NATIONAL DAIRY DEVELOPMENT BOARD ANAND GUJARAT

ANIMAL HEALTH UPDATES

Animal Health Group

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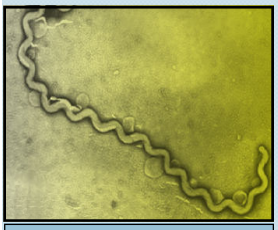
Disease - Bovine Leptospirosis

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.



Magnified image of a leptospire which is a gram negative spirochaete.
Source: www.bol.ucla.edu

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.
- More efficacious vaccines.
- Example is serovar *pomona* infection in cattle.

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 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 :

Sl.no	Species	Avg. prevalence range (%)
1	Cattle	11-62
2	Buffalo	11-35
3	Goat	13-75
4	Sheep	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° C or higher than 34-36° C is detrimental. Water is the most important factor governing its persis-

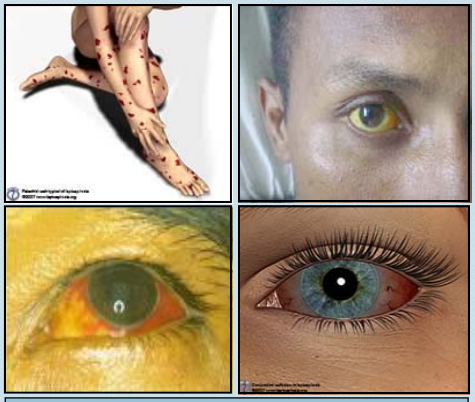
tence in soil or bedding. It can persist for at least 42 days in soil under average conditions and as long as 15 days in water.

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.

The average 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:

S.no	Population description	Avg. prevalence range (%)
1	Veterinarians	7-14
2	Farm workers	30-100
3	Farmers	21-32
4	Sewer workers	13-39
5	Animal handlers	21-41
6	Butchers	30
7	Patients with fever of unknown origin	33
8	Patients with hepato-renal dysfunction of unknown origin	34-80
9	People in flood prone areas	15-34
10	Common population	5-28



Body rashes, Icterus, conjunctival suffusion and uveitis are some of the symptoms of leptospirosis in humans. (Clockwise from top left)
Sources :(Clockwise from top left)
1.www.leptospirosis.org
2.www.microbewiki.kenyon.edu
3.www.leptospirosis.org
4.www.virutes.hu

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 India. The Regional Medical Centre under the Indian Council of Medical Research (ICMR) in Port Blair, Andaman and Nicobar Islands, has now been redesignated 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.

Interstitial nephritis manifested by small white cortical foci in later stages of the disease
Source : www.inta.gov.ar

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

a. 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.

b. 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. Some other tests like the **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:

Acknowledgement : The AH Group is grateful to Dr. Govindarajan, PhD., Professor and Head, Leptospirosis Reasearch Laboratory, Centre for Animal Health Studies, for providing his valuable comments.

S.no	Test	Available at
1	MAT	1.Indian Veterinary Research Institute (IVRI), Izatnagar, UP. 2.WHO- CCDRTL, Port Blair, Andaman and Nicobar Islands. 3. Leptospirosis Research Laboratory. Centre for Animal Health Sciences. TamilNadu Veterinary and Animal Sciences University, Chennai.
2	ELISA	-Do-
Note: The list is not comprehensive		

Sources:

1.http://rmrc.res.in
2.OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2006).
3.PubMed
4.The Merck Veterinary Manual,8th Edition, Merck and Co., Inc. USA.
5.Veterinary Medicine,9th Edition , (Radostits et al 2000). A textbook of the diseases of cattle, sheep, pigs, goats and horses. W.B. Saunders Company Ltd.
6. Veterinary Science Database, CAB International

OIE - Significant animal diseases reported to OIE in Dec'07– Jan'08

Sl.No	Disease Outbreak	Countries reporting
1	Foot and Mouth Disease	Laos, Nigeria
2	Anthrax	Romania
3	Brucella melitensis	Croatia
4	African Swine Fever (ASF)	Russia, Zambia, Azerbaijan
5	Blue Tongue	Spain
6	Highly Pathogenic Avian Influenza	Benin, Poland, Israel, UK, India, Iran, Thailand, Germany, Turkey, Ukraine.
7	Low Pathogenic Avian Influenza	Dominican Republic
8	New Castle Disease	Italy
9	Scrapie	Israel (Source : www.oie.int)

New fluorescence microscopy technique

The new technique, known as Photo-Activated Localisation (PAL) microscopy has been developed by *Carl Zeiss which* enables proteins to be located in a cell to a resolution of 20nm - an order of magnitude higher than conventional fluorescence techniques such as confocal microscopy achieve.

The technique was developed by scientists at Howard Hughes Medical Institute in Virginia, USA.

Assembling individual images into a single image enables a resolution to be achieved that is close to that of an electron microscope.

Source : www.labtechnologist.com

Nanotechnology in Food

Nanotechnology refers to the control of matter at an atomic or molecular scale of between one nanometer (nm) (one millionth of a millimetre) and 100 nm.

Despite still being in its infancy, current estimates on the value of products using nanotechnology is in the range of 7billion US dollars.

Currently, nanotechnology is being used in a range of applications, from computer chip layouts, to new polymers. The main commercial applications using it are in cosmetics - but the potential for food is not small.

More 600 nanofood products are already available on the global market, according to new data from the Helmut Kaiser Consultancy (HKC).

In packaging, embedded nanosensors will alert consumers before the food goes stale.

There are also some concerns particularly in relation to absorption and reaction, and the possibility of nanoparticles crossing natural barriers and membranes.

However, there are no regulations or labelling requirements to allow consumers to make an informed choice at present.

Source :www.foodnavigator.com

New biochip technology to reduce animal testing during drug safety studies

Toxicity testing has traditionally relied on animal testing procedures to predict whether a drug candidate is toxic or not, but these expensive animal tests do not always accurately mimic human responses to compounds.

The new biochip technology, developed by a team of researchers at Rensselaer Polytechnic Institute, the University of California at Berkeley, USA and Solidus Bioscience will be described in an upcoming early edition of the online journal *Proceedings of the National Academy of Sciences (PNAS)*.

The Datachip comprises over 1000 three dimensional tissue cultures that mimic how cells would be arranged in the body to provide researchers with a fast screening system capable of predicting the potential toxicity of a drug candidate to various organs.

The Metachip was described in a previous paper in the same journal and mimics the metabolic reactions of the human liver where seemingly benign chemicals can become highly toxic.

Scientists developed the MetaChip and DataChip to deal with the two most important issues that need to be assessed when examining the toxicity of a compound - the effect on different cells in the human body and how toxicity is altered when the compound is metabolised in the body.

By varying the ratios of enzymes on the MetaChip, scientists could develop personalised chips that predict a patient's response to a particular compound.

Source: www.labtechnologist.com

Epidemiological evidence for MAP as a cause of Crohn's disease

Mycobacterium *avium* subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease, a chronic enteritis in ruminants. Recent studies have shown that a high percentage of people with Crohn's disease (CD) are infected with MAP; whether the association of this bacterium and CD is causal or coincidental is not known. The search for risk factors in CD has been frustrating. Epidemiologists now claim to have gathered enough information that points to an association between MAP and CD. The article appeared in the Oct '07 edition of *Epidemiology and Infection* journal.

Source: www.ncbi.nlm.nih.gov

Protein fibres in semen enhance ability of HIV to infect cells many fold

Collaborating research teams in Germany have shown that fragments of prostatic acidic phosphatase (PAP) protein found in human semen form tiny fibre-like structures called amyloid fibrils which enhance the infection rate by several orders of magnitude, usually more than 50-fold and, under certain conditions, even more than 100,000-fold.

HIV-1, which causes AIDS, has infected about 60 million people and caused over 20 million deaths worldwide. The majority of these people were infected through sexual intercourse and yet the factors influencing the infectiousness of HIV in semen are poorly understood, according to the researchers.

Once they had identified PAP, researchers then verified that synthetic PAP fragments also enhanced HIV, confirming it as the active ingredient. Although individual PAP fragments are inactive, once they clump together into fibrils, they can capture virions and promote their physical interaction and fusion with target cells, including immune system T cells and macrophages. The scientists dubbed the fibres 'Semen-derived Enhancer of Virus Infection' (SEVI).

The enhancing activity of SEVI is most pronounced when the levels of infectious virus are low, resembling the conditions of sexual HIV-1 transmission, they reported in the most recent issue of the journal *Cell*.

The high potency of SEVI in promoting viral infection together with its relatively low cytotoxicity suggests that it may not only play a relevant role in sexual HIV transmission, but could also help to improve vaccine approaches and gene delivery by lentiviral vectors.

Source : www.labtechnologist.com

Bovine Spongiform Encephalopathy (BSE) detected in Canada in Dec'07

The Canadian Food Inspection Agency confirmed the diagnosis of BSE in a 13-year-old cow from Alberta in Canada in December 2007.The animal was identified by a national surveillance program, which targets cattle at risk. The World Organization for Animal Health (OIE) has already categorized Canada as a Controlled Risk country for BSE .

Source : www.foodproductiondaily.com

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