Leptospirosis : A Re-emerging Disease

Mahajan, S[1] and Chhabra, Daljeet

Department of Veterinary Microbiology,
College of Veterinary Sciences and A.H, Mhow (M.P.)

Leptospirosis is emerging as an important public health problem, there is a sudden upsurge in the number of reported cases in past few years. It is an acute anthropozoonotic infection of worldwide significance cause by spirochaetes of species *Leptospira*.

History :- It is little more than 100 years, since weil, professor of medicine at Heidelberg (1886) had first described the disease in human. *Leptospira* had been seen at that time, but were not cultured and were named *spirocheta interrogans* by Siimson as early as 1907. Naguchi proposed the name '*Leptospira* ' (means thin spirals) in 1918, following detailed microscopical and cultural observation, Yanagawa and Faine (1966) showed that leptospires were analogues to other bacteria in structure and that characteristic antigens are associated with structural elements. As the time passed the researchers become more concerned with serological classification, based on absorption and cross agglutination of antisera, ELISA, method to analyse antigens and monoclonal antibodies were used to identify epitopes involved in Immunity or for classification.

Distribution**:-** The disease has been recorded through out the major continents of the world. In India, the disease has been reported from various states-A.P,M.P,Punjab, Maharashtra, Bihar, Haryana, Kerala, Tamil nadu, U.P, Delhi, Jammu and Kashmir, Islands of Andaman and Nicobar. The disease has been a serious problem in Mumbai and Southern states. Alkaline pH of these states provides a favorable environment for the survival and multiplication of leptospires.

In India the disease has been recorded in Cattle, Buffalo, Horse, Sheep, Goat and Dog (Arora, 1977; Uppal and Singh 1982). Among the earliest reports of leptospirosis has been recorded in madras in 1932 by Ayyar. Sawnney and Saxena (1967) from M.P. Rajasekhar and Nanjiah (1971) from Mysore. Triapthi (1977) from Orissa, Verma (1982) from Punjab and Dwivedi (1988) from Uttar Pradesh.

Etiology **:-** Leptospirosis is a bacterial disease caused by Genus *Leptospira* which are aerobic or microaerobic gram-negative and members of the order spirochaetales. There are more than 200 serologically distinct leptospriae. The new classification of genus *Leptospira* relies on generic relatedness of the organism. There are currently 7 genotype, 28 serotypes and numerous serovar. The predominant serotypes in India are *L. hebdomadis* and *L. semaranga.*

Leptospira as encountered in various species are as follows :-

1.**Cattle** :- *L. ponoma, L. grippotyphosa, L. canicola, L. icterohaemorrhagiae*
2.**Dogs** :- *L. icterohaemorrhagiae, L. pomana, L. canicola*
3.**Horse** :- *L. pomana, L. grippotyphosa, L. canicola, L. bratisalva* (Emerged in 2000)
4.**Pig** :- *L. canicola, L. bratisalva, L. pomana*
5.**Sheep and Goat** :- *L. pomona, L. grippotyphosa*

Zoonotic Aspect **:-** Leptospirosis thought to be the most widespread zoonosis in the world, it is a direct occupational anthropozoonosis with significant economic importance due to its wide range of host susceptibility and world wide distribution. Leptospirosis is primarily a monsoon disease which affects the domestic and wild animals as well as man. Rats are the most common source of infection for humans and act as a reservoir host. Rural farm workers are at high risk, man gets infection accidentally through direct or indirect contact with the infected animal and considered to be a "*dead end host* ". Several epidemics of leptospirosis have been reported world wide during the past century, where as in India leptospirosis is endemic in most of the southern and western parts, where epidemic

1. M.V.Sc. Scholar

occurs after flooding caused by heavy seasonal rainfall. Some important outbreaks of leptospirosis that had been reported in India in recent past by World Health Organization (WHO), National Institute of Communicable Diseases (NICD) Delhi, National Leptospirosis Reference Centre, Regional Medical Research Centre (ICMR) Port Blair, are in August 2004, affecting Valstad, Nasari and Surat district of Gujrat (WHO). A post dengue out break of leptospirosis in Mumbai in 2005, In July 2003 in kerla and Tamil nadu (WHO). *Leptospira* outbreak after cyclone hits Orrisa in October-November 1999 (ICMR), 27 October 1997 Valsad and Surat in Gujrat (NICD). In 1997 Kerla (WHO), Diglipura of north Andaman during October-November 1993 (ICMR).

Mode of Transmission**:-** Transmission of *Leptospirae* takes place through direct contact with urine of infected animal or ingestion of urine contaminated water or food. Human and animal may contract infection through flood or while swimming in contaminated water. Transplacental and venereal routes are considered to be an important route of infection in dog, cat, cattle, pig and man. Dog may acquire infection by ingestion of infected carcass. Vectors like ticks may transmit disease mechanically from one animal to other.

Antigenicity and Immune Response**:-** The organism have a complex antigen structure. The somatic antigen is genus specific. The surface antigens is a polysaccharide and is serover specific. The outer membrane is a potent immunogen, LPS in nature. It is the major antigen and the target of antibody and complement- mediated bactericidal activity. The immunological response to *Leptospira* is both humoral and cell mediated. Both B and T-cell dependent area's are stimulated on entry of organism in the body. The initial elimination is done by phagocytosis. Most of the *Leptospira* are digested in the vacuoles of macrophages. There is peak level of IgM antibody which appears first, followed by IgG and which presist longer than IgM. IgA antibodies appear's at the last stage and presist upto nine month.

Pathogenesis **:-** Leptospiraes are presumed to enter via small abrasion on the skin, although organism can invade lungs through aerosols but the most potent, common source of infection is water infected with the urine, organism can pass through placenta at any stage of pregnancy and cause septicemia in foetus. *Leptospira* usually establish septicemia in 4 to11 days and spread systemically to internal organs including liver and kidney. The primary lesions in leptospirosis is disruption of the integrity of cell membrane of endothelial cells lining small blood vessel in all part of the body. These effects are attributed to glycoprotein (GLP) toxin of leptospires. Liver and kindly are mostly affected, changes in liver leads to focal necrosis and cellular infiltration with marked icterus. The lesion seen in kidney are in glomerulus and tubules. Interstitial nephritis and fibrosis results in chronic cases. The animal may excreat leptospires intermittenly or regularly for period of months or for their life time.

Clinical Signs

a) Cattle and Buffalo :- In pre-acute / acute form the incubation period varies from 1 to 10 days there is elevation of body Temp (2 to 5 oF), anorexia, lassitude, haemoglobinuria, dyspnoea, icterus, abortion, anaemia along with icterus has also bean recorded. In lactating animals yellow clotted milk fallowed by agalactia, the condition is ascribed as "*cold mastitis*" due to obvious reason of no swelling. In chronic form there is weakness, emaciation, intermittent fever along with sub-icterus and haemorrhoges.

b) Horse **:-** The incubation period ranges from several days to three weeks the disease may appear as per-acute, acute or chronic form, the symptom are anorexia, depression, tachycardia, polynaea, haemonglobinuria, along with changes in the blood that is decrease in RBC count and haemoglobin level in per-acute and acute forms. Whereas in chronic form there is anorexia, intermittent fever, emaciation, Jaundice.

c) Pigs **:-** Pig usually show sub-clinical form with no apparent clinical signs.In acute form there is abortion, jaundice, conjunctivitis, anorexia, temperature, Meningo-encephalitic signs like hyperirritability, epileptic form of convulsion, circling, inco-ordination, paralysis may appear as clinical manifestation in some pigs.

d) Sheep and Goat **:-** Per-acute form is characterized by sudden onset resulting to death. Acute cases shows anaemia, haemoglobinuria and jaundice with high temperature. Abortion may be one of the important signs in goat besides icterus.

e) Dog and Cat **:-** The signs in dog appears as per-acute, acute, sub-acute and chronic. In per-acute cases the onset is sudden and is characterized by haemorrhages of mucous membrane and death in one to few days. The main signs of acute form are attributable to gastritis and nephritis. Ulceration of

the buccal mucosa and tonsillar involvements cause foul breath called Halitosis. There is parenchymatous nephritis may lead to lethal uraemic condition. Fibrotic Kidney in older dog is considered to be sequlae to it. The sub-acute form show slight icterus and tenderness of sub-lumber region due to involvement of Kidney. Ocular manifestation characterized by scleral congestion and ocular discharge is not uncommon.

Diagnosis **:-** Due to diverse clinical presentation of the disease, it is essential for the laboratory to play a role in its diagnosis. History and clinical symptoms play a major role in tentative diagnosis or presumptive diagnosis of the disease. Since isolation rate of the microorganism from clinical specimens is low due to prior indiscriminate use of antibiotics, serological techniques remains the cornerstone of diagnosis.

A) Laboratory Diagnosis:-

i) Collection of the sample **:-** In living subject, blood, urine, CSF, tissue scrapings/emulsions whereas in case of animal suspected to be dead from Leptospirosis, samples from Kidney, liver, brain, aborted foetus, etc. can be collected.

ii) Laboratory culture **:-** Sample if collected before administration of antibiotics can be used for culturing of leptospires and its isolation, if suitable media is provided for growth.

1. Media containing serum like Korthof;s medium, Stuart's media, Fletcher's semi-solid medium, etc.
2. Media containing bovine albumin fraction V and polysorbate 80/between 80 EM medium, EMJH (Ellinghausen and mc-cullough, Johnson and Harris) medium, protein free medium, Ellis medium.

iii) Examination of organism **:-**

a) Dark-field microscopy:- It is the simplest method and organism is observed with dark-field microscopes in clinical sample. But it may not be positive if there are few bacteria in the sample.

b) Phasecontrast microscopy:- is useful for visualizing *Leptospira* but because of its technical limitation. It has no practical purpose when ever dark-field microscopy is available.

c) Staining method:-Silver staining/ silver deposition technique is used to stain the smear of tissue containing *Leptospira* weil's stained preparation show black spriochaetes in pale yellow or brown tissue element. This method has the same limitation as stated above.

d) Immunofluoresence staining :- Immunofluoresence staining of leptospries are often preferable to silver staining because it is easy to see leptospries especially in small number.

iv) Animal inoculation :- Young animals are preferable over the older because older may resist the infection. Guinea pig, hamsters, young rabbits, swiss white mice and 1 to 3 days old chicks may be used.

B) Serological Methods **:-**

i) Macroscopic agglutination test **:-** A rapid macroscopic slide agglutination test can be used to screen human and animal serum sample. The best method is Galton's macroscopic slide agglutination test.

ii) Sensitized erythrocyte test **:-** *Leptospira*e extracts (lipopolysaccharides) and erythrocytes sensitising substance (ESS) are used to sensitized sheep and human red blood cells. Two types of reaction occurs when ESS mixed with sera containg homologus antibodies :-

a) Haemagglutination (HA)
b) Hemolysis (HL)

iii) Microcapsule agglutination test (MCAT) **:-** The test is developed in 1982 for serodiagnosis of leptospirosis based on the positive agglutination of synthetic polymer carrier sensitized with mixed antigens of sonicated leptospires by *Leptospira*l antibody.

iv) Latex agglutination test **:-** This test depends on the sensitization of commercially available latex particles with a *Leptospira* antigen. Antiserum will react with the antigen to cause agglutination of the particles.

v) ELISA Test **:-** Most widely used genus specific screening test used in man and animals. Other than simple ELISA, simplified dot ELISA test with antigen prepared from *L.biflexa* is as sensitive as multi-antigen (MAT). Most common used ELISA test for and evaluation of leptospirae species are :-

a) dot ELISA
b) Serotype specific ELISA
c) Lepto dipstick ELISA

vi) Microscopic agglutination test (MAT) **:-** The MAT is slow, tedious, potentially biohazardous, painstaking and subjective, but it is very sensitive and reliable assay when used by skilled people. MAT is carried out with suspension of living culture or of culture killed by addition of neutralized formaldehyde.

C) Molecular Diagnosis **:-**

i) DNA restriction enzyme analysis (REA) **:-** DNA

REA involve the extraction of DNA from a homogenous population of organism, digestion of the DNA with a restriction endonuclease and electrophoresis of the digested DNA in an agarose gel. The DNA finger prints thus generated are highly specified for each type of leptospirae.

ii) Nucleic acid probes and hybridization **:-** The nucleic acids that contain specified sequence are isolated, cleaved and labelled with a reporter molecule such as radioactive (32P or 35S) or non-radioactive (biotin, digoxigen) molecules. The labeled DNA in the single-stranded form is then hybridized to ssDNA in tissues or in solution.

iii) Polymerase chain reaction (PCR) **:-** PCR involve invitro enzymatic amplification of a target DNA sequence through a series of polymerization carried out by a thermostable DNA polymerase, primed with a pair of short DNA fragement, which bind specifically to the sequence of interest.

iv) Pulsed field gel electrophoresis (PFGE) **:-** PFGE is a variation of agrose gel electrophoresis that permits analysis of bacterial DNA fragment over an order of magnitude longer than that with conventional REA.

v) Ribotyping **:-** Recently, ribosomal ribonucleic acid gene restriction patterns have been used for the identification of species or for epidemiological typing bacteria for any phylogenetic comparision.

References

1. Arora, B.M. (1997): Ph.D. Thesis, Punjab Agriculture University, Ludhiana.
2. Edward, A. and Hodder, S. (1990): Leptospirosis, Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 8th Edn., Vol. ,3: 619.
3. Rao. R. S., Gupta, N., Bhalla, P. and Aggarwal, S.R. (2003): *Brazilian, J. Infectious Disease* Vol. 7 No. 3.
4. Rodastits, O.M., Blood, D.C. and Gay, C.C. (1994): Veterinary Medicine, 8th Edn. Bailliere Tindal.
5. Sawhney, A.N. and Saxena, S.P. (1967): *Indian, Vet. J.* 44 : 1008.
6. Uppal, P.K. and Singh, S.P. (1982): *Indian J. Animal Science* 52 : 1247.

* * * *

Bangladesh reports its first human H5N1 case

Bangladesh has become the 15th country to have a human case of H5N1 avian influenza. The Ministry of Health, Bangladesh has confirmed its first case of human infection with H5N1 avian influenza. The case was identified retrospectively as part of seasonal surveillance activities run by the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRB). The case is a 16-month-old male from Komalapur, Dhaka . He developed symptoms on 27th January 2008 and subsequently recovered. The case was confirmed as being infected with A(H5N1) by the WHO H5 Reference Laboratory, US Centers for Disease Control and Prevention (CDC). The case was exposed to live and slaughtered chickens at his home. Specimens have been collected from his family members and neighbours. All remain healthy to date.

Source: http://www.who.int/csr/don/2008_05_28/en/index.html

H5N1 outbreaks in Korea, Japan seen as linked

Two more wild swans infected with H5N1 avian influenza were found in Japan this week, and authorities in South Korea said H5N1 viruses found in chickens there closely matched an earlier isolate from swans in Japan . In late April, four H5N1-infected swans were found on Lake Towada in Akita prefecture, which neighbors Aomori . In the ensuing 2 weeks, two more infected swans were found on Japan 's northern island of Hokkaido . In South Korea , the National Veterinary Research and Quarantine Service said this week that an H5N1 virus from chickens in South Jeolla province was 99.7% genetically identical to an isolate from the swans in Akita prefecture, according to a report from the newspaper Chosun Ilbo. The finding suggests that the recent poultry outbreaks in South Korea may have stemmed from migratory birds, the story said. Kim Jae-Hong, a veterinary professor at Seoul National University , told the newspaper that viruses more than 99% identical are considered the same strain. This substantiates assumptions that migratory birds spread the virus on their way north in March and April after spending the winter in Southeast Asia . The report said similar observations were made when South Korea and Japan had avian flu outbreaks in 2003 and 2006. South Korea has had 33 H5N1 outbreaks in poultry since Apr 1, leading to the culling of 663,034 birds, according to a report the country filed with the World Organization for Animal Health (OIE) on May 20. http://www.cidrap.umn.edu/cidrap/content/influenza.