MANDATORY SCREENING OF BREEDING BULLS FOR INFECTIOUS DISEASES

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In India, 6 diseases are identified which need to be screened in cows and/or bulls regularly to prevent their spread and to maintain the reproductive health status of animals to an optimum level. The following diseases are included in this group.

1. TUBERCULOSIS

Tuberculosis is a chronic bacterial disease caused by *Mycobacterium tuberculosis* and *M. bovis* and characterized by presence of tubercle nodules in lungs, spleen and lymph nodes.

Pathogenesis

Disease spreads through contact with infected animals or their discharges or morbid tissues. The infected animals release the organisms through sputum at coughing and contact animals get infection through droplet inhalation. Besides sputum, faeces, urine, vaginal discharges, semen, milk, lymph and wound discharges may act source of infection. It is also transmitted through contaminated instruments, utensils and beddings. After entry through inhalation or ingestion, bacilli localizes at the point of entry and produce typical tubercle in associated lymph nodes. Mostly pharyngeal and mesenteric lymph nodes are affected. From lymph nodes, infection may extend across body cavities, blood vessels and lymph vessels. The inhaled organism enter in the bronchial tree after lodging in bronchi a tissue reaction takes place. Neutrophilic infiltration is there and when neutrophils are necrosed, macrophages come to destroy the organism but they fail then many macrophages becomes elongated to form epithelioid cells, the hallmark of granulomatous inflammation. Some macrophages are joint together to form giant cells to destroy the acid fast bacilli. As a consequence of this some bacilli are phagocytosed and are destroyed. A zone of lymphocytes, macrophages, epithelioid cells, giant cells and fibrous connective tissues are formed around the central necrosed area. In CADRAD, over the years a total of 8091 cattle and buffaloes were tested for tuberculosis and 3.28% were found as positive reactors.

Characteristic symptoms

- Low grade fever
- Progressive wasting/weakness, loss of production
- Coughing

Macroscopic features

- Consolidation of lungs
- Nodules of tubercle present in lungs containing cheesy mass
- Granulomatous lesions in spleen, lymph nodes, liver and intestines.
- Tubercle on pleura and mesentery (*pearly disease*).

Microscopic features

- Granulomatous lesions characterized by the presence of tubercles in different organs
- Tubercle consists of central caseative necrosed area surrounded by macrophages,
lymphocytes, epithelioid cells and giant cells

- In older cases central necrosed area is calcified and surrounded by fibrous tissue capsule.

Tuberculosis in animals:
- (1) Granulomatous lesions in spleen,
- (2) in lungs,
- (3) Microscopic features of tuberculosis:
  - A. Calcification,
  - B. Caseation,
  - C. Macrophages and
  - D. Fibrous covering,
- (4) A. Caseation and B. Calcification and giant cell (arrow),
- (5) Tubercle and
- (6) A. Giant cells, B. Caseation and C. Calcification.
**Diagnosis**
1. Symptoms and lesions
2. Tuberculin testing of animals
3. Immunodiagnostic tests for demonstration of antigen /antibody
   - ELISA
4. Demonstration of acid fast bacilli in impression smears of tubercle or in tissue sections of lungs and lymph nodes.

**Management of Tuberculosis**

**SCREENING TEST DETAILS**

<table>
<thead>
<tr>
<th>Name of the test</th>
<th>Primary screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmatory test</td>
<td>Intradermal comparative test in positive reactors, gamma-IFN/LST</td>
</tr>
<tr>
<td>Reagents</td>
<td>Bovine tuberculin PPD, Avian tuberculin PPD, Kit for Gamma-IFN</td>
</tr>
<tr>
<td>Source of reagents</td>
<td>IVRI, Izatnagar/Private firms</td>
</tr>
<tr>
<td>Testing laboratory</td>
<td>Animal farm, RDDLs, CDDL</td>
</tr>
<tr>
<td>Positive result criteria</td>
<td>Increase in skin thickness more than double the normal skin thickness with presence of odema and induration at the site, 72 hours post-inoculation.</td>
</tr>
</tbody>
</table>

**Final positive result criteria**
Increase in skin thickness at the bovine tuberculin site more than 4 mm greater than the reaction at site of avian tuberculin injection, positive gamma-IFN assay/LST.

**ELIGIBLE ANIMALS FOR TEST**
All animals above 6 months of age.

**FREQUENCY OF TESTING**

<table>
<thead>
<tr>
<th>Positive herd</th>
<th>Minimum 60 days after isolation and segregation of last positive animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative herd</td>
<td>Annual testing.</td>
</tr>
</tbody>
</table>

**ACTION ON FINDING A POSITIVE ANIMAL**

- **Animal**
  - Immediate isolation and segregation of cattle at separate farm. Culling of buffalo, sheep and goat.

- **Semen**
  - Test semen by PCR/Isolation; if negative, semen can be used in AI (hygienic semen/certified semen).
  - If positive, destroy the semen by autoclaving.

- **TUBERCULOSIS FREE HERD**
  - Herd found negative on two consecutive tuberculin tests at an interval of 6 months, the first being performed 6 months after the segregation of last affected animal.

**QUARANTINE**

<table>
<thead>
<tr>
<th>Duration of quarantine</th>
<th>Minimum 90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test schedule</td>
<td>Two tuberculin tests (SID and comparative), minimum interval of 60 days between intradermal tests.</td>
</tr>
<tr>
<td>TRAINING</td>
<td>Train the person concerned on SID for uniformity in results.</td>
</tr>
</tbody>
</table>
2. JOHNE’S DISEASE / PARATUBERCULOSIS

Johne’s Disease is a chronic bacterial disease of bovines, ovines and caprines caused by *Mycobacterium paratuberculosis* and characterized by dehydration, emaciation, chronic diarrhoea and thickening of the intestine (*corrugations*).

**Pathogenesis**

Faeces containing the organism is primary source of infection which is acquired by ingestion of contaminated feed and water. It has very long incubation period (15-18 months). The organism has also been isolated from genitalia and semen of infected bulls. Following infection, organism localizes in intestinal mucosa and its associated lymph nodes. The organism penetrate the intestinal mucosa and set up residence within macrophages. The organism multiplies intracellularly without killing host cells and are resistant to intracellular digestion. They grow inside macrophages and distributed through out body. The primary site for bacterial multiplication is terminal ileum and the large intestine leading to decreased absorptive surface, chronic diarrhoea and malabsorption. A total of 7222 animals were tested and 3.5% were found positive reactors.

**Characteristic symptoms**

- Chronic diarrhoea
- Progressive wasting/weakness, loss of production
- Hide bound condition

**Macroscopic features**

- Emaciation, cachexia
- Thickening of the intestinal wall
- Presence of ‘*rugae*’ or transverse folds thickened due to chronic inflammatory changes
- Corrugations cannot be removed even after stretching of intestinal wall
- Lymph nodes (Mesenteric) are also enlarged.

**Microscopic features**

- Infiltration of macrophages, epithelioid cells and lymphocytes in mucosa and sub mucosa
- Presence of acid fast bacilli in epithelioid cells
- Absence of caseative necrosis and calcification in cattle. However, it is present in sheep and goat.
- Nests of epithelioid cells in mesenteric lymph nodes.

**Diagnosis**

1. Symptoms and lesions
2. Demonstration of acid fast bacteria in rectal pinch or faecal sample
3. Johnin testing of animals
4. Immunodiagnostic tests for demonstration of antigen /antibody
   - ELISA, AGID
5. Demonstration of organisms in tissue sections using special stains and immunoperoxidase technique.
Paratuberculosis in cattle and sheep: (1) Corrugations in large intestine, (2) Enlargement of mesenteric lymphnodes, (3) Microscopic lesions in intestine - M. Macrophages and Giant cells (arrow) and (4) Acid fast bacilli in tissue section (arrow).
**Management of Johne’s Disease**

**SCREENING TEST DETAILS**

| Name of the test | Bovine: Single intradermal test, fecal examination, ELISA  
| Reagent | Sheep & Goat: Single intradermal test (Skin test), AGID, ELISA  
| Source of reagents | Johnin PPD, ELISA kits, antigen for AGID  
| Testing laboratory | IVRI, Izatnagar; ELISA kit from CIRG/private firms.  
| Positive result criteria | 1. Increase in skin thickness twice the normal with edema and induration at the site of injection, 72 hours post-inoculation.  
| | 2. ELISA (depends on the cut off value).  

**ELIGIBLE ANIMALS**

| Bovine | All animals above one year.  
| Sheep & Goat | All animals above six months.  

**FREQUENCY OF TESTING**

| Positive herd | Minimum 60 days after segregation of last positive animal  
| Negative herd | Annual test is minimum. Six months (±1 week) after last whole herd negative testing.  

**ACTION ON FINDING A POSITIVE ANIMAL**

| Animal | Immediate isolation and segregation of cattle at separate farm. Culling of buffalo, sheep and goat.  
| Semen | Bulls: Test by culture/ PCR, if negative, semen can be used for AI. If positive, destroy the semen by autoclaving.  
| | Sheep & goat: Don’t use semen, destroy the semen by autoclaving.  

**QUARANTINE**

| Duration of quarantine | Minimum 90 days  
| Test schedule | Bovine: Two Johnin tests and fecal examination/ ELISA, minimum interval of 60 days between tests.  
| | Sheep & goat: Two Johnin tests and fecal examination/ AGID, minimum interval of 60 days between tests.  

**TRAINING**

Train the person concerned on SID and fecal examination for uniformity in results.

**3. BRUCELLOSIS**

Brucellosis is an infectious bacterial disease of animals caused by *Brucella* sp. and characterized by abortion in late gestation and formation of granulomatous lesions in genital organs, joints and fetal liver.

**Etiology**

- *Brucella abortus*
- *Br. ovis*
- *Br. melitensis*
- *Br. canis*

**Transmission and Pathogenesis**

The brucella organism spreads through ingestion of contaminated feed and water with aborted foetal contents or foetal membrane. It is also transmitted through inhalation and may pierce the intact or abraded skin or conjunctivae. Congenital infection may also occur.
After entry bacteria multiply in reticuloendothelial cells in regional lymph nodes. The organism enters and travels through intestinal epithelial cells overlaying Peyer’s patches by endocytosis and then enters into lymphatics. The brucella organism has specific affinity with female and male reproductive organs, placenta, foetus and mammary glands. The organism can also localize in other organs like lymph nodes, spleen, liver, joints and bones. The bacteria proliferate intracellularly and it has high affinity with erythritol sugar which is present in abundance in placenta and foetus. This organism causes abortions in animals in late gestation. About 7-8% positive animals were recorded over the years in different farms out of more than 20,000 animals tested.

**Characteristic Symptoms and Lesions**
- Orchitis
- Accumulation of fluid in scrotum
- Abortion in late gestation (7-9 month)
- Retention of placenta
- Oedema and thickening of chorion
- Oedema of foetus, serosanguinous fluid in body cavity
- Pneumonia, necrotic foci in liver
- Induration of mammary gland in cows

**Macroscopic features**
- Oedema of chorion, thickened and leathery chorion/placenta
- Oedema of foetus, serosanguinous fluid in body cavity
- Pneumonia, necrotic foci in liver
- Enlargement of scrotum in males
- Induration of mammary gland in cows.

**Microscopic features**
- Infiltration of phagocytic cells, epithelioid cells and lymphocytes surrounded by fibrous tissue proliferation
- Fetal broncho pneumonia
- Organism in chorionic epithelial cells
- In males, proliferation of fibrous tissue which compresses or replaces the epididymis.

**Diagnosis**
- Symptoms and lesions
- Immunodiagnostic tests for demonstration of antigen /antibody- CFT, SAT, ELISA, DIA
- Isolation of bacteria
- Demonstration of organisms in tissue sections using special stains.
Brucellosis in animals: (1) Orchitis in ram, (2) Enlargement of festicle, (3) Granulomatous lesions in spleen, (4) Microscopic features in spleen including caseation (N) and Zone of inflammatory cells (Z), and (5) Microscopic features of placenta-oedema, congestion.
Management of Brucellosis

SCREENING TEST DETAILS

Name of the test: MRT, RBPT, ELISA, STAT
Sample: Serum, Milk
Source of reagents: IVRI, Izatnagar / PD ADMAS / Private firms
Testing laboratory: RDDLs, CDDL, PD ADMAS

Positive result criteria for:
- STAT: In vaccinated animals: 200 IU or more is positive
- In non-vaccinated animals: 100 IU or more is positive

ELIGIBLE ANIMALS
- All animals above one year of age
- In females paired serum samples 0 and 21 days after calving or abortion

FREQUENCY OF TESTING
- Positive herd: 30 to 60 days after finding last positive animal
- Negative herd:
  - Cattle: One year (± 1 week) after last whole herd negative testing
  - Sheep & Goat: Six months after last whole herd negative testing

ACTION ON FINDING A POSITIVE ANIMAL

Animal:
- Cattle: Immediate isolation and segregation, castration of bulls (retesting not recommended)
- Buffalo, Sheep & Goat: Destroy animals and dispose properly.

Semen: Destroy semen since last negative test after autoclaving.

BRUCELLOSIS FREE HERD: Herd found negative on two consecutive six monthly tests.

QUARANTINE

Duration of quarantine: Minimum 30 days
Test schedule: Two tests, serum ELISA, interval of 21 days between tests. Only negative animals to be allowed to mix with the rest of the herd.

ADDITIONAL TESTING AT SEXUAL MATURITY

RBPT, Serum ELISA, STAT before bulls are used for semen collection and distribution for AI.

PREVENTION

Cattle & Buffalo: Vaccination of females at 4-6 month with \textit{B. abortus} S-19. Males should not be vaccinated. If incidence of disease is very high at a farm than adult vaccination with a low dose of \textit{B. abortus} S-19 is advised.

4. CAMPYLOBACTERIOSIS

Campylobacteriosis is an infectious disease of cattle and sheep caused by \textit{Campylobacter foetus} and characterized by early abortion, supplicative metritis, cervicitis and vaginitis.

Transmission and Pathogenesis

The bacteria are natural inhabitant of reproductive tract of bovines. In case of males, it is confined to prepucial cavity and mucosa of glans penis and distal portion of urethra. In females, lumen of vagina, cervix, uterus and oviduct harbor the bacteria. Infection spreads through coitus or AI due to use of infected semen. Bull to bull transmission occurs through artificial vagina at breeding centers. After entry in females, the bacteria multiply in the cervix and reach to uterine horn and oviduct within 7-14 days. In the cervix, the organism metabolizes amino acids and other organic acids. The organism is protected from phagocytes because it is covered with mucous. This organism causes abortion at 5 month of gestation because the organism impairs the supply of oxygen and some nutrients (amino acids) required for implantation of embryo leading to abortion.
Characteristic Symptoms and Lesions

- Mucopurulent vaginal discharge
- Retention of placenta
- Abortions at 5-6 month of gestation (C. foetus), 6-7 months (C. jejuni)
- Purulent exudate in uterine discharges - yellowish
- Congestion of vagina
- Oedema in foetus
- Necrotic foci in liver
- Oedematous placenta

Diagnosis

- Isolation of causative agent

Management of Campylobacteriosis

SCREENING TEST DETAILS

Name of the test: Bacterial isolation and identification
Sample: Preputial washing, semen, vaginal mucous
Testing laboratory: RDDLS, refer CDDL for confirmation.

ELIGIBLE ANIMALS
All male breed able animals.
All repeat breeder female animals with early embryonic death (prolonged estrous cycle).

PREVENTION
Male animal sheath lavage with antibiotics/antiseptics.

FREQUENCY OF TESTING
Positive herd: 30 days after finding of positive animal.
Negative herd: One year (± 1 week) after last whole herd negative testing.

ACTION ON FINDING A POSITIVE ANIMAL
Animal: Isolate, segregate the positive females and isolate, segregate and castrate the positive bulls.
Semen: Destroy all the semen doses since last negative after autoclaving.

QUARANTINE
Duration of quarantine: Minimum 30 days
Test schedule: One test, if age is less than 6 months, else 3 consecutive tests at weekly intervals.

5. INFECTIOUS BOVINE RHINOTRACHEITIS

Infectious bovine rhinotracheitis is an infectious viral disease of cattle caused by herpes virus bovine and characterized by respiratory disease, conjunctivitis, encephalitis in calves and abortions, vulvovaginitis and mastitis in cows and balanoposthitis in bulls.

Transmission and Pathogenesis

The main source of infection is nasal exudate and coughed up droplets, genital secretions (seminal plasma), semen and foetal fluid/ tissues. Aerosol or droplet infection causes respiratory disease. In genital form, venereal transmission occurs. In respiratory disease, after entry virus multiplies in nasal cavity and upper respiratory tract resulting in rhinitis, laryngitis and tracheitis. There is extensive loss of cilia in trachea that adversely
affects the respiratory immunity. The virus spreads from nasal mucosa through trigeminal ganglion, resulting in non-suppurative encephalitis. Through peripheral leucocytes, virus may reach to placenta and foetus in pregnant animals causing abortion. Foetus is highly susceptible to the herpes virus infection.

**Characteristic Symptoms and Lesions**

- Fever (106°F), Nasal discharge
- Abortions in late gestation
- Conjunctivitis
- Infectious pustular balanoposthitis in bulls
- Infectious pustular vulvovaginitis in cows
- Rhinotracheitis, pneumonia, mucopurulent exudate in trachea
- Pustules in vulva/vagina, glans penis and prepuce
- Ulcers in vulva/vagina
- Necrotic lesions in liver of aborted foetus

**Macroscopic features**

- Rhinotracheitis, pneumonia, mucopurulent exudate in trachea.
- Pustules in vulva/vagina, glans penis and prepuce
- Ulcers in vulva/vagina
- Necrotic lesions in liver of aborted foetus

**Microscopic features**

- Hyaline membrane pneumonia
- Erosions/ulcers in mucosa of upper respiratory tract
- Ulcers on vulval mucous membrane
- Infiltration of lymphocytes
- Presence of intranuclear inclusions in mucosal epithelium
- Necrotic lesions in liver, spleen and kidneys of aborted foetus

**Diagnosis**

- Symptoms and lesions
- Immunodiagnostic tests for demonstration of antigen /antibody- ELISA, VNT/SNT
- PCR and Isolation of virus
Bovine herpes virus-1 infection in cattle- (1) Granular vaginitis, (2) Aborted foetus, (3) Granular balaeno-posthitis and (4) Hyaline membrane bronchopneumonia in aborted foetus.
**Management of IBR**

**SCREENING TEST DETAILS**

Name of the test: ELISA for screening and Serum Neutralization Test (SNT) for confirmation

Sample: Serum (history of vaccination to be given)

Source of regents: PD ADMAS/Private firms

Testing laboratory: RDDLs, CDDL

**ELIGIBLE ANIMALS**

All animals above 6 month of age.

**FREQUENCY OF TESTING**

Positive herd: Whole herd test, 30 to 60 days after finding of positive animal. Six monthly, after the herd become negative.

Negative herd: Exactly one year. Where the disease has been maintaining a low profile (less than 5% positive), quarterly or six monthly samples could be collected to minimize losses.

**ACTION ON FINDING A POSITIVE ANIMAL**

Animal: For confirmation seropositive animals should be subjected to PCR/ virus isolation from semen in 3 ejaculates collected at one week interval. If positive for virus, bull should be castrated and segregated.

Semen: Destroy all semen doses since last negative by autoclaving.

**IBR FREE HERD**

Whole herd tested negative on two consecutive occasions at an interval of 2 to 12 months between tests.

**QUARANTINE**

Duration of quarantine: Minimum 30 days

Test schedule: Two tests, serum ELISA or SNT, interval of 21 days between tests. PCR for semen.

**ADDITIONAL TESTING AT SEXUAL MATURITY**

Serum ELISA or SNT, before bulls are used for semen distribution in field AI programmes. PCR for semen.

**PREVENTION**

No vaccination in bulls.

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**6. TRICHOMONOSIS**

Trichomonosis is a protozoan parasitic disease of animals caused by *Trichomonas foetus* characterized by early abortion, vaginitis, metritis and balanitis in cattle.

**Transmission and Pathogenesis**

Following coitus, cow gets infection from bull that causes vaginitis, endometritis, placentitis, foetal infection and abortion in early gestation. Abortion is followed by pyometra due to invasion of secondary bacterial infection.

**Characteristic Symptoms and Lesions**

- Retention of placenta
- Sterility, Pyometra- Metritis, Vaginitis
- Abortion during 3-5 month of gestation
- Balanitis in bulls

**Diagnosis**

- Characterization of causative agent
Collection, Preservation and Dispatch of Samples for Laboratory Diagnosis

- Freshly collected 5 ml blood in EDTA in sterilized vials
- Freshly collected semen (2-5 ml) in sterilized vials
- Serum samples in sterile vial (5 ml)
- Preputial washings collected by using 20 ml of sterile PBS (pH 7.2) divided into two equal parts

a. For Campylobacteriosis: In first part transport medium (10 ml) is added.

**Transport medium:** Mullar Hinton broth 21 gm/lit, Bacteriological charcoal 5 gm/lit dissolved in one litre of distilled water and autoclaved. After cooling Vancomycin (40 mg), Polymyxin B sulphate (10000 IU), Cycloheximide (100 mg), Trimethoprim (20 mg) and 5 Fluorouracil (500 mg) are added. Filter sterilized 2 ml solution of 250 mg each ferrous sulphate, sodium metabisulphite and sodium pyruvate are also mixed.

b. For Trichomoniasis: Second part of preputial washing is centrifuged. Discard the supernatant. Sediment is collected and mixed into 10 ml transport medium.

**Transport medium:** PBS pH 7.2 + 5% fetal calf serum or 5% skimmed milk + Penicillin 100 units/ml and Streptomycin 100 µg/ml.

**Management of Trichomonosis**

**SCREENING TEST DETAILS**

<table>
<thead>
<tr>
<th>Name of the test</th>
<th>Agent isolation and identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Preputial washing, vaginal discharge</td>
</tr>
<tr>
<td>Testing laboratory</td>
<td>RDDLs, CDWL.</td>
</tr>
</tbody>
</table>

**ELIGIBLE ANIMALS**

All breed able males, teaser and females with reproductive disorders.

**FREQUENCY OF TESTING**

Annual

**ACTION ON FINDING A POSITIVE ANIMAL**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Isolation, segregation and castration of the bull</th>
</tr>
</thead>
<tbody>
<tr>
<td>semen</td>
<td>Destroy the collections since last negative test by autoclaving.</td>
</tr>
</tbody>
</table>

**QUARANTINE**

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<tr>
<th>Duration of quarantine</th>
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<td>Test schedule</td>
<td>One test, if age is less than 6 months, otherwise 3 consecutive tests at weekly intervals.</td>
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</tbody>
</table>

**ADDITIONAL TESTING AT SEXUAL MATURITY**

Protozoa isolation before bulls are used for semen distribution for AI.

**PREVENTION**

Breeding rest for positive females for a period of six months and retest before breeding.

**Further Reading**