NATIONAL DAIRY DEVELOPMENT BOARD ANAND GUJARAT

# ANIMAL HEALTH UPDATES

## Animal Health Group

VOLUME I ISSUE III (For Private circulation only) Oct-Nov 2007

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## Disease - Bovine brucellosis

Brucellosis is an important disease of livestock and a zoonosis worldwide.

Some of the animal species affected by various brucella species are given below:

| Host | B.abortus | B.melitensis | B.suis | B.canis | B.ovis |
|---|---|---|---|---|---|
| Cattle | + | + | + (rare) | - | - |
| Buffalo | + | + | - | - | - |
| Sheep | +(rare) | + | + (possible) | - | + |
| Goat | +(rare) | + | - | - | - |
| Pig | +(rare) | +(rare) | + | - | - |
| Camel | +(rare) | + | - | - | - |
| Horse | + | +(rare) | +(rare) | - | - |
| Dog | + | + | +(rare) | + | |

Marine mammal brucellosis due to two new proposed brucella species represents a new zoonotic threat but pathogenicity for humans has not been clearly established.

### Animal brucellosis in India

In India, many authors have reported a higher incidence of brucellosis in organized farms compared to unorganized farms. *(Vaid et al., 1991; Mehra et al., 2000; De et al., 1982)*. Within organized farms, those owned by the government have been reported to have a lesser incidence compared to the privately owned ones. *(Nawathe & Bhagwat.,1984)*

Studies in cattle and buffalo herds in India have shown a much higher prevalence (25%) in herds using artificial insemination (AI). *(Chatterjee et al., 1985 )*

The incidence of brucellosis has also been reported higher in cattle than in buffaloes, whether in mixed or separate herds.

The sero-prevalence of brucellosis as per the annual reports of Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), has risen from 8.46% during 1994-2004 (10 years cumulative) to 20.89 in 2004 -05 and 22.2% in 2005-06.

Cattle:

Infection in cattle is mainly caused by *B.abortus*. The disease occurs in cattle of all ages but is most common in sexually mature animals.

An aborted foetus due to brucellosis

Large numbers of organisms are excreted in the aborted foetus, foetal membranes and uterine discharges of infected animals.

### Transmission

- Contact with aborted foetus and infected newborns.
- Contact of conjuctiva or intact skin with the tail heavily contaminated with infected uterine discharges.
- Ingestion of contaminated raw milk. Excretion of organisms in milk is usually intermittent and is more common during late lactation and can persist for several years.
- Ingestion of organisms through pasture, feed stuffs and water supplies contaminated by uterine discharges and foetal membranes from infected cows.
- In-utero infection may occur.
- Contamination of the udder during milking from infected to uninfected cow through milker or milking machine.
- Though infected bulls discharge organisms in semen they are unlikely to transmit the disease but chances are increased if semen is used for AI.

### Pathogenesis

*B.abortus* has a predilection for the pregnant uterus, udder, testicles, accessory male sex glands, lymph nodes, joint capsules and bursae. Congenital infection may occur as a result of in-utero infection in new born calves. Infection remains latent in a small proportion of congenitally infected calves which remain sero-negative in early life until parturition, when it sheds the organism. The frequency of latent infection may range from 2.5-9% of the total infected cases.

In adult non-pregant cow, localization occurs in udder and uterus. When the uterus becomes gravid, it becomes infected from periodic bacteraemic phases originating from the udder. Infected udders are clinically normal but are important

**Average incidence (%)of brucellosis in apparently normal herds in various regions of India***

| Regions | Cattle | Buff | Sheep | Goat | Pig | Camel | Mithun | Equines |
|---|---|---|---|---|---|---|---|---|
| North | 9 | 10 | 13 | 11 | 11 | 1 | | 38 |
| South | 5 | 6 | 4 | 6 | 7 | | | |
| East | 19 | 19 | 5 | 8 | 8 | | 15 | |
| West | 10 | | | | | | | |
| Others | 13 | 3 | | | | | | |
| **Country** | **13** | **7** | **11** | **10** | **8** | **1** | **15** | **38** |

*- Consolidated from a review of literature over the last three decades on the subject by our group.

as a source of infection both for calves and humans drinking milk from it.

Few infected cows ever recover from infection completely and should be considered as permanent carriers whether they have aborted or not.

**Clinical findings**

In non-vaccinated pregnant cattle, abortion after 5th month of pregnancy is a typical feature of the disease. The foetus is carried to full term in subsequent pregnancies although 2nd or 3rd abortions may occur in the same cow. Retention of placenta and metritis are common sequelae to abortion. In a susceptible herd it is common for infection to spread rapidly and for an abortion storm to occur which may last for a year or more. In recent years, particularly in areas where vaccination is extensively practiced, an insidious form of the disease may develop which spreads more slowly and abortion is less common.

Joint swelling due to brucellosis

Other symptoms like still birth, mastitis, joint swelling, repeat breeding also occur.

In infected bulls, orchitis and epididymitis occur. They are also potential spreaders of the disease if they are used for AI. Some infected bulls which are negative on serum agglutination can be detected only by isolation of organisms from semen or diagnostic tests on seminal plasma.

**Average incidence (%) of brucellosis in herds with abortions and other symptoms of brucellosis in various regions of India***

| Regions | Cattle | Buff | Sheep | Goat | Equines |
|---|---|---|---|---|---|
| North | 33 | 14 | 74 | | |
| South | 16 | | | 55 | |
| East | 53 | | | | |
| West | 38 | 19 | | | |
| Others | 48 | | | | 40 |
| **Country** | **36** | **16** | **74** | **55** | **40** |

*- Consolidated from a review of literature over the last three decades on the subject by our group.

**Zoonotic implications**

*B.melitensis* infection is most frequently reported and causes severe disease in humans. *B.suis* has a much more restricted occurrence but can be as severe as the first. Though *B.abortus* is the most widespread cause of infection, the severity is much lesser than the above two.

In India, fewer than 10% of the human cases of brucellosis may be clinically recognized and treated or reported. Screening of family members of index cases of brucellosis in an endemic area will help to pick up additional unrecognized cases.

Arthritis in humans caused by brucellosis

Brucellosis exists in the general population in India and high clinical suspicion must be made in patients when there is history of animal contact or consumption of unpasteurized milk.

**Average incidence (%) of brucellosis reported in human populations in India***

| Vets | Para-vets | Abattoir workers | Patients with fever of unknown origin | Patients with fever and other symptoms | Occupationally exposed | General population |
|---|---|---|---|---|---|---|
| 25 | 32 | 49 | 0.8 | 35 | 12 | 2.6 |

*-Consolidated from a review of literature over the last three decades on the subject by our group.

The disease is acute in about half the cases, with an incubation period of 2-3 weeks. In the other half, the onset is insidious, with the symptoms developing over a period of weeks to months from the infection. There is no vaccine available for humans.

In an outbreak of polyarthritis with pyrexia reported in Kanvari village, Churu district, Rajasthan , 91.6% of 48 persons presented were positive for brucellosis by Rose Bengal Test (RBT).

**Diagnosis**

A. OIE prescribed tests

Office International des Epizooties (OIE) has prescribed certain tests as suitable for purposes of international trade of animals and its products. However, no single serological test is appropriate in all epidemiological situations.

1. Rose bengal test (RBT)

This is a very sensitive and simple spot agglutination test using antigen stained with rose bengal and buffered to a low pH of 3.65 ± 0.05. Equal volumes of serum and antigen are mixed (25-30 µl of each) thoroughly on a white tile to produce a circular or oval zone approximately 2 cm in diameter. The mixture is agitated gently for 4 minutes and is read immediately after this period. Any visible reaction is considered positive.

Rose Bengal Test (RBT)
Source : www.who.int

The serum samples and antigen should be brought to room temperature before the test. Only the required quantity of antigen sufficient for a day's testing should be removed from the refrigerator.

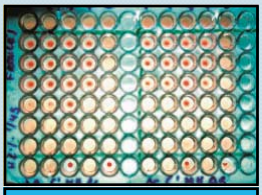
False negative reactions rarely occur, mostly due to prozoning (zone of relatively high antibody concentration within which no reaction occurs) and can be eliminated by diluting the serum sample or retesting after a given time.

False positive reaction can be a result of vaccination with S-19 vaccine.

This test is adequate for detecting infected herds and is used widely throughout the world.

2. Complement fixation test (CFT)

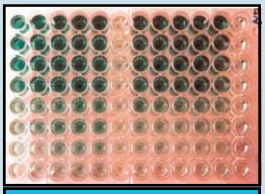
Though it is a widely accepted confirmatory test, it is complex to perform. CFT is very specific but could give positive result due to S-19 vaccination. The test is not used very widely nowadays.



Complement Fixation Test (CFT)
Source : www.who.int

3. Indirect Enzyme Linked Immunosorbent Assay (I-ELISA)

There are numerous variations of this test. This is a highly sensitive test but is not capable of differentiating antibody resulting from S-19 vaccination. This test is used widely throughout the world.



Indirect ELISA test (I-ELISA)
Source : www.who.int

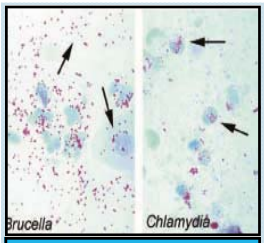
4. Fluoresence polarization assay (FPA)

It is a simple technique for diagnosis of bovine brucellosis and may be performed in a laboratory or in the field. The sensitivity and specificity is almost identical to ELISA. This test is not used widely.

B. Other tests

1. Modified Ziehl-Neelsen staining:

Presumptive evidence of brucellosis is provided by demonstration of *brucellae* in aborted material or vaginal discharge, especially if supported by serological tests. The results, whether positive or negative, should be confirmed by culture. Brucella organisms are seen as short rods




Modified Ziehl-Neelsen staining
Source : www.who.int

measuring 0.6 to 1.5µm in length and 0.5 – 0.7µm in width. They are usually arranged singly and less frequently in pairs or small groups. The morphology is fairly constant. It is also difficult to differentiate it from other organisms like *Chlamydophila abortus* (previously known as *Chlamydia psitacii*) or *Coxiella burnetti* which also cause abortion in cattle.

2. Culture

This is the most satisfactory method. The most valuable samples for isolation of *brucellae* include stomach contents, spleen and lungs of aborted foetuses, foetal membranes, vaginal secretions (swabs), milk, semen and arthritis or hygroma fluids. From carcases, the preferred tissues are head, mammary and genital lymph nodes and, spleen.

All samples should be cooled immediately after they are taken and transported rapidly to laboratory.

Semi-automatic bacteriological methods like BACTEC 9204 have considerably shortened the time taken for detection of *brucellae* in suspected samples.

DNA probes and PCR techniques are also being used for identification of *brucellae*.

3. Brucellin skin test

This test can be used for screening unvaccinated herds. It has a high specificity but low sensitivity. The test is not much in use.

4. Serum agglutination test (SAT)

This test has been used with success for many years in surveillance and control programmes for bovine brucellosis. The test is performed either in tubes or microplates. This test has now generally regarded as being unsatisfactory for the purposes of international trade by OIE. This has largely been replaced by simpler tests like ELISA.

5. Milk tests

a. Milk I-ELISA

Milk I-ELISA is a sensitive and specific test and can be used to test large herds. It is reported to be around 22 times more sensitive than the milk ring test (MRT).

b. Milk ring test (MRT)

Although it is very useful in identifying herds with low prevalence of *B.abortus* infection, it becomes less reliable in large herds (above 100 lactating cows). False positive reactions occur in cattle vaccinated less than four months prior to testing, with colostrum or in case of mastitis.

**Availability of various antigens/tests in India**

| S.No | Test/Antigen | Available at |
|---|---|---|
| 1 | RBT antigen | (1) Indian Veterinary Research Insti tute (IVRI), Izatnagar, UP.<br>(2) Some State Institutes of Animal Health and Veterinary Biologicals (IAH&VB) |
| 2 | MRT antigen | - Do- |
| 3 | SAT antigen | - Do- |
| 4 | Serum ELISA kits | Project Directorate on Animal Disease Monitoring and Surveillance |
| 5 | Milk ELISA Kits | -Do- |
| 6 | Serum ELISA testing | (1) Centre for Animal Disease Research and Diagnosis (CADRAD), Izatnagar, UP.<br>(2) State Regional Disease Diagnostic Laboratories (RDDL)<br>(3) Indian Immunologicals Limited, Hyderabad. (Uses imported kits) |
| 7 | PCR testing | -Do- |
| Note: The list is not comprehensive | | |

**Vaccines for animals**

1. *Brucella abortus* Strain-19 vaccine

- Optimum age of vaccination is between 4 and 8 months.
- Only female cattle and buffalo calves are to be vaccinated.
- A single dose is to be administered subcutaneously.
- Vaccinated animals have high degree of protection against abortion for 5 or more subsequent lactations under field conditions and 65-75% resistance to most kinds of exposure.
- Remaining 25-35% of vaccinated animals may become infected if continuously exposed, but usually do not abort.
- Multiple or late vaccination have no appreciable advantage. Moreover it increases the incidence of post-vaccinal positive agglutination reactions.
- Vaccination of bulls is of no value in protecting them against infection and should not be practiced.
- The vaccine may infect humans, particularly after accidental inoculation.

**Adult vaccination** in infected herds with a reduced dose is efficacious in controlling abortion and thereby useful in "abortion storms". This also has certain disadvantages like residual vaccine titres, persistant milk ring test, persistant strain 19 infection in a small percentage of animals and a problem of them being identified with infected herds. Such reduced dose vaccines are not readily available but have to be tailor made by making appropriate dilutions of the available vaccine.

*Bruvax*, a S-19 vaccine manufactured by Indian Immunologicals Limited (IIL), Hyderabad, is the only freeze dried vaccine available in India. Other vaccine producers (IVRI & some State IAH & VB) make the vaccine in liquid form.

2. Strain RB51 vaccine

- Optimum age of vaccination is between 4 and 12 months.
- Only female cattle and buffalo calves are to be vaccinated.
- To be administered sub-cutaneously.
- A booster of the same dose can be given from 12 months of age onwards.
- Vaccinates do not produce positive results in serological diagnostic tests.
- Can be given to cattle and buffalo females already vaccinated with S-19.
- It has been approved by OIE as an alternative to S-19 vaccine.
- It is less hazardous to humans than S-19 vaccine if accidental inoculation occurs.

**Differential Diagnosis**

Trichomoniasis, Leptospirosis, Vibriosis, Infectious Bovine Rhinotracheitis (IBR), Mycoses, Neosporiasis, Listeriosis, Epizootic viral abortion and nutritional factors.

**Control of brucellosis in farms**

- Carry out RBT of all animals, confirmed by serum ELISA once in 3 months and cull positive reactors. The farm is considered free if there are no positive reactors for two consecutive tests.
- If positives are detected, rear negative in-contact animals separately for at least 120 days and test at 30 day interval for any positive reactors.
- Test pregnant animals in the last trimester of pregnancy and, 0 & 21 day post calving. Also test the calf at 21 days of age.
- Vaccinate all female calves (not the male calves) at the prescribed age group depending on the type of vaccine.
- In quarantine, test animals at 30-45 days interval, and if any positives are detected, cull them and impose fresh quarantine for negative animals with testing interval of 30-45 days.
- In case of abortion history in the farm, test all animals every two months and cull positive reactors until there are no further reactors detected.
- Isolate the aborted/infected animal immediately until all genital discharges cease.

*(Continued on page 4)*

**OIE - Significant animal diseases reported to OIE in Oct-Nov'07**

| Sl.No | Disease Outbreak | Countries reporting |
|---|---|---|
| 1 | Brucellosis | Japan |
| 2 | Foot and Mouth Disease | Namibia, Botswana, Cyprus, Ecuador |
| 3 | Blue Tongue | Denmark, Spain, Switzerland, France, Czech Republic |
| 4 | Anthrax | Azerbaijan |
| 5 | Rift Valley Fever | Sudan |
| 6 | Glanders (*Burkholderia mallei*) | Iran |
| 7 | Equine Influenza | China, Mongolia |
| 8 | African Swine Fever | Mauritius |
| 9 | Classical Swine Fever | Russia |
| 10 | Rabies | Belgium, Uruguay, Chile, Finland |
| 11 | Myxomatosis | Switzerland |
| 12 | Porcine Reproductive and Respiratory Syndrome Virus | Russia |
| 13 | Highly Pathogenic Avian Influenza | Mynmar, UK, Saudi Arabia, Romania |
| 14 | New Castle Disease | Estonia, Greece |
| 15 | Low Pathogenic Avian Influenza | Republic of Korea |
| 16 | Aujeszhy's disease | Romania |
| 17 | European bat Lyssa virus | France source :www.oie.int |

## WTO to launch SPS MIS

The SPS (sanitary and phytosanitary) Information Management System (SPS IMS) allows users to track and obtain information on measures that member governments have submitted to the WTO.

The submissions include information on new export and food safety requirements, specific trade concerns that governments have raised, documents of the WTO's SPS measures committee, member governments' national enquiry contacts, and the authorities who handle notifications.

Specific areas also deal with supplements and what equivalent food safety measures are accepted by trading partners.

The WTO has released a public version of the system to help member governments and others find the information according to their specific needs.

The information system is part of an effort by the WTO to break down trade barriers relating to foods. Regulations on food safety can often serve as a barrier to competitive exports from other countries.

Source: www.foodproductiondaily.com

## New foot - controlled PC mouse aims to increase researchers' efficiency

The 'footmouse' developed by Bili Incorporated, aims to remove the need to constantly remove gloves and wash hands to avoid contaminating electronic equipment in laboratories. A foot-controlled mouse

Footmouse
Source : www.biopharma-reporter.com

uses an optical 'slipper' mouse to control the cursor and a user-programmable pedal that enables users to tie command sequences to specific buttons.

The footmouse works with Windows systems and connects directly into any available USB port.

Source : www.biopharma-reporter.com

*(Continued from page 3)*

- Burn, or bury (at least 2-3 feet deep) aborted foetus, placenta and bedding soiled with discharges from infected cow after sprinkling lime.
- Do not use calves from seropositve dams for breeding purposes.
- Disinfect floors & walls of the animal shed where the animal has aborted by using any of the following: (1) 1% hot caustic soda (NaOH) (2) 5% Phenol (3) 1-2% Formaldehyde solution (4) 5% Sodium Hypochlorite solution.
- Do not serve the cow which has aborted for at least 2 months from the day of abortion.
- Do not handle aborted foetuses with bare hands.
- Disinfect exposed areas like hands with a suitable disinfectant like chlorhexidine (0.5%).
- Ensure that farms from where animals are procured practice periodic screening for brucellosis .
- Select calves preferably from MRT negative villages.
- Lift identified calf only if calf and its dam are negative by ELISA.
- Embryo transfer is a safe procedure of salvaging genetic material from infected females.
- Control of vermin in the farm may be beneficial since it has been reported that 32-60% of the trapped/killed rats from many pig farms surveyed were carriers of brucellosis. (*Avylov Ch., 2001*)

*Sources :*
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5) Mehra KN, Dhanesar NS, Chaturvedi VK. *Sero-prevalence of brucellosis in bovines of Madhya Pradesh.* Indian Vet J. 2000;77(7):571-73.
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7) *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2006).*
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11) *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.*
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13) *www.who.int*

## New technology turns tap water into powerful detergent

*The Tennant company* claims its *Ech2o* system has significant advantages over chemical cleaners, including lower costs and ease of use. It is also eco-friendly. The system works by unlocking the energy stored in the water molecule. The electrically charged water attacks the dirt, breaks it into smaller particles and suspends it off the floor's surface enabling easy removal . In about 45 seconds after it was created, the cleaning solution returns to plain water. While similar technology is currently in use in some niche applications, this is the first one to be introduced for commercial cleaning.

Source: www.foodqualitynews.com

## Human stem cells from reprogrammed skin cells

Two sets of scientists, one in the US and another in Japan have used a retrovirus to carry four genes to the fibroblast cells, converting them to pluripotent cells that exhibit the essential characteristics of embryonic stem cells.

The ability to generate embryonic stem cells without using an embryo will negate much of the ethical debate that has dogged the field over the years. Coupled with the fact that these cells are much easier to generate and the end result is likely to be more scientists using stem cells both to screen new drugs and eventually, as a therapy by itself.

The results of the US and Japanese researches have been published in the journals *Science* and *Cell* respectively.

Source : www.in-pharmatechnologist.com



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