NATIONAL DAIRY DEVELOPMENT BOARD ANAND GUJARAT

ANIMAL HEALTH UPDATES

Animal Health Group

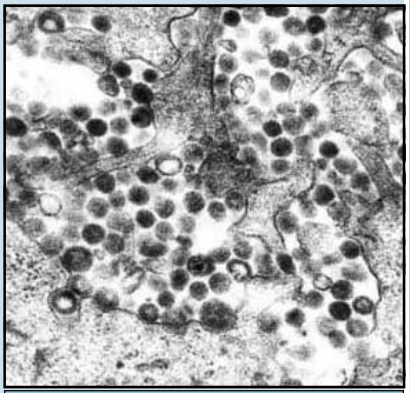
VOLUME II ISSUE II (For Private circulation only) Jul– Sep'08

In this issue :

Disease - Infectious Bovine Rhinotracheitis (IBR)

Etiology

Infectious Bovine Rhinotracheitis (IBR)/Infectious Pustular Vulvovaginitis (IPV) is caused by bovine herpes virus -1(BoHV-1). It is a disease of domestic and wild cattle as well as buffalo. The virus has ubiquitous distribution. There are at least three distinct BoHV-1 subtypes : (i) Respiratory- BoHV-1.1 (ii) Genital-BoHV-1.2 -which is further divided into BoHV-1.2a , which cause abortion and, BoHV-1.2b , that does not cause abortion and, (iii) Encephalitic-BoHV-1.3, now known as BoHV-5.



Electron micrograph of BoHV-1 in cell culture. Source: niah.naro.affrc.go.jp

Differentiation of the subtypes can be done by various immunoassays using monoclonal antibodies and, restriction endonuclease analysis.

Epidemiology

The genital carrier state is important in the maintenance of venereal IBR virus and in the occurrence of sporadic infectious pustular vulvovaginitis (IPV) and infectious pustular balanoposthitis (IPB). The disease has been eradicated from Norway, Sweden, Finland, Denmark, Switzerland, Austria and Italy (Bolzano region). With stricter control measures in artificial insemination (AI) centres, outbreaks of IBV and IBP have been controlled in Britain.

I. Prevalence in India

Surveys throughout the world have estimated a 10-50% sero-prevalence or even more in cattle. The average sero-prevalence percentages of IBR in cattle and buffaloes in India as estimated by a review of past three and a half decades of published literature done by the AH group is given in the table below.

Sero-prevalence of IBR/IPV in India

S.no	Details	Cattle	Buffaloes
1	Prevalence range (%) in normal herds	11-65%	4-52%
2	Prevalence range (%) in herds where abortions, repeat breeding , respiratory disease and conjunctivitis have been reported	11-82%	28-70%

Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS) has estimated an overall IBR prevalence of 37% in bovines in the country.

II. Morbidity and case fatality

The uncomplicated form of respiratory disease in cattle is not highly fatal, most losses being mainly due to secondary bacterial bronchopneumonia. The case fatality in the systemic form of the infection in newborn calves is almost 100%.

PD_ADMAS have estimated high morbidity rates in India with the case fatality rates of about 3-5%.

III. Methods of transmission

The main source of infection are the nasal exudates and coughed up droplets, genital secretions, semen, foetal fluids and tissues. Aerosol infection is the method of spread of the respiratory form. The genital form spreads by venereal transmission. Survival of BoHV-1 virus up to 1 year in frozen semen at -196°C has been reported.Introduction of new animals or use of contaminated semen often precedes an outbreak.

IV. Risk Factors

All ages and breeds are susceptible but infection occurs more commonly in animals over 6 months of age. An unvaccinated herd of breeding cattle is highly susceptible to epidemics of respiratory disease and abortion. Newborn calves are highly susceptible to systemic form of infection if the level of specific antibodies to the virus in the colostrum fed to the calf is inadequate.

Only rarely do the respiratory and genital forms of the disease occur together.

The outcome of BoHV-1 infection can vary from subclinical to a systemic infection in neonatal calves that is often highly fatal. A subclinical infection of bulls can last several months, even in vaccinated animals.

Pathogenesis

The virus enters the animal via the nose and replicates to high titres in mucous membranes of the upper respiratory tract and in tonsils. It subsequently disseminates to conjunctivae and by neuronal transport, reaches the trigeminal ganglion.

After genital infection, BoHV-1 replicates in the mucous membranes of vagina or prepuce and becomes latent in sacral ganglia. The viral DNA remains in the neurons of the ganglia, probably for the entire life of the host. Stress, such as transport,

parturition, inclement weather and treatment with steroid can induce reactivation of the latent infection. Consequently, the virus may be shed intermittently to the environment.

The incubation period is usually 2-4 days. Uncomplicated cases of respiratory or genital disease lasts 5 -10 days. Many infections also run a sub-clinical course. After initial infection, nasal viral shedding is detected for 10-14 days.

The semen of an infected bull may contain the virus and thus can be transmitted by natural mating or AI.

I. Respiratory disease

The BHV-1 virus infects the nasal cavities and upper respiratory tract, resulting in rhinitis, laryngitis and tracheitis. Spread from nasal cavities to the ocular tissues probably occurs by the way of lacrimal ducts and causes conjunctivitis with oedema and swelling and multifocal plaque formation, peripheral corneal oedema and deep vascularisation.

II. Encephalitis

Brain becomes infected by spread of virus presumably from the nasal mucosa via the trigeminal nerve to the trigeminal ganglion, resulting in non-suppurative encephalitis.

III. Abortion

Systemic invasion of the virus is followed by localization of the virus in several different tissues. The virus may be transported by leukocytes to the placenta and transferred to the foetus causing abortion. The foetus is highly susceptible to the virus, which causes a peracute infection that is usually fatal. Infection in the last trimester of gestation may result in mummification, abortion, stillbirth or birth of weak calves.

The systemic form of infection in new born calves is characterized by severe inflammation and necrosis of the respiratory and alimentary tracts, nephritis and encephalitis.

IV. Latency

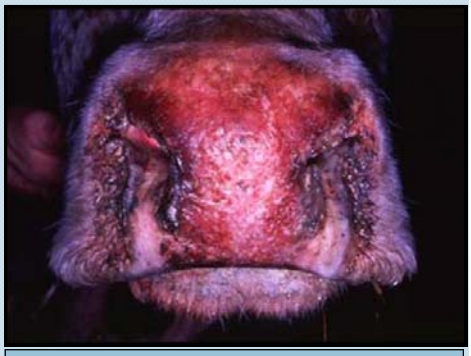
The BoHV-1 virus can become latent following a primary infection from field or following vaccination with an attenuated strain. Colostral antibodies in calves do not prevent initial virus replication. Latency can persist even if calves are seronegative.

The practical aspect of latency is that all cattle from endemic herds must be considered as potential sources of BoHV-1 virus and capable of spreading infection. Reactivation and shedding can occur in known carrier bulls at the time of mating. The placenta may harbour the virus in a latent stage for up to 90 days without transmission to foetus.

Clinical findings

I. Rhinitis, tracheitis and conjunctivitis (red nose)

There is considerable variation in the clinical signs following natural infection. Many animals in a herd become affected within a few days. The disease is usually mild, characterized by inappetance, coughing, profuse bilateral serous nasal discharge, excessive salivation, hyperaemia of nasal mucosa and focal necrosis of mucous membrane of nasal septum visible just inside the external nares, moderate fever and moderate drop in milk production which recovers in a few days. Several animals may have the corneal form of the disease with corneal oedema, conjunctivitis and profuse ocular discharge. The affected animals do not return to full production for 10-14 days. The outbreak of respiratory disease will be usually followed by abortions up to 90 days after the index case occurred.



Typical red nose in an animal suffering from IBR

II. Ocular form of IBR

Conjunctivitis is a common finding in typical red nose but outbreaks of conjunctivitis may occur as the major clinical finding. One or both eyes may be affected, which is easily misdiagnosed as infectious keratoconjunctivitis (pink eye) caused by *Moraxella bovis*. However, in IBR there are no lesions on cornea except diffuse oedema. Calves below 6 months may develop encephalitis which is marked by incoordination, excitement alternating with depression and a high mortality rate.

III. Systemic disease in newborn calves

In calves below 10 days of age, the systemic form of the disease is severe and highly fatal.

IV. Abortion

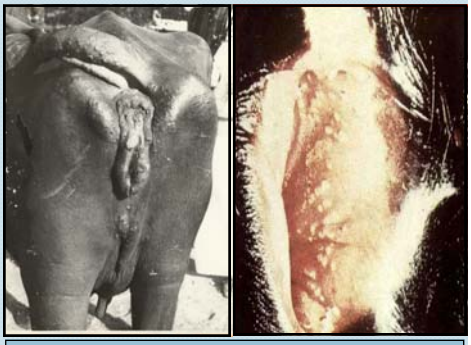
Abortion is a common sequel and occurs some weeks after the clinical illness or parenteral vaccination of non-immune pregnant cows with modified live virus (MLV) vaccine of bovine tissue culture origin.

Abortion may occur up to 90 days following vaccination if the virus becomes latent in the placenta and infects the foetus much later.

Abortion is common at 6-8 months of pregnancy. Retention of placenta follows, but residual infertility is unimportant. However, endometritis, poor conception, and short oestrus can occur after insemination with infected semen.

V. Infectious pustular vulvovaginitis (IPV)/ Infectious pustlar vulvovaginitis (IPB)

IPV is characterized by frequent urination, elevation of tail and a mild vaginal discharge. The vulva is swollen and small papules are seen which later form erosions and then become ulcers. Mucosal ulcers may coalesce and sloughing of necrotic tissue may occur. Recovery usually occurs in 10-14 days unless there are complications.IPB is characterized by similar lesions of the glans penis and preputial mucosa.



Typical lesions seen in Infectious Pustular Vulvovaginitis

Diagnosis

Office International des Epizooties (OIE) has prescribed certain tests for diagnosis of IBR/IPV for purposes of international trade.

I. OIE prescribed tests

1. Virus Isolation from semen

For virus isolation from semen, 0.5ml straw of extended semen or 0.02 ml of raw semen should be tested with two passages in cell culture. For extended semen, an approximation should be made to ensure that an equivalent of 0.05ml of raw semen is examined. The isolation of virus from semen requires some special adaptations because seminal fluids contain enzymes and other factors that are toxic to the cells and inhibit viral replication.

2. Real-time polymerase chain reaction (Real-time- PCR)

This method has been developed to detect BoHV -1 in extended bovine semen intended for trade. Real-time-PCR differs from the standard PCR in that the amplified PCR products are detected directly during amplification cycle using a hybridization probe. Real-time PCR assays use only one pair of primers and are able to

provide high sensitivity with a much lower risk of contamination. The amplification and detection of target is done simultaneously. Since there is no post amplification PCR product handling, the risk of contamination is greatly reduced. Moreover, it is also possible to perform quantitative analysis with real-time PCR systems.

Facilities are available at Research and Development (R&D) Laboratory, Indian Immunologicals Limited (IIL), Hyderabad, to carry out virological diagnosis of IBR using cell culture, PCR and real-time PCR.

3. Virus Neutralisation (VN)

The most relevant diagnostic tools for detection of BoHV-1 are still VN test or ELISA. Preferably paired sera samples are tested, one at the onset of the disease and other, after 21 days. VN tests are performed with various modifications. Of these variables, the virus/serum incubation period has the most profound effect on the antibody titre. A 24-hour incubation period may score up to 16 fold higher antibody titres than a 1 hour incubation period, therefore the former is recommended for purposes of international trade.

4. Enzyme Linked Immunosorbant Assay (ELISA)

ELISAs for the detection of antibody against BoHV-1 in serum appear to be replacing VN tests. There are a number of variations in the ELISA procedures. Several types of ELISA are commercially available, including indirect and blocking ELISAs, some of which are also suitable for detecting antibodies in milk.

Facilitites for both VNT and ELISA are available at R&D Laboratory, IIL, Hyderabad.

II. Other tests

1. Virus isolation from tissue samples

Samples to be collected:

(a) Live animals: Nasal swabs from several (5-10) affected cattle in the early phase of the infection which have serous rather than muco-purulent discharge.

In case of vulvovaginitis or balanoposthitis, swabs are taken from genitals. Swabs should be vigourously rubbed against the mucosal surfaces. The prepuce can also be washed with saline and the fluid collected. The specimens are to be suspended in transport medium (cell culture medium containing antibiotics and 2-10% foetal bovine serum), cooled at 4°C and rapidly submitted to laboratory.

(b) Necropsy: Mucous membrane of respiratory tract, portions of tonsil, lung and bronchial lymph nodes are collected for virus detection. In cases of abortion, foetal liver, lung, spleen, kidney and placental cotyledon are examined. The samples should be sent to the laboratory as quickly as possible, on ice.

Tissues collected post-mortem can be examined for the presence of BoHV-1 antigen by the immunofluorescence test on frozen sections. Immunohistochemistry and ELISAs may also be used for direct rapid detection. There are presently four OIE reference laboratories in the world for IBR/IPV; two of which are in Canada, and one each in the UK and Germany.

Vaccination

An infection normally elicits an antibody and a cell-mediated response within 7-10 days. The immune response is presumed to persist for life although it may fall below the detection limits of some tests.

Conventional, subunit and marker vaccines are available currently throughout the world against BoHV-1.

I. Conventional vaccines

1. Modified Live Virus (MLV) vaccines

There are two types, one given parenterally and the other, intranasally. The advantages of this vaccines are:

(a)Induce a rapid immune response.

(b)Provide relatively long duration of immunity.

(c)Also induce local immunity (intranasal).

The major disadvantages of MLV vaccines are:

(a)Vaccination is not effective if the animal snorts out the vaccine (in case of intranasal).

(b)It is abortigenic and cannot be used on non-immune pregnant cattle.

(c)The virus can become latent following vaccination.

(d)The vaccine is relatively unstable on storage.

A temperature sensitive (TS) MLV vaccine (intranasal) has also been developed which contains a strain whose growth is limited to the upper respiratory tract and is safe for use in pregnant cattle.

2. Inactivated vaccines

The advantages of inactivated vaccines are:

(a)It does not cause abortion or latency, though it does not prevent latency by field strains.

(b)Safe for use in pregnant animals and do not cause viral shedding.

(c)Relatively stable on storage.

The major disadvantages of inactivated vaccines are:

(a)It is not as efficacious as MLV vaccines.

(b)Require two doses 10-14 days apart, and protection not observed until 7-10 days following the second dose of vaccine.

A major drawback of conventional vaccines with the exception of TS-MLV is that vaccinated and naturally infected animals cannot be differentiated.

II. Sub-unit and Marker vaccines

A sub-unit vaccine contains only one or part of the viral antigen necessary to evoke protective immunity and lacks components that may cause undesired effects. Marker or DIVA (differentiation of infected from vaccinated animals) are attenuated or inactivated vaccines based on deletion mutants (lacks glycoprotein E (gE)). This marker vaccine is used along with a companion diagnostic test (gE ELISA).

These vaccines do not have any disadvantages of the conventional vaccines and, vaccinated animals can be differentiated from naturally infected ones. However, none of the above vaccines are presently available in India. Research is ongoing at R&D Laboratory, IIL, Hyderabad on the following IBR vaccines: (a) conventional tissue culture inactivated vaccine (b) DNA vaccine and, (c) gE marker vaccine.

Control

- Impose a 2-3 week quarantine period for newly introduced cattle. Only seronegative cattle are then admitted to the herd. Periodical screening of animals in the farm and culling of positive animals generally helps to maintaining a disease free herd.
- Vaccinate calves as soon as passive immunity disappears.
- Vaccines usually prevent the development of severe clinical signs and reduce the shedding of the virus after infection, but do not prevent infection.
- Vaccination also does not provide protection against the establishment of latent infection by a wild strain nor does it inhibit re-excretion of a wild strain that was in latent form at the time of vaccination.
- Since no vaccines are available in India, the first option is the only feasible one presently available.

OIE guidelines for a free herd

To qualify as free from IBR/IPV, a herd must satisfy the following requirements:

1. All animals in the herd subjected to a diagnostic test for IBR/IPV on a blood sample on 2 occasions (3 occasions with individual milk samples in a dairy herd) with negative results, at an interval of not less than 2 months and not more than 12 months.
2. Ensure that introduced animals have been :

(a) From an IBR/IPV free herd; or

(Continued on page 4)

OIE - Significant animal diseases reported to OIE during Jul – Sep'08

Sl.No	Disease Outbreak	Countries reporting
1	Foot and Mouth Disease-(Strain in parenthesis)	Columbia (A), Namibia (SAT 2), Botswana (SAT 2), Malawi
2	Brucellosis (Sheep)	Croatia
3	Anthrax	Romania, Kazakhstan, Azer-
4	Bovine Viral Diarrhoea	Israel
5	Highly Pathogenic Avian	Nigeria, Benin, Laos, Togo
6	Peste des Petits Ruminants	China, Morocco
7	Scrapie	Portugal
8	Sheep & Goat Pox	Chinese Taipei
9	Classical Swine Fever	Slovakia, El Salvador, Brazil
10	Rabies	Nigeria
11	Equine Infectious Anaemia	France
12	Rift Valley Fever	Swaziland
13	Blue Tongue	Portugal, Hungary, Denmark,
14	Equine Influenza Virus-A	Egypt
15	Equine Piroplasmosis	USA
16	Porcine Reproductive and	Bhutan
17	Low Pathogenic Avian	USA
18	Glanders	Brazil
19	Equine Viral Arteritis	Croatia
20	Q Fever	Finland
21	West Nile Fever	Italy, France
22	African Horse Sickness	Ethiopia
23	Avian Infectious Laryn-	Peru
24	New Castle Disease	Dominican Republic, Peru
25	Myxomatosis	Luxembourg

Ghrelin and Orotic acid in sub-clinical mastitis (SCM)

Ghrelin and orotic acid accelerate wound healing as well as control inflammation and immunity. Research revealed that both occur in high concentrations in milk and serum during SCM, and may be also used as indicators of SCM. Ghrelin is a novel gastrointestinal peptide secreted from the A-like cells of the gastric fundus. This hormone does not only promote the release of growth hormone, but also stimulates food intake, gastric motility and cardiac output. Orotic acid (vitamin B13) is not really recognized as a vitamin and is manufactured by the intestinal flora.

Source : www.ncbi.nlm.nih.gov

(Continued from page 3)

(b)Placed in isolation for a period of 30 days during which it has been subjected to a diagnostic test for IBR/IPV on a blood sample on 2 occasions with negative results, at an interval of not less than 21 days.

3. Ensure that introduced fresh bovine semen:

(a)Is from donor animals kept in an IBR/IPV free herd at the time of collection of semen; and

(b)Was collected, processed and stored in conformity with the provisions laid down for the same in the Terrestrial Animal Health Code (TAHC) of OIE.

4. Ensure that introduced frozen semen:

(a)Is from donor animals kept in an IBR/IPV free herd at the time of collection of semen; or

(b)From donor animals that have been held in isolation during, and for a period of 30 days following collection, during which it has been subjected to a diagnostic test for IBR/IPV on a blood sample taken at least 21 days after semen collection, with negative results; or

(c)If from a bull of unknown or positive serological status, an aliquot of each semen collection has been subjected to virus isolation with negative result; and

(d)Was collected, processed and stored in conformity with the provisions laid down for the same in the TAHC of OIE.

5. Ensure that introduced embryos/ova were collected, processed and stored in conformity with the provisions laid down for the same in the TAHC of OIE.

Sources :
1. 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.
2. www.oie.int
3. Veterinary Science Database, CAB International
4. Annual Report 2005-06 .PD_ADMAS

Heat Shock Proteins (HSPs) and its therapeutics

Protective heat shock proteins present in every cell have long been known to counteract stress. HSPs are found in all forms of life, keep a wide variety of cellular processes running smoothly. These proteins pick up tell tale 'fingerprints' of each cell's contents which has allowed them to evolve a critical role in immune responses to cancer or pathogens. HSPs also deliver antigens from diseased cells to immune system's antigen presenting cells which in turn present it to T-cells. Therapies include inhibitors and enhancers of their various natural functions. Inhibitors of HSPs are able to block the functioning of HSPs that would normally help a cancer dell, virus-infected cell or pathogenic bacterium to survive.Heat or chemicals are able to induce a patient's own HSPs to protect an organ during surgical or other treatments.Antigenic HSP-peptide complexes that are purified are introduced into the body to stimulate an immune response to a tumour or pathogen. Patented products for all the three modes of action are presently available in the market for treatment of various tumours.

Source : Scientific American India , July 2008

Melamine in foods

Dairy products made in China which were contaminated with melamine have sickened at least 54,000 babies and killed four. Following this, many countries, including India, have banned import of Chinese dairy products. Melamine is a nitrogen-based compound used in commercial and industrial plastics. It is also used as a fertilizer in Asia. When large amounts of melamine are ingested, it causes the formation of kidney stones, as well as the organs' failure. Melamine added to food spikes its nitrogen level, making these products appear artificially to have more protein on testing. Source: www.sciam.com

For further details please contact : Dr.A V Hari Kumar , Manager (AH), NDDB, Anand, Phone : 02692 226244 E mail:avhk@nddb.coop