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SEROPREVALENCE OF INFECTIOUS BOVINE RHINOTRACHEITIS IN CROSSBRED CATTLE OF KERALA

By

RAJESH, J. B.

THESIS

Submitted in partial fulfilment of the requirement for the degree of

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Veterinary Epidemiology and Preventive Medicine
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

I hereby declare that this thesis entitled **"SEROPREVALENCE OF INFECTIOUS BOVINE RHINOTRACHEITIS IN CROSSBRED CATTLE OF KERALA"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy,
26-04-2001

RAJESH, J.B.

CERTIFICATE

Certified that the thesis, entitled **"SEROPREVALENCE OF INFECTIOUS BOVINE RHINOTRACHEITIS IN CROSSBRED CATTLE OF KERALA"** is a record of research work done independently by **Sri. Rajesh, J.B.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Dr. P.V. Tresamol
(Chairperson, Advisory Committee)
Assistant Professor
Department of Veterinary Epidemiology & Preventive Medicine
College of Veterinary and Animal Sciences, Mannuthy

Mannuthy,
26-04-2001

CERTIFICATE

We, the undersigned members of the Advisory committee of **Sri. Rajesh, J.B.,** a candidate for the degree of Master of Veterinary Science in Preventive Medicine, agree that the thesis entitled **"SEROPREVALENCE OF INFECTIOUS BOVINE RHINOTRACHEITIS IN CROSSBRED CATTLE OF KERALA"** may be submitted by Sri. Rajesh, J.B., in partial fulfilment of the requirement for the degree.

26/04/01

Dr. P.V. Tresamol
(Chairperson, Advisory Committee)
Assistant Professor
Department of Veterinary Epidemiology & Preventive Medicine
College of Veterinary and Animal Sciences, Mannuthy

26.04.01

Dr. M.R. Saseendranath
(Member, Advisory Committee)
Associate Professor and Head
Department of Veterinary Epidemiology & Preventive Medicine

Dr. V. Vijayakumaran
(Member, Advisory Committee)
Associate Professor & Head
Cattle Breeding Farm
Thumburmuzhy

26/04/01

Dr. Koshy John
(Member, Advisory Committee)
Assistant Professor
Department of Microbiology

24.5.01

External Examiner
J. Ramakrishna
Professor Dept of Preventive medicine
Madras Vety College
Chennai

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Affectionately dedicated to
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Introduction

1. INTRODUCTION

Art and science of Animal Husbandry lies in the efforts of man striving to optimize the interaction between his animals and environment to his advantage. India possess a large livestock population numbering 222.3 millions which includes 201.5 million cattle (Banerjee, 1999). Livestock rearing in India is carried out under a variety of adverse climatic and environmental conditions. Cattle occupies an important position in the national economy which is premodinantly dependent on agriculture. While female progeny supplies milk, male progeny continues to be the principal source of draught power for agriculture and for rural transport.

In all parts of world, disease is an important constraint to increased production of animal food for human consumption. In the absence of effective disease control measures, an expanded population of unproductive animals are created, which can not fulfill its genetic potential in the utilization of feed for growth and reproduction. Control of animal diseases in countries like India, where livestock forms the backbone of agriculture, is an essential prerequisite to any systematic campaign for the improvement of the nation's economy.

Emerging diseases like infectious bovine rhinotracheitis is a serious problem in developing countries like India. The infection has assumed greater

economic importance, as India has emerged as the largest milk producer in the world.

Infectious bovine rhinotracheitis (IBR) is an important emerging viral disease caused by bovine herpes virus 1 (BHV-1). IBR has a world wide distribution. The disease was first noticed in the beef feedlots of Colorado, USA. After that the disease was reported from almost all nations of the world. BHV-1 infection is manifested as various syndromes viz., infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB), abortions, eye infections, encephalitis and enteritis in cattle. IBR can cause considerable economic losses to the cattle farmers. In dairy cows due to IBR infection, milk output may be diminished for three to four days and in fattening cattle there may be weight loss up to 30 kilograms.

One of the peculiarity of IBR infection is its latency when there is immunosuppression, reactivation can occur and that animal will be a source of BHV-1 infection for other animals. Morbidity may reach 100 per cent and mortality may reach up to 10 per cent in IBR infection.

Laboratory diagnosis of IBR can be done by virus isolation or by serologic tests.

Serologic tests like neutralisation tests, passive haemagglutination test and ELISA were employed for detection of BHV-1 antibodies. Sensitivity and

specificity of ELISA in detecting antibodies against BHV-1 is more when compared with other serological tests. Avidin-Biotin ELISA is cheaper, easier to perform, less time consuming and more sensitive and specific. Control of IBR infection can be done by seromonitoring of herds at regular intervals and detection and elimination of reactors.

In India, isolation of BHV-1 was first done by Mehrotra (1977) at Punjab from a case of keratoconjunctivitis. After that different workers isolated the etiological agent of IBR in different states. Seroprevalence studies showed that BHV-1 has gained ground and established in bovine population of the country (Aruna and Suribabu, 1982; Renukaradhya *et al.*, 1996 and *Suresh et al.*, 1999).

In Kerala the seroprevalence of IBR was first reported by Sulochana *et al.* (1982). After that no systematic study has done to detect the seroprevalence of IBR in Kerala.

The present study was undertaken with the objective of assessing the seroprevalence of Infectious Bovine Rhinotracheitis (IBR) in crossbred cattle of Kerala using Avidin-Biotin ELISA.

Review of Literature

2. REVIEW OF LITERATURE

2.1 History

Infectious bovine rhinotracheitis was first noticed in beef feedlots in Colorado in 1950 and was known by the synonyms rednose, dust pneumonia, necrotic rhinotracheitis and necrotic rhinitis (Miller, 1955).

The disease emerged as a major economic problem in dairy herds and beef feedlots in California and Colorado, USA in 1954 (Mc Kercher *et al.*, 1955a).

The disease was designated as infectious bovine rhinotracheitis or IBR by US Livestock Sanitary Association in 1955 (Mc Kercher *et al.*, 1955b).

Kendrick *et al.* (1958) and Mc Kercher (1963) recognised this disease in cattle and later found that it is associated with infectious pustular vulvovaginitis (IPV).

Owen *et al.* (1964) and Wilson (1974) reported that the disease can cause abortion.

Michalaski and Hsuing (1975) confirmed that IBR virus possess oncogenic potential.

Gibbs and Rweyemamu (1977) recorded IBR as one of the most important emerging disease of livestock causing balanoposthitis in bulls;

abortion, repeat breeding and infertility in cows and other infections of eye and nervous system.

Wiseman *et al.* (1979) reported that Bovine Herpes Virus-1 (BHV-1) is the most important virus responsible for great economic loss in livestock industry.

Kahrs (1981a) reported that IBR can cause mild inapparent infection or a wide variety of clinical manifestations having effects on feed efficiency, milk production and reproduction.

Van Der Maaten and Miller (1985) stated that BHV-1 can cause infertility in heifers infected at the time of breeding.

Bovine Herpes Virus-1 infection cause pathophysiologic changes in the uterus and cause early embryonic death in latently infected and acutely infected cows (Miller and Van Der Maaten, 1987).

Donkersgoed and Babiuk (1991) recommended vaccination for the effective control of IBR/IPV.

Gajendragad *et al.* (1997) reported that BHV-1 infection in Karnataka causes huge economic losses to farmers in the form of reproductive inefficiency, impaired production and sometimes mortality.

Danner *et al.* (1999) stated that because of suppression of latent infection by vaccination the virus shedding can be prevented and control of transmission of BHV-1 infection can be achieved.

2.2 Virus

The viral etiology of the disease was confirmed by Madin *et al.* (1956) who first isolated the virus in tissue culture.

The virus can produce Cowdry type A intranuclear inclusions in infected cells (Cheatham and Crandell, 1957).

Griffin *et al.* (1958) observed that virus is stable between pH 6.0-9.0 but labile at pH 4.5-5.0.

The virus was identified as a herpes virus by Armstrong *et al.* (1961).

Stewans and Groman (1963) found that IBR virus is extremely susceptible to lipid solvents such as ethyl ether, acetone, ethyl alcohol and chloroform.

Virus was inactivated within 21 minutes at 56°C, within 10 days at 37°C and within 50 days at 22°C and the virus was stable for upto one month at 4°C and for 9 months at –60°C (Snowdon, 1964).

Russel and Crawford (1964) reported that the genome of Bovine Herpes Virus-1 or BHV-1 contains double stranded DNA.

The capsid is of icosahedral symmetry and comprised of 162 capsomeres and an envelope surrounds the nucleocapsid (Cruickshank and Berry, 1965).

Straub and Mackle (1965) tested the susceptibility of BHV-1 to various disinfectants and found that the virus was rapidly inactivated by 0.5 per cent NaOH, 0.01 per cent HgCl, 1 per cent chlorinated lime, 1 per cent phenolic derivatives, 1 per cent quarternary ammonium bases and 1 per cent Lugol's iodine. Also formalin at 5 per cent inactivates BHV-1 within one minute.

Virus can be cultivated in cell lines of bovine kidney, Madin Darby Bovine Kidney (MDBK), HeLa (Bagust, 1972).

Bovine Herpes Virus-1 harboured for long periods in the ganglia of central nervous system and thus establish latency (Ackermann *et al.*, 1982; Pastoret *et al.*, 1984).

During primary infection with BHV-1, there is an IgG-1 and IgM antibody response; during secondary infection with BHV-1 there is an IgG-1 and IgG-2 antibody response (Guy, 1985).

Miller and Van Der Maaten (1987) observed typical herpes virus type intranuclear inclusions in affected cattle's adrenal gland.

The virus can produce necrotizing endometritis, necrosis of the corpus luteum and embryonic death in pregnant cows (Miller, 1991).

Drunen *et al.* (1993) reported the use of recombinant glycoprotein gIV for the preparation of subunit vaccine to confer protection against BHV-1 infection.

Viral latency is a common feature in BHV-1 and once an animal is infected it must be considered as a virus carrier for years and thus a potential source of infection (Roll and Mayr, 1993).

Danner *et al.* (1999) reported that humoral antibodies will not prevent latency and it will neutralise freely circulating virus but BHV-1 will not be eliminated from body.

2.3 Incidence

2.3.1 Global

Infectious pustular balanopostitis (IPB) infection in bulls were described by Studdert *et al.* (1964) in America.

Allan *et al.* (1975) reported the natural venereal infection of heifers with infectious bovine rhinotracheitis virus in Australia.

Payment *et al.* (1979) detected antibodies to BHV-1 in 146 serum samples of the total 250 samples tested in Canada.

Approximately 56.5 per cent of seroprevalence of IBR was recorded in cattle by Samal *et al.* (1981) in India.

In Tunisia among 2656 serum samples collected from indigenous, imported and crossbred cattle, 45.44 per cent samples were positive for IBR antibodies (Cherif and Rabet, 1984).

Werner (1985) in a study observed 12 per cent seroprevalence of IBR in cattle of Harburg region.

Serological study in Spain from 1986 to 1988 in dairy cattle revealed 54 per cent of seropositiveness (Espuna *et al.*, 1988).

Neilson and Grace (1988) after examination of 42,000 serum samples in NewZealand reported 50 to 70 per cent prevalence in different herds.

Of the 1592 cattle sera tested in Ethiopia 1060 samples (67 per cent) were serologically positive for IBR infection (Bekele *et al.*, 1989).

Madic *et al.* (1989) screened serum samples for the presence of antibodies of bovine respiratory syncytial virus, bovine viral diarrhoea virus and BHV-1 in Yugoslavia and detected 27 per cent prevalence of IBR.

Prevalence of antibodies to BHV-1 in cattle was 37.8 per cent in a study conducted in Canada (Durham and Hassard, 1990).

Behymer *et al.* (1991) detected three per cent seroprevalence of IBR among beef cattle of USA.

In Italy of the 6793 serum samples (from 686 farms) examined at Parma and the 17740 serum samples (from 954 farms) examined at Pavia over 11 year period revealed 51.58 per cent and 48.25 per cent prevalence of BHV-1 antibodies respectively (Cavirani *et al.*, 1991).

Durham *et al.* (1991) in a serological study in calves of six to nine months of age in a bull station in Canada detected a 20 per cent prevalence of IBR antibodies.

Prevalence of antibodies to bovine herpes virus 1 in Tanzanian cattle was found to be 42 per cent (Lyaku *et al.*, 1991).

Yilmaz, (1994) observed BHV-1 antibodies in 44.25 per cent in calf sera samples screened in Turkey.

Serum samples collected from 835 Friesian cattle from three farms in Slovenia in 1994 revealed 86 per cent prevalence of IBR (Hostnik and Grom, 1997).

Thai Dairy farms revealed 23.3 per cent seroprevalence of IBR in a study conducted during 1995 (Virakul *et al.*, 1997).

Wergifosse *et al.* (1997) detected 47.2 per cent IBR seropositivity in Belgium from 6153 animals of 70 farms examined.

In New Zealand serum samples screened from 272 dairy herds during 1995 revealed 66.54 per cent prevalence of antibodies to IBR (Motha and Hansen, 1998).

Rusvai amd Fodor (1998) reported the occurrence of some viruses and bacteria involved in respiratory disease of ruminants in Hungary in addition to 45 per cent of seroprevalence of IBR in three to eight months old calves.

2.3.2 India

In India, BHV-1 was first isolated from a case of keratoconjuctivitis in a calf as early as 1976 by Mehrotra (1977) at Punjab.

Samal *et al.* (1981) reported 50.7 per cent seroprevalence of IBR in animals without history of abortion and 65.2 per cent prevalence in animals with history of abortion in among Indian cattle.

Suribabu and Mallick (1983) isolated BHV-1 from cases of abortion in cattle of Andhra Pradesh.

A sero positiveness of 64.72 per cent was found in a study where 1684 cattle samples were tested in Izatnagar (Suribabu *et al.*, 1984).

Manickam and Mohan (1987) in a sero epidemiological study on viral abortion in cows in Tamil Nadu detected that 23 per cent of abortion were due to IBR.

A seroprevalence of 77.46 per cent was recorded in a serological survey of BHV-1 infection in bovines of Andhra Pradesh (Satyanarayana and Suribabu, 1987).

A seroprevalence of 51.6 per cent to IBR was observed in three southern states of India during a study conducted by Renukaradhya *et al.* (1996).

Shome *et al.* (1997) in a seroprevalence study of IBR in the Andaman and Nicobar group of islands showed 89 per cent seropositive cattle among 203 animals tested.

In a recent serosurvey, Suresh *et al.* (1999) detected 38.01 per cent of seroprevalence of antibodies to BHV-1 in India among 3428 bovines from 18 states and the union territory of Andaman and Nicobar islands.

2.3.3 Kerala

A preliminary serological survey on the occurrence of IBR in Kerala was done by Sulochana *et al.* (1982). Among 367 bovine serum samples tested 47.43 per cent of the clinically normal animals and 56.84 per cent of animals with history of reproductive disorders were found to be seropositive for IBR.

2.4 Epidemiology

2.4.1 Host range

2.4.1.1 Cattle and buffaloes

Several workers have reported that the virus can infect cattle resulting in different clinical manifestations (Miller, 1955; Mc Kercher *et al.*, 1955a; Gibbs and Rweyemamu, 1977; Kahrs, 1981b).

Seroprevalence studies suggested that the disease is widespread all over the world in cattle and buffaloes (Krause, 1988; Lyaku *et al.*, 1991; Suresh *et al.*, 1992a; Kadohira *et al.*, 1996; Virakul *et al.*, 1997; Suresh *et al.*, 1999).

2.4.1.2 Horse

In an experimental study conducted by Marolt and Brudnjak (1965) found that intra-ocular inoculation of BHV-1 in horses can produce recurrent ophthalmitis.

2.4.1.3 Sheep and goat

Mohanty *et al.* (1972) reported the isolation of BHV-1 from naturally occurring respiratory disease and conjunctivitis in goats in USA.

Trueblood *et al.* (1978) isolated BHV-1 from sheep with respiratory disorder.

In an experimental study BHV-1 was isolated from goats showing clinical symptoms like circling, ataxia, recumbency and death (Tolari *et al.*, 1990)

Bovine Herpes Virus-1 isolated in bovine foetal kidney cell mono layers from the lungs of two lambs showing hepatization of the lungs at the abattoir (Cavalli *et al.*, 1994).

In an experimental study clinical symptoms like transient pyrexia, coughing and nasal discharge reported in lambs inoculated with BHV-1 via intra-nasal and intra-tracheal routes (Sandeep Guliani and Sharma, 1995).

Hage *et al.* (1997) stated that BHV-1 infection in sheep is possible, but sheep do not have a major role in BHV-1 transmission.

2. 4.1.4 Pig

Bovine Herpes Virus-1 is relatively avirulant in piglets but may have a predilection for the genital tract of mature pigs and for porcine foetuses (Derbyshire and Caplan, 1976).

The disease can affect swine naturally in both respiratory and genital form (Radostits *et al.*, 1994).

2.4.1.5 Wild animals

Chow and Davis (1964) detected presence of neutralising antibodies to BHV-1 in male deer (*Odocoileus hemionus*).

Antibody to BHV-1 was detected in white tailed deer (*Odocoileus virginianus*) by Friend and Halterman (1967).

Significant BHV-1 antibody levels were found in African buffalo (*Syncerus caffer*), hippopotami and eland by Kamninjolo and Paulsen (1970).

Hoff *et al.* (1973) isolated BHV-1 from Pronghorn antelope (*Antilocapra americana*) in USA.

Karstad *et al.* (1974) isolated BHV-1 from cases of pustular vulvovaginitis in wildbeest (*Connochaetes taurinus*).

2.4.2 Transmission

Washing of the preputial orifice of the bulls with infected sponges or clothes can transmit the IBR infection (Knoblauch, 1962).

Straub and Bohm (1963) reported that close physical contact with infected cows can lead to transmission of infection.

Infectious pustular vulvovaginitis (IPV) may arise from the use of infected semen for artificial insemination (Straub and Mackle, 1965).

Collings *et al.* (1972) observed that infectious pustular vulvovaginitis or infectious balanoposthitis is normally transmitted by coitus and possibly by mechanical transmission of virus by insects.

Stress may cause cattle vaccinated with attenuated vaccines to excrete BHV-1 through secretions (Sheffy and Rodman, 1973).

Donkersgoed and Babiuk (1991) in their study found that respiratory form of the IBR can spread through nasal secretions or aerosol droplets containing the virus.

Latest infections can be reactivated by stress, steroids or other stimuli and virus can be shed in respiratory, ocular or genital secretions including semen (Miller, 1991).

Renukaradhya *et al.* (1996) reported that the transmission of BHV-1 from bulls through semen used for artificial insemination is a distinct possibility.

Franken (1997) stated that bought in cattle which carry the infection is a great risk of infectious bovine rhinotracheitis.

Noordegraaf *et al.* (1998) opined that BHV-1 can be transmitted by direct contact between animals, indirect contact through fomites, air borne transmission and venereal or iatrogenic transmission.

Schaik-G-Van (1998) stressed the importance of transmission of BHV-1 infection by contact between animals, and the risk of acquiring infection through purchases or cattle shows.

Suresh *et al.* (1999) reported that BHV-1 infection in bulls assume great epidemiological importance as artificial insemination is now practiced all over the country.

2.4.3 Managemental practices

Kahrs (1981a) reported that feedlot cattle seem to have higher attack rates, and higher case fatality rates than do range or dairy cattle.

Donkersgoed and Babiuk (1991) were of the opinion that feedlots, crowding and mixing of animals, high levels of stress and exposure to microbes enhances the susceptibility of animals to BHV-1 infection.

Bovine Herpes Virus-1 infection is more in large, concentrated cattle populations such as feedlots or intensively managed dairy operation (Miller, 1991).

Fenner *et al.* (1993) opined that change in managemental practices such as dietary changes may enhance the risk of BHV-1 infection.

Kadohira *et al.* (1996) stated that occurrence of IBR in farm level is mainly exhibited a large amount of clustering.

Mc Dermott *et al.* (1997) in Kenya reported that seroprevalence of IBR significantly decreased on small sized zero grazing farms.

Seroprevalence of IBR was more in large cattle herds in a study conducted in Hungary (Tekes *et al.*, 1999).

2.4.4 Season

Singh *et al.* (1985) in India observed higher seropositivity to BHV-1 in animals aborted in rainy season than in those aborted in summer season showing that virus is less active in summer.

Higher incidence of IBR infection was observed in UK in the fall and winter months when large numbers of susceptible animals were assembled (Radostits *et al.*, 1994).

2.4.5 Breed

Masolla *et al.* (1981) observed that Holstein Friesian had significantly higher BHV-1 seropositivity than Jersey cattle.

Serological survey by Singh *et al.* (1985) showed higher incidence of BHV-1 in exotic cattle than the indigenous cattle.

Manickam and Mohan (1987) found that crossbred cattle were highly susceptible to BHV-1 infection.

Infectious Bovine Rhinotracheitis was more in exotic cattle and crossbred cattle than in indigenous cattle in a serological survey conducted by Satyanarayana and Suribabu (1987).

Murrah and Murrah cross buffaloes are more susceptible to BHV-1 infection than native breeds. (Aruna and Suribabu, 1992; Suresh *et al.*, 1992a)

Engle *et al.* (1999) reported that Angus calves are more susceptible to BHV-1 infection than Simmental calves.

2.4.6 Age

Frey *et al.* (1974) opined that the incidence of IBR in animals younger than six months of age is less because of presence of maternal antibodies which reduce the severity of infection.

Kirby *et al.* (1974) noticed that older animals were showing more seropositiveness to BHV-1 infection than younger animals.

Satyananrayana and Suribabu (1987) observed higher antibody titres to BHV-1 in the animals of above three years of age.

Infectious Bovine Rhinotracheitis BR occurs more in animals older than six months of age (Donkersgoed and Babiuk, 1991).

There is an increase in the number of animals susceptible to BHV-1 infection among animals aged above three years (Suresh *et al.*, 1992a)

Aruna and Suribabu (1992) observed a higher seropositiveness to IBR among buffaloes of three to eight years of age in Andhra Pradesh.

Seroprevalence study in Kenya revealed that occurrence of IBR is more in aged animals (Mc Dermott *et al.*, 1997).

2.5 Economic Significance

Mc Kercher (1968) reported an economic loss to the beef cattle industry from respiratory tract disease of BHV-1 as $25 million each year in USA.

A loss of $500 estimated in an out break of IBR in a dairy farm in UK and mentioned a loss of $25 to $50 per cow in large milk herds (Townley, 1971).

Infectious Bovine Rhinotracheitis infected animals may suffer from secondary bacterial pneumonia, endometritis, epididymitis which adds to the treatment cost which cause severe economic loss (Kaminjolo *et al.*, 1975).

Fenner *et al.* (1993) concluded that BHV-1 infection is responsible for serious economic loss to cattle farmers throughout the world.

2.6 Morbidity and mortality

Morbidity may reach 100 per cent and mortality may reach 2 to 12 per cent in severe genital form of IBR infection in cattle (Mayr, 1988).

The morbidity and case fatality rates in dairy cattle are about eight per cent and three per cent respectively, while in feedlot cattle the morbidity rate is usually 20 to 30 per cent in unvaccinated cattle and may rarely reach 100 per cent (Radostits *et al.*, 1994).

2.7 Pathogenesis

2.7.1 Respiratory form

Several workers reported that in IBR infection, hyperemia of nasal turbinates and muzzle and white necrotic debris in nasal musoca can occur (Studdert *et al.*, 1964 and Kahrs, 1977).

Initial multiplication of the BHV-1 occur in the tissues of upper respiratory tract and extended apparently via lacrimal duct to the occular tissues. (Gibbs and Rweyemamu, 1977).

Congestion, oedema, focal haemorrhages and sloughing of epithelium are observed in nasal passages of animals affected with respiratory form of IBR (Kiorpes *et al.*, 1978).

Samal *et al.* (1981) reported that BHV-1 can cause transient respiratory illness in young calves which can be reactivated in the adult.

Bovine Herpes Virus-1 infection can cause ulcerative patches on nasal mucosa, dryness and crackedness of muzzle which is easily peeled. (Mohankumar *et al.*, 1994).

Bitgel (1997) detected BHV-1 antigens by immunoflourescent tests in cattle with respiratory disorders in Turkey.

Youssef (1997) detected antibodies to BHV-1 from calves of age between two weeks and six months in which there were some reported cases of pneumonia.

Narita *et al.* (2000) could induce pneumonia by endotracheal inoculation of calves with BHV-1.

2.7.2 Occular form

Inflammation of conjunctiva and occasional opacity can occur in BHV-1 infection (Timoney, 1971).

In occular form of IBR infection, pustules or plaques of white necrotic debris on conjunctiva can be seen (Rebhun *et al.*, 1978).

Foldi *et al.* (1992) stated that BHV-1 infection can cause development of corneal opacity which developed centrally and later became diffuse and in most cases only one eye is affected.

2.7.3 Genital Form of IBR

In BHV-1 infection the genital mucosa shows barely visible elevated nodules to rather extensive vesicular, erosive or occasionally ulcerative lesions (Kendrick, 1958).

Placentitis has been reported in cases of abortion due to IBR (Kennedy and Richards, 1964).

Discrete cream coloured pustular lesions developed in the genital mucosa of BHV-1 infected animals (Studdert *et al.*, 1964).

Gibbs and Rweyemamu (1977) opined that placenta infected during the viremic phase of the maternal infection, and the disease of foetus is of short duration and terminates in death of the foetus which stimulates abortion in BHV-1 infection

Miller and Van Der Maaten (1985) reported that BHV-1 infection during oestrus may cause severe oopheritis characterised by diffuse corpus luteum necrosis and necrotic follicles.

In pregnant heifers detachment of foetal membrances from the endometrium and expulsion through the cervix into the vagina reported in IBR infection (Miller and Van Der Maaten, 1987).

Salwa and Stryszak (1995) reported 50.1 per cent of positive cases of IBR in a study of infectious pustular vaginitis, in cows.

Vaginal discharge, conjuctivitis and pustular vulvovaginitis were the clinical manifestations in an outbreak of infectious pustular vulvovaginitis in a UK dairy herd (Cook, 1998).

2.7.4 CNS infection

Barenfus *et al.* (1964) reported that occasionally in young cattle, infection with BHV-1 results in non-purulent meningitis and encephalitis.

Bovine Herpes Virus-1 entrance to mid brain was followed by generalisation of infection throughout the brain and results in encephalitis (Hall *et al.*, 1966; Narita *et al.*, 1976).

Bagust and Clark (1972) observed that within four to five days of infection, from nasal, pharyngeal and tonsilar regions BHV-1 spread to brain through maxillary, mandibular branches of trigeminal nerve.

Experimental inoculation of BHV-1 in gnotobiotic calves resulted in meningoencephalitis (Edington *et al.*, 1972).

2.8 Clinical Signs

The outcome of natural infection is probably determined multifactorially by the strain of virus, dose and route of exposure or inoculation, immunologic status of the exposed animal and environmental influences (Schultz *et al.*, 1976)

Change in epidemiologic pattern can occur due to changes in husbandry or multiple infections (Rogers *et al.*, 1978)

2.8.1 Respiratory tract disease

Studdert *et al.* (1964) reported that in IBR visible portions of the nasal mucosa frequently reveals adherent white necrotic debris that results from the coalescence of pustules.

Dyspnoea in IBR infection is associated with mucopurulent material in the nasal passages and trachea, dilated nostrils and partial blockage of airways (Kahrs and Smith,1965).

Rosner, (1968) reported that the case fatality rate from respiratory form of IBR is slow unless it is complicated with secondary bacterial infections or super-imposed viral infections.

Abortions may occur when the respiratory form of IBR develops in a herd that includes pregnant cattle (Wilson, 1974).

Gibbs and Rweyemamu (1977) observed that IBR is characterised by pyrexia (40.5-42.0 °C), increased respiratory rate, persistent harsh cough, slight anorexia, depression and in milking cows a severe drop in milk production.

Profuse nasal discharge in early stages of IBR infection become mucopurulent, later hyperemia and reddening of the nasal turbinates and muzzle were the basis for the non defunct synonym "red nose" (Kahrs, 1981b).

Wizigmann (1987) reported that progression of the respiratory form of IBR may result in purulent bronchopneumonia.

2.8.2 Conjuctivitis and occular lesions

Conjunctivitis, either unilateral or bilateral associated with profuse lacrimation is a common clinical sign in cattle with typical IBR (Mc Kercher *et al.*, 1959).

Conjuctivitis may appear as a principal clinical sign of IBR infection (Quin 1961,Timoney and O' Connor, 1971).

Conjunctivitis may develop in some animals with respiratory form of the disease (Ferris *et al.*, 1964, Mc Kercher and Wada, 1964).

Taylor and Hanks (1969) isolated BHV-1 from 15 cases of eye tumours from cattle slaughtered at an abattoir in Nevada, USA.

Bovine Herpes Virus-1 isolated from cases of squamous cell carcinoma of eye (Anderson, 1970).

Bowen *et al.* (1970) isolated BHV-1 from cases of infectious keratoconjunctivitis.

Conjunctivitis, ocular discharges and occasionally corneal opacity appear as the principal manifestation of IBR infection (Timoney and O'Connor, 1971; Rebhun *et al.*, 1978).

Foldi *et al.* (1992) isolated BHV-1 from cases of conjunctivitis where profuse lacrimation, photophobia, severe conjunctivitis, corneal opacity, coughing and serous nasal discharge were the other clinical symptoms.

Al-Bana *et al.* (1998) isolated BHV-1 from calves affected with kerato-conjunctivitis.

2.8.3 Alimentary tract disease

Bovine Herpes Virus-1 was recovered from abomasal fluid of a foetus aborted by an experimentally inoculated dam (Owen *et al.*, 1959).

Baker *et al.* (1960) demonstrated lesions of IBR and the virus in the forestomach of a four day old calf inoculated with BHV-1.

Characteristic lesions of IBR were noted in the ruminal mucosa of a 10 day old calf by Van Kruiningen and Bartholomew (1964)

Diarrhoea may be a clinical sign of generalized and fatal form of IBR in calves (Curtis *et al.*, 1966).

Bovine Herpes Virus-1 was isolated from the Peyer's patch of a calf (Gratzek *et al.*, 1966) and from the intestine of cattle affected with Bovine Viral Diarrhoea (Tyler, 1968).

Bovine Herpes Virus-1 was isolated from adult cattle faeces without enteritis (Crandell, 1974) and with enteritis (Wellemans *et al.*, 1974)

Kahrs (1981b) reported diarrhoea with intestinal epithelial ulcerations as accompanying clinical signs of IBR infection.

Bovine Herpes Virus-1 isolated from 19 cases of pneumo enteritis in a study of enteritis in calves of 7-10 month old. (Shehab *et al.* 1996).

2.8.4 Central nervous system

Barenfus *et al.* (1964) reported that IBR can cause non-purulent lepto meningitis and encephalitis in young cattle.

Bovine Herpes Virus-1 can cause encephalitis (Bartha *et al.*, 1969 and Gouch and James, 1975) recumbency and death in calves (Mc Kercher *et al.*, 1970).

Encephalomyelitis characterised by incordination, occasional circling or licking at the flank region were noticed in IBR infection in calves (Beck, 1975).

Gibbs and Rweyemamu (1977) reported that IBR affected cattle may initially exhibit slight incoordination, progressing to ataxia, periods of depression followed by periods of excitement, stumbling, falling, clonic spasm of leg, neck and lumber muscles, opisthotonus, blindness, coma and death.

Experimental infection of neonatal calves with neurovirulent BHV-1 caused severe encephalitis and associated neurological signs. (Belknap *et al.*, 1994).

Horiuchi *et al.* (1995) isolated BHV-1 from calves died with fatal encephalitis. In a study of meningoencephalitis in calves less than 18 months old revealed that 17 per cent of animals were positive to BHV-1 (Marin and Campero, 1999).

2.8.5 Mastitis

Bovine Herpes Virus-1 may produce mastitis if inoculated into udder (Greig and Bannister, 1965; Corner *et al.*, 1967).

Gourlay *et al.* (1974) and Roberts *et al.* (1974) isolated BHV-1 from clinical cases of mastitis.

Bovine Herpes Virus-1 infection cause significant reduction of milk production (Hage *et al.*, 1998).

2.8.6 Disease of the skin

Bwangamoi and Kaminjolo (1971) reported dermatitis of the perineal skin of bulls infected with BHV-1.

2.8.7.1 Disease of reproductive system

Mc Kercher (1963) and Saxegaard (1970) reported BHV-1 infection of the vaginal and vulval mucosa, manifested by pustules and mucopurulent discharge and the disease has been known as blarchenansschlag in Europe for many years.

Purulent or necrotizing endometritis was found in cows infected with BHV-1 (Bouters *et al.*, 1964).

Infertility and shortened oestrous cycle reported in cows inseminated with semen containing BHV-1 (Kendrick and Mc Entee, 1967; White and Snowdon, 1973; Loretu *et al.* 1974)

Parsonson and Snowodon (1975) observed that BHV-1 can cause endometritis and temporary failure of conception

Lomba *et al.* (1976) isolated BHV-1 from cases of metritis in which cows had fever, metritis, mucopurulent uterine discharge and a crepitous feel to the uterus on palpation per rectum.

Infectious Bovine Rhinotracheitis infection can cause pustules or plaques of white necrotic material on the mucosa of the vulva and vagina and odorless material on the floor of vagina (Kahrs, 1977).

Cystic corpus luteum was found in cows infected with BHV-1 (Miller and Van Der Maaten, 1984)

Van Der Maaten and Miller (1985) opined that BHV-1 infection at oestrus may cause severe oopheritis characterized by diffuse corpus luteum necrosis and necrotic follicles.

The straining caused by the pain associated with extensive lesions of IBR in the reproductive tract can lead to uterine prolapse (Miller, 1991).

Exposure of bovine oocytes enclosed by follicular epithelial cells to BHV-1 caused a severe cytopathic effect on the cells (Tsuboi and Imada, 1997).

Mohini Saini *et al.* (1999) stated that BHV-1 was found to cause apoptosis in bovine peripheral blood mononuclear cells.

2.8.7.2 Abortion

Pregnant heifers affected with BHV-1 frequently aborted (Jensen *et al.*, 1955).

Ormsbee (1963) isolated BHV-1 from aborted cows.

Placentitis has been reported in aborting cows due to IBR infection (Kennedy and Richards, 1964).

Foetuses exposed to IBR virus at any stage of gestation can be aborted (Owen *et al.*, 1964; Kahrs *et al.*, 1973; Stubbings and Camerson, 1981).

Interval between exposure and abortion can range from eight days (Saunders *et al.*, 1972 and Wilson, 1974) to several months (Dellers, 1975).

In some cases of IBR abortion, manual removal of aborted foetus is necessary and retention of placenta may occur and usually blanched and degenerated cotyledons are found (Gibbs and Rweyemamu, 1977).

In IBR infection foetuses aborted can be dead in utero for several days before expulsion and there will be autolysis, brownish stained friable tissues, a lack of gross lesions and fluid in both body cavities (Kahrs, 1981b).

2.8.7.3 Infection in bulls

Bovine Herpes Virus-1 can cause infectious pustular balanoposthitis (IPB) and orchitis in bulls (Bouters *et al.*, 1960).

Studdert *et al.* (1964) described pustules on the mucosa of the penis and prepuce, formation of extensive adhesions, annular constrictions, penile distortion, loss of libido and painful erection and ejaculation as the characteristic findings in IBR affected bulls.

Insemination of susceptible cattle with semen containing IBR virus can lead to endodmetritis (Kendrick and Mc Entee 1967) shortened oestrus periods and marked reduction in conception rates (Parsonson and Snowdon, 1975).

Kaminjolo *et al.*, (1975) isolated BHV-1 from cases of epididymitis in bulls.

Weiblen *et al.* (1992) isolated BHV-1 from preputial swabs and semen of 11 bulls with balanoposthitis in an artificial insemination centre.

Rocha *et al.* (1998) detected BHV-1 in semen of naturally infected bulls by PCR.

2.8.8 Systemic form of IBR

Young calves infected with BHV-1 shows acute febrile reaction and usually end fatally (Baker *et al.*, 1960; Reed *et al.*, 1973).

Systemic form of IBR appears in calves infected in-utero during late gestation or shortly after birth (Dellers, 1975).

Kahrs (1981a) reported respiratory distress, white necrotic lesions on mucosa of mouth, tongue, oesophagus and all four stomach, compartments and diffuse peritonitis in calves affected with IBR.

2. 8.9 Combined disease

Concurrent IBR and IPV/IPB outbreaks were recorded infrequently (Hyne and Johnson, 1964 and Collings *et al.*, 1972).

Kahrs and Smith (1965) reported a concurrent outbreak of IBR, IPV and abortion.

Pritchard *et al.* (1997) reported an outbreak of IBR with clinical symptoms like pyrexia, milk drop, bilateral conjunctivitis and pustular vulvovaginitis.

2.9 Diagnosis

Laboratory conformation of suspected IBR can be obtained by serologic tests, virus isolation or flourescent antibody (FA) staining of tissues (Buening and Gratzek, 1967 and Carbray *et al.*, 1971).

2.9.1 Viral antigen detection

Rapid diagnosis of abortion and neonatal calf death due to BHV-1 can be done by detection of viral antigen in tissues such as liver, brain and foetal spleen using immunoflourescence (Reed *et al.*, 1971, Shimizu *et al.*, 1972, Kirkbride *et al.*, 1973; Reed *et al.*, 1973).

The use of electron microscope to identify virus particles present in clinical materials has been described as a rapid method for the diagnosis for IPV and IPB (Collings *et al.*, 1972).

Cutaneous reactions to viral antigen occur with various herpes virus infections including BHV-1 (Darcel and Dorward, 1972).

Guesdon *et al.* (1979) developed enzyme immuno assays of high sensitivity by exploiting the biotin-avidin interaction for BHV-1 detection.

Most widely applied antigen detection has been immunoflourescence, either on smears of cells from the nasal or ocular epithelium (Nettleton *et al.*, 1983; Silim and Elazhary, 1983) or on crystal sections of tissues collected at post-mortem (Terpstra, 1979).

Immunoenzymatic techniques have been described for the labelling of infected cells (Edwards *et al.*, 1983) and for detection of soluble antigen in diluted nasal mucus (Faye *et al.*,1979; Nettleton *et al.*, 1982; Collins *et al.*, 1985).

Many workers used antigen capture ELISA for the detection of BHV-1 from feedlot cattle (Collins *et al.*, 1985; Collins *et al.*, 1988)

Polymerase chain reaction has been used to detect small quantities of viruses in nasal secretions and in tissues (Gama, 1989).

Immunoperoxidase staining of fixed lung tissue was demonstrated to be a rapid and specific method of diagnosing IBR (Wyler, 1990).

Vilcek (1993) detected bovine herpes virus, (BHV-1) genome by polymerase chain reaction.

Xia *et al.* (1995) compared dot blot hybridization and polymerase chain reaction for detecting BHV-1 in artificially infected bovine semen and PCR is found to have more sensitivity.

Bulut *et al.*(1998) did demonstration and localization of bovine herpes virus antigens in cell culture by immuno peroxidase staining.

2.9. 2 Serological tests

2.9. 2.1 Passive haemagglutination test

Samal *et al.* (1981) detected antibodies to BHV-1 using indirect haemagglutination test and found it as a sensitive and inexpensive diagnostic test.

Manickam and Mohan (1987) used micro-indirect haemagglutination test for detecting antibodies to BHV-1 and found it as a useful test.

In a serological survey of BHV-1 infection in bovines in Andhra Pradesh, Satyanarayana and Suribabu (1987) used indirect haemagglutination test.

A modified passive haemagglutination test for the detection of BHV-1 antibodies in bovines was done by Suresh *et al.* (1992b) and judged it as a rapid method.

2. 9. 2. 2 Serum neutralisation test

Allan *et al.* (1975) studied the effects of BHV-1 on reproduction in heifers using serum neutralisation test and found it as a sensitive test.

Zhou-Chang-Feng *et al.* (1988) evaluated the efficacy of serum neutralisation test and concluded it as a sensitive test for detecting BHV-1 antibodies.

In an evaluation of immunogenicity of IBR vaccine in Indian tropical climate, Kilari *et al.* (2000) made use of serum neutralisation test.

2.9.2.3 Virus neutralisation test

Detection of specific antibodies to IBR virus in bovine serum using virus neutralisation test was done by Schilow *et al.* (1985).

Juhasz *et al.* (1991) reported the use of virus neutralisation for monitoring ISCOM type and control vaccines against BHV-1 infection.

Zakutskii *et al.* (1997) used virus neutralisation test for detecting the effectiveness of cultured inactivated vaccine against infectious bovine rhinotracheitis.

2.9.2.4 Immunoprecipitation test

Aruna and Suribabu (1992) used immuno diffusion (ID), immuno electrophoresis (IEP) and counter immuno electrophoresis (CIEP) and found CIEP as a quicker, easy, sensitive and specific test to detect antibodies to BHV-1.

2. 9.2.5 ELISA

Payment *et al.* (1979) developed ELISA for detection of IBR antibodies and found that it is highly sensitive

A micro-enzyme linked immunosorbent assay for the detection of antibodies to IBR was developed by Herring *et al.* (1980).

Beccaria *et al.* (1982) used macro and micro ELISA for the rapid detection of antibodies to IBR and found ELISA as a useful diagnostic test.

Durand *et al.* (1984) monitored the antibodies against BHV-1 with ELISA and suggested ELISA as the preferred serological technique.

Schilow *et al.* (1985) demonstrated the value of ELISA in detecting specific antibodies to IBR/IPV virus in bovine serum.

The ELISA has been widely used for screening of BHV-1 antibodies and the test found to be sensitive, specific, inexpensive and the reagents are stable (Florent and Derjmameffe, 1986; Riterer and Schuller, 1988; Waran and Abraham, 1991).

Behymer *et al.*, (1991) used ELISA for mass screening of cattle sera against 14 infectious agents including IBR and ELISA was advocated for mass screening of livestock sera for the application in epidemiological methods for disease control in food animals.

Kita *et al.* (1991) screened serum samples from 604 cows for IBR antibodies using ELISA and declared that this method of screening is useful to assess the current epidemiological situation in the country.

Gajendragad *et al.* (1997) developed an indirect sandwich ELISA for detection of IBR antibodies and found to be highly sensitive

2.9.2.5.1 Avidin-Biotin ELISA (AB ELISA)

Renukaradya *et al.* (1996) used avidin-biolin enzyme linked immunosorbent assay (AB-ELISA) for studying the antibody prevalence of IBR virus in Southern India and the procedure found to be highly sensitive and specific.

Shome *et al.* (1997) detected antibodies to IBR in Andaman and Nicobar Islands using AB-ELISA.

Suresh *et al.* (1999) detected IBR antibodies in cattle using avidin-biotin ELISA and noted that AB-ELISA offered an additional benefit of integrating high and specific binding between avidin and biotin into the conventional ELISA technique. They also found that it is economical, as it allows the use of immunoconjugates at a remarkably high dilution and is also very sensitive and specific and less time consuming.

2.10 Comparison of ELISA with other serological tests for the detection of BHV-I antibodies

Solsona *et al.* (1980) detected antibodies to BHV-1 by ELISA technique and passive haemagglutination test and found that the ELISA test as rapid, reliable and more sensitive than the passive haemagglutination test.

Fedida *et al.* (1982) compared the efficiency of ELISA with other serological tests for the detection of BHV-1 antibodies and found that passive haemagglutination and neutralisation without complement were less sensitive.

Cho and Bohac (1985) compared ELISA and serum neutralisation test (SNT) and ELISA was considered to be a technically superior routine diagnostic test.

Durham and Sillars (1986) evaluated an indirect ELISA procedure against SNT and found the ELISA procedure was quicker, cheaper and detected more reactors than SNT.

Riegel *et al.* (1987) screened the cattle sera for BHV-1 antibodies by virus neutralisation test (VNT) and ELISA and ELISA was found to be rapid, highly reproducible, more sensitive and inexpensive than VNT.

The efficiency of VN and ELISA tests in the serodiagnosis of IBR/IPV in cattle were tested by Salwa and Donderska (1991) and concluded that ELISA is more sensitive and adequate for detection of IBR antibodies.

Comparative studies on the detection of BHV-1 antibodies in blood serum by ELISA and VN was done by Tekes *et al.*, (1991) and concluded that ELISA is more sensitive and adequate.

Rattan *et al.* (1992) studied the efficacy of serum neutralisation (SN) and ELISA for detecting antibodies to BHV-1 in cattle sera and concluded that

ELISA was more sensitive as it detected more positive among the sample found negative by serum neutralisation.

Comparative study of serological methods like neutralization tests, indirect immunoflourescence test, passive haemagglutination and ELISA for detecting antibodies to BHV-1 shown that highest sensitivity is with ELISA (Perrin *et al.*, 1993).

Graham *et al.* (1998) compared virus neutralization, haemagglutination inhibition and ELISA for detecting antibodies to BHV-1 and concluded that ELISA is having high sensitivity and that may be used to detect seroconversion to BHV-1.

Materials and Methods

3. MATERIALS AND METHODS

The study was carried out in the department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy during 1999-2000.

3.1 Materials

3.1.1 Glasswares/plastic wares and reagents

All glasswares/plastic wares used were of Tarson, Laxbro or Corning brand. Chemicals were of analytical or guaranteed reagent grade. The materials were processed using standard methods (Hoskins, 1967) and sterilised either in hot air oven or autoclave depending upon the material to be sterilized.

3.1.2 Sera Samples

A total of 719 serum samples were randomly collected from cattle of all districts of Kerala. Samples were collected from apparently healthy animals and animals with symptoms suggestive of IBR from different farms, Veterinary hospitals, slaughter houses and from the field (Annexure-I).

3.1.3 Avidin-Biotin ELISA

Avidin-Biotin ELISA was performed using the kit supplied by ADMAS, Bangalore.

3.1.3.1 Reagents required

3.1.3.1.1 Antigen

Madin Darby Bovine Kidney cell culture grown BHV-1 purified by ultracentrifugation, sonicated and freeze dried, stored at –20°C.

Reconstituted the freeze dried contents of the vial with one ml of the reconstitution diluent (double glass distilled water). Stored the antigen stock at -20°C (in the freezer).

3.1.3.1 2 Control sera

a. Strong anti BHV-1 antibody positive (C++)
b. Weak anti BHV-1 antibody positive (C+)
c. Anti BHV-1 antibody negative (C-)

All are bovine sera, freeze dried and stored at –20°C. Reconstituted the freeze dried contents of a vial each of control serum (C++, C+ and C-) separately with one ml of reconstitution diluent and stored at –20°C.

3.1.3.1.3 Immunoconjugates

(i) Biotinylated anibovine IgG (B-antibovine IgG)

Freeze dried contents of the vial is reconstituted with one ml of the reconstitution diluent and mixed gently until completely dissolved. Stored the biotinylated antibovine IgG stock at +4°C.

(ii) Avidin-Horse radish peroxidase (A-HRP)

Freeze dried contents of the vial mixed with one ml of the reconstitution diluent and stored at +4°C.

3.1.3.1.4 Buffers

3.1.3.1.4.1 Antigen coating buffer

Phosphate buffered saline (PBS) of 0.01 M, pH 7.4 ± 0.2

Sodium chloride	7.02 g
Disodium hydrogen phosphate	1.1 g
Potassium chloride	0.2 g
Sodium dihydrogen phosphate $2H_2O_2$	0.35 g
Distilled water	1000 ml

Weighed and dissolved the contents in distilled water.

3.1.3.1.4.2 Blocking buffer

(i) Tween 20 (liquid) (ii) Bovine gelatin (crystals)

Prepared 0.1 M PBS. Took 100 ml of 0.1 M PBS. Added one g of bovine gelatin (Rallis India Ltd., Bangalore) and 0.1 ml of Tween-20.

3.1.3.1.4.3 Wash buffer

Phosphate buffered saline (PBS) of 0.002 M of pH 7.4 ± 0.2. First prepared 0.01 M PBS and took 200 ml from the prepared PBS and added to 800 ml of distilled water.

3.1.3.1.5 Substrate

Three per cent hydrogen peroxide liquid (H_2O_2). Stored at +4°C.

3.1.3.1.6 Reconstitution diluent

Double glass distilled water. Stored at +4°C.

3.1.3.1.7 Chromogen

O-phenylene diamine dihydrochloride (OPD) tablets stored at +4°C. Dissolved one 30 g tablet of O-phenylene diamine dihydrochloride (OPD) in 75 ml of distilled water and stored at 4°C in a glass bottle wrapped with aluminium foil.

3.1.3.1.8 Stopping solution

Sulphuric acid 1 M

Slowly added 5.5 ml of concentrated sulphuric acid to 94.5 ml of distilled water and stored at room temperature.

3.2 Methods

3.2.1 Collection of test sera

Collected five ml of blood aseptically through the jugular vein puncture using a 16 G or 18 G sterile hypodermic needle in test tubes having a capacity of 15 ml. After labelling kept the tubes at an angle for clotting. After one hour breaking of the clot was done with help of a long needle. Kept the tubes at

37°C for 30 minutes. Transferred the tubes to refrigerator having a temperature of +4°C. After 12-18 hours separated the serum and centrifuged at 1000 G for 10 minutes. Inactivated at 56°C for 30 minutes to destroy the non specific factors. After that kept the serum in cryovials of two ml capacity and labelled. Stored the samples at –20°C (in deep freezer).

3.2.2 Assay Procedure (Annexure II)

As per the method described in the laboratory manual provided by ADMAS, Bangalore (Rajasekhar, 1998).

3.2.2.1 Coating of microplates

Gently mixed the vial of reconstituted antigen stock for uniform dispersion.

Preparation of working dilution of antigen

For coating one plate added 60 µl of antigen stock to six ml of coating buffer. Mixed well and dispensed 50 µl of the working dilution of antigen into all 96 wells of the microplate. Tapped the sides of the plates to ensure even distribution over the bottom of each well.

Covered the microplates using sealing tape and incubated at 37°C for one hour. Returned the remainder of the antigen stock to –20°C.

3.2.2.2 Addition of test sera and control sera

Agitated the vials of test sera and control sera (C++, C+ and C-) gently to ensure homogenicity.

Preparation of sera for test

Control sera and test sera are diluted 1/100 in blocking buffer (Five µl of each added to 500 µl of blocking buffer) separately in perspex plate.

Returned the remainder of the control sera to –20°C for further use.

Washing the plate and addition of sera

Removed the antigen coated microplates from the incubator. Discarded the contents by inverting the microplates and taped the inverted microplates on to a lint free absorbent towel to remove all residual contents. With the help of wash bottle filled all 96 weeks of the microplates with wash buffer, discarded, and dried by tapping the microplates onto a lint free absorbent towel. Repeated the same procedure two more times.

Dispensed 50 µl of the diluted test sera and control sera to respective wells and added 50 µl of blocking buffer to conjugate control wells (Annexure-III).

Covered the microplates and placed on an orbital plate shaker housed in an incubator at 37°C and incubated for one hour with continuous shaking to cause thorough mixing without spillage of contents or movement of the plate.

3.2.2.3(a) Addition of Biotin-antibovine IgG

Working solution of Biotin antibovine IgG for one microplate is prepared by adding 60 µl of stock Biotin-antibovine IgG to six ml of blocking buffer.

Returned the conjugate stock to +4°C.

Washing the plate and addition of conjugate

After one hour of serum incubation, removed the microplates from the incubator and washed them with wash buffer three times and dried the microplates by tapping onto a lint free absorbent towel.

Added 50 µl of the working dilution of Biotin-antibovine IgG conjugate to all 96 wells of the microplates and taped the sides of the microplates to ensure uniform dispersion over the bottom of each well.

Covered the microplates and incubated for one hour at +37°C on an orbital shaker.

b. Addition of Avidin-Horse radish peroxidase (A-HRP) conjugate

Preparation of working solution

Added 60 µl of stock A-HRP to six ml of blocking buffer to use in one microplate.

Returned the conjugate stock in 4°C.

Washing the plate and addition of conjugate

After one hour of Biotin-antibovine IgG conjugate incubation, removed the microplates from the incubator and washed them with wash buffer three times and dried the microplates by tapping onto a lint free absorbent towel.

Added 50 µl of the working dilution of A-HRP to all 96 wells of the microplates.

Covered the microplates and incubated for 20 minutes at 37°C on an orbital shaker.

3.2.2.4 Addition of substrate/chromogen solution

Preparation of working solution of the substrate chromogen

Added 24 µl of three per cent H_2O_2 (substrate stock) to six ml of chromogen solution for the use in one microplate.

Returned the substrate and chromogen stock to +4°C.

Washing the plate and addition of substrate/chromogen

After 20 minutes of A-HRP inclubation, removed the microplates from the incubator and washed them with wash buffer three times and dried the microplates by tapping onto a lint free absorbent towel.

Added 50 µl of working solution of the substrate/chromogen to all 96 wells of the microplates.

Kept the plates at room temperature for 10 minutes.

3.2.2.5 Addition of stopping solution

Added 50 µl of the stopping solution (1M H_2SO_4) to all 96 wells of the microplate and gently tapped the microplates to ensure thorough mixing.

(All wells should contain 50 µl of substrate/chromogen solution and 50 µl of stopping solution).

3.2.2.6 Preparation of blanking plate

A clean microplate not coated with antigen used as blanking plate. Added 50 µl of stopping solution (1M H_2SO_4) to the wells of the first column of blanking plate.

3.2.2.7 Measurement of colour development

The microplate ELISA reader is turned on and allowed to warm up for 15 minutes before reading to ensure uniformity of reading for all plates. Wiped the bottom of plate with a clean cloth to remove condensation and smudges.

Placed the blanking plate first followed by test microplate in the carriage of the microplate reader and initiated sequence.

Interpretation (Annexure-IV)

Readings are used in two types of data analysis.

1. Per cent positivity (PP) values used for Quality Assurance (QA)

$$PP = \frac{\text{Replicate OD value of each control}}{\text{Median OD value of C++}} \times 100$$

2. Per cent positivity (PP) values used for acceptance of test sera data and for diagnostic interpretation

$$PP = \frac{\text{Replicate OD value of test serum}}{\text{Median OD value of C++}} \times 100$$

Acceptance of control data

The data expressed in OD values and PP values for the C++ control and the data expressed in PP values for the three other controls (C+, C- and Cc) are used to determine whether or not the test has performed within acceptable limits of variability.

Acceptance criteria for control data

		Upper control limit (UCL)	Lower control limit (LCL)
C++	OD values	1.2	0.6
C++	PP values	120	70
C+	PP values	55	35
C-	PP values	16	0
Cc	PP values	8	0

Microplate acceptance (First level)

Strong positive (C++ control serum)

Compared the two intermediate OD values of the C++ control to the lower and upper control limits. Both values fell within limits and plate accepted.

Micropolate acceptance (second level)

Strong positive (C++), weak positive (C+), Negative (C-) and conjugate control (Cc).

Compared the replicate PP values for the C++, C+, C-, and the CC controls to the UCLS and LCLS.

Microplate accepted (or in within UCL and LCL range) because all controls met the PP acceptance criteria.

Diagnostic threshold PP values

Methods for the determination of diagnostic threshold PP values are (a) double the mean PP of the disease free group, (b) mean PP plus 3 standard deviations, (c) median PP value of the 100^{th} percentile. The diagnostic threshold PP value determined as 28 (standard PP value for the Kit provided).

Acceptance of test sera data

Test sera having mean PP values equal to or greater than the calculated threshold PP value are considered as positive. Test sera having mean PP values less than calculated threshold PP value are considered as negative.

Statistical analysis

The results of prevalence of BHV-1 antibodies in cattle were subjected to statistical analysis as per the procedures of Snedecor and Cochran (1994).

Results

4. RESULTS

A total number of 719 sera samples were collected from cattle of different districts of Kerala comprising of different age groups, parity, breeds, managemental practices and health status and were subjected to Avidin-Biotin ELISA for detection of antibodies to BHV-1. Of these 107 (15%) samples showed a positive result.

4.1 Seroprevalence of IBR in different managemental systems

Seroprevalence of IBR under various managemental practices are given in Table 1 and Figure 1.

Four hundred and ninety five samples were collected from organised farms. Out of which 65 (13.13%) samples were found positive.

Two hundred and twenty four samples were from cattle reared under rural farming system among which 42 (18.75%) samples were found positive.

Eventhough the seroprevalence is more under rural farming system than organised farms, no statistical difference was observed between the above two managemental systems.

4.2 Seroprevalence of IBR in animals of different health status

Out of total 719 serum samples 427 were of apparently healthy animals and 292 were from animals with history of disease. Among apparently healthy

groups 41 (9.6%) animals showed positive result and 66 (22.6%) animals of diseased group showed positive reaction (Table 2 and Figure 2). Result showed highly significant difference between ($P \leq 0.01$) the above two groups.

4.3 Seroprevalence of IBR among different breeds of cattle

The seroprevalence of IBR among various breeds of cattle are given in Table 3 and Figure 3.

The serum samples were collected from crossbred cattle of Holstein Friesian (CBHF), Brown Swiss (CBBS), Jersey (CBJ) and from crossbreds between the above animals (others). Out of 282 serum samples from CBHF, 51 (18.09%) samples were positive. Among 137 animals of CBBS, 17 (12.41%) animals were found positive. Out of 286 animals of CBJ, 36 (12.59%) animals were positive. Fourteen samples were from other crossbreds among which three (21.43%) samples were found positive.

On statistical analysis no significant difference of seroprevalence was observed between any of the above breeds of cattle.

4.4 Seroprevalence of IBR among cattle of different age groups

The seroprevalence of IBR among various age groups are given in Table 4 and Figure 4.

Sixteen samples were collected from animals below one year of age and one (6.25%) animal was found positive. Among forty three animals between

one year and three years of age three (6.97%) animals were positive. One hundred and ninety nine samples were from animals of 3-5 years of age and 24 (12.06%) animals were positive. Four hundred and twenty samples were from animals of 5-10 year age and 72 (17.14%) animals were positive. Forty one samples were collected from animals above 10 years of age and seven (17.07%) animals were positive.

Statistical analysis revealed significant difference ($P \leq 0.05$) between animals of 1-3 years of age and animals of 5-10 years of age.

4.5 Sex-wise seroprevalence of IBR

Ten males were tested for BHV-1I antibodies and none were found positive. Out of 709 female animals 107 (15.09%) animals were positive (Table 5).

4.6 Seroprevalence of IBR in different farms

Seroprevalence of IBR in various farms are presented in Table 6 and Figure 5.

Among the different organised farms, the highest seropositivity was found in Osho Farm, Pattikadu (38.33%) followed by University Livestock farm, Mannuthy (25%), St. Benedictine Monastery farm (18.75%), Regional Agricultural Research Station, Pilicode (14.28%), Cattle Farm, Vellayani (14.28%), LRS Thiruvazhamkunnu (8.09%), Jersey farm, Vithura (5.45%)

Pookot Dairy Project (4.15%) and Cattle farm, KCAET, Thavanur (0%). None of the animals from KCAET, Thavanur showed positive reaction to BHV-1 antibodies.

Statistical analysis revealed highly significant difference ($P \leq 0.01$) between Osho Farm Pattikadu, and Pookot Dairy Project, Jersey Farm, Vithura, Cattle Farm, College of Agriculture, Vellayani and Cattle farm, Thavanur. Significant difference ($P \leq 0.05$) noted between Regional Agricultural Research Station, Pilikode and Osho Farm, Pattikadu and between Cattle Farm, Pattambi and University Livestock Farm, Mannuthy, Cattle Farm, College of Agriculture, Vellayani and Livestock Research Station, Thiruvazhamkunnu.

4.7 Seroprevalence of IBR in animals with different clinical manifestations

Different clinical manifestation in IBR seropositive animals are given in Table 7 and Figure 6.

Among 54 animals with a history of respiratory infection 18 (33.33%) animals showed antibodies to BHV-1. Two hundred and eight animals had history of reproductive disorders including abortion, retention of placenta, metritis, cervicitis, anoestrum, suboestrum and repeat breeding. Among these, 41 (19.71%) animals showed positive reaction. One animal among three (33.33%) animals with eye lesions showed antibodies to BHV-1. Among six animals with arthritis two (33.33%) showed positive result. Fourteen samples

were from case of mastitis among which four (28.57%) animals were positive for antibodies to BHV-1. None of the animals with a history of enteritis had antibodies to BHV-1.

On statistical analysis no significant difference was found between any of the above group with respect to seroprevalence of IBR.

4.8 Seroprevalence of IBR in animals with different reproductive disorders

Out of the total 208 animals with reproductive disorders, 41 animals were positive (19.71%) for antibodies to BHV-1. Ten animals were having history of abortion among which two animals (20%) were positive for IBR antibodies. Four animals were with history of retention of placenta (ROP) and one animal (25%) among them was positive. One hundred and ninety four animals were with different reproductive disorders like endometritis, cervicitis, anoestrum, suboestrum and repeat breeding and thirty eight animals (19.58%) among them were positive (Table 8 and Figure 7).

4.9 Seroprevalence of IBR in different parity

Seroprevalence of IBR in different parity were given in Table 9 and Figure 8.

Out of 201 animals of second parity tested, 46 were found positive (22.89%). Hundred and three animals of third parity were tested in which

twenty animals (19.41%) showed positive reaction. Five (19.23%) animals were positive from 26 animals after 4th parity.

On statistical analysis there is high significant difference ($P \leq 0.01$) between (a) heifers and animals after two parity (b) animals after one parity and two parity.

There is significant difference ($P \leq 0.05$) between (a) heifers and animals after three parity (b) animals after one parity and three parity.

4.10 Seroprevalence of IBR in different districts of Kerala

Number of serum samples collected from each district and number of seropositive animals are given in Table 10 and Figure 9.

Highest seropositiveness was observed in Idukki district followed by Ernakulam, Thrissur, Kozhikode and Alapuzha. None of the sera samples collected from Malappuram district showed positive reaction.

Table 1. Seroprevalence of IBR among cattle reared under different managemental practice

Managemental practice	Number of animals tested	Number of positive animals	Percentage
Organised farms	495	65	13.13 ^{NS}
Rural farming	224	42	18.75 ^{NS}

NS – Non significant

Table 2. Seroprevalence of IBR among cattle with different health status

Health status	Number of animals tested	Number of positive animals	Percentage
Apparently healthy animals	427	41	9.6
Animals with history of disease	292	66	22.6**

** Highly significant (P≤0.01)

Table 3. Breed wise seroprevalence of IBR among cattle

Breed	Number of animals tested	Number of positive animals	Percentage
CBHF	282	51	18.09 ^{NS}
CBBS	137	17	12.41 ^{NS}
CBJ	286	36	12.59 ^{NS}
Others	14	3	21.43 ^{NS}

NS – Non significant

Table 4. Age-wise seroprevalence of IBR among cattle

Age group	Number of animals tested	Number of positive animals	Percentage
0-1	16	1	6.25
1-3	43	3	6.97
3-5	199	24	12.06
5-10	420	72	17.14*
10 and above	41	7	17.07

*Significant (P≤0.05)

Table 5. Sex-wise seroprevalence of IBR among cattle

Sex	Number of animals tested	Number of positive animals	Percentage
Male	10	0	0
Female	709	107	15.09**

** Highly significant (P≤0.01)

Table 6. Seroprevalence of IBR among cattle of different farms

Farm	Number of animals tested	Number of positive animals	Percentage
St. Benedictine Monastery Farm, Kappad	16	3	18.75
Livestock Research Station, Thiruvazhamkunnu	247	20	8.09
Pookot Dairy Project	24	1	4.15
Cattle farm, KCAET, Thavanur	15	0	0
Regional Agricultural Research Station, Pilicode	14	2	14.28
Jersey Farm, Vithura	55	3	5.45
University Livestock Farm, Mannuthy	16	4	25.0*
Cattle Farm, College of Agriculture, Vellayani	35	5	14.28
Cattle Farm, Pattambi	13	2	15.38
Osho Farm, Pattikadu	60	23	38.33**

* Significant (P≤0.05)

** Highly significant (P≤0.01)

Table 7. Seroprevalence of IBR among cattle with different clinical manifestations

Form of infection	Number of animals tested	Number of positive animals	Percentage
Respiratory form	54	18	33.33 ^{NS}
Genital form	208	41	19.71 ^{NS}
Eye lesions	3	1	33.33 ^{NS}
Arthritis	6	2	33.33 ^{NS}
Mastitis	14	4	28.57 ^{NS}
Enteritis	7	0	0

NS – Non significant

Table 8. Seroprevalence of IBR among cattle with different reproductive disorders

Form of infection		Number of animals tested	Number of positive animals	Percentage
Genital form	Abortion	10	2	20.00 ^{NS}
	ROP	4	1	25.00 ^{NS}
	Endometritis Cervicitis Anoestrum Suboestrum Repeat breeding	194	38	19.58 ^{NS}
	Total	208	41	19.71 ^{NS}

NS – Non significant

Table 9. Seroprevlance of IBR in females according to their parity

Parity	Number of animals tested	Number of positive animals	Percentage
Calves	13	1	7.69
Heifers	91	7	6.69
Pregnant animals	13	1	7.69
1	234	22	9.4*
2	201	46	22.89**
3	103	20	19.41*
4	26	5	19.23
5	10	1	10.0
6	10	3	30.0
7 and above	8	1	12.5

** Highly significant (P≤0.01)
* Significant (P≤0.05)

Table 10. Seroprevlance of IBR in different districts of Kerala

Parity	Number of animals tested	Number of positive animals	Percentage
Thiruvananthapuram	90	9	10
Kollam	30	4	13.33
Alapuzha	24	5	20.83
Pathanamthitta	22	3	13.64
Kottayam	17	3	17.65
Idukki	34	11	32.35
Ernakulam	10	3	30.00
Thrissur	85	25	29.40
Palakkad	255	22	8.56
Malappuram	15	0	0
Kozhikode	18	4	22.22
Wyanad	30	3	10.00
Kannur	76	13	17.11
Kasaragode	13	2	15.38

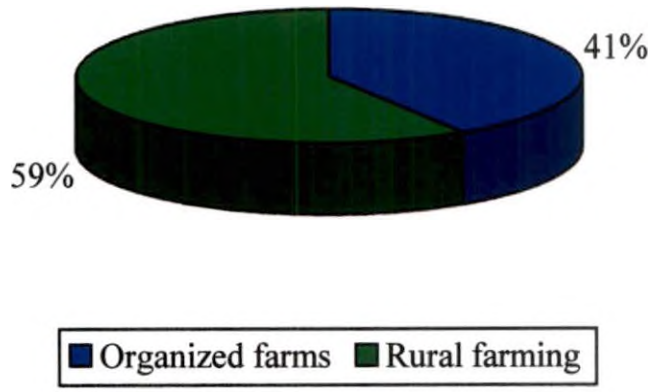


Fig. 1 Seroprevalence of IBR among cattle reared under different managemental practice

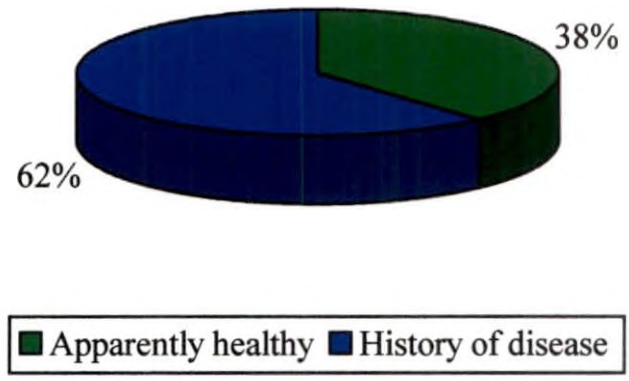


Fig. 2 Seroprevalence of IBR among cattle with different health status

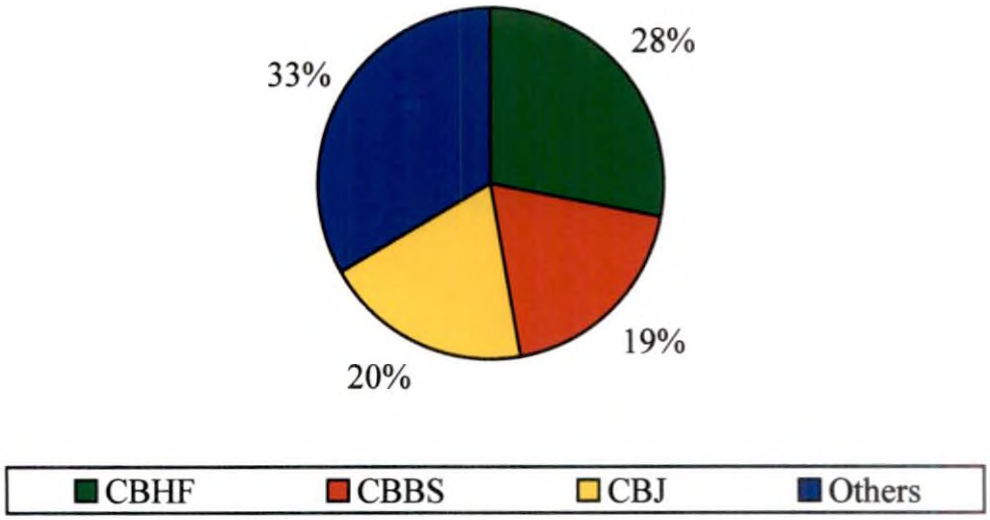


Fig. 3 Breed-wise seroprevalence of IBR among cattle

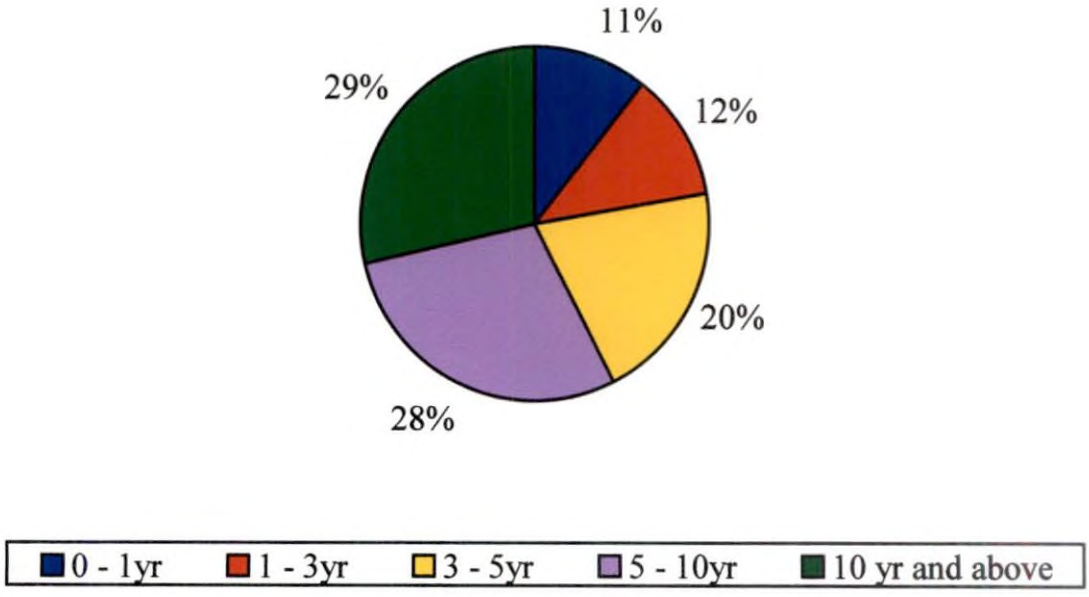
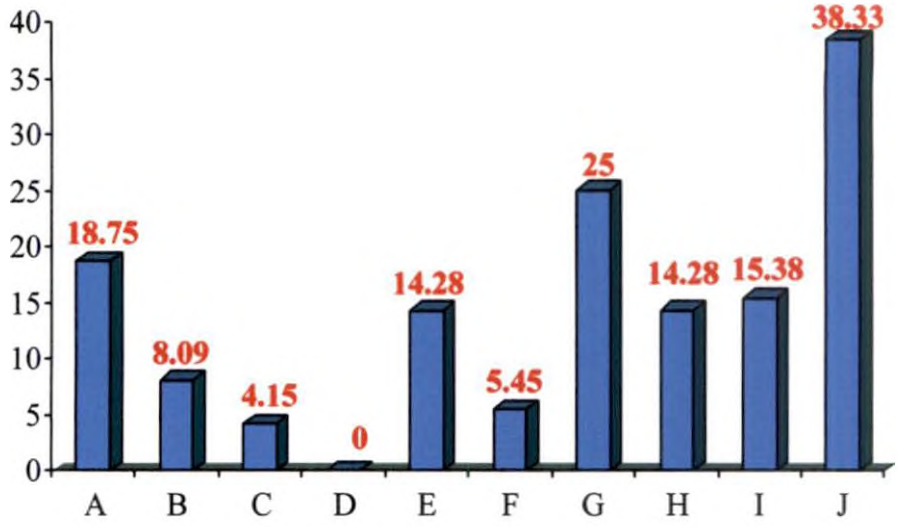


Fig. 4 Age-wise seroprevalence of IBR among cattle



A – St. Benedictine Monastery farm, Kappad
B – Livestock Research Station, Thiruvazhamkunnu
C – Pookot Dairy Project
D – Cattle farm, KCAET, Thavanur
E – Regional Agricultural Research Station, Pilicode
F – Jersey farm, Vithura
G – University Livestock farm, Mannuthy
H – Cattle farm, Vellayani
I – Cattle farm, Pattambi
J – Osho farm, Pattikadu

Fig. 5 Seroprevalence of IBR among cattle of different farms

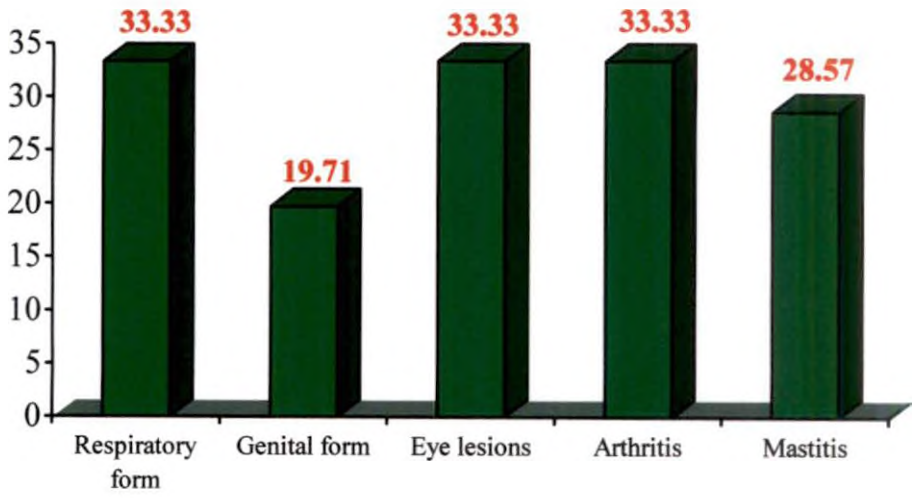


Fig. 6 Seroprevalence of IBR among cattle with different clinical manifestations

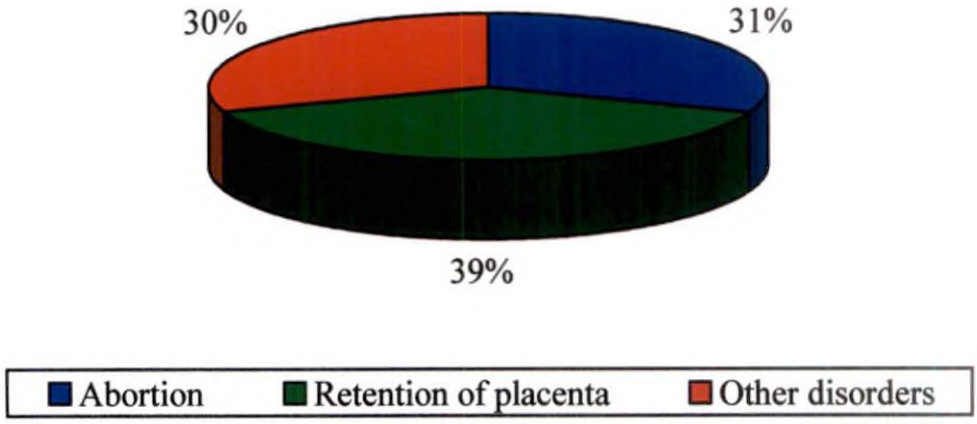


Fig. 7 Seroprevalence of IBR among cattle with different reproductive disorders

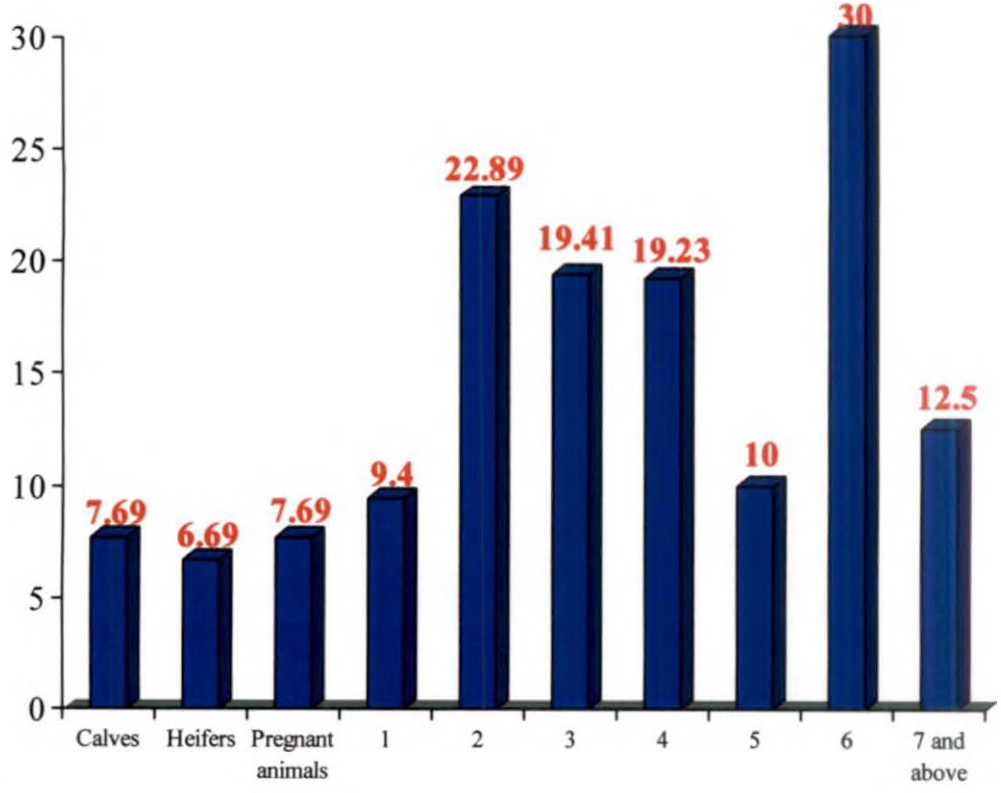


Fig. 8 Seroprevalence of IBR in females according to their parity

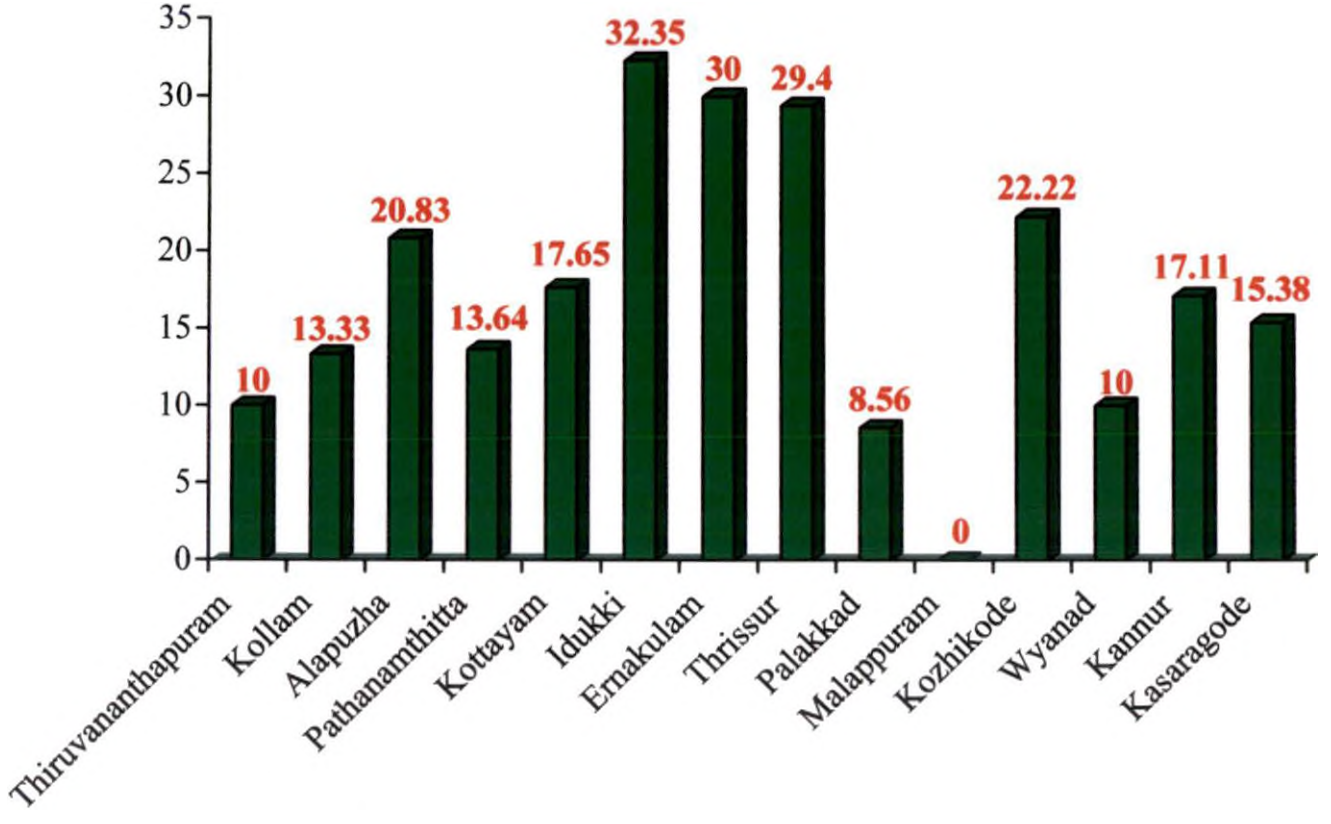


Fig. 9 Seroprevalence of IBR in different districts of Kerala

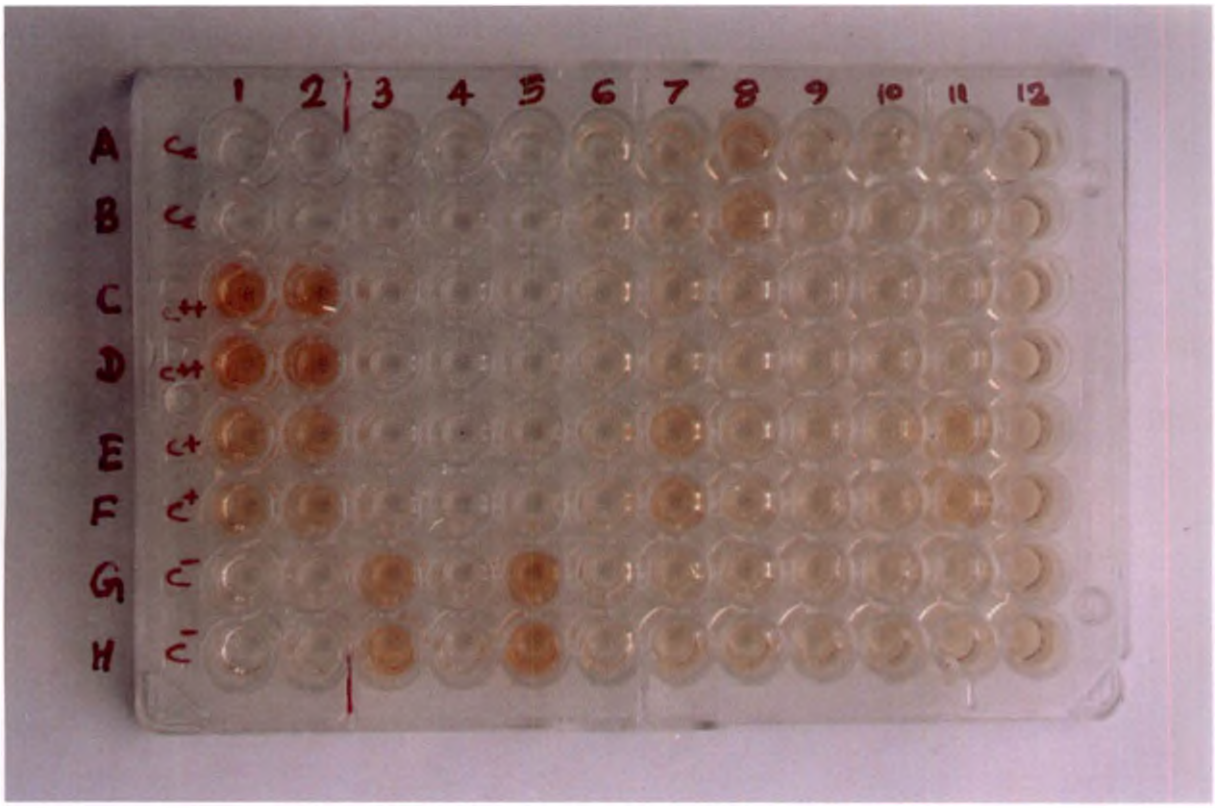


Plate 1. Avidin - Biotin Enzyme Linked Immunosorbent Assay (AB-ELISA) - Test Plate

Conjugate Control (Cc)	- A1, A2, B1 and B2
Strong Positive Control (C^{++})	- C1, C2, D1 and D2
Weak Positive Control (C^{+})	- E1, E2, F1 and F2
Negative Control (C^{-})	- G1, G2, H1 and H2
Test Serum Samples	- A3 to H12
Positive Serum Samples	- 4 (G3 and H3) 12 (G5 and H5) 19 (E7 and F7) 21 (A8 and B8) 35 (E11 and F11)

Discussion

5. DISCUSSION

In the present study seroprevalence of IBR among crossbred cattle of Kerala was assessed using AB-ELISA. Results revealed a prevalence rate of 14.88 per cent of BHV-1 antibodies. The preliminary study on serological survey of IBR in Kerala by Sulochana *et al.* (1982) revealed a prevalence rate of 49.86 per cent BHV-1 antibodies among 367 samples tested using passive haemagglutination test. The moderate prevalence figures reported in the present study (14.88 per cent) could be attributed to the large sample size and the inclusion of significant number of samples from healthy animals. Workers like Renukaradhya *et al.* (1996) also reported similar findings.

Cattle movement from the neighbouring states to Kerala might be one of the factors for the occurrence of IBR infection in the State, as Suresh *et al.* (1999) have reported wide spread seroprevalence of IBR among bovine population of most of the states of India including the neighbouring states such as Andhra Pradesh, Tamil Nadu and Karnataka.

5.1 Seroprevalence of IBR among cattle reared under different managemental practice

In the present study serological evidence of infection was observed both in organised herds and in cattle population maintained by rural farmers (Table 1 and Figure 1). Percentage of seroprevalence was more in animals reared under

rural farming (18.75 per cent) than in animals of organized farms (13.13 per cent). This is contradictory of earlier findings where a more seroprevalence of IBR found in organised farms (Radostits *et al.*, 1994). However on statistical analysis there was no significant difference between these two groups of animals with regard to seroprevalence of IBR.

In organised farms there is more chance of spread of virus from infected to susceptible animals since the source of infection are nasal exudate, coughed up droplets, genital secretion, semen, foetal fluids and tissues (Donkersgoed and Babiuk, 1991).

In rural farming system there is chance of spread of infection through natural breeding with infected bulls. In such cases semen will be contaminated with virus and that can transmit the disease. This was also suggested by Kahrs (1981b).

5.2 Seroprevalence of IBR among cattle with different health status

Bovine Herpes Virus-1 antibodies were detected from healthy animals and from animals having history of disease such as respiratory infections, genital infections, abortion, mastitis, conjunctivitis and arthritis (Table 2 and Figure 2). Such findings were also reported by workers like Sulochana *et al.* (1982), Satyanarayana and Suribabu (1987), Aruna and Surababu (1992) and Renukaradhya *et al.* (1996). The result showed highly significant difference in seroprevalence ($P<0.01$) between the healthy and diseased animal group.

The presence of IBR antibodies in the sera of animals without any history of suggestive symptoms of IBR might be due to the persistence of the virus in a latent form, probably after the establishment of infection in the calfhood (St. George *et al.*, 1967). Persistence of BHV-1 antibodies for a period of 17 months after natural exposure is also a reason for this finding (Kahrs and Smith, 1965). Higher percentage of positive reaction among animals with a history of respiratory infection, genital infection, abortion, conjunctivitis, mastitis and arthritis suggests the possible association of IBR virus with such conditions.

5.3 Breed-wise seroprevalence of IBR among cattle

Present study revealed a more seroprevalence of IBR in animals originated from the crossbred cattle population (Table 3 and Figure 3). Crossbred cattle and exotic cattle are more susceptible to BHV-1 infection than indigenous cattle. This finding is in agreement with the observations of Masolla *et al.* (1981) and Satyanarayana and Suribabu (1987).

Crossbreds of Holstein-Friesian had higher seroprevalence (18.09 per cent) than crossbreds of Brown-Swiss or Jersey. Masolla *et al.* (1981) found that Holstein-Friesian had significantly high seropostivity than Jersey cattle.

Crossbreds of Brown-Swiss and Jersey had more or less same seroprevalence percentage and no significant difference was observed.

Variation in the seroprevalene of IBR with different breeds of cattle was also reported by Singh *et al.* (1985).

5.4 Age-wise seroprevalence of IBR among cattle

From the percentage of seroprevalence it is clear that as the age advances percentage of seropositivity also increases (Table 4 and Figure 4). Seroconversion is more in animals above three years of age. This is probably due to exposure to various degrees of stress. This observation agrees with the findings of previous workers (Satyanarayana and Suribabu, 1987; Suresh *et al.*, 1992 and Mc Dermott *et al.*, 1997).

Seroprevalence of IBR is less in 0-1 year age group (6.25 per cent) and 1-3 year group (6.97 per cent). Younger animals generally possess maternal antibodies which prevents the infection. This was supported by the finding of Donkersgoed and Babiuk (1991). Stress factors like pregnancy, lactation etc, reduce the immunity and that may lead to onset of infection in adult animals. Such stress factors are very less in younger animals which also contributes to the lesser prevalence in this group (Radostits *et al.*, 1994).

5.5 Sex-wise seroprevalene of IBR

Of the 709 females tested 15.09 per cent animals were positive for BHV-1 antibodies (Table 5). None of the male animals tested were positive for antibodies to BHV-1 infection.

The higher rate of occurrence in females may be due to the use of semen from an infected bull to many females in the form of artificial insemination. Suresh *et al.* (1992a) also had the same opinion. In rural areas when infected bulls were used for natural breeding there will be transmission of infection to many cows (Kahrs, 1981b). This is also a reason for more prevalence in females. Pregnancy can act as a stress factor which can lead to onset of infection (Manickam and Mohan, 1987).

5.6 Seroprevalence of IBR among cattle of different farms

Seroprevalence of IBR is more in private farms like St. Benedictine Monastery farm, Kappad and Osho farm, Pattikadu (Table 6 and Figure 5). The variation in the seroprevalence between different farms may be due to difference in managemental practice such as over-crowding, unhygienic environment, absence of isolation and quarantine measures which allows the easy spread of BHV-1 infection. Higher incidence in Osho farm may also be contributed to recent purchase of animals from Tamil Nadu where a higher percentage (45.7 per cent) of seroprevalence of IBR was reported (Renukaradhya *et al.*, 1996). Introduction of infected animals into a group of cattle often leads to outbreak of IBR infection. This was also pointed out by Donkersgoed and Babiuk (1991). Sudden changes in managemental practices such as dietary changes may enhance the risk of BHV-1 infection, as suggests by Fenner *et al.* (1993).

5.7 Seroprevalence of IBR among cattle with different clinical manifestations

Serum samples were collected from animals with respiratory form of infection, genital form of infection, eye lesions, arthritis, mastitis and enteritis (Table 7 and Figure 6).

High seropositivity (33.33 per cent) to IBR infection observed in samples collected from animals having respiratory form of infection. This may be attributed to the fact that all ages of cattle are susceptible to respiratory infection unlike the genital infection which is reported mainly in adults. Crowding and mixing of animals in farms allow high rate of transmission of respiratory form of infection. More seroprevalence in respiratory form of infection was also noted by Donkersgoed and Babiuk (1991) and Renukaradhya *et al.* (1996).

Percentage of seropositivity in genital form of infection is 19.72. Association of BHV-1 with genital infection was also reported by many workers (Salwa and Stryszak, 1995 and Cook, 1998).

Three samples were from cases of eye infection with symptoms such as lacrimation, presence of pus and reddening of conjunctival mucous membrane. Out of three samples, one sample was positive for IBR infection. Ability of BHV-1 to cause conjunctivitis was reported by workers like Foldi *et al.* (1992).

Six samples were from animals having arthritis. Two samples were positive for IBR infection (33.33 per cent). There are no report of arthritis with BHV-1 and this may be an accidental finding.

Seropositivity of 28.57 per cent was noted in samples collected from animals that are having mastitis. Bovine Herpes Virus-1 can cause mastitis, and that was proved by Roberts *et al.* (1974) who isolated IBR virus from a mastitis case. Hage *et al.* (1998) also identified BHV-1 as a cause of mastitis in cattle.

There are reports of association of BHV-1 with enteritis. But in the present study out of the seven samples tested from cases of enteritis none were positive for BHV-1 antibodies. Enteritis in these animals may be due to other etiological agents.

Bovine Herpes Virus-1 may prevail in a form causing transcient respiratory illness and keratoconjunctivitis in young ones and may cause abortion after being established in adults (Miller, 1991).

5.8 Seroprevalence of IBR among cattle with different reproductive disorders

In the present study seroprevalence of IBR observed in animals with history of abortion, retention of placenta, endometritis, cervicitis, anoestrum, suboestrum and repeat breeding (Table 8 and Figure 7). Association of BHV-1 with such reproductive disorders also described by workers like Gibbs and Rweyemamu (1977).

Abortion occur in pregnant cows, due to IBR infection especially in the third trimester because of the degree of pregnancy stress, exposure to multiple pathogens and also due to conducive uterine environment during the later stages of gestation for the rapid multiplication of virus following recent infection or latent infection which in turn cause foetal death and abortion (Sheffy and Davies, 1972 and Darsel and Dorward, 1975).

5.9 Seroprevalence of IBR in females according to their parity

Percentage of seroprevalence of IBR is more in older animals or animals having one parity or more (Table 9 and Figure 8). Result reveals high significant difference ($P \leq 0.01$) between the seroprevalence of IBR of (a) heifers and animals after two parity and (b) animals after one parity and two parity. Also there is significant difference ($P \leq 0.05$) in the seroprevalence of IBR between (a) heifers and animals after one parity and (b) animals after one party and three parity.

The disease occurs mostly in animals over six months of age probably because of their greater exposure to different stress factors and also due to the presence of maternal antibodies in younger animals (Donkersgoed and Babiuk, 1991 and Radostits *et al.*, 1994). Prevalence is more in animals having one parity or more and this may be due to degree of pregnancy stress and also exposure to different pathogens (Sheffy and Davies, 1972). The occurrence of higher rate of infection in the first lactation might be due to the lack of adequate

protective immunity during the first lactation as reported by Manickam and Mohan (1987).

5.10 Seroprevalence of IBR in different districts of Kerala

Seroprevalence of IBR was recorded from almost all districts of Kerala (Table 10 and Figure 9). Seroprevalence ranged from zero per cent in Malappuram to 32 per cent in Idukki district with an average value of 14.88 per cent. Number of samples collected from Malappuram was very less and the samples were from one organised farm. Good hygienic conditions and managemental practices may be the reason for the absence of infection in that farm. In Idukki many samples were from slaughter house and from diseased animals. Most of the samples from slaughter house were of older animals. More seroprevalence of IBR reported from older animals (Suresh *et al.* 1992a and Mc Dermott *et al.*, 1997).

Transmission of BHV-1 infection through semen is a possibility in rural areas where there is practice of natural breeding as suggested by Kahrs (1977) . Movement of animals within the state may be the reason for spread of infection throughout all districts of Kerala. Previous workers also supported this view (Sulochana *et al.*, 1982). Bovine Herpes Virus-1 infection may occur in cattle herds which were originated from infected herds. This possibility was reported by Tekes *et al.* (1999).

Summary

6. SUMMARY

Infectious bovine rhinotracheitis (IBR) is caused by Bovine herpes virus 1 (BHV-1). Infectious Bovine Rhinotracheitis exhibited in a variety of clinical manifestations.

First isolation of BHV-1 in India was done by Mehrotra (1977). Seroprevalence studies shown that disease is prevalent throughout the country.

Seroprevalence of infectious bovine rhinotracheitis in crossbred cattle of Kerala were studied. Samples were collected from all the districts of Kerala.

Samples were subjected to Avidin-Biotin ELISA for detecting antibodies against BHV-1 infection. Results were subjected to statistical analysis.

Seroprevalence of IBR was more in animals reared under rural farming system.

Animal with a history of disease showed more seroconversion to BHV1 and there is high significant difference ($P<0.01$) between apparently healthy animals and animals with a history of disease.

Seropositivity of BHV1 was more in crossbred cattle than indigenous cattle.

Seroprevalence of IBR was more in animals of above three years of age; and percentage of seropositivity increased as the age advances.

Calves and heifers showed less seroprevalence of IBR. Animals with one parity and above had a high seroprevalence of IBR.

Private farms had a high seroprevalence of IBR than other farms and there is significant difference.

High percentage of seroprevalence of IBR detected in animals with respiratory form of infection.

Percent of seroprevalence of IBR in cattle of Kerala observed as 14.88 per cent.

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Appendices

Annexure - I

Places from where samples collected

Places	Number of samples
St. Benedictine Monastery farm, Kappad	16
Livestock Research Station, Thiruvazhamkunnu	247
Pookot Dairy Project	24
Cattle Farm, KCAET, Thavanur	13
Regional Agricultural Research Station, Pilicode	14
Jersey Farm, Vithura	55
University Livestock Farm, Mannuthy	16
Cattle Farm, College of Agriculture, Vellayani	35
Cattle Farm, Pattambi	15
Oshofarm, Pattikadu	60
Slaughter Houses, Hospitals Field	224

Annexure-II

Avidin-Biotin ELISA

Coating the plate with BHV-1 antigen

↓

Addition of control and test sera

↓

Addition of Biotinylated antibovine IgG

↓

Addition of Avidin-Horse radish peroxidare

↓

Addition of Substrate/chromogen solution

↓

Addition of stopper

↓

Measurement of colour development

Annexure III

Avian-Biotin ELISA plate layout

	Controls		Serum samples (in duplicate)									
	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	Cc	1	5	9	13	17	21	25	29	33	37
B	Cc	Cc	1	5	9	13	17	21	25	29	33	37
C	C++	C++	2	6	10	14	18	22	26	30	34	38
D	C++	C++	2	6	10	14	18	22	26	30	34	38
E	C+	C+	3	7	11	15	19	23	27	31	35	39
F	C+	C+	3	7	11	15	19	23	27	31	35	39
G	C-	C-	4	8	12	16	20	24	28	32	36	40
H	C-	C-	4	8	12	16	20	24	28	32	36	40

Cc : Conjugate control
C++ : Strong positive control
C+ : Weak positive control
C : Negative control

Numbers –
From 1 to 40 : Serum samples (in duplicate)

Annexure IV

Avidin-Biotin ELISA

Result Sheet-Layout

IBR (A-B ELISA)	:	Plate Status	:
Plate name	:	Test date	:
Created by	:	Test time	:
Filter	:	Technician	:
Blanking value	:	Kit Batch	:

Controls: Acceptable OD range C++ 0.600-1.200 Threshold: PP>=28% Outside control limits: OD (#) PP (*)

ID	Status	OD1	OD2	OD3	OD4	PP1	PP2	PP3	PP4	LCL	UCL
C++										70	120
C+										35	55
C-										0	16
Cc										0	8

The average of two intermediate C++ value is : Test samples

ID WELLS DESCRIPTION STATUS OD1 OD2 Odav PP1 PP2 Var Ppav

1
2
3
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40

SEROPREVALENCE OF INFECTIOUS BOVINE RHINOTRACHEITIS IN CROSSBRED CATTLE OF KERALA

By

RAJESH, J. B.

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Veterinary Epidemiology and Preventive Medicine
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

2001

ABSTRACT

Seroprevalence of IBR in 719 crossbred cattle of Kerala was studied using Avidin-Biotin ELISA. Sera samples were collected from cattle belonging to different places of Kerala covering all the districts. Samples were collected randomly from cattle of different age, managemental practice, breed, sex, parity, different health status and also from different farms.

Samples were subjected to Avidin-Biotin ELISA for detecting antibodies against BHV-1 infection. Out of 719 sera samples tested 14.88 per cent gave positive result for BHV-1 antibodies.

Animals reared under rural farming system showed high seropositivity (18.75%) to BHV-1 infection than animals under organised farms.

Seroprevalence of BHV-1 antibodies was more in animals with some history of disease (22.6%) than apparently healthy animals. This result is highly significant ($P<0.01$).

Prevalence of BHV-1 antibodies is found to be more in crossbred cattle when compared to non-descript cattle.

Seroprevalence of IBR in various age group revealed that percentage of positiveness to BHV-1 was more in animals above three years of age. Serological evidence of IBR is very less in younger animals. Also animals after

one parity and above had a high percentage of seroprevalence than heifers and calves.

Animals from different farms were tested for antibodies against BHV-1 infection. Private farms had a high seroprevalence of IBR infection than University farms and Government farms. There is significant difference between private farms and other farms.

Seroprevalence of IBR among cattle with different clinical manifestations were tested. High percentage of seroprevalence noted in respiratory form of infection (33.33%).

Seroprevalence of IBR in crossbred cattle of Kerala detected as 14.88%.