NDDB

Leptospirosis:

A desk study

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Etiology

Leptospirosis is a contagious disease of nearly 160 animal species including cattle, goat, sheep, dogs, horses, pigs and marine mammals. The pathogenic leptospires are classified into one species of Leptospira *interrogans* containing nearly 300 serovars, arranged in 25 serogroups which affect various species. It is also an important zoonosis, endemic in India. It is an occupational hazard to butchers, farmers, sewer workers and veterinarians. It could also be an important cause of febrile illness in patients from urban slums during monsoon and post-monsoon season.

Epidemiology

Epidemiology of leptospirosis is easily understood by classifying them into two categories: a) host – adapted and b) non- host adapted. An animal infected with host-adapted serovar is a **maintenance or reservoir host**. Exposure of susceptible animals to non- host adapted serovars results in **accidental or incidental disease**.

A maintenance host is characterized by:

- A high susceptibility to infection.
- Endemic transmission within the host species.
- Relatively low pathogenicity.
- Tendency to cause chronic rather then acute disease, producing insidious economic loss through reproductive losses.
- Persistence of the serovar in kidney and sometimes reproductive tract.
- A low antibody response to infection, with difficulties in diagnosis.
- Low efficacy of vaccination in prevention of infection eg.*hardjo* serovar in cattle.

In contrast, an incidental host is characterized by:

- Relatively low susceptibility but high pathogenicity.
- A tendency to cause acute, severe rather than chronic disease.
- Sporadic transmission within the host species and acquisition of infection from other species, sometimes in epidemic form.
- A short kidney phase.
- A marked antibody response against infection.
- Vaccines are more efficacious for protecting incidental host infection (eg. serovar *pomona* infection in cattle)
- Swine serve as incidental hosts for the other serovars.

Occurrence and prevalence of infection

Most leptospiral infections are subclinical. *Leptospira interrogans* serovar *pomona* (L.*pomona*) is the commonest infection in all farm animals.

Cattle are the only reservoir for L.*hardjo* and is an important cause of abortion. It is

also the commonest leptospiral infection in man. In cattle the morbidity rate for the clinical disease may vary from 10-30% and fatality is relatively low at about 5%.

Cattle is also maintenance host to L.*hardjo-bovis* which has low pathogenicity but could cause epidemics of agalactia, milk drop syndrome and a major cause of infertility.

On the other hand, cattle are incidental hosts to L.*pomona* which causes abortion and fatal haemolytic disease in calves.

Transmission

The source of infection is an infected animal which contaminates the pasture, drinking water and feed through infective urine, uterine discharges and aborted fetus. But urine leptospiruria is the chief source of contamination. In cattle, leptospiruria may persist for a mean period of 36 days even after clinical recovery.

The average percentage prevalence of leptospirosis in farm animals in India as estimated based on a review of published literature over the past few decades is given below:

S.no	**Source**	**Cattle**	**Buffalo**	**Goat**	**Sheep**
1	(Sharma et al.)	40			
2	(Sivaseelan, Rani R.U, and Kathiresan)			13	7
3	(Mrunalini and Ramasastry P)	11	11		9
4	(Biswal et al.)				
5	(Tripathi et al.)	24			
6	(Natarajaseenivasan and Ratnam)	62		75	56
7	(Ratnam, Everard, and Alex C)	44	35	47	54
8	(Natarajaseenivasan et al.)	53			
9	(Ratnam, Sundararaj, and Subramanian)	68			
10	(Mariya, Srivastava, and Thangapandian)	25			Uttaranchal
		19			TN
		16			UP
11	(Dwivedi, Mahesh, and Srivastava)	13			
12	(Srivastava et al.)	18			
14	(Mariya et al.)	21			
15	(Jyothi et al.)	15			
16	(Debnath et al.).	13			

17	(Jai et al.)			16	
18	(Pushpa and Kumari).		18		17
19	(Srivastava and Kumar)	16		14	15
20	(Sivaseelan, Rani, and Kathiresan)			28	28
	Incidence range (%)	**11-68**	**11-35**	**13-75**	**7-56**

Entry portals

Entry of the organisms into the body occurs mostly through skin abrasions and mucous membranes, and to a lesser extent by ingestion. Transplacental transmission is uncommon.

Survival

The organism is susceptible to drying and a pH lower than 6 or higher than 8. Temperatures lower than 7-10^{o} C or higher than 34-36^{o} C is detrimental. Water is the most important factor governing its persistence in soil or bedding. It can persist for at least 42 days in soil under average conditions and as long as 15 days in water.

Zoonotic implications

Clinical cases in humans by acquiring infection from animals are uncommon. However, farmers who milk cows are highly susceptible to L.*hardjo* and L.*pomona*. There is no vaccine available for humans. Infection in humans is most likely to occur by contact with contaminated urine or uterine contents.

Leptospirosis is endemic in Tamil Nadu, Kerala and Andamans and is being increasingly reported from other parts of India. It usually affects people who work in agriculture and causes severe morbidity and mortality.(*John*)

It may have a mortality as high as 40% in its icteric form (weil's disease) (*Dutta and Christopher*). However the presentation of non-icteric forms of leptospirosis are often non-specific and may be missed unless there is a high index of suspision.*(Rajajee, Shankar, and Dhattatri*)

The ICU mortality in patients suffering from leptospirosis has been recorded to be higher (52%) than compared to the total ICU mortality (31%).(*Chawla, Trivedi, and Yeolekar*)

The average percentage prevalence of leptospirosis in various human populations in India as estimated by a review of published literature over the past few decades is given below:

Sl.No	Source	Vets	Farm Workers	Farmers	Sewer workers	Animal handlers	Butchers	Patients with fever of	Patients with hepato-renal dysfunction of	People in flood prone areas	In people following	Children	Common population
1	*(Biswal et al.)*		100										
2	*(Natarajaseenivasan and Ratnam)*		73										27
3	*(Ratnam et al.)*												28
4	*(Sharma et al.)*		63		39	38	30						15
5	*(Angnanai, Pathak, and Mishra)*		36	32				33	39				
6	*(Chawla, Trivedi, and Yeolekar)*												7
7	*(Ambekar et al.)*				17								
8	*(Karande et al.)*											34	
9	*(Sethi et al.)*												5
10	*(Kaur et al.)*											15	
11	*(Karande et al.)*											32	
12	*(Natarajaseenivasan et al.)*		68										
13	*(Ratnam et al.)*				33								
14	*(Sharma et al.)*								34				
15	*(Vajiravelu et al.)*	7				21							
16	*(Govindarajan et al.)*					22			80				
17	*(Koteeswaran)*	14			13								
18	*(Savalia and Pal)*		30	21		41							
19	*(Jyothi et al.)*												7
20	*(Srivastava and Kumar)*												15
21	*(Ratnam, Sundararaj, and Subramanian)*										47		

It was not until the late 80s that the Andaman Haemorrhagic Fever (AHF) with majority cases showing pulmonary involvement was identified as leptospirosis and severe pulmonary haemorrhage was shown for the first time as a complication of leptospirosis from Andamans, India. This pulmonary form has a case fatality rate of 10-15%.*(Vijayachari et al.*)

The Regional Medical Centre under the Indian Council of Medical Research (ICMR) in Port Blair, Andaman and Nicobar Islands, has now been re-designated as WHO Collaborating Centre for Diagnosis, Reference and Training in Leptospirosis. (WHO-CCDRTL).

Pathogenesis

After penetration of skin or mucosa, the organisms multiply in the liver and migrate to peripheral blood. During early period of septicaemia, haemoglobinuria is common in young calves but unlikely in adults. In the acute phase, animal may die from septicaemia and/or haemolytic anaemia. If they survive this phase, they may succumb to uremia caused by interstitial nephritis.

Clinical findings

Clinically, the disease may be acute, subacute or chronic and is usually caused by L.*pomona* or L.*hardjo*.

A. Infection due to L.pomona

1. Acute

In calves:

Calves up to 1 month are most susceptible. The symptoms are high fever, petechiation of mucosa, acute hemolytic anaemia with haemoglobinuria and jaundice. The fatality rate is high.

In adults:

Abortion may occur and is most commonly seen in the second half of pregnancy but may occur at any time after 4 months. Abortion without prior clinical illness may also occur. Milk production is markedly reduced and is red tinged or contains blood clots. There may be no apparent physical change in the udder. Severe lameness or necrotic dermatitis is reported in some animals.

2. Sub acute

It differs from acute form only in degree. Abortion usually occurs 3 – 4 weeks later than in the acute form of the disease. Marked drop in milk production is a characteristic finding with appearance of blood – stained or yellow – orange, thick milk in all four quarters without apparent physical change in udder. This form occurs in all species of animals and is common in adult cattle.

3. Chronic

Symptoms are mild and may be restricted to abortion. Severe storms of abortion occur most commonly in groups of cattle which are in the same stage of pregnancy and occur during the last trimester. Apart from abortion, there is no depression of reproductive efficiency. Many animals develop positive agglutination titres without clinical illness. There are occasional reports of meningitis also.

B. Infection due to L.hardjo

Signs are usually restricted to infertility and lower milk yield. There is sudden onset of fever, anorexia, immobility and agalactia. The milk is yellow to orange and may contain clots. The udder is flabby without any heat or pain. The sudden drop in milk production may affect up to 50 % of cows at a time but production returns to normal within 10 – 14 days.

Leptospiruria may be present in up to 30% of affected cows without any changes in udder or milk. Cattle may shed leptospires in urine between 26 - 32 weeks.

Abortion may occur 3-10 weeks after initial infection and may be the only evidence of the disease. Furthermore, many cows with subclinical infections may show only a fall in milk yield.

Aborted bovine fetuses are usually autolysed to the point where no lesions or bacteria can be obtained. Even from a fresh foetus, it is difficult to culture these organisms, especially L.*hardjo*.

Diagnosis

Laboratory diagnosis of leptospirosis can be complex and involves tests which fall into two groups: One group of tests is designed to detect anti-leptospiral antibodies and the other group, to detect leptospires, leptospiral antigens, or leptospiral nucleic acid in animal tissues or body fluids.

A.Serological tests

Acute and convalescent sera taken 7- 10 day apart should be submitted from each clinically affected animal or from those with a history of abortion. Sera should also be taken from some of the apparently normal animals. If possible, results of the tissues samples from rodents which are known to inhabit the farm may be compared with those obtained in farm animals.

1. Microscopic agglutination test (MAT)

This is the only alternative test prescribed by OIE for the purposes of international trade. The MAT is primarily used as a herd test. At least 10% or ten animals, whichever is greater should be tested and vaccination history documented, if carried out. As an individual test, MAT is very useful for diagnosing acute infection.

The MAT is particularly useful in diagnosis of disease caused by incidental, non-host adapted serovars or acute disease caused by host-adapted serovars. It is less useful in diagnosis of chronic disease in maintenance hosts since antibody response may be negligible in chronic infections.

Titres after infection are generally higher and persist longer than vaccination titres. MAT titres above 100 is considered significant in cattle and a four fold increase in the titre on a paired sample taken two weeks apart is diagnostic. In abortion caused

by incidental serovars, titres are often above 3000.

Paired sera are of limited value in chronic infections. In chronic *hardjo* infections, a recently aborting cow with a titre of above 300 has about 60%,above 1000 an 80%, and above 3000, 90% chance of foetal infection. Several aborting cows having high titres (above 300) is evidence of leptospirosis in unvaccinated herds.

Bulls destined for AI are to be free of antibody to serovars *hardjo, grippotyphosa, canicola, pomona, sejroe* and *icterohaemorrhagiae* at a final serum dilution of 1:100 in MAT.

2. Enzyme Linked Immunosorbent Assay (ELISA)

This is a more accurate test. For diagnosis of leptospiral abortion in cattle, a titre of 3000 is proposed as the threshold for *pomona* but no similar figure is available for *hardjo*. For a herd diagnosis of disease due to *hardjo,* a total of 10 animals from different age groups ie. yearlings, first calf heifer, second - calf cow and adult cow groups should be tested.

B. Other tests

1. Isolation of leptospires: Isolation of leptospires from clinical material are time consuming and done only in specilaized laboratories.

2. Fluorescent antibody test (FAT):

a. FAT of urine is a fast and accurate diagnostic method for identifying serotypes. It detects degenerated as well as intact organisms. Antibodies also appear in milk.

b. FAT of tissue may also be adopted but false positives are common unless done by an experienced eye.

3. Silver Staining: Organisms may be visible in silver stained sections, especially in the proximal convoluted tubules of the kidney.

4. DNA probes and Polymerase Chain Reaction (PCR): These are sensitive and rapid tests for the detection of leptospires tissues and in urine of cattle which has become infected subsequent to vaccination and is superior to bacteriological culture and FAT. It is possible that serologically negative but infected bulls may contain leptospires. This could be overcome by adopting PCR to detect pathogenic leptospires in the semen and urine of infected bulls.

5. Immunoperoxidase techniques: They are highly useful in demonstration of leptospirae in formalin fixed tissues, although not serovar specific.

6. Other tests: Dipstick test, **Latex agglutination test** (indigenously developed) etc, are available at WHO- CCDRTL, Andaman and Nicobar Islands.

Differential diagnosis:

Acute leptospirosis must be differentiated from diseases causing haemolytic anaemia with or without haemoglobinuria. They include: Babesiosis, Anaplasmosis, Post parturient haemoglobinuria & Bacillary haemoglobinuria.

Chronic leptospirosis causing abortion must be differentiated from all other causes of abortion such as: Brucellosis, Trichomoniasis, Protozoal abortion, Campylobacteriosis, IBR, Mycotic abortion & BVD.

In case of chronic leptospirosis with milk drop syndrome it must be differentiated from milk drop due to change of feed or management or epidemic infection such as Bovine Respiratory Disease.

Treatment:

The primary aim of treatment is to control the infection before irreparable damage occurs to the liver and kidney. Treatment with Dihydrostreptomycin or tetracyclines as soon as possible after the signs appear is recommended.

The secondary aim of treatment is to control leptospiruria of carrier animals thereby rendering them safe to remain in the group.

Control strategy for farms:

- Conduct MAT at periodic intervals as a herd test on 10% of the animals and cull or treat positive reactors.
- If reactors are found, screen the entire herd by MAT or any other suitable test and cull or treat any other reactors.
- Screen a cow which fails to carry a calf to term, produces a dead or weak calf, or exhibits any other signs of the disease by a suitable test and treat or cull if found positive.
- Investigate signs of infection like mastitis and high numbers of abortion.
- Paired sera samples taken 7- 10 day apart during acute and convalescent phases should be submitted from each suspected case.
- Paired sera samples from a few apparently normal animals also need to be submitted along with the above.
- Paired sera are of limited value in chronic infections.
- Use veterinary gloves while assisting cows in calving.
- Keep animals away from effluent ponds.
- Do not spray pastures with effluent stored in ponds during the wet season.
- Dry out pasture sprayed with effluent before allowing grazing.
- Properly seal and drain effluent disposal tanks.
- If pigs are kept on the farm, their effluent should be kept inaccessible to cattle.
- Treat suspected bulls to reduce the level of urinary shedding.
- Control of rodents in the farm is important.

Vaccination:

- The disease can be controlled by a combination of vaccination and antimicrobial therapy. But there are no vaccines available in India. Monovalent, bivalent and pentavalent vaccines are available abroad. In areas were vaccination is practiced:
- Cattle over 6- 9 months of age are to be vaccinated.
- Annual revaccination is recommended.
- Majority of vaccinated animals do not have MAT antibodies 20 weeks after vaccination and is not necessarily an indication that protection is wanting. They are protected from natural infection for many months after MAT titres become undetectable.

Tests available in India:

Sl.no	Test	Available at
1	MAT	• Indian Veterinary Research Institute (IVRI), Izatnagar, UP. • WHO- CCDRTL, Port Blair, Andaman and Nicobar Islands. • Leptospirosis Research Laboratory. Centre for Animal Health Sciences. TamilNadu Veterinary and Animal Sciences University, Chennai.
2	ELISA	-Do-

Note: The list is not comprehensive

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