NATIONAL DAIRY DEVELOPMENT BOARD ANAND GUJARAT

ANIMAL HEALTH UPDATES

Productivity Systems - Animal Health Group

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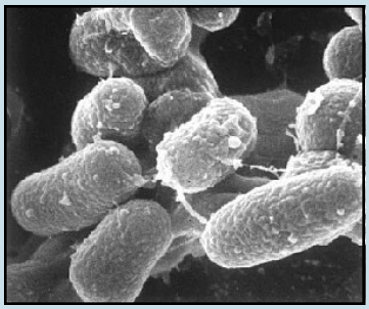
Disease - Paratuberculosis (Johne's disease) in Bovines

Etiology

Johne's disease (pronounced "yo-knees") is a contagious, chronic infection that affects primarily the small intestine of ruminants. All ruminants are susceptible to Johne's disease (JD). Johne's disease is caused by *Mycobacterium avium subspecies paratuberculosis (MAP). MAP* is 99 % genetically related to *Mycobacterium avium*, but infects only mammals. The disease occurs worldwide most commonly in cattle and to a lesser extent in sheep and goats. The incidence is greater in intensively reared animals.

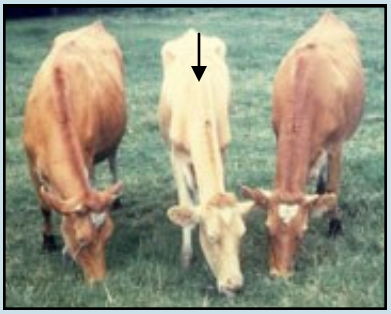


Electron micrograph of Johne's Bacillus
Source: www.johnes.org

Any role of *MAP* in Crohn's disease of humans still remains unproven.

Morbidity

The incidence of clinical disease in an infected herd is very low and rarely exceeds 5% of adult animals. But the economic losses can be significant. For every clinical case of Johne's disease in a herd, there are 15-25 additional infected animals in various stages of clinical and subclinical infection and, 10-14 with latent infection. Due to its insidious nature, it is considered to be a hidden threat to the livestock industry.



MAP positive animal (centre) with two MAP negative animals
Source: www.communigate.co.uk

Transmission

⇒Mainly by faecal – oral route

⇒***Infected animals can excrete the organisms 15-18 months before clinical signs appear.***

⇒Pre natal infection.

⇒Consumption of contaminated colostrum and milk soon after birth.

⇒Natural service or artificial insemination (AI) (*Ayele et al.,2004)*)

⇒Mechanically by beetles and certain blowflies.

Resistance

⇒*MAP* is capable of occasional survival in colostrum and milk subjected to commercial pasteurization.

⇒Survival for about a year in dry, fully shaded environment.

⇒Moisture and application of lime to soil do not affect survival.

Economic importance

The economic losses due to both clinical and subclinical infection include:

⇒Reduced feed efficiency

⇒Decreased milk production (up to 4% in subclinical to 19.5% in clinical cases)

⇒Decreased milk fat and protein

⇒Reduced slaughter weight at culling

⇒Decreased fertility

⇒Premature culling

⇒Increased incidence of mastitis

Pathogenesis

Following oral ingestion, the organism localizes in the mucosa of small intestine and its associated lymph nodes. The primary site of bacterial multiplication is the terminal part of the small intestine and the large intestine. Three different groups of animal can emerge.

1.Infected-resistant:

Animals develop resistance quickly, control the infection and do not shed the bacteria.

2.Intermediate:

Infection is not completely controlled, some animals shed the organism intermittently, others those are incubating may become heavy shedders.

3.Clinical :

The organism persists in the intestinal mucosa and clinical cases develop from these animals. There is infilteration of a large number of macrophages

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in the intestinal mucosa resulting in decreased absorption, chronic diarrhoea and leakage of protein resulting in muscle wasting, hypoproteinemia and oedema.

Clinical Signs

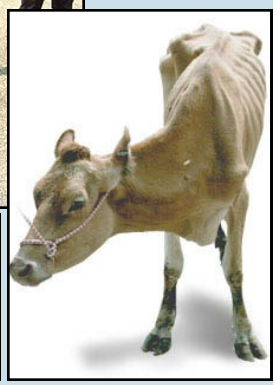
Four stages have been described in cattle:

⇒**Silent/latent infection** –Cattle up to 2 years of age do not exhibit any clinical signs but may shed the organism. Clinicopathologic tests cannot detect the infection but demonstration of organism in tissues and faeces may be possible.

⇒**Subclinical infection** – Subclinical infection is commonly seen in carrier adults which may have other ailments such as mastitis or infertility. No apparent clinical signs of the disease are visible. In absence of culling, the infection is likely to advance. Infected bull calves could be a source of infection later when used for AI or natural service, since *MAP* is shed in semen. (*Ayele et al.,2004)*

⇒**Clinical disease** – Clinical disease does not appear before 2 years and is most common in 2-6 years age group. There is a gradual loss of body weight despite normal appetite and after several weeks, diarrhoea also develops concurrently.

⇒**Advanced clinical disease** – Emaciation is most obvious and usually accompanied by intermandibular oedema.The diarrhoea is characteristic by a fluid 'waterhose' or 'pipestream' passage of faeces .



Advanced clinical Johne's disease in cows
Source:www.johnes.org

Differential Diagnosis

⇒The chronic nature of diarrhoea and ensuing emaciation are usually sufficient to differentiate it from the other common enteritis of cattle.

⇒Salmonellosis – Acute diarrhoea

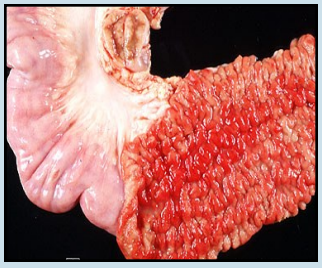
⇒Coccidiosis and gastrointestinal helminthiasis – Occur in younger animals and are distinguishable on faecal examination for oocyts and helminth eggs.

⇒Bovine virus diarrhoea

⇒Secondary copper deficiency (due to chronic molybdenum poisoning) – affects large number of animals and responds well to administration of copper.

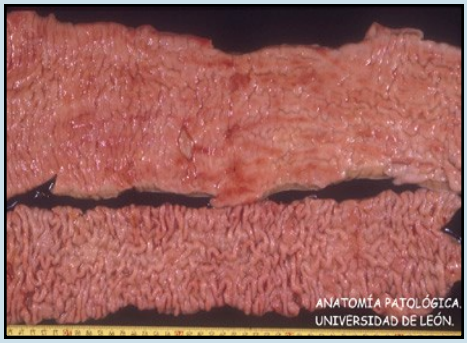
⇒Malnutrition,chronic reticuloperitonitis, hepatic abscess, pyelonephritis, lymphosarcoma and amyloidosis are other diseases that cause emaciation.

Lesions in Johne's disease are confined to posterior part of alimentary tract and its associated lymph nodes.



Thickened and corrugated intestinal mucosa in Johne's disease
Source :www.johnes.org

The intestinal wall becomes 3 -4 times thicker and exhibits corrugations in the mucosa.



Normal (above) and corrugated intestinal mucosa (below) in Johne's disease
Source:www3.unileon.es

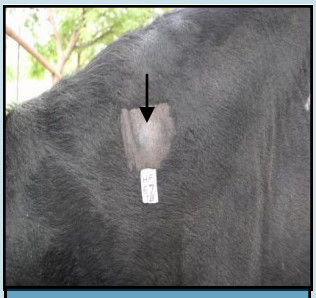
Disease Diagnosis

A. OIE recommended tests in cattle

The Office Internationale des Epizooties (OIE) has recommended two tests, namely Delayed Type Hypersensitivity (DTH) test and Enzyme Linked Immunosorbent Assay (ELISA) for international trade purposes.

1. Delayed Type Hypersensitivity (DTH) test

It is an allergic test wherein Purified Protein Derivative (PPD) is injected intradermally on the side of the neck. Intradermal test using Johnin (PPD from *MAP*) is used as a preliminary test to identify positive herds. A dose of 0.1ml Johnin is given intradermally on the side of the middle third of the neck. The reaction is read 72 hrs after inoculation. An increase in skin thickness of over 4 mm is considered positive. The same person should measure the skin thickness before the injection and 72 hrs after injection. Proper control and positioning of animal are important to avoid irregularities in reading test results.



An animal positive on Delayed Type Hypersensitivity (DTH) Test

Avian tuberculin (PPD from *M.avium*) can also be used in place of Johnin since its sensitivity and specificity are comparable.

False positive results may occur due to cross reactivity with other organisms and in recovered resistant animals.

False negative results can also occur as a result of tolerance and anergy which occurs most often during the terminal stages of clinical disease or at any stage of chronic infection.

The test is being replaced by cytokine assays like gamma interferon (IFN-γ) test. Diagnostic kits are presently not available in Indian market .

Johnin (*MAP* PPD) is available from IVRI, Izatnagar, UP. Avian PPD is presently not available in India.

2. Enzyme linked immunosorbent assay (ELISA)

Though different ELISAs are available, the absorbed ELISA is one of the most accurate serological test available for detection of subclinical infection.

Milk ELISA has also been used for detection of exposure to the organism in cows but lacked correlation with

serum ELISA.

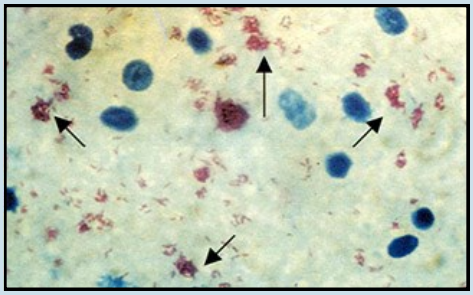
The ELISA tests are available both at IVRI, Izatnagar, UP, and IIL, Hyderabad.

B. Other tests :

1. Microscopic examination:

Distal ileum, colon, ileo-caecal lymph node and its direct smears, rectal pinch and faecal smears can be used for Ziehl-Neelsen (acid – fast) staining.

Ziehl-Neelsen stained faecal smears can identify the presence of the organism within an hour. But it may be difficult to differentiate Johne's bacillus from other acid-fast organisms present in faeces. A diagnosis can be made if clumps (three or more organisms) of small (0.5-1.5 µm), strongly acid-fast bacilli are found. It may also be necessary to examine smears on several occasions to obtain a positive result. The rectal pinch biopsy is of no great advantage in early detection as it is only in the late clinical stages that the rectal mucosa is invaded.



Ziehl-Neelsen stained tissue section showing MAP bacilli
Source : www.technovet.uchile.cl

2. Faecal culture:

It is the most reliable method with 100% specificity and sensitivity and can detect infection in cattle 1 -3 years prior to appearance of clinical signs. But procedures are laborious and require 8-16 weeks for isolation. Current techniques also allow recovery of even one organism per gram of faeces.

MAP colonies
Source: www.leine.no

Culture of milk: Milk can be cultured in subclinically infected cows. The presence of MAP in milk is highest in heavy shedders.

A technique for detection of *MAP* in faeces and other specimens through culture (BACTEC MGIT system)* is available at IIL, Hyderabad.

*- Mycobacteria Growth Indicator Tube (MGIT) manufactured by BD Diagnostic Systems.

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

2. Serological tests

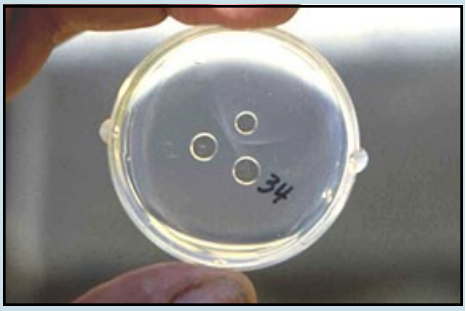
a) Complement fixation test (CFT)

Most widely used till recently. It has a diagnostic sensitivity is 90% and specificity of 70% in clinical cases. It cannot be used as a single test for definitive identification of cattle with subclinical infection.

b) Agar gel immunodiffusion (AGID)

It has a diagnostic sensitivity of 96% and specificity of 94% in clinical cases. Results are available in 48 hrs.

Tests for different situations



Agar Gel Immunodiffusion test (AGID)
Source :www.ohioline.osu.edu

1. Confirmation of clinical cases

Since more than 85% of the clinical cases with diarrhoea and weight loss will be sero-positive, the most rapid, accurate and least expensive test to confirm a clinical case is by ELISA.

2. Confirmation of subclinical cases :

Any animal positive by ELISA or DTH but not showing any clinical signs could be confirmed by faecal culture. This would be desirable especially in case of valuable animals. A positive faecal culture of a ELISA/DTH positive animal would indicate a positive confirmed case.

3. Screening of healthy animals and estimation of prevalence :

Rapid and easiest method for screening is to test all animals over 2 years of age with ELISA. The true prevalence in a herd is twice the apparent prevalence of infection as determined by ELISA. The prevalence of infection will be underestimated in herds which have been culling clinical cases. IFN-γ test has been reported to be good for screening cattle between 1-2 years of age.

The DTH test is also used widely as a primary screening test but must be used in combination with other tests to arrive at a conclusion. Further, it must not be done in cows and calves within 6 weeks of birth /delivery or 60 days of a previous DTH test.

The use of pooled faecal samples from 10 cows for culture of *MAP* has been reported as a cost effective tool for herd screening which provides a good estimate of percentage of MAP infected cows in herds with low prevalence.

Disease control

1. Screening tests

First step in a control programme is to test all animals above 2 years of age by ELISA. After ELISA positive animals are culled, a second ELISA can be done on the herd again. An alternative is faecal culture which is more expensive but will detect animals missed by ELISA. It is suggested that every third or fourth test should be faecal culture. Animals are to be retested half yearly or annually.

DTH can also be used as a screening test but preferably be used in combination with other tests to arrive at a conclusion.

2. Pre-purchase testing

Ideally, purchase of animals should be made from JD free farms. The next best option is to purchase from farms which have no recent history of clinical JD. ELISA is recommended for purchased animals above 12 months of age to know the serological status. If animals are below 12 months, they are unlikely to be sero-positive and faecal culture would be a better option. DTH test alone may be of limited value.

3. Prevention

A basic prevention strategy formulation to control *MAP* infection in farms should consider the following :

⇒Remove calves from the dams immediately after birth to reduce the exposure of newborns to dam's ma-

(Continued on page 4)

OIE - Significant animal diseases reported to OIE in Aug-Sep'07

Sl.No	Disease Outbreak	Countries reporting
1	Foot and Mouth Disease (FMD)	UK , Turkey
2	Blue Tongue	Netherland, Luxembourg, Tunisia, Portugal,UK
3	African Swine Fever (ASF)	Georgia, Armenia, Nigeria
4	Classical Swine Fever (CSF)	Russia, Guatemala
5	Highly Pathogenic Avian Influenza	Canada
6	Low Pathogenic Avian Influenza	Portugal
7	Equine Influenza	Australia, Japan
8	West Nile Fever	Israel
9	Porcine Reproductive and Respiratory Syndrome	Sweden
10	African Horse Sickness	Senegal
11	New Castle Disease	Romania

Research News: Plant produced protein 'CD 14' eases coliform mastitis in dairy cows

Researchers have developed a new approach to thwart bacteria that cause coliform mastitis, which is mainly caused by the *E.coli* . Scientists have endowed a potato virus with a gene that—when introduced into a host plant—prompts the plant to produce a therapeutic protein called 'CD14'. This beneficial protein can be extracted from the plant and used to treat mastitis.

The researchers chose the tobacco plant to be their CD14-producing factory by inoculating the young plants with laboratory-produced RNA of the recombinant virus by rubbing a small drop of the RNA onto the plants' leaves.

Once the viral RNA entered the plants through small tears in the leaves, it spreads and the CD14 gene begins to make the protein. The protein can then be extracted from mashed-up leaves.

CD14 is known to help the immune system fight infection, but it is present in the cow's mammary gland at low levels. CD14 binds to the endotoxin (lipopolysaccharide) located on *E. coli's* outer membrane. The scientists hypothesized that increasing the level of CD14 in the milk would enhance protection. When inserted into the mammary gland through the teats, CD14 binds to the *E. coli* and triggers the cow's immune response. This process helps to neutralize and clear toxins produced by the bacteria, lessening the chances of an excessive immune response since coliform mastitis is caused by the effects of endotoxin in the mammary gland and the formation of inflammatory mediators within the udder and their subsequent release into the systemic circulation.

The researchers were able to purify about 1,000 micrograms of CD14 from 10 grams of leaf tissue taken from one plant. That means each of these plants provides enough protein to potentially treat about 10 cows with a dosage of 100 micrograms each. Fifty plants would yield purified protein to treat a herd of 500 cows.

The CD14-based product may eventually be commercially developed for use by dairy farmers as a treatment to prevent cows from becoming infected during their dry period, during which they are most susceptible to coliform infections. This report was published in the Sep'07 issue of Agricultural Research magazine. *Source : www.usda.gov*

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nure.

⇒Avoid manure contamination of feed and water.

⇒Use unpooled colostrum from Johne's negative animals.

⇒Avoid natural nursing and milk feeding whenever possible.

⇒Calf handling and feeding to be done before contact with adult dairy stock or by a different person.

⇒Clean the udder and teats thoroughly before collection of the colostrum to avoid manure contamination.

⇒Cull or separate offspring of infected dams as soon as possible.

⇒Mix calves with adult cattle only when they reach breeding age, e.g. 18 to 20 months.

⇒Immediately cull cattle with chronic diarrhoea not responding to treatment .

⇒Isolate unhealthy cattle till a definite diagnosis is made.

⇒It must be emphasized that no single test will detect all infected cattle.

Vaccination:

Live attenuated (Vallée's) and killed (Sigurdsson) vaccines are suggested in literature but are not available presently in India.

Vaccination against *MAP* infection is an aid to the prevention of clinical disease, but does not necessarily prevent infection. Vaccination is done only in calves less than 1 month of age.

A major drawback of vaccinated animals is that they become positive on DTH test (for both *MAP* and *TB* PPD) and develop serum antibodies. Therefore, for diagnosis of infection in vaccinated animals, only tests to detect *MAP* in faeces can be used.

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) OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2006).

3) Veterinary Science Database, CAB International.

4) Ayele et al., 2004. Distribution of MAP in organs of naturally infected bull calves and breeding bulls. Veterinary Microbiology.103 (3-4): 209-217

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