:

The patients, suspected for leptospirosis were evaluated on the basis of their case history, epidemiological risk factors, laboratory findings and clinical findings as per criterion (Table 1). Samples were collected from recognised patients which had been used as test sample. Several samples were also collected from community include urban slum area or insalubrious slum area which is detrimental for health, were taken as a control.

Table 1: Modified Faine’s criterion

Clinical features (A)	Score	Fever
2 Headache		2
Temperature > 39°C	2	Myalgia
4 Conjunctival suffusion	4	Meningism
4 Jaundice		1
Albuminuria/ elevated BUN	2	Epidemiological
factors (B)	Rainfall	
5 Contaminated environment	4	Animal
contact	1	Laboratory
criteria (C)	Culture	
Diagnosis certain ELISA IgM		
15 MSAT		15
MAT- single positive high titer	15	MAT- rising
titer (paired sera)	25	

Laboratory procedure:

Serum was separated from blood sample by using centrifuge machine; then serum was taken into another clear eppendorf and stored at -20°C until it was tested. Serum was tested for qualitative detection of leptospira specific IgM antibodies using leptospira IgM Microtisa test is an enzyme immunoassay based on "Indirect ELISA". Once the assay has started, full procedure was completed without any interruption. Briefly for ELISA, tested serum samples were gently mixed with Rf absorbent in 1:11 dilution in separate tubes (10 µl serum samples + 100 µl Rf absorbent) and incubated for 10 mins in room temp. 25 µl of this mixed sample from each tube were used for the ELISA procedure. 100 µl of sample diluent (Buffer containing protein stabilizers) were added in each antigen coated microwell including the positive and negative control wells; kept it for 30 mins at 37°C. Each wells were carefully washed 5 times with wash buffer solution. 100 µl antihuman IgM labelled with horseradish peroxidase were added in each well and incubated for 30 mins at 37°C. Again washed it 5 times; then 100 µl of TMB substrate were added and incubated at RT for 30 mins. 50 µl of 1N sulphuric acid (stop solution) were added. The absorbance was taken at 450 nm/630nm. The reactivity of serum samples were construed on the basis of calculation on of the leptospira IgM unit. Leptospira IgM unit were derived from calculation process of cut off value as follows:

Cut off value = mean value of negative control + 0.500
 Ratio of sample O.D. = sample O.D. / cut off value
 Leptospira IgM units = Ratio of sample O.D. x 10

When sample leptospira IgM unit is > 11; it was interpreted as a sample is reactive but when the unit is < 9 then it was interpreted as the sample is nonreactive. For sample showing the unit lies between 9 -11 that determined as equivocal result; another blood sample was taken from same person after a period of 10 days.

RESULT

The sample was collected from patients with age group 13 to 100 years. It was distributed among 6 different ranges and positive cases among that was calculated separately. Total of 459 samples of suspected cases comparing other parameters and clinical and radiological suspicion was collected maintain proper inclusion criteria and informed patient consent. Out of which 83 turnout to be serologically positive. Among the age groups 13-25 years shown to be 7 positives, 26-40 years age group 21 positives, 41-55 years age group 25 positive, 56-70 years age group 19 positives, 71-85 years age group 11 positives.

Table 1: Age wise sample and positivity distribution of total test samples

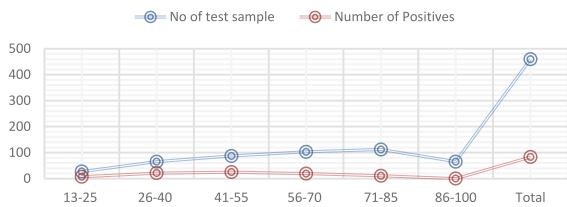
Age	No of test sample	Number of Positives
13-25	27	7
26-40	65	21
41-55	87	25

56-70	103	19
71-85	112	11
86-100	65	00
Total	459	83

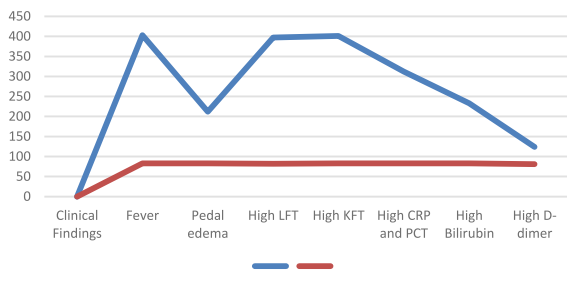
Table 2: Clinical findings wise distribution of test samples

Clinical Findings	No of Samples	Number of Positives
Fever	403	83
Pedal edema	212	83
High LFT	397	82
High KFT	401	83
High CRP and PCT	312	83
High Bilirubin	233	83
High D-dimer	124	81

Age group wise number of sample and positives comparative assy



Clinical and Biochemical Findings comparative assy among Positive Patients and Suspected patients screened



DISCUSSION

The sample was collected from patients with age group 13 to 100 years . It was distributed among 6 different ranges and positive cases among that was calculated separately. Total of 459 samples of suspected cases comparing other parameters and clinical and radiological suspicion was collected maintain proper inclusion criteria and informed patient consent. Out of which 83 turnout to be serologically positive. All the patients had common systemic signs and symptoms like fever , Pedal edema , High Kidney Function Test results mostly high urea and creatinine. Even C reactive Protein , Procalcitonin and Hyper bilirubinaemia is seen in all positive patients. Maximum patients except few are also have high Lifer Function Tests value and High D Dimer Value.

REFERENCES

- Suvarna P, Ningal, Manoj B, Kothule, Nilesh Y, Jadhav, Sagar D, Kadam, Yogesh S, Katare, Sandip A. Hapse A REVIEW ON LEPTOSPIROSIS WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES SJIF Impact Factor 5.210 Volume 4, Issue 09, 1531-1543 Review Article ISSN 2278 – 4357
- Faine, S.; Adler, B.; Bolin, C.; Perolat, P. *Leptospira* and Leptospirosis. 2. Medisci; Melbourne, Australia: 19
- Adler B, Moctezuma A. *Leptospira* and leptospirosis. *Vet Microbiol* 2010;140(3-4):287–296. Overview of the biology of *Leptospira* and how recent progress in genetic research will contribute to our understanding of *Leptospira* pathogenesis. [PubMed: 19345023]
- Bharti AR, Nally JE, Riccaldi JN, et al. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis* 2003;3(12):757–771. [PubMed: 14652202]
- Brenner DJ, Kaufmann AF, Sulzer KR, Steigerwalt AG, Rogers FC, Weyant RS. Further determination of DNA relatedness between serogroups and serovars in the family *Leptospiraceae* with a proposal for *Leptospira alexanderi* sp. nov.

- and four new *Leptospira* genomospecies. *Int J Syst Bacteriol* 1999; 49(2): 839–858. [PubMed: 10319510]
- Levett P. Leptospirosis. *Clin Microbiol Rev* 2001;14(2):296–326. [PubMed: 11292640]
- Karen V Evangelista, Jenifer Coburn. *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiol*. 2010 September; 5(9): 1413–1425. doi:10.2217/fmb.10.102.
- Palaniappan R, Ramamujam S, Chang Y. Leptospirosis: pathogenesis, immunity, and diagnosis. *Curr Opin Infect Dis* 2007;20(3):284–292. [PubMed: 17471039]
- Saha Dalal Bibhas, Roy Swagnik, Dasgupta Rajat, Saha Dalal Barun. LEPTOSPIROSIS IN KOLKATA- A RETROSPECTIVE STUDY OVER EIGHT YEARS IN A MEDICAL COLLEGE AND HOSPITAL. *J. Evolution Med. Dent. Sci./eISSN- 2278-4802, pISSN- 2278-4748/ Vol. 6/ Issue 87/ Oct. 30, 2017. Page 6066*
- Sara Chandyl, Lokeshwaran Kirubanandhan2, Priya Hemavathy3, Anees Mohammad Khadeeja3, Siby Jacob Kurian3, Krishnan Venkataraman2, Kristine Mørch4, Dilip Mathai5, Anand Manoharan. Serovar prevalence of *Leptospira* in semirural India and the development of an IgM-based indirect ELISA/ *Infect Dev Ctries* 2017; 11(3):234-241. doi:10.3855/jidc.8067
- David A. Haake , Paul N. Levett. Leptospirosis in Humans. *Curr Top Microbiol Immunol*. 2015 ; 387: 65–97. doi:10.1007/978-3-662-45059-8_5.
- HarajiHarajiMohammed CohenNozha KaribHakim, Fassouane Abdelaziz and Belahsen Rekia. LEPTOSPIRA: Morphology, Classification and Pathogenesis *Journal of Bacteriology & Parasitology* · September 2011 DOI: 10.4172/21559597.1000120https://www.researchgate.net/publication/2341163768
- Thompson JC, Manktelow BW (1986) Pathogenesis and red blood cell destruction in haemoglobinuric leptospirosis. *J Comp Pathol*, 96: 529-540.
- Hui Yi L, Sing Hwa N, Chia Ying T, Abdullah A, Azmi F, Samsuki N, et al. Leptospirosis. *WebmedCentral INFECTIOUS DISEASES* 2011;2(12): WMC 00277 doi: 10.9754/journal.wmc.2011.002772
- Ricardo Pereira Igreja. Infectious disease associated with caves https://doi.org/10.1016/j.wem.2011.02.012
- KumaresanVedhagiri, Sridhar Velineni, John F. Timoney, Santhanam Shanmughapriya, PaluruVijayachari, Ramasamy ,Narayanan Kalimuthusamy Natarajaseenivasan Detection of LipL32-specific IgM by ELISA in sera of patients with a clinical diagnosis of leptospirosis DOI10.1179/204773213Y.0000000088 *Pathogens and Global Health* 2013 VOL. 107 NO. 3.
- Enrique Canal, Simon PPollett, Kristen Heitzinger , Michael Gregory et al . . Detection of human leptospirosis as a cause of acute fever by capture ELISA using a *Leptospira interrogans* serovar Copenhageni(M20) derived antigen. Canal et al. *BMC Infectious Diseases*3:438 http://www.biomedcentral.com/1471-2334/13/438
- Cumberland P, Everard CO, Levett PN: Assessment of the efficacy of an IgM-elisa and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. *Am J Trop Med Hyg* 1999, 61(5):731–734.
- Levett PN, Mandel GL, Mandell D: Mandell, Douglas & Bennett's Principles and Practice of Infectious Diseases. 7th edition. Philadelphia, PA: Elsevier Publishing Co; 2010. Chapter 216
- Vinetz JM: Ten Common Questions About Leptospirosis. *Infect Dis Clin Pract* 2000, 9(2):59–65.
- Maria Ines Rosa , Maria Fernandes dos Reis , Carla Simon , Eduardo Dondossola , Maria Cecilia Alexandre. IgM ELISA for leptospirosis diagnosis: a systematic review and meta-analysis. DOI: 10.1590/1413-812320172212.14112016.
- Rao R. Sambasiva, Gupta Naveen, Bhalla P and Agarwal S.K. Leptospirosis in India and the Rest of the World *The Brazilian Journal of Infectious Diseases* 2003;7(3):178-193 © 2003.
- Dhanze Himani, M. Kumar Suman and B. G. Mane. Epidemiology of leptospirosis: an Indian perspective *Journal of Foodborne and Zoonotic Diseases* | July-September, 2013 | Vol 1 | Issue 1 | Pages 6-13©2013.
- Rajdeep Saha, Soma Sil (Mullick) and . Rajyasri Guha Thakurta. INCIDENCE OF LEPTOSPIROSIS IN INDIA: A CROSS SECTIONAL STUDY. *Ejpmr*, 2016, 3(7), 502-504. ISSN 2394-3211 EJPMPR
- Andra DechnerA retrospective analysis of the leptospirosis research in Colombia *J Infect Dev Ctries* 258-264. doi:10.3855/jidc.3123
- S. Faine, N. D. Stallman (1982) Amended descriptions of the genus *Leptospira* Noguchi 1917 and the species *L. interrogans* (Stimson 1907) Wenyon 1926 and *L. biflexa* (Wolbach and Binger 1914) Noguchi 1918. *Int. J. Syst. Bacteriol* 32:461–463.
- Johnson RC, Faine S (1984) *Leptospira*, In NR Krieg and JG Holt (ed) *Bergey's manual of systematic bacteriology*. Williams & Wilkins, Baltimore 1: 62–67.
- Shenberg E, Gerichter B, Lindenbaum I. Leptospirosis in man: Israel 1970-1979. *Am J Epidemiol* 1982;115:352-8.
- Revital Kariv, Robert Klempfner, Ada Barnea, Yechezkel Sidi, and Eli Schwartz The Changing Epidemiology of Leptospirosis in Israel, *Emerging Infectious Diseases* 990 Vol. 7, No. 6, November-December 2001.
- Didier Musso , Bernard La Scola. Laboratory diagnosis of leptospirosis: A challenge 1684-1182/\$36 Copyright © 2013, Taiwan Society of Microbiology. Published by Elsevier Taiwan L.L.C. All rights reserved.http:// dx.doi. org/ 10.1016/j.jmii.2013.03.001
- ASHVIKA SARWANKAR, SUMMAIYA MULLA. PREVALENCE OF LEPTOSPIROSIS IN VARIOUS RISK GROUPS OF SOUTH GUJARAT, INDIA *International Journal of Advances in Science Engineering and Technology*, ISSN: 2321-9009, Vol-5, Iss-2, Spl. Issue-2 Jun.-2017 http://araj.in.
- Sameer Sharma, Paluru Vijayachari, Attayoor P. Sugunan, alimuthusamy Natarajaseenivasan, and Subhash C. Sehgal. Seroprevalence of Leptospirosis among high-risk population of Andaman islands, india. *Am. J. Trop. Med. Hyg.*, 2006; 74(2): 278–283
- Human leptospirosis: Guidance for diagnosis, surveillance and control. WHO Library Cataloguing-in-Publication Data. World Health Organization, 2003.
- Dr. D. Rani Prameela 1, Dr. Arun2, Dr. D. Sreenivasulu3, Dr. P. Eswara Prasad4 and Dr. S. Vijaya Lakshmi, STUDIES ON PREVALENCE OF LEPTOSPIROSIS IN WILD ANIMALS. *International Journal of Science, Environment* ISSN 2278-

- 3687 (O) and Technology, Vol. 4, No 6, 2015, 1576 – 1580 2277-663X (P)
35. Sedigheh Zakeri ,* Neda Sepahian , Mandana Afsharpad et al.Molecular Epidemiology of Leptospirosis in Northern Iran by Nested Polymerase Chain Reaction/Restriction Fragment Length Polymorphism and Sequencing Methods. *Am. J. Trop. Med. Hyg.*, 82(5), 2010, pp. 899–903 doi:10.4269/ajtmh.2010.09-0721 Copyright © 2010 by The American Society of Tropical Medicine and Hygiene
 36. Kupek E, de Sousa Santos Faversoni MC, de Souza Philippi JM. The relationship between rainfall and human leptospirosis in Florianopolis, Brazil, 1991–1996. *Braz J Infect Dis* 2000;4:131–4
 37. Karande S, Kulkarni H, Kulkarni M, et al. Leptospirosis in children in Mumbai slums. *Indian J Pediatr* 2002;69:855–8.
 38. Sanders EJ, Rigau-Perez JG, Smits HL, et al. Increase of leptospirosis in dengue-negative patients after a hurricane in Puerto Rico in 1996. *Am J Trop Med Hyg* 1999;61:399–404.
 39. Kerri Pedersen, Theodore D. Anderson, Rachel M. Maisson et al, LEPTOSPIRA ANTIBODIES DETECTED IN WILDLIFE IN THE USA AND THE US VIRGIN ISLANDS DOI: 10.7589/2017-10-269 *Journal of Wildlife Diseases*, 54(3), 2018, pp. 450–459 Wildlife Disease Association 2018
 40. Takayoshi Yamaguchi, Naomi Higai, Nobuhiko Okura, Arina Matsumoto, Idam Hermawan et al. Characterizing interactions of *Leptospira interrogans* with proximal renal tubule epithelial cells Yamaguchi et al. *BMC Microbiology* (2018) 18:64 <https://doi.org/10.1186/s12866-018-1206-8>.
 41. Picardeau M. Virulence of the zoonotic agent of leptospirosis: still terra incognita? *Nat Rev Microbiol.* 2017;15(5):297–307
 42. Toma C, Okura N, Takayama C, Suzuki T. Characteristic features of intracellular pathogenic *Leptospira* in infected murine macrophages. *Cell Microbiol.* 2011;13(11):1783–92
 43. Eshghi A, Lourdault K, Murray GL, Bartho T, Sermswan RW, Picardeau M, Adler B, Snarr B, Zuerner RL, Cameron CE. *Leptospira interrogans* catalase is required for resistance to H₂O₂ and for virulence. *Infect Immun.* 2012;80(11):3892–9.
 44. Ramesh Holla ,1 Bhagwan Darshan ,1 Latika Pandey,2 Bhaskaran Unnikrishnan, et al Leptospirosis in Coastal South India: A Facility Based Study *Hindawi BioMed Research International* Volume 2018, Article ID 1759125, 5 pages <https://doi.org/10.1155/2018/1759125>
 45. D. Deodhar, M. John, and Leptospirosis., "Experience at a tertiary care hospital in northern India," *The National Medical Journal of India*, vol. 24, no. 2, pp. 78–80, 2011.
 46. S. Sethi, A. Sood, S. Pooja Sharma, C. Sengupta, and M. Sharma, "Leptospirosis in northern india: a clinical and serological study," *The Southeast Asian Journal of Tropical Medicine and Public Health*, vol. 34, 822, no. 4, p. 825, 2003.
 47. A. F. B. Victoriano, L. D. Smythe, N. Gloriani-Barzaga et al., "Leptospirosis in the Asia Pacific region," *BMC Infectious Diseases*, vol. 9, article 147, 2009.
 48. Krishna Prasad Dahal, Supriya Sharma, et al *Detection of Anti-Leptospira IgM Antibody in Serum Samples of Suspected Patients Visiting National Public Health Laboratory, Teku, Kathmandu* *Hindawi Publishing Corporation International Journal of Microbiology* Volume 2016, Article ID 7286918, 4 pages <http://dx.doi.org/10.1155/2016/7286918>
 49. André Alex Grassmann1, Frederico Schmitt Kremer1, Júlia Cougo dos Santos et al Discovery of novel leptospirosis vaccine candidates using reverse and structural vaccinology, *Frontiers in immunology* April 2017, volume 8, article 463
 50. Allison M. White a, Carlos Zambrana-Torrelío a, Toph Allen a, Melinda K. Rostal Hotspots of canine leptospirosis in the United States of America *The Veterinary Journal* 222 (2017) 29–35
 51. Edwards GA, Domm BM: Leptospirosis. *Med Times* 1966;94(9):1086-95
 52. Terpstra W. Human Leptospirosis: Guidance for Diagnosis, Surveillance, and Control. Geneva: World Health Organization 2003.
 53. PS Singh, V Tiwari Acute fulminant leptospirosis with multi-organ failure: Weil's disease. *Journal, Indian Academy of Clinical Medicine* 1 Vol. 13, No. 4 1 October-December, 2012, 332
 54. Charlotte van de Werve, MD, Alice Perignon, MD, Travel-Related Leptospirosis: A Series of 15 Imported Cases 2013 *International Society of Travel Medicine*, 1195-1982 *Journal of Travel Medicine* 2013; Volume 20 (Issue 4): 228–231
 55. Pappas G, Papadimitriou P, Siozopoulou V, Christou L, Akritidis N. The globalization of leptospirosis: worldwide incidence trends. *Int J Infect Dis* 2008;12(4):351–357. [PubMed: 18055245]
 56. Victoriano A, Smythe L, Gloriani-Barzaga N, et al. Leptospirosis in the Asia Pacific region. *BMC Infect Dis* 2009;9:147. [PubMed: 19732423]
 57. McCurry J. Philippines struggles to recover from typhoons. *Lancet* 2009;347(9700):1489. [PubMed: 19891040]
 58. Gale N.B., Alexander A.D., Evans L.B., et al. An outbreak of leptospirosis among US army troops in the canal zone. *Am J Trop Med and Hyg.* 1966;(15): 64-70.
 59. Cacciapuoti B., Nuti M., Pinta A., Sabrie A.M. Human Leptospirosis in Somalia: A serological survey: *Trains Roy Soy of Trop Med & Hyg* 1982
 60. Cacciapuoti B., Vellucci A., Ciceroni L., et al. Prevalence of Leptospirosis in man: A pilot survey. *Eur J Epidemiol* 1987;3(2):137-42.
 61. Assimina Zavitsanou 1, Fotoula Babatsikou, LEPTOSPIROSIS: EPIDEMIOLOGY AND PREVENTIVE MEASURES Leptospirosis: epidemiology and preventive measures 75 pp:75-82 ISSN:1108-7366 *Health Science Journal* © All Rights Reserved