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INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS OF CATTLE AND BUFFALOES

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INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

OF CATTLE AND BUFFALOES

by

Saw Plei Saw

A thesis submitted
in partial fulfilment of the requirement
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UPM

This thesis attached hereto, entitled "Infectious Bovine Rhinotracheitis Virus of Cattle and Buffaloes" prepared and submitted by Saw Plei Saw in partial fulfilment of the requirements for the degree of Doctor of Philosophy, is hereby accepted.

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ABSTRACT

Infectious bovine rhinotracheitis virus infection in cattle has been reported throughout the world. The study of infectious bovine rhinotracheitis in buffalo was very limited. There was no report on infectious bovine rhinotracheitis in cattle and buffalo in Malaysia.

A serological study on the prevalence of infectious bovine rhinotracheitis in cattle and buffalo in 10 states of Peninsular Malaysia showed that 52.52 per cent of the cattle and 65.07 per cent of the buffaloes had neutralizing antibodies to infectious bovine rhinotracheitis virus. Neutralizing antibodies were detected in 532 out of the 1013 serum samples from cattle and in 298 out of the 458 serum samples from buffaloes. The titre of the serum neutralizing antibodies ranged from 1:4 to 1:256.

Four cattle and four buffaloes with neutralizing antibodies to infectious bovine rhinotracheitis virus were treated with dexamethazone at 0.1 mg. per kilogram of body weight for 7 consecutive days. Treatment of these animals with dexamethazone resulted in shedding of virus. Viruses were isolated from nasal cavities and vaginas of the cattle and buffaloes in bovine embryonic kidney cells. The isolates were identified as infectious bovine rhinotracheitis virus by virus neutralization test. The viruses isolated from nasal cavity and vaginal mucosa of buffaloes were designated UPM BB 1 and UPM BB 2 respectively while the viruses

isolated from nasal cavity and vaginal mucosa of cattle were designated UPM BC 1 and UPM BC 2 respectively. The 4 virus isolates had infective titres ranging from $10^{6.8}$ to $10^{7.8}$ Pfu per ml.

The four infectious bovine rhinotracheitis virus isolates were then subjected to 3 cycles of plaque purification. The cloned infectious bovine rhinotracheitis viruses were further purified by rate zonal ultracentrifugation in 10 to 40 percent potassium tartrate. Examination of purified viruses under the electron microscope revealed virus morphology similar to that of herpes virus. The four purified virus preparations were also identified as infectious bovine rhinotracheitis virus by immune electron microscopy.

Further study of physical and chemical characteristics of the four viruses were carried out. Heat stability test revealed that the viruses were inactivated at 65°C in 15 minutes and at 56°C in 1 hour. Their half life at 37°C lasted for 8 to 16 hours. All 4 viruses were sensitive to ether and chloroform. They were stable at pH 7 but the infective titre dropped considerably when treated at pH 4 for 1 hour. Haematoxylin and eosin-stained bovine embryonic kidney cells infected with the virus isolates showed eosinophilic intranuclear inclusion bodies of Cowdry type A. Acridine orange staining of virus-infected bovine embryonic kidney cells revealed yellow green intranuclear inclusions indicating the presence of double-stranded deoxyribonucleic acid. Since virus particles has been seen in intranuclear location under EM, the DNA were probably of virus origin.

Morphological study on plaques did not show any significant difference between the four viruses. The plaque sizes at 4 days post

infection of bovine embryonic kidney cell measured at 0.54 to 0.71 mm. The plaques were rounded with well defined boundry and clear centre. A wide range of cell cultures were found to be susceptible to the isolated viruses. The growth of viruses in bovine embryonic kidney cells showed an eclipse period of 4 to 8 hours with rapid growth until 24 hours. The maximum titre of all 4 virus strains were observed in 48 hours with slight decline until 72 hours. A marked decrease in virus titre was observed after 72 hours of virus infection to the cell.

The study of morphological, physical and chemical characteristics of the virus isolates supported the serological findings that the 4 virus isolates were infectious bovine rhinotracheitis virus. It was confirmed that infectious bovine rhinotracheitis occured in cattle and buffaloes in West Malaysia. Suggestions for further research on infectious bovine rhinotracheitis especially in buffaloes were given.

INTRODUCTION

Infectious bovine rhinotracheitis virus (IBRV) which belongs to herpes virus vroup usually affects cattle of all ages with diverse clinical manifestations. Infectious bovine rhinotracheitis (IBR) may occur as enzootics in certain places or assume an epizootic form throughout thecountry. IBR may be introduced into a clean herd when new stocks are brought in. The morbidity of the disease may vary from a few cases to as high as 100% with the mortality ranging between 2 - 10% of the animals infected. The mortality rate may increase considerably if the general hygienic conditions of the farms are poor and when a secondary infection exists. IBR was first observed in 1950, in Colorado feed lot cattle and was later reported in dairy cattle in Los Angeles County in California (Miller 1955). After IBR has been reported, many researchers studied the clinical history of the disease and they suspected a virus to be the cause. The causal organism was later isolated by Madin, York and McKercher in 1956 from the nasal washings of clinically affected cattle. Since then IBR has been reported in Canada, United States of America, South America, AFrica, Australia, Europe and some parts of Asia (Gibbs and Rweyemamu 1977). IBR is claimed to be of world wide distribution. Although cattle are the principle reservoir of IBRV, the infection has been reported in goats (Mohanty et al 1972), swine (Derbyshire and Caplan 1976), water buffalo and some wild animals (St. George and Philpott 1972).

IBR is highly infectious and is characterised by fever, depression, polypnoea accompanied at times by respiratory dyspnoea, rhinitis, foamy salivation and inflammation of the upper respiratory tract. In severe cases with bacterial complication, there is muco-purulent exudate and mucosal necrosis of the anterior part of the turbinates and extending into the sinus and trachea. The course of the disease depends on the severity of infection. Besides respiratory infection, IBRV has predilections for different parts of the body. Infection of the vaginal and vulval mucosa is manifested by pustules and mucopurulent discharge and it is referred to as infectious pustular vulvo-vaginitis (IPV). The disease may spread from IPV infected cows to sniffing cattle. Bulls that breed cows with IPV may contract the disease in the form of balanoposthitis. Bulls with infectious pustular balanoposthitis (IPB) do not loose their fertility, but they continue to spread the disease to breeding cows and temporary infertility among such cows may be observed. Abortion is one of the important sequele after IBRV infection. Bovine foetus in all trimesters are highly susceptible to IBRV and abortion may occur within 60 days after the infection. Keratoconjunctivitis which is characterised by pink vascularised oedematous area raised above the cornea with complete opacity has been associated with IBRV infection. Young cattle infected with the virus may develop encepha-lomyelitis characterised by incoordinations circling or licking of the flanks, recumbency and death. IBRV may cause diarrhoea or it may also be associated with a few clinical cases of mastitis in cows.

Besides the clinically apparent form, IBRV can persist in the infected animals as a latent or subclinical type of infection.

Cattle which have recovered from IBR may acquire a certain antibody level while at the same time harbour the virus in dormant stage in trigeminal ganglions and lumbosacral spinal cords and their ganglions. When latently infected animals have undergone stress conditions such as adverse climate, distant hauling, overcrowding, vigorous handling and poor nutrition, the disease is reactivated. Cows that are latently infected prior to conception may abort as a consequence of the stress of pregnancy. Experimental studies showed consistent reexcretion of IBRV from latently infected cattle following corticosteroid treatment. The presence of IBR antibody does not protect the animals from reactivated virus and they develop the clinical symptoms as those cattle which contract the disease for the first time. Infected cattle develop antibody in detectable quantity after 8 to 10 days and lasted for 12 to 18 months. Calves also acquired a passive immunity from their dams, but the duration is short lived. Antibodies are easily detectable from serum of convalescent animals by serum neutralization test.

IBRV consists of a central core of double stranded deoxyribonucleic acid (DNA) surrounded by a protein capsid and enclosed by an outer envelope. The complete virion measures from 130 - 180 nm in diameter. The viral capsid is about 100 nm in size and icosahedral in shape. IBRV grows well in cell cultures of bovine origin and replicates in the cell nucleus forming the intranuclear inclusion bodies. After a few hours of latent period, the virus growth is detectable by its cytopathic effect (CPE) in susceptible cell. Maximum growth is obtained within 24 to 48 hours. CPE is characterised by foci of rounded cell and disruption of the monolayer

which is totally destroyed at a later stage. IBRV is relatively thermolabile and its infectivity is destroyed by ether and chloroform. The virus is labile at pH 4 to 5 stable at pH 6 to 9.

The cattle industry in Malaysia mainly involves smallholder farmers. The livestock development programmes emphasize an expanding cattle and buffaloes production to an extent where the country would be self sufficient in milk and meat requirements. The existing total cattle population of 469,000 is estimated to increase at a rate of 4% annually (Country report 1980). The increase of cattle population on the other hand favours the spread of diseases. The occurence of IBR in cattle has not been reported in Malaysia and its role of economic importance in this country has not been defined. However there are frequent reports of respiratory infections, calf mortality, abortion and infertility among cattle. It is possible that a certain proportion of the problem may be associated with IBRV infection.

The population of domesticated water buffaloes (Bubalus bubalis) in the world is estimated at 130 millions and 90% of them are found in Asia. The buffaloes besides being used as draft animals also is a major source of meat and milk. Low reproductive efficiency and high calf mortality can be accepted as the major problems in buffalo production. There are a number of reasons underlying these animals and diseases could be one of them. There are limited reports on the incidence of IBR in buffaloes. In 1967, St. George et al reported the detection of antibodies to IBRV in serum of feral and domesticated buffaloes in Australia.

Infectious diseases of buffalo in Malaysia are poorly defined. Besides foot and mouth disease and haemorrhagic septicemia, very little is known about other infectious diseases of buffalo. IBRV is known to cause a number of clinical manifestations in cattle and these include temporary failure of conception and calf mortality (Kahrs 1977). It is not known if a similar situation occurs in buffaloes. In Peninsular Malaysia there is no report regarding the prevalence of IBRV infection among buffaloes. Recently Ibrahim et al (1983) reported the isolation of IBRV from a buffalo.

IBR is one of the major problem in intensive farming where the herd population is to be maintained in full capacity for maximum profit. The economic loss due to IBR is of great importance in cattle production. There is a possibility of similar effect in buffaloes. Animals infected with IBRV loose weight and require longer feeding period before they can be marketed. There is a great loss in milk yield in dairy herds affected with the disease. Abortion and calving mortality further contribute to the economic loss.

The objective of this project is

1. to study the prevalence of IBRV infection in cattle and buffaloes in Malaysia.
2. to isolate IBRV from cattle and buffaloes.
3. to characterize IBRV isolated from cattle and buffaloes.
4. to study the replication of IBRV, isolated from cattle and buffaloes, in tissue culture.

REVIEW OF LITERATURE

Identification of disease and virus isolation

Schroeder and Moys (1954) first reported an acute respiratory infection of dairy cattle in which they suspected a virus to be responsible. A respiratory tract disease appeared in Weld County, Colorado, in the year 1950 and virus was presumed to be the primary causal agent (Miller 1955). In the same year McKercher et al (1955) reported a similar disease and their study confirmed that the disease was caused by virus. The disease was identical with a cattle disease syndrome known in Colorado as rhinotracheitis. It was recommended that the disease be referred to as 'infectious bovine rhinotracheitis' (McKercher et al 1955). Kendric et al (1958) mentioned that a disease called Blaschenausschlag or pustular coital exanthema had been recorded by Witte since the latter part of 19th century. This genital disease known also as infectious pustular vulvovaginitis (IPV) is caused by IBRV (McKercher 1963).

IBRV was first isolated in 1956 from nasal washings collected from the clinically affected cattle (Madin, York and McKercher 1956). York et al (1957) later isolated a virus in bovine embryo tissue culture from cattle having IBR. Chow and his associates (1955) reproduced IBR in 13 out of 15 experimental cattle by using bacteria-free inocula from nasal and tracheal secretions and exudates, sera and splenic tissue from field cases of bovine rhinotracheitis.

IBR-IPV had been reported in New Zealand (Webster and Manktelow 1959), Australia (French 1962a, Parsonson 1964, Snowdon 1964 and St. George 1965), Canada (Studdert et al 1961), Africa (Provost and Borrendon 1965), Europe (Dawson et al 1962, Darbyshire and Shanks 1963, Wiseman et al 1979) and Japan (Shimizu et al 1972). The world wide distribution of IBRV infection in cattle was reviewed by Gibbs and Rweyemamu (1977).

Nelson et al (1972) conducted a serological survey in pigs and found that their sera contained significant antibody level against IBRV. The virus had also been isolated from stillbirths in swine (Derbyshire and Caplan 1976). Experimentally infected goats responded with pyrexia, harboured the virus and developed antibodies (McKercher 1959). Mohanty et al (1972) reported a natural infection with IBRV in goats. IBR antibody was widely distributed in water buffaloes in Australia and IBRV was isolated from prepucial swabs of these animals (St. George and Philpott 1972). Evidence of IBRV infection was found in captured mule deer with high prevalence of antibody and these animals were also susceptible to experimental infection with IBRV (Chow and Davis 1964).

Clinical manifestations

IBR is associated with a number of clinical manifestations in cattle and these include

1. Respiratory tract disease. Miller (1955) described the disease as 'red nose' 'dust pneumonia' and 'necrotic rhinotracheitis' and

'necrotic rhinitis' all of which were associated with respiratory problems. Kahrs (1977) noted that IBR played a prominent role in the cause of many undifferentiated bovine respiratory diseases. A classical IBR was described to be characterised by fever, coughing, hyperpnoea, depression, slight anorexia and dropped milk yield. A clear nasal discharge developed within one or two days which later turned mucopurulent. Some animals showed excessive salivation. Some animals showed highly inflammed nasal mucosa, external nares and muzzle and the condition was termed red nose disease (Madin et al 1956, McKerche 1959, Gillespie et al 1959 and York 1968). Young calves which were experimentally infected with IBR-IPV virus showed pathological changes in mucosa of the mouth, oesophagus, forestomach, liver, spleen, kidney, lymph nodes and virus concentration was correlated with the pathological findings (Baker et al 1960).

2. Conjunctivitis and keratoconjunctivitis. Classical respiratory form of IBR was often associated with conjunctivitis (Abinanti and Plummer 1961, Ferris et al 1964, McKercher and Wada 1964). A general unilateral and few bilateral conjunctivitis associated with IBR was reported in one case of keratitis (Dawson et al 1962). One of the common sign of IBR was conjunctivitis followed by keratitis (Hughes et al 1964). A national survey conducted in Australia showed that the most common clinical signs were occular discharge (43.9%), corneal opacity (9.9%) or both (46.1%) (Slatter et al 1982a). Infectious keratoconjunctivitis had been reported from Nigeria, but the authors failed to identify the causal agent (Griffin et al 1965). Following Griffin's report, Provost and Borrendon (1965) claimed

that they had succeeded in demonstrating that IBRV was responsible for infectious keratoconjunctivitis occuring in Northern Nigeria and the neighbouring countries. In one IBR outbreak in a herd, 15 out of 19 infected cattle developed keratoconjunctivitis and IBRV had been isolated from occular swabs (St. George 1965). Sykes et al (1962) isolated a virus from infectious bovine keratoconjunctivitis with similar physical and chemical characteristics to that of IBRV. During the period of 1975 - 1979, Slatter et al (1982b) found that out of the total 505,000 cattle surveyed, 370,190 (73.3%) were affected by infectious kerato-conjunctivitis.

3. Diseases of the reproductive system. Kendric et al (1958) isolated a virus from genital tracts of cows with vesicular disease. They believed the name 'infectious pustular vulvovaginitis' was appropriate for the salient characteristics of the disease they have observed. The clinical sign of PIV was marked by hyperaemic area which developed into nodules, vesicles and pustules. In some cases pustules coalesced to form yellow white fibrinous membrane which detached and formed ulcers (Gillespie et al 1959, Saxegaard 1970). Other characteristic features observed during IPVV infection were the swollen vulva, the cow exhibited pain by arching the back, switching the tail and frequent urination (Saxegaard 1970, Collings et al 1972). Afshar (1965) observed that vaginitis could be recognized by dry discharge adhering to the hairs of the tail level with the lower angle of the vulval floor.

There was a general agreement that under natural breeding condition, IPVV spread from bulls to cows and vice versa (Saxegaard

1970, Huck et al 1973, Allan et al 1975). The disease has also been reported to be transmitted through artificial insemination (Saxegaard 1970, Parsonson and Snowdon 1975). It was found that the disease produced by artificial insemination was associated with reproductive problems but the disease caused by natural breeding did not interfere with fertility (Saxegaard 1970, Allan et al 1975).

IBRV infection was also reported to be associated with metirtis (Lomba et al 1976) infertility in artificial insemination (Saxegaard 1970, Allan et al 1975) and abortion in pregnant cows and post abortion infertility (Kahrs and Smith 1965, Afshar 1965). There were also reports on abortion of pregnant cows during IBRV infection (Crane et al 1964, McKercher and Wada 1964) and following IBR vaccination (McKercher & Wada 1964, Kelling et al 1973). Kendric (1973) reported that susceptible pregnant cows infected with IBRV either abort or continue their pregnancy as there were no intermediate stage of the disease. An experiment conducted by Durham et al (1975) showed that pregnant cows maintain their normal gestation. It was noted that abortion occured as a sequele to IBR but not to IPV because the virus could reach the foetus only by hematogenous transport from the infected respiratory passages (McKercher 1963).

In one concurrent respiratory and genital disease both IBRV and IPVV have been isolated (Collings et al 1972). Kahrs and Smith (1965) have also isolated both IBRV and IPVV from 2 cases of IPV in a New York dairy herd. Inspite of their differences in clinical manifestations, IBRV and IPVV were proved to be identical (Gillespie et al 1959, McKercher et al 1959, Dawson et al 1962 and

Snowdon 1964). House (1972) studied 12 strains of IBR-IPV viruses and he found minor differences between them, but he could not differentiate between the genital and non-genital strain. On the other hand Buening and Gratzek (1967) demonstrated that serum neutralization kinetics produce minor antigenic differences between IBRV strains ISU-IBR 1 and 3 other IBRV strains such as ISU IBR 2, Colorado and Los Angeles strains. Potgieter and Mare (1974) also found that IBRV vaccine strain could be distinguished from IBR-IPV virus by neutralizing kinetics method using late 19s rabbit antibodies.

4. Disease of the central nervous system. An outbreak of meningo-encephalitis with high morbidity and mortality in calves in Australia was reported by Johnston et al (1962). Further investigation revealed that intracerebral inoculation of brain tissue into 3 calves produced a disease similar to naturally occuring IBR (Johnston et al 1964). French (1962b) reported that a virus isolated from cases of encephalomyelitis of cattle was serologically related to IBRV. A cytopathogenic agent isolated in primary bovine embryonic kidney cell culture from various tissues of calves with meningoencephalitis was identified as IBRV by reciprocal cross serum virus neutralization tests (Barenfus et al 1963). IBRV isolated from a case of bovine meningoencephalitis produce a fatal disease in the experimental calves (Hall et al 1966). Beck (1975) isolated IBRV from brain tissue and spinal cord of heifer with severe neurological lesions. IBRV infected calves showed neural changes with lesions in trigeminal ganglions (Narita et al 1981).

5. Diseases of the alimentary system. IBRV. had been isolated from faeces of cattle with clinical symptoms of IBR (Crnadle 1974). Diarrhoea was one of the common clinical sign associated with IBRV infected calves (Curtis et al 1966, Gratzek et al 1966). Lesions of the gastro intestinal tract as a result of IBR was similar to those of bovine virus diarrhoea mucosal disease complex (Peter et al 1966). In one IBR outbreak among a group of beef herd in Oxfordshire, several animals had diarrhoea in conjunction with other symptoms (Dawson et al 1962).

6. Mastitis. Herpes virus of IBR may produce mastitis in lactating cows under experimental conditions (Greig and Bannister 1965, Corner et al 1967). IBRV has been isolated from an outbreak of mastitis (Gourlay et al 1974) and from the milk of a cow with mastitis (Roberts et al 1974). Whether such natural infections are the primary or a secondary cause is uncertain.

Morbidity and Mortality

Observations on IBR showed the morbidity to be varied from a low percentage to 100 percent depending on the severity, and the mortality range from 2% to 10% of the animals affected (Miller 1955). Survey conducted in Colorado feedlot disclosed the morbidity to be 10.6% with 3% mortality (Chow et al 1956). In a few incidence 5% of the animals at risk had either died or been culled and on one particular farm 19 out of 280 (7%) bullocks were lost (Wiseman et al 1979). In one disease which involved 13,108 cattle, 1,002 (7.6%) became infected and 30 cattle (3%) died (Schroeder and Moys 1954).