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Chapter 8

Herpesvirus in Bovines: Importance of Bovine Herpesvirus Type 1

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/63157

Abstract

The chapter aims at providing readers with the overview of the herpesvirus infection in bovines. It includes the detailed etiology of the infection, discussing the virus characteristics, virus structure, genome, viral proteins, types and subtypes of the virus. Then, the chapter discusses the transmission and pathogenesis of the virus, which are very important as they help to control the virus spread, viral latency and clinical signs observed in the affected animals. Later, the chapter discusses the diagnosis of herpesvirus infection in bovines to help the readers to gain knowledge about the techniques used earlier and nowadays for the diagnosis of the infection. Then, the chapter provides information on the procedures to be adopted for the prevention and control of the infection in bovines. Thus, the chapter provides complete information about the herpesvirus infection in bovines.

Keywords: bovines, herpesvirus, etiology, epidemiology, diagnosis, prevention, control

1. Introduction

Viruses have either RNA or DNA as their genome which is one of the criteria for the classification of viruses. Among the DNA virus families is the Herpesviridae family. It is the largest DNA family and derives its name from the Greek word, "herpein", which means "to creep". The viruses belonging to this family affect a number of species of mammals, birds, reptiles, fishes and amphibians. This chapter discusses the herpesvirus infection in bovines.

There are many bovine herpesviruses belonging to different genus viz. Bovine herpesvirus-1 (BHV-1), BHV-2, BHV-4, BHV-5 in the family Herpesviridae. BHV-2 causes skin lesions, BHV-4

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often produces subclinical infection and BHV-5 produces meningoencephalitis. This chapter is written with an aim to focus on the most important disease caused by herpesviruses in bovines; infectious bovine rhinotracheitis caused by BHV-1.

BHV-1 is one of the important viruses responsible for great economic loss to the livestock industry. It causes losses in terms of drop in milk yield, abortion and repeat breeding. BHV-1 is associated with different clinical conditions like respiratory infections, conjunctivitis, vulvovaginitis, abortion and balanoposthitis in males. It is also associated with encephalitis and generalized systemic infections [1] too. The virus is readily transmitted and has world-wide distribution. The virus can remain latent in the ganglia of the infected, but clinically normal, animals [2]. The virus is excreted through nasal and ocular secretions, placenta of aborted animals and through semen. The virus transmission via semen is special concern to cattle breeders.

Mayfield et al. [3] identified 3 strains of BHV-1 namely I, II, III depending on the restriction pattern. Studdert [4] reclassified them as BHV-1.1 (respiratory form), BHV-1.2 (genital form) and BHV-1.3 (encephalitic form). BHV-1 consists of 25–33 polypeptides of which 11 are glycosylated [5–7] and associated with virus envelope [5, 6]. The three sets of enveloped glycoproteins gI, gIII and gIV were characterized earlier which are involved in the immune response [7–12]. Babiuk et al. [13] showed that gIV produces the highest serum-neutralizing antibody titres with gI being the least immunogenic. The strains of BHV-1 with different tissue affinities may exist in the field conditions and these different clinical strains can be differentiated by restriction endonuclease strategies [14]. The monoclonal antibodies specific for the major BHV-1 glycoproteins GVP6/11a/16, GVP7, GVP3/9 and GVP11/b are used to determine the glycoproteins and identify the epitopes involved in neutralization and antibody complement lysis [15]. Both humoral and cell-mediated immune responses are generated against BHV-1, but cell-mediated immune response is the major means of defence mechanisms [16]. The disease can be diagnosed by clinical signs and lesions and by a variety of virologic and immunologic techniques [17].

This chapter is written with an aim to provide information on herpesvirus in bovines to the readers.

2. Taxonomy and nomenclature

In 1973, the International Committee for the Taxonomy of Viruses (ICTV) named the virus as bovid herpesvirus type-1 [18] on the basis of the family of virus and the host it infects. The nomenclature system was further discussed in the International Committee for the Taxonomy of Herpesvirus (ICTH) in 1976 [19]. Then, Gibbs and Rweyemamu [1] adopted a system based on sub-family of Bovidae and named the virus to be BHV-1.

According to ICTV, the Herpesviridae family includes three subfamilies. The three subfamilies are Alphaherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae. Alphaherpesviruses replicate and spread in the host, rapidly destroying the host cells. They establish the latent

infection in the sensory ganglia of the host. Betaherpesviruses replicate and spread in the host slowly. They cause infected cells to enlarge, hence they are commonly named as cytomegaloviruses. They become latent in secretory glands and lymphoreticular cells of the host. Gammaherpesviruses infect T and B lymphocytes of the host and produce latent infections in these cells. Some of Gammaherpesviruses also replicate in epithelial and fibroblastic cells causing cytolysis. Many of these viruses lead to neoplastic transformation of lymphocytes.

There are nine genera included in the family Herpesviridae. The genera included in the subfamily Alphaherpesvirinae are Simplexvirus (includes BHV-2), Varicellovirus (includes BHV-1 and BHV-5), Mardivirus and Iltovirus. The genera included in the subfamily Betaherpesvirinae are Cytomegalovirus, Muromegalovirus and Roseolovirus. The genera included in the subfamily Gammaherpesvirinae are Lymphocryptovirus and Rhadinovirus (includes BHV-4). There is a 10th genera including Ictalurid herpes-like viruses which includes herpesviruses of fish. BHV-1 is also known as infectious bovine rhinotracheitis virus; BHV-5 is also known as bovine encephalitis virus and BHV-4 is also known as Movar virus. There is a proposal in ICTV to create the BHV-6 in the new genus Macavirus in the subfamily Gammaherpesvirinae.

Herpesviruses of mammals, birds and reptiles share extensive genetic relationship among themselves. Herpesviruses of fish and amphibians are also related to each other but are related only marginally to herpesviruses of mammals, birds and reptiles. No herpesvirus-specific gene is conserved between the classes, but one gene which encodes the putative ATPase subunit of a DNA-packaging terminase is conserved among all herpesviruses.

3. Virus structure and genome

BHV-1 has icosahedral symmetry approximately 150–200 nm in diameter [1, 20]. The structure of the virus consists of four parts viz.

1. The core which includes the genome having linear double-stranded DNA molecule of 139 Kbp [21, 22] in the form of a torus. The G+C content of the genome ranges from 31 to 75% and contains 60 to 120 genes [23]. Herpesvirus genes are not arranged in operons and in most cases have individual promoters. The genome termini are not covalently closed or covalently linked to a protein. All herpesvirus genomes contain lengthy terminal repeats, both direct and inverted.

2. The core is surrounded by the icosahedral capsid made up of 162 capsomeres.

3. The virus consists of an envelope which forms the outer layer of virion. The lipid bilayer envelope is derived from the nuclear membrane of the host by budding [24, 25]. It consists of unique viral glycoproteins which appear as short spikes embedded in the envelope.

4. Between the capsid and the envelope is a proteinaceous layer known as tegument. It consists of the viral enzymes needed to take control of host cell chemical processes for virion production and also to defend itself from the host cell responses.

The glycoproteins are critical to the virus for attachment to [26] and penetration of the susceptible cells [27] and to the host for virus neutralization [28] by the production of neutralizing antibodies. The glycoproteins are the major targets of immune response both at humoral and cellular level [29]. The major BHV-1 proteins that are involved in the immune response are gI, gIII and gIV enveloped glycoproteins [11].

Replication of herpesvirus occurs in the nucleus of the host cell and the envelope is derived from the nuclear membrane of the host cell. The virions are released by exocytosis. Intranuclear inclusion bodies are formed, which are the characteristic of herpesviruses. Herpesvirus genomes replicate by circularization and production of concatemers, followed by cleavage of unit-length genomes during packaging into capsids [30].

For the replication to initiate, the virion attaches to host cell (heparan sulfate moieties of cellular proteoglycans) with the envelope glycoprotein, gC. The viral envelope fuses to the plasma membrane of the host cell so that the nucleocapsid enters the cytoplasm. The glycoproteins involved during this process are gB, gD and gH. The capsid travels along the cytoskeleton to a nuclear pore where the viral DNA is released. The linear genome enters the nucleus and circularizes. Once in the nucleus, the viral DNA is transcribed into mRNA by cellular RNA polymerase II. The viral gene expression is tightly regulated. First, the tegument protein associates with two cellular proteins and the complex transactivates transcription of immediate-early (IE or alpha) genes. The immediate-early genes encode regulatory proteins and initiates transcription of the early (E or beta) genes. These proteins are enzymes needed for viral replication. Then, the late (L or gamma) genes are activated for production of viral structural proteins. After transcription in the nucleus, all mRNA transcripts are translated into protein in the cytoplasm. Subsequently, the proteins can go to the nucleus, stay in the cytoplasm, or become a part of the membrane bilayer. Capsid proteins assemble in the nucleus to form empty capsids. In these capsids, full-length viral DNA is packaged to form nucleocapsids. The nucleocapsids associate with segments of the nuclear membrane where tegument and glycosylated envelope proteins have bound. This association triggers the formation of envelope by budding through the nuclear membrane. Enveloped virions accumulate in the endoplasmic reticulum. Mature virions are released by exocytosis.

4. Physico-chemical and antigenic properties of BHV-1

The virus is sensitive to ether, chloroform, acetone, alcohol and bile salts. It is inactivated within 10 min at 56°C and in 4 min at 65°C. The virus is labile at pH 4.5 to 5.0, but stable between pH 6 to 9. Virus is extremely susceptible to 0.5% sodium hydroxide, 0.01% $HgCl_2$, 1% phenol and 1% quaternary ammonium compounds. Also, 5% formalin can inactivate the virus in 1 min. Thus, the virus is fragile and sensitive to detergents and lipid solvents. It is unstable in the environment, but can survive in the frozen semen indefinitely.

Antigenically, there is only a single serotype of BHV-1 recognized. There are three strains of BHV-1 (I, II, III) described on the basis of endonuclease pattern of viral DNA [3]. Studdert [4] reclassified them as BHV-1.1 (respiratory subtype), BHV-1.2 (genital subtype) and BHV-1.3

(encephalitic subtype). BHV-1.3 has been reclassified as a distinct herpesvirus designated as BHV-5. BHV-1 is antigenically related to equine rhinopneumonitis virus (EHV-1) in complement fixation test [31]. It also shares one-way relationship with Pseudorabies virus and goat herpesvirus [32]. The virus also shares a common antigen with Marek's disease virus [33] and Burkitts lymphoma virus.

5. Epidemiology and pathogenesis

The virus is host-specific. All ages and breeds of cattle are susceptible. Although cattle is the natural host for infectious bovine rhinotracheitis infection, antibodies against BHV-1 have also been demonstrated in sheep, goat, pigs and wild animals [34]. The disease mostly occurs in animals over 6 months of age. Prevalence of BHV-1 increases with age and is less prevalent in cows under 2 years of age. The virus does not discriminate between sex and breed; however, feed lot cattle are more frequently infected than dairy cattle [35].

The virus is readily transmitted and has worldwide distribution. Transmission of the virus occurs by aerosol route. The virus is also secreted through nasal and ocular secretions, genital secretions and placenta of aborted animals and also in milk [1]. The virus is also transmitted through infected semen during artificial insemination and embryo transfer. It is the most common viral pathogen found in bovine semen [36]. The extensive use of artificial insemination in cattle has facilitated the exchange of genetic characteristics. Therefore, contamination of semen and dissemination of bovine pathogens via semen are of primary concern to cattle breeders. It also poses a potential threat to cattle industry as it can cause a variety of genital tract disorders including endometritis, infertility and abortion [37]. Thus, semen should ideally be collected from bulls that are serologically negative for BHV-1 infection. After primary respiratory or genital infections, animals become lifelong carriers of BHV-1, despite the development of neutralizing antibodies. Reactivation of latent BHV-1 in carrier animals occurs periodically and has been due to stress such as intercurrent diseases, transportation, cold, crowding and corticosteroid treatment.

Pathogenesis depends upon the subtype of virus and route of entry of the virus. If the virus (BHV-1.1) enters by aerosol route, it replicates in the mucous membrane of the upper respiratory tract. Thus, the virus is shed in nasal secretions. The virus enters local nerve cell endings and becomes latent in trigeminal ganglion. Secondary bacterial infection leads to systemic infection and death of the animal. Abortion occurs due to fetal infection. Genital infection is caused by subtype BHV-1.2. In this case, the virus replicates in the mucosa of vagina or prepuce and becomes latent in sacral ganglion. Focal necrotic lesions occur on genital mucosa, which later on forms ulcers. Abortion does not occur in the animals affected by BHV-1.2.

6. Clinical signs

Infectious bovine rhinotracheitis initially had several names such as "red nose", "dust pneumonia", "necrotic rhinitis" or "necrotic rhinotracheitis" based on the signs and symptoms manifested by the virus in different parts of the world [38]. The reproductive form of the disease was reported as early as 1938 and was called "epivag", which means causing epididymitis in males. In 1955, at a meeting of US Livestock Sanitary Association it was designated as infectious bovine rhinotracheitis (IBR) [39].

BHV-1 causes various clinical manifestations in cattle and buffaloes. Type of disease caused by the virus depends mostly on the route of the entry of virus. The incubation period of the disease is 2 to 4 days. In case of the respiratory form of infection, the virus multiplies initially in the tissues of respiratory tract and is extended via the lacrimal ducts to the ocular tissues where a secondary site of infection is established. There is sudden onset of anorexia, fever, severe hyperaemia of the nasal mucosa, serous discharge from the eyes and nose, increased salivation and excitation. There is sharp drop in milk yield. Animal has respiratory distress and sudden death occurs within 24 hours after the first signs appear due to obstructive bronchitis. In prolonged cases, the nasal discharge becomes profuse and purulent and death occurs due to secondary bronchopneumonia. There is either bilateral or unilateral conjunctivitis or keratoconjunctivitis with profuse lacrimation. Various workers [40, 41] have isolated IBR virus from the conjunctival and nasal discharge of calves showing symptoms of the disease. The BHV-1 type causing respiratory form of the disease causes abortions usually during 4–8 months of gestation. Abortion occurs due to fetal infection which results in severe visceral damage, gradual cessation of placental circulation and placental degeneration, leading to detachment and abortion.

Kendrick et al. [42] isolated herpesvirus from genital infection of cows which they termed as infectious pustular vulvo-vaginitis (IPV) caused by the genital form (BHV-1.2) of the virus. The initial signs in the genital form of the disease include reddening of the vaginal mucosa and pustule formation, which lead to mucopurulent vaginal discharge. No abortion is seen in this form of the disease. The respiratory and genital forms of the disease are rarely seen at the same time in a herd. Bouters et al. [43] isolated herpesvirus from bulls with infectious pustular balanoposthitis and orchitis. Thus, in males, there is inflammation of preputial lining with pustule formation. Singh et al. [44] isolated IBR/IPV virus from the semen of Jersey bull and from the placenta and cotyledons of Jersey cow.

7. Diagnosis

Any disease control programme has a prerequisite of diagnosis at the earliest. The serological assays for antibodies to BHV-1 have been widely used for the diagnosis of acute infections, epidemiological studies and the detection of latent carriers in relation to control schemes and international trade. Gibbs and Rweyemamu [1] reviewed the diagnostic tests available for BHV-1. These included the conventional clinical diagnosis based on signs and symptoms; but

as the virus has numerous clinical manifestations, diagnosis is difficult and requires laboratory confirmation. So, the specific diagnosis of IBR has been traditionally based on the isolation of the causal agent in the cell culture, together with the detection of active antibody response in the host animal. In the cell culture system, the detection of IBR virus is based on the cytopathic effects (CPE) characterized by rounding of individual cells with shrinkage of some with increased granularity, which progresses rapidly to a characteristic "bunch of grapes". The detection of viral antigen in clinical samples like nasal swabs, conjunctival swabs, vaginal discharge, semen, preputial washings, placenta and aborted fetus can be rapid and economical alternative to cell culture. The most widely applied antigen detection technique has been immunofluorescence, either on smears of cells from nasal or ocular epithelium [45] or on cryostat sections of tissues collected at post-mortem. Electron microscopy is another rapid, reliable method of diagnosis of the antigen, but it is difficult to distinguish all herpesviruses [1]. Immunoenzymatic techniques have been described for the labeling of infected cells [46] and for the detection of soluble antigen in diluted nasal mucus [9]. The fluorescent antibody technique (FAT) is frequently used to diagnose respiratory and reproductive forms of BHV-1 infections. Frank et al. [47] described FAT as the test of choice for diagnosis of IBR/IPV virus. The virus can also be identified by neutralization test with mono-specific antiserum or with the use of monoclonal antibodies. Monoclonal antibodies can differentiate between BHV-1 subtypes like BHV-1.1 and BHV-1.2 [20]. Further characterization can be done by DNA restriction enzyme analysis. Enzyme-linked immunosorbent assay (ELISA) to capture antigen can also be done.

The serological investigations are the faster means of detecting the prevalence of antibody in cattle population. The virus neutralization test is highly specific and reliable for the detection of BHV-1 antibodies. The other serological techniques used for the detection of antibodies include passive hemagglutination test, indirect hemagglutination test, agar gel diffusion test (AGID), counter immunoelectrophoresis and complement fixation test (CFT). ELISAs for antibody detection are also being used frequently. Payment et al. [48] compared serum neutralization test (SNT) with ELISA and reported that the antibody titres detected were 100 times higher than those seen in SNT, which showed the increased sensitivity of enzyme immunoassay. The rapid diagnosis of recent infection to BHV-1 was carried out using ELISA by detecting virus-specific IgM by Edwards et al. [49]. Edwards and Gitao [50] reported that antigen detection using avidin-biotin ELISA increased by 50-fold than compared to simple sandwich ELISA. Renukaradhya et al. [51] and Suresh et al. [52] demonstrated the use of avidin-biotin ELISA for the epidemiological studies against IBR antibodies. A gE ELISA is based on the use of two different monoclonal antibodies against gE of BHV-1. Thus, this ELISA detects antibodies against two different epitopes on gE [53]. The gE ELISA can detect antibodies in milk and in bulk milk samples [54]. The milk samples tested by ELISA have advantages over the SNT for mass field surveys [55]. Perrin et al. [56] reported that paired sample of serum and milk indicated that IBR antibody concentration in serum was about 200 times higher than in milk; hence, negative ELISA results in bulk milk samples would not exclude the possibility of infection in up to 25% animals. Since the antibody concentration in the milk is lower than serum, the antibody in the milk should be concentrated by the addition of rennet and ammonium sulfate and the ELISA should be carried out to a solution of the

precipitate. To differentiate between the vaccinated and naturally infected animals (DIVA strategy), gE blocking ELISA can be used [54].

Isolation of the IBR virus was done for the first time by Madin et al. [57]. Their earlier attempts to recover the virus in chicken embryo, in weaning and suckling mice and in guinea pigs were unsuccessful. The failure was followed by successful isolation of the virus in tissue cultures of bovine embryonic kidney. Mehrotra et al. [58] isolated the virus in primary calf kidney cell culture and the virus produced intranuclear inclusions and CPE. The virus was also isolated from semen, placenta and cotyledons employing bovine embryonic kidney and bovine turbinate cell lines [44]. Mehrotra et al. [59] isolated BHV-1 in primary cow calf kidney (PCCK) cell culture and used Madin Darby bovine kidney (MDBK) cell line for subsequent passages and characterization of virus isolate. BHV-1 was isolated from the conjunctiva of affected cow after serial passages in MDBK cell line by Mohan Kumar et al. [60].

Nucleic acid detection methods also have widespread application in routine diagnosis nowadays and they have the advantage of being sensitive and quicker than virus isolation. Engelenburg et al. [61] developed polymerase chain reaction (PCR) assay to detect BHV-1 in bovine semen using a purification method to eliminate interfering components. The results of PCR were achieved in a day compared with virus isolation, which takes 7 days and is less laborious. Wiedmann et al. [62] used a nested PCR assay targeting a portion of the glycoprotein IV gene to detect BHV-1 DNA. Kibenge et al. [63] amplified strains of BHV-1 by PCR with primers in the thymidine kinase gene region. Vilcek et al. [64] employed PCR assay on samples like nasal swabs, lung, lymph nodes and tracheal mucosa. Masri et al. [65] used a rapid nested PCR for detection of BHV-1 in semen of infected bulls in which dexamethasone was given for reactivation of BHV-1 from latency. Santurde et al. [66] detected BHV-1 using PCR amplification of a highly conserved DNA fragment within the glycoprotein gI sequence (323 bp between nucleotide 1491 to nucleotide 1814) of the virus. Ashbaugh et al. [67] used nested PCR for simultaneously detecting and discriminating between BHV-1 and BHV-5 using type-common primers derived from gC sequences. Nested PCR is more sensitive than PCR. Rocha et al. [68] detected BHV-1 in the tissues of a naturally aborted bovine fetus by nested PCR assay. Further, real-time PCR can be used for detection and characterization of BHV-1 virus. The real-time PCR reveals the results in the real time during the time the PCR is running. It is a highly sensitive, specific and reliable assay.

The authors developed monoclonal antibodies against BHV-1 [69] using hybridoma technology. The technology is detailed below:

7.1. Propagation of virus in MDBK cell culture

The virus BHV-1 isolate was propagated in MDBK cell line using Dulbecco's minimum essential medium with 10 and 2% FCS for growth and maintenance, respectively, for preparing antigen in bulk quantity.

7.2. Purification of the virus

The cell culture fluid, after three cycles of freezing and thawing, was centrifuged at 7000 rpm for 20 min at 4°C to collect supernatant. The supernatant was subjected to ultracentrifugation at 105 × g for 1 hour to pellet the virus. The pellet was resuspended in 500 μl of phosphate buffered saline and subjected to 20–40% (w/v) sucrose density gradient centrifugation (the sucrose gradient was prepared in 200 mM tris 50 mM NaCl buffer, pH 8.0) at 105 × g for 2 hours and 30 min to collect the virus at the junction of 20–40% sucrose. The presence of virus was confirmed by agar gel precipitation test (AGPT) as per Le Yeune et al. [70] and the protein content was estimated [71].

7.3. Immunization of BALB/C mice

BALB/C mice of 6–7 weeks of age procured from the National Institute of Nutrition, Hyderabad, India, were used for immunization. Four hundred microgram of purified virus was mixed with equal amount of Freund's complete adjuvant to prepare an emulsion and injected into mice *via* intraperitoneal route. Booster was administered after 14 days with 100 μg of purified virus alone *via* intraperitoneal route. Three days after the second injection, all the mice were tail bled to collect serum which was subjected to ELISA to determine the antiviral antibodies. For this, the ELISA plate was coated with purified virus (10μg/ml) and incubated overnight at 4°C. About 100 μl of each dilution (1:20 and 1:40) of the test serum was added in duplicate wells and the normal serum served as negative control. The plates were incubated at 37°C for 2 hours. After washing the plate three times, 100 μl of anti-mouse IgG-horse radish peroxidase conjugate (1:10,000) was added in each well and further incubated the plate at 37°C for 2 hours. After washing, 100 μl of substrate solution was added to each well and incubated the plate at room temperature for 10 min. After stopping the reaction with 50 μl of 1M H2SO4, optical density was read at 450 nm wavelength.

7.4. Myeloma cell line

Non-secretory myeloma cell line SP2/O/Ag-14, procured from the National Centre for Cell Science, Pune, India, was subcultured and maintained in RPMI-1640 medium with 10% fetal calf serum containing 0.2% streptopenicillin (500×) and 1% glutamine. A day prior to fusion, myeloma cells were subcultured in growth medium containing 2% 8-azaguanine. Myeloma cells growing in log phase of growth were used for fusion.

7.5. Preparation of feeder spleen cell layer

Albino mice procured from Punjab Veterinary Vaccine Institute, Punjab Agricultural University, Ludhiana, India, were used for the preparation of feeder layer. One day prior to fusion, splenocyte feeder cell layer was laid down in the 24 well tissue culture plate in HAT medium having 106 cells/ml/well. The plates were incubated in a humid incubator at 37°C with 5% CO_2 tension.

7.6. Preparation of polyethylene glycol (PEG)

PEG (molecular weight 3000–4000) was autoclaved and immediately suspended in RPMI-1640 medium aseptically by keeping the vial at 56°C in water bath.

7.7. Fusion protocol

Myeloma cells in log phase of growth were brought into suspension by tapping and the suspension was transferred into calibrated centrifuge tube and final volume made to 10 ml with serum-free media. The immunized BALB/C mouse was etherized and disinfected by dipping into 70% alcohol. The spleen was removed into a petri plate and washed with 2 ml of serum-free media twice. The splenocytes were released into the medium by flushing with the medium into the spleen and the cells were collected by centrifugation. The splenocytes and myeloma cells were separately centrifuged at 4000 rpm for 10 min. The supernatant was discarded and pellet was brought into suspension by tapping. Equal number (107) of spleen cells and myeloma cells were mixed and co-pelleted by centrifugation at 4000 rpm for 10 min. To the pellet, 1 ml of PEG was added slowly along the side of tube over 1 min, taking care to swirl the tube gently while adding PEG so as to ensure proper mixing of PEG solution. The tube was incubated at room temperature for 1 min to facilitate fusion of myeloma and lymphocyte cells. Later, PEG was diluted by adding 10 ml of growth medium over 10 min and centrifuged at 4000 rpm for 3min. After discarding the medium, the final pellet was dissolved in 48 ml of HAT medium, and the cell suspension was dispensed in a quantity of 1 ml in two 24 well plates, which had already been seeded with normal mice spleen feeder cells a day before. The plates were incubated at 37° C in 5% CO2 tension. The plates were left undisturbed for 3–4 days and examined daily for signs of growth. When the hybrids started showing growth, the supernatants were collected aseptically and screened for anti-BHV-1 antibody secretion by indirect plate ELISA [72].

7.8. Cloning

After identification of single clone (B6.1), the cells were dislodged and ten-fold serial dilutions were made starting from 10^{-1} to 10^{-6} and dispensed in 1 ml quantity into each well which had feeder layer one day before seeding.

7.9. Isotyping of antibodies

Isotyping of monoclonal antibodies in cell culture supernatants was performed with the indirect ELISA-based isotyping kit.

7.10. Immunoblotting

The specificity of the monoclonal antibody for a particular protein of BHV-1 was determined by immunoblot. For this, discontinuous system of polyacrylamide gel electrophoresis (PAGE) [73] was followed. Electrophoresis on 10% resolving gel with 4% stacking gel in tris glycine buffer was carried out at 140 volts for 3 to 4 hours. Following electrophoresis, the protein was blotted to nitrocellulose membrane (NCM) in transfer buffer. Transfer of pro-

tein was carried out in transblot apparatus at 100 volts for 1 hour. A total of 600 ml of infected cell culture fluid was used for purification of virus.

The importance of monoclonal antibodies lies in the fact that they are the antibodies that can be used to detect the single epitope (protein) of the virus. Thus, they can be used in different assays like ELISA, immunofluorescence, virus neutralization test, AGID, CFT for detecting the virus along with differentiating between the different types of the virus. This can be achieved by producing the monoclonal antibodies against the protein of the virus which differentiates between the types of the virus.

8. Prevention and control

Prevention and control of BHV-1 are based on intensive farm management which includes following hygienic measures at the farm, vaccinating the animals at the right time and detection and removal of infected animals. The practice should be made at the farm to keep the newly introduced cattle in quarantine for the period of 4 weeks and the cattle that are BHV-1 sero-negative should be introduced to a herd. The practice of natural mating should be avoided and artificial insemination should be done using semen from BHV-1 negative bulls. Eradication programmes should be followed. These include test and removal programs in which the infected animals should be detected and culled. Carrier cattle should also be identified and removed from the herd.

Vaccines usually prevent the development of clinical signs and markedly reduce the shedding of virus after infection, but do not completely prevent infection. Vaccination campaigns should be a part of eradication programmes. Since BHV-1 is a highly contagious virus, vaccination is recommended as soon as passive immunity in calves disappears, usually around 4–6 months of age. Currently available vaccines for IBR include modified-live-virus vaccines and inactivated vaccines. The timing of vaccination is also as important as the choice of vaccine. Since maximum protection does not generally occur until approximately 3 weeks after vaccination, calves should be vaccinated 2–3 weeks before weaning at which time they start to be at risk of infection. Single vaccination will reduce the severity of disease, but not provide complete protection. Thus, booster vaccination should be done. The use of marker vaccines can help to distinguish between vaccinated and naturally infected animals.

9. Conclusions

The chapter concludes that the BHV type 1 is an important virus of cattle and buffaloes. As the virus can survive for long in frozen semen, it should be of special concern during artificial insemination and also during national and international trade.

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