

DIAGNOSIS AND CONTROL OF INFECTIOUS BOVINE RHINOTRACHEITIS, (IBR).*

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Infectious bovine rhinotracheitis (IBR), a contagious disease characterized by severe inflammatory changes in the anterior part of the respiratory system of cattle, has been reported from most, if not all, parts of the world since its first description in USA in 1950 (1,2,3,4). The disease is caused by bovine herpesvirus type 1, commonly known as IBR virus (5,6).

Cattle of all breeds and ages, unless having passively or actively acquired immunity, are susceptible to the virus and respond in many clinically different forms depending on the site of infection. The virus was shown to produce both frank and inapparent forms of infection. The infection, however, in feedlots and dairy farms, where animals are kept under intensive conditions, could become economically important.

Heavy economic losses reported from IBR infection is due mostly to the mortality among infected animals and to the abortion which follows both mild and severe cases of the infection among pregnant cows.

Fortunately in an IBR infection a fairly strong immunity develops and thus the heavy losses caused by the virus infection could satisfactorily be prevented if a reliable rapid means of diagnosis is available and a potent safe vaccine together with a good management is applied.

Diagnosis procedures

IBR may be diagnosed by clinical symptomatology alone in certain cases.

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However, depression, nasal and lacrimal discharges and other symptoms associated with rhinitis and conjunctivitis at early stages of the infection are not always sufficiently characteristic to differentiate the infection from some other cattle diseases such as calf diphtheria, shipping fever complex, mucosal disease and even rinderpest. For this reason any clinical diagnosis should be confirmed by either virus isolation or by the demonstration of an antibody response to IBR virus. In the case of abortion definite diagnosis depends mainly on the virus isolation.

Virus isolation.

IBR virus could readily be isolated from the infected animals providing suitable samples are collected at the proper time and shipped to the laboratory under a good condition.

A number of cell cultures especially kidney and testicle cell cultures of bovine, ovine, and rabbit origin could successfully be used for the virus isolation. The following procedure is used at the Razi Institute for IBR virus isolation and the virus identification. The technique is appeared to be the cheapest and the most reliable means of IBR diagnosis and could be practiced wherever the cell culture technique is routinely used.

1) Nasal and conjunctival secretions from animals in the acute stage of the infection are collected by using sterile cotton swabs. The swabs are immediately placed into screw capped bottles each containing 2 ml. of culture medium with antibiotics and are transported to the laboratory in a thermos flasks filled with ice cubes.

Specimens from lung, liver, tonsil, spleen and tracheal or nasal mucosa of dead or sacrificed animals and a piece of placenta and samples from amniotic fluid, pericardial or pleural fluids, lung, kidney, or spleen of aborted foetuses are also collected and brought to the laboratory under the same conditions.

2) The secretion absorbed into the cotton is extracted into the culture medium from the swabs by pressing them with a pair of sterile pincers. The extracts are then centrifuged at 6,000 r.p.m. for 20 minutes and the supernatant fluid is kept at -70C until used as inoculum for virus isolation.

Similarly the extracts from tissues are prepared from a 10 per cent suspension of each tissue specimen in culture medium and are kept at -70 C. until used for virus isolation.

3) Primary or secondary cultures of bovine embryonic kidney (BEK) cells in rolling tubes is used for virus isolation.

The fluid medium of the culture tubes is discarded and then the cell sheet in each tube is washed twice with 2 ml. of culture medium. 0.2 ml. of each inoculum is used to inoculate each of 4 cell culture tubes.

At least 4 cell culture tubes are kept as "cells control".

A 2 hours adsorption period is allowed and then. 1.5 ml. of culture medium containing 2% foetal bovine serum is added to each tube.

4) The cells (in 3) are reincubated at 37 C. and are examined microscopically everyday for the appearance of cytopathic effect (CPE).

5) If no (CPE) appears in any of the inoculated cell culture tubes (in 4), within 7 days incubation, a second passage in BEK is performed as follows:

Cells and the fluid of tubes inoculated with each sample are pooled after being frozen and thawed. The mixture is centrifuged at 2500 r.p.m. for 15 minutes and the obtained supernatant is used for inoculation of a new series of BEK cell culture tubes as already mentioned (in 3).

The sample is considered to be negative if no CPE appears in the second passage.

6) Appearance of CPE in the inoculated cells, providing the "cell controls" have kept their healthy normal appearance during the observation period, serves as an indication of the presence of a virus in the used specimen.

IBR virus, like other member of the herpesviruses, produces a very characteristic CPE consisting of rounding, shrinking and clumping of infected cells in BEK cell cultures. The infection develops first in small disseminated foci but spreads rapidly and affects the whole cell sheet which results in the complete destruction of the cells. Therefore formation of any CPE resembling to the above cytopathic alteration strongly suggests that the virus could be an IBR virus strain.

The fluid containing virus is harvested as before (in 5) and is kept at-70 C. for further passages and virus identification.

7) Several Leighton tubes containing coverslip with uniform monolayer of BEK cells are inoculated with the virus suspension (in6) as described before (in3).

8) Equal volume of virus suspension and its 1/10 and 1/100 dilutions are mixed with IBR antiserum.

To a separate set of virus dilutions equal volumes of inactivated normal rabbit serum is added.

Both series of virus-serum mixtures are incubated at 37 C. for 90 minutes.

9) From each virus-serum mixture 4 cell culture tubes are inoculated as previously described.

10) Everyday, for 3 to 4 days, one or two coverslips (in 7) are collected and stained by haematoxylin eosin.

11) Appearance of CPE in culture tubes (in 9) are recorded.

The isolated virus is identified as a strain of IBR virus if intranuclear inclusion bodies are formed in infected cells(in 10) and the CPE has been prevented by the IBR antiserum (in 11).

Demonstration of specific IBR antibody.

IBR virus persists in the nasal and lacrimal secretions only for a short period of time following the onset of clinical symptoms of the infection. Therefore, in many cases virus isolation method could not be utilized as a reliable means for the diagnosis of the disease. In such cases the diagnosis are made strictly by the demonstration of antibody response of the recovered animals to the virus infection.

Among serological procedures, serum neutralization test (SNT) and indirect haemagglutination test (IHAT) are the most practical and reliable procedures currently used for the diagnosis of IBR (13,19,17,22).

Antibodies to IBR virus could be demonstrated by the (SNT) as early as 10 to 17 days, and by (IHAT) as early as 7 to 9 days after the onset of symptoms. The antibodies titer attained its maximum within 3 to 6 weeks and persists in a demonstrable level for years (19,23).

(SNT), whether conducted by the plaque or tube method, is performed by the "constant virus serum dilution" technique. The test probably has been the most widely used procedure for the serological diagnosis of IBR.

(SNT) has the disadvantage of being relatively cumbersome, expensive and time consuming to conduct.

(IHAT), on the other hand, being as specific as (SNT) for the detection of IBR antibody, has the advantage of being about 10 times more sensitive and rapid and much less costly method that can be performed without sophisticated equipment.

Both tests can be used, however, as a valuable aid for the diagnosis of IBR providing paired serum samples from each animal are available. The samples should be collected within 2 to 3 weeks intervals at the early stage of the disease or soon after recovery.

In determining the IBR antibody of serum samples by (SNT) for diagnosis purpose, the results are considered to be positive when the first serum sample shows no antibody titer and the second serum sample have a titer of 1/4 or greater against 100 TCID₅₀ of a known IBR viurs. If both serum samples contain antibody, the IBR infection is diagnosed when the antibody titer of the second sample is at least 4 times more than that of the first one.

The interpretation of the results of (IHAT) in the diagnosis of IBR is similar to the above except that titers of 1/8 or greater are considered positives.

Control and prevention

Since IBR does not appear to be a highly contagious disease, separation of sick animals and quarantine regulation may be used as a means to limit further spreading of the disease inside the farms and to prevent the introduction of the infection to the clean areas. However, in order to control the disease more efficiently, besides the above sanitary measures, an immunoprophylactic scheme is of absolute necessity.

Natural or experimental exposure of cattle to IBR virus results to a very good immunological response which appears soon after recovery and persists for a long period of time.

The immunity, in addition to the humoral antibody, in some extent, is related to the "cell mediated and cellular immunity" and to the production of interferon and nasal antibody in infected animals.

Immunity against IBR could also be produced by vaccination of cattle with live or killed-virus vaccines.

Prevention of IBR by modified live-virus vaccine has been outstandingly successful since it was first practiced in 1957 (8). Modification of the virus has been acheived through successive passaging of IBR virus in calf kidney, rabbit kidney, dog kidney, and swine kidney cell cultures by several workers (7,9,10, 16,18,20).

Vaccine is given by intramuscular inoculation or by intranasal administration. Nasal administration of the vaccine has the advantage of stimulating the secretory immune system in the respiratory tract of cattle to produce nasal interferon and antibody much better than the vaccine inoculated intramuscu-

larly. The immunity following nasal administration of modified live-virus vaccine appears within 48 to 72 hours after administration of the vaccine.

However, the production of humoral antibody and the cell mediated immunity appears to be similar in both ways of vaccination and thus calves vaccinated nasally or intramuscularly are equally immune and refractory to experimental challenge, for at least 2 years, from 7 to 10 days-post vaccination.

The immunity following vaccination with killed-virus vaccines, on the other hand, is related mostly to the production of humoral antibody. The antibody level, in comparison with antibody produced following administration of live – virus vaccines, is low but high enough to protect the cattle against natural and experimental exposure to virulent IBR virus. To induce a solid immunity with killed-virus vaccines, however, 2 vaccination at 3 to 5 weeks intervals is recommended.

Live-virus vaccine, regardless of the route of administration has the disadvantage of causing a high rate of abortion, up to 70 per cent, especially in cows from 5th through 7th month of gestation (11,12,14,15,21).

Each kind of vaccine, however, has its own particular advantage and could be used, depending on the circumstances involves, for the control of IBR as follows:

1) Live-virus vaccine is recommended for vaccination of calves in feedlots. Vaccine could be administered either nasally or intramuscularly. Vaccination by intramuscular route can be carried out with much more simplicity and accuracy, and thus it appears to be the route of choice under ordinary conditions. In some situation, e.g. during an outbreak of IBR among animals in feedlot or neighbouring farms, when an early immunity is desirable, vaccination via intranasal is strongly recommended.

2) When calves have to be immunized while nursing their susceptible pregnant dams, nasal vaccination must be avoided as this may results in infection of the cows and possible abortion.

On the other hand, nasally administration of the vaccine is preferable in the case of young calves of 4 to 6 month-old, whose passively acquired nasal antibody has been declined but still possess circulation antibody, as only in this way the vaccine could stimulate the production of antibody without the interference of circulating antibody.

3) In dairy farms cows must be vaccinated before pregnancy. In emergency situation, however, susceptible unvaccinated pregnant cows could be vaccinated with a killed-virus vaccine, as administration of live virus vaccines may result to a high rate of abortion.

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