STUDIES ON SEVERE INFECTIOUS BOVINE RHINOTRACHEITIS IN BRITAIN

TWO VOLUMES

VOLUME 1

by

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I declare that the work presented in this thesis has been carried out by me. The clinical aspects of the investigation were carried out in conjunction with Drs. A. Wiseman and I.E. Selman, Department of Veterinary Medicine, the pathology in conjunction with Drs. E. Allan and H.M. Pirie and virology in conjunction with Dr. H.J.C. Cornwell, Department of Pathology.

Some of the material in this thesis has already been published in the following papers:-

(1) Allan, E.M., Obi, T.U., Wiseman, A., Cornwell, H.J.C., Selman, I.E., Msolla, P.M. and Pirie, H.M. (1978). The isolation of Mycoplasma bovis from pneumonic calves in Scotland. Vet. Rec., 103, 139.

(2) Msolla, P.M., Wiseman, A., Selman, I.E., Pirie, H.M. and Allan, E.M. (1979). Vaccination against infectious bovine rhinotracheitis. Vet. Rec., 104, 535-536.

(3) Wiseman, A., Msolla, P.M., Selman, I.E., Allan, E.M., Cornwell, H.J.C., Pirie, H.M. and Imray, W.S. (1978). An acute severe outbreak of infectious bovine rhinotracheitis: Clinical, epidemiological, microbiological and pathological aspects. Vet. Rec., 103, 391-397.

(4) Wiseman, A., Selman, I.E., Msolla, P.M., Pirie, H.M. and Allan, E.M. (1979). Infectious bovine rhinotracheitis. Vet. Rec., 104, 40-41.

(5) Wiseman, A., Selman, I.E., Msolla, P.M., Pirie, H.M. and Allan, E.M. (1979). The financial burden on infectious bovine rhinotracheitis. Vet. Rec., 105 (in press).

(6) Wiseman, A., Selman, I.E., Msolla, P.M., Pirie, H.M. and Allan, E.M. (1979). Role of markets in the spread of severe IBR. The Scottish Farmer, 49.

SUMMARY

A severe form of infectious bovine rhinotracheitis was confirmed in recently purchased fattening cattle in the north east of Scotland during the winter-housing period of 1977-78. During the remainder of 1978 and the subsequent winter-housing period, many similar incidents were investigated.

The first signs of illness noticed by the farmer were slight dullness, reduced appetite, serous ocular and nasal discharge, an increased rate and depth of respiration and the drooling of saliva. Pyrexia (103 - 107.5°F) was always detected on clinical examination. The serous lachrymation, which had resulted from conjunctivitis, became mucoid or mucopurulent as the disease progressed. Conjunctival oedema and granular lesions on the conjunctiva were seen in severe cases. The most common clinical sign was serous nasal discharge which became mucoid or mucopurulent in severe cases. Initially, congestion of the nasal mucosae was present but then yellowish-brown diphtheritic plaques developed. In these cases halitosis was always detected. Tachypnoea, hyperpnoea and frequent, soft, coughing were common. In particularly severely affected individuals, inspiratory respiratory distress developed as a result of obstruction of the upper respiratory tract. Pneumonia was rarely diagnosed except in terminal cases. Although drooling of saliva was also a common feature, the only abnormal finding was congestion of the oral mucous membranes. In addition to the respiratory and ocular signs, there was a sudden drop in milk yield, and even agalactia, when lactating dairy cattle became affected. Mildly affected animals recovered after about a week while severely affected individuals took longer to recover. The clinical response of severely affected cases to antibiotic therapy was variable.

The morbidity rate in many incidents was over 90 per cent. In a number of incidents fatalities did not occur, while in the others the average mortality rate was 2-3 per cent. At necropsy, inflammation of the upper respiratory tract with a variable degree of a diphtheritic pseudomembrane formation and numerous haemorrhages were characteristic features. In some cases the yellowish-brown necrotic debris was present in sufficient quantities to produce almost total obstruction of the upper respiratory tract and larynx. An acute exudative pneumonia involving the cranio-ventral lobes was present in every case. Thrombosis of the pulmonary veins and renal infarction were often present.

Virus was isolated more frequently from nasal swabs (75%) than from ocular swabs (67%) in the early stages of the disease. In 3 of the 4 outbreaks from which paired sera were obtained, a large number of animals had developed serum neutralising antibodies within a few weeks of the outbreak of disease.

Following a serological investigation in which 1152 sera from 114 herds, mainly in the Grampian and Strathclyde regions, were examined, antibodies were found in 140 samples (12%) and in 58 herds (51%). This figure is considerably greater than the prevalence of antibodies found in previous serological surveys. In the Grampian region, Holstein cattle had been imported into 5 herds and the prevalence of antibodies in these herds was significantly greater than in the other 35 herds.

The cultural characteristics of the new isolate (Strichen strain) were found to be similar to those of the prototype Colorado and Oxford strains. Although the mean plaque sizes of the Strichen and Colorado strains were significantly greater than that of the Oxford strain, the Strichen strain was considerably more resistant to the neutralising effect of circulating antibodies than either of the other 2 strains.

When susceptible cattle of varying ages were challenged intranasally with the Strichen strain of virus, clinical signs similar to, but less severe than, those observed in the field incidents developed. The clinical disease was most severe in the 2 week and 5 week old calves and fatalities occurred in both age groups. Although the Colorado and Oxford strains also induced clinical disease in susceptible cattle, the syndromes were less severe than that produced by the Strichen strain. When susceptible heifers were challenged intravaginally with the Strichen strain, infectious pustular vulvovaginitis developed. The vaccination of susceptible cattle with a pentavalent inactivated vaccine containing antigens derived from infectious bovine rhinotracheitis virus failed to confer a detectable degree of protection against challenge with the Strichen strain of virus.

The recent incidents of infectious bovine rhinotracheitis were considerably more severe than those previously recognised in this country. The most recent virus isolate, which had probably been imported into Britain with Holstein cattle, had biological characteristics different from the Oxford strain of the virus. These properties could explain, in part at any rate, the greater virulence of the Strichen strain of virus.

ABBREVIATIONS

All abbreviations used in different appendices or tables are shown at the beginning of the appendix or at the end of the table respectively.

REFERENCES

In the reference section, the contractions for the various journals quoted are those given in the World List of Scientific Periodicals, published by Butterworths, London.

STATISTICAL METHODS

The statistical methods used were the Chi-squared test, "Student's" t test and the coefficient of correlation. The calculations were carried out on an "Olivetti programma 101" desk computer (British Olivetti Ltd., Berkley Square, London). Unless otherwise stated, when a difference is described as "significant" (S) this implies that the probability of its resulting from chance is less than 5 per cent ($p < 0.05$). When a difference is described as "highly significant" (HS) this implies that the probability of its resulting from chance is less than 1 per cent ($p < 0.01$). When a difference is described as "very highly significant" (VHS) this implies that the probability of its resulting from chance is less than 0.1 per cent ($p < 0.001$).

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) is a severe upper respiratory tract disease characterised by anorexia, depression, pyrexia, nasal discharge and coughing. Lachrymation and conjunctivitis are often present. This disorder, which was first recorded in the United States of America in 1950's (Shroeder and Moys, 1954; Miller, 1955), was found to be most severe on large intensive dairy and beef units. Following these reports, the disease was diagnosed with increasing frequency in North America and in other parts of the world (Gibbs and Rweyemamu, 1977).

Infectious bovine rhinotracheitis was first recorded in Britain in 1962 in bullocks which developed conjunctivitis and nasal discharge (Dawson, Darbyshire, Loosemore, Paterson and Faull, 1962). The incidence of virus infection throughout the country was later found to be low (Dawson and Darbyshire, 1964) and it was generally accepted that IBR was a mild and economically unimportant disease. However, this pattern changed dramatically in 1977 when there was a sudden increase both in the incidence of disease and in its severity.

The aims of the investigations undertaken for this thesis were fourfold: to define the clinical, epidemiological, pathological and virological aspects of severe IBR, to ascertain if there had been an increase in the infection rate with IBR virus in the cattle population at large, to define the cultural characteristics and antigenic structure of the most recent isolate of IBR virus (Strichen strain) and then to compare its properties with those of the Colorado and Oxford strains, and finally to study the pathogenicity of the Strichen strain of IBR virus in susceptible cattle.

As a result of this work, it has been established that the recent severe outbreaks of IBR are characterised by anorexia, depression, nasal and ocular discharge as well as conjunctivitis, frequent coughing and the drooling of saliva. The morbidity rate has usually been over 90 per cent while the average mortality rate has been 2-3 per cent. This form of the disease predominantly affected recently purchased, beef animals although all classes of cattle were affected. The Strichen strain of IBR virus was antigenically distinct from the Colorado and Oxford strains and it produced a more severe clinical and pathological disease in susceptible cattle. Although the 3 strains behaved in a similar manner in tissue cultures, the Oxford strain produced significantly smaller plaques than the other 2 strains.

CHAPTER I

REVIEW OF THE LITERATURE ON INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS INFECTIONS

There has been a dramatic increase in the demand for livestock-derived foodstuffs, particularly beef and milk, within the last fifty years in the westernised countries. This has resulted in ever increasing numbers of animals being kept under more and more intensive conditions. It is not surprising, therefore, that this has led to the emergence of new disease problems which either had not been recognised hitherto or, although present, were considered to have been economically unimportant. Since many of the new intensive systems of cattle management were pioneered in the United States of America, it was only to be expected that many of these "new" disease syndromes were first recognised in that country.

Several disorders affecting both the digestive and respiratory tracts of cattle were recognised at about the same time; these were termed collectively "mucosal diseases" (Huck, 1957) or the "new pseudorinderpests" (Scott, 1964). One such syndrome which affected young cattle (two to eighteen months of age) was characterised by diarrhoea and gastroenteritis (Olafson, MacCallum and Fox, 1946); this condition was called "virus diarrhoea - New York". Later Pritchard, Taylor, Moses and Doyle (1956) reported a similar syndrome in Indiana; they called their disorder "virus diarrhoea - Indiana". Although these two "virus diarrhoea" syndromes closely resembled rinderpest, cross-immunity tests showed that neither was related aetiologically to rinderpest. In 1951 a "mucosal disease of cattle" characterised by profuse salivation, erosions and ulcerations of the oral mucosa and with a variable degree of diarrhoea was described in Iowa by Ramsay and Chivers (1953). Many of the severely affected animals also had a bilateral mucopurulent nasal discharge. Initially, the New York, Indiana and Iowa "mucosal diseases" were considered to be separate disease entities. However, the lesions were basically similar, although differing in degree, and cross-protection tests in cattle subsequently confirmed that the isolates were identical (Gillespie and Baker, 1959).

Salivation and a mucopurulent nasal discharge were the most obvious clinical signs of an upper respiratory disorder of dairy cows reported from California (Shroeder and Moys, 1954). A similar upper respiratory tract condition of feedlot cattle had been recognised as early as 1950 in Colorado where it was known colloquially as "red nose", "dust pneumonia", "necrotic rhinotracheitis" or "necrotic rhinitis" (Miller, 1955). Initially, Shroeder and Moys (1954) had considered

the condition they had seen in dairy cattle to be the "bovine virus diarrhoea - mucosal disease" complex but this was subsequently rejected as diarrhoea was not a prominent clinical feature. In addition, the diseases described by Olafson and others (1946) and by Ramsay and Chivers (1953) were transmitted by the parenteral administration of blood and splenic suspensions whereas the conditions reported by Shroeder and Moys (1954) and by Miller (1955) could only be transmitted to susceptible animals by exposing them intranasally to nasal secretions from affected cattle (McKercher, Moulton, Kendrick and Saito, 1955). Further work indicated that there were two distinct groups of "new" diseases, the first, which mainly affected the digestive tract (mucosal disease and virus diarrhoea) and the second which mainly affected the respiratory tract. The term "infectious bovine rhinotracheitis" (IBR) was adopted officially for the latter syndrome in 1955 (McKercher and others, 1955).

The condition described by Shroeder and Moys (1954) was characterised by a sudden drop in milk production, pyrexia (104-108°F) tachypnoea (Respiratory Rate = 30-60 per minute), drooling saliva, mucoid to mucopurulent nasal discharge and explosive coughing. Although early workers considered IBR to be alien to the United States (Shroeder and Moys, 1954; McKercher, Moulton and Jasper, 1954; Miller, 1955), the identification of specific antibodies in a stored sample of blood, which had been collected in 1941, indicated that the infection, and perhaps also the disease, existed prior to these initial reports (Gillespie, Lee and Baker, 1957). Following the isolation of the virus of IBR by Madin, York and McKercher (1956) and the development of specific serological tests, it was soon established that infection was widespread throughout the country particularly in areas where the livestock density was greatest i.e. the west and eastern parts of the United States. As the disease came to be recognised throughout the country it became obvious that the severity of the respiratory involvement could vary markedly and that other systems could be affected with IBR virus even in the absence of overt respiratory disease. The various syndromes associated with IBR virus infection are summarised in Tables 1 and 2.

The main clinical signs of IBR have been summarised and are presented in Table 1. This condition has occurred most frequently in feedlot cattle which were noticed to be dull, anorexic and markedly pyrexic (104-108°F). One of the most common presenting signs was a bilateral nasal discharge which was serous initially but changed to

mucopurulent as the disease progressed. Close examination of the nares in the early stages usually revealed an acute inflammation with ulceration of the mucous membranes. Yellowish-brown diphtheritic lesions or plaques were often seen in severe cases. Hyperpnoea and tachypnoea developed as well as sporadic coughing often with the expulsion of tracheal casts. Halitosis and mouth-breathing were stated to be a feature of both acute and chronic stages of the disease (Miller, 1955; Dawson and others, 1962; Curtis, Van Dreumel and Ditchfield, 1966). On auscultation of the chest the typical harsh respiratory sounds were considered to have been referred from the trachea (Curtis and others, 1966).

There was usually conjunctivitis with profuse lachrymation which resulted in the matting of the hair on the cheeks. In severe cases there was oedema of the conjunctiva as well as punctate haemorrhages and granular lesions. The drooling of saliva was observed in some animals without there being any visible lesions.

The clinical signs were usually more severe in young animals than in adult (Shroeder and Moys, 1954; Curtis and others, 1966). In dairy cows, in addition to slight depression, pyrexia and tachypnoea, there was a sudden drop in milk production which usually returned to normal within 1-2 weeks. When abortions occurred, they were uncomplicated except for the retention of the placenta in extremely pyrexic animals. In both dairy and feedlot cattle, recovery was usually complete within 5 to 10 days provided the animals did not develop secondary bacterial infection and pneumonia. In protracted cases, there was almost invariably a marked weight loss.

The morbidity rate ranged from about 20 per cent up to almost 100 per cent in severe outbreaks. This tremendous variation was considered to be dependent upon the circumstances surrounding each particular outbreak because, in general, the morbidity rates were found to be much higher in intensive operations of feeder and dairy animals. However, that IBR was not exclusively a disorder of intensively-kept animals was confirmed by McKercher and Straub (1960) who isolated the virus from range beef cow and calves which had developed ocular discharge and congestion of the muzzle. In contrast to the relatively high morbidity rate, the mortality rate is usually low, ranging from nil to 10 per cent (average 2-3) (McKercher, Moulton, Madin, Kendrick, 1957; McKercher, 1959; Curtis and others, 1966).

It has been claimed that there is a seasonal incidence of disease since the number of clinical cases is highest during the autumn and early winter months (Jensen and Mackey, 1979). However, it is at this time that the greatest numbers of susceptible animals enter the feedlots and, furthermore, it has been amply demonstrated that IBR can affect susceptible animals under natural conditions of exposure irrespective of the time of year. While it is possible that the severity of IBR may not be markedly increased during the severe winter weather experienced under feedlot conditions, it has been suggested that "stress factors" can result in the excretion of virus by carrier animals and this may be responsible for the initiation of new outbreaks (McKercher, 1959).

The clinical picture is considerably more severe in feedlot cattle than in those kept in less intensive systems (Jensen and Mackey, 1979; McKercher, 1959). With this in mind, Gibbs and Rweyemamu (1977) suggested that the feedlot system of husbandry was ideally suited for the perpetuation and transmission of IBR virus. Under feedlot conditions young stock are continually being introduced at an age when they have lost any passive immunity and, therefore, are fully susceptible. This constant introduction of susceptible animals also maintains active infection and the close contact between individuals facilitates the transmission of virus. The collection of cattle from diverse backgrounds in terms of previous exposure to disease also ensures the continual introduction of a wide variety of pathogens including IBR virus.

Infectious bovine rhinotracheitis is not a fatal disease unless severe stress conditions result in extensive lesions or complications arise following secondary bacterial infections. In mild and early cases, there was a moderate degree of hyperaemia and oedema of the nasal mucosa accompanied by a small quantity of serous exudate in the nasal cavity. In severe cases, the inflammation was much more intense and widespread extending into the pharynx, larynx and trachea. The exudate became more copious and assumed an increasingly catarrhal nature. Later, it became fibrinous and adhered to the walls of the nasal passages, collecting on the larynx and to some extent in the trachea, forming a yellowish-brown pseudo-membrane of variable thickness. This pseudo-membrane partially occluded the nasal passages. Petechial and ecchymotic haemorrhages were commonly found in the frontal sinuses, nasopharynx and trachea. In severe advanced cases, there was extensive necrosis of the upper respiratory tract mucosa and macroscopically this

appeared as a diphtheritic membrane covering the mucosa (Miller, 1955; McKercher and others, 1957; Kahrs, 1977). This pseudomembrane also partially filled the palatine and submaxillary sinuses (Shroeder and Moys, 1954; McKercher, 1959; Curtis and others, 1966; Jensen and Mackey, 1979). In addition to the above, Miller (1955) reported that there was a widespread purulent pneumonia, alveolar emphysema, minute caseation necrosis in the sinuses, ulceration of the mucosa of the abomasum and severe enteritis. In chronic cases abscesses were frequently seen in the lungs and liver.

Microscopical changes in the early and mild cases were characteristic of an acute catarrhal inflammation with excessive amounts of mucous present on the epithelium of the upper respiratory tract. The submucosa was found to be oedematous and infiltrated with fibrinous exudate; neutrophils, lymphocytes and mononuclear macrophages became abundant. In advanced and acute cases, some areas of epithelium were found to be degenerated and desquamated (Jensen, Griner, Chow and Brown, 1955; McKercher, 1959). Bronchopneumonia was a common finding in the majority of fatal cases; the bronchioli were filled with purulent exudate while the alveolar air spaces were filled with oedema containing fibrin, leucocytes and erythrocytes. In addition to lesions in the respiratory tract, ulceration of the mucosa of the rumen, abomasum and a severe enteritis have been reported in fatal cases of IBR examined fully at necropsy (Miller, 1955; Van Kruiningen and Bartholomew, 1964; Curtis and others, 1966; Jensen and Mackey, 1979). Multiple pin-point foci of necrosis have been found scattered throughout the liver parenchyma (Curtis and others, 1966; Van Kruiningen and Bartholomew, 1964). In some cases, pathological lesions have also been found in the brain, kidneys and spleen (Gibbs and Rweyemamu, 1977).

The virus causing IBR was first isolated from the nasal washings of experimental calves which had been infected with the nasal washings of naturally infected cases (Madin and others, 1956). Since the virus isolate had growth and morphological characteristics indistinguishable from those of herpes simplex virus, it was classified as a herpesvirus (Armstrong, Pereira and Andrewes, 1961). Initially the herpesvirus study group of the International Committee for the Taxonomy of Viruses suggested that all herpesviruses for which the usual natural hosts are members of the family Bovidae should be

designated Bovid herpesvirus 1, 2, 3 etc. (Roizman, 1973). However, since the family Bovidae is diverse consisting of 128 species, most of which are ruminants of no veterinary importance, McKercher (1973) and Gibbs and Rweyemamu (1977) have opted for a different system of classification to avoid unnecessary confusion. Under the latter system, the name bovine herpesvirus 1 (BHV 1) has been given to all virus isolates serologically related to the virus of IBR.

Bovine herpesvirus 1 possesses most of the properties of the herpesvirus group which are deoxyribonucleic acid viruses. The virion consists of an enveloped icosahedral nucleocapsid composed of 162 elongated hollow, protein subunits (Capsomeres) which surround the nucleic acid. The diameter of the enveloped particle ranges from 120-180nm. The envelope, which is acquired as the nucleocapsid buds through the nuclear membrane, is important for infectivity and confers upon the virus the physical properties of ether, chloroform, acid and disinfectant sensitivity (Armstrong and others, 1961; Curtis and others, 1966; Straub, 1965). The virus is stable between pH 6.0-9.0 but labile at pH 4.5-5.0 (Griffin, Howels, Crandell and Mauerer, 1958) and has a buoyant density ranging from 1.249-1.254g.cm^{-3} (Bagust, 1972).

Cell cultures of bovine origin are highly susceptible to this virus and extensive cytopathic effects are produced in cells derived from embryo kidney (Madin and others, 1956; Greig, Bannister, Mitchell and Baker, 1958; Gillespie and others, 1959), embryo skin (Greig and others, 1958), embryonic lung and lymph node (McKercher, Straub, Saito and Wada, 1959b), testis (Bagust, 1972; House, 1972), kidney, adrenal gland, pancreas, thymus and thyroid (Chaetam and Crandell, 1957; Schwartz, York, Zirber and Estela, 1957; Armstrong and others, 1961; Dawson and others, 1962; French, 1962a; Snowdon, 1964).

The site of viral replication is in the nucleus and virus particles can be identified from 4 to 6 hours after infection. The virions are released from the nucleus into the cytoplasm and lie in proximity to the cytoplasmic membrane from which an additional lipoprotein coat is derived. Infected cells become rounded, frequently ballooned, with small refractile syncytia being formed and, as the cytopathic effect progresses, strands of cytoplasm can be seen linking the affected cells. On microscopic examination of tissue cultures, large intranuclear, often eosinophilic (Cowdry type A), inclusion bodies can be identified in infected cells (Cheatam and Crandall, 1957).

However certain isolates appear to lack this ability (Madin and others 1956; York and others 1957).

Since the severity of the clinical syndrome varies greatly (Miller 1955; Dawson and others 1962) and BHV 1 causes syndromes other than respiratory disease (Table 2), it was suggested that the various isolates differed antigenically (Gillespie and others 1959; McKercher and Wada 1964; Mohanty and Lillie 1970). However, Buening and Gratzek (1967) and House (1972), who compared the neutralisation kinetics of four BHV 1 isolates, found only minor antigenic differences and the behaviour of the four viruses in tissue culture was identical.

Infectious bovine rhinotracheitis was considered to be one of the most important causes of economic loss in cattle fattening operations (Jensen, Griner, Chow and Brown, 1955; McKercher, 1959), and consequently considerable efforts were made to develop vaccines. Initially, the virus was inactivated but later attenuated live virus vaccines became available (Kendrick, York and McKercher, 1956; York and Schwartz, 1956). The main disadvantage of these early live vaccines was that they caused abortions when pregnant females were vaccinated between the sixth and eighth months of gestation. Consequently, their use was restricted to feedlot animals. However, this abortifacient property was useful in that it allowed young in-calf range heifers to be aborted on admission to the feedlot. Whereas abortion was the main obvious disadvantage of live vaccines, the potential hazard resulting from vaccinates shedding vaccine virus, or remaining latent carriers, made it necessary to examine alternative types of vaccine and immunisation protocols. Despite these difficulties, when routine vaccination against IBR was undertaken on a large scale in North America it was found that there was a drop in the incidence of respiratory disorders which were frequently diagnosed as shipping fever. This finding implied that IBR was part of the shipping fever complex (Jericho, Magwood and Stockdale 1976).

Infectious bovine rhinotracheitis is, to all intents and purposes, a disease of cattle only. However there is a single report of a natural infection in goats (Mohanty, Lillie, Corselius and Beck, 1972). Two weeks after being shipped to a milking herd for breeding, several goats developed severe respiratory illness. A virus identified as IBR by neutralisation method was isolated from both the nasal and ocular discharges. The IBR/IPV virus has also been isolated from cases of vaginitis and balanitis in swine and from healthy swine (Saxegaard and Onstad, 1967).

As has already been stated, infection with BHV 1 has also been associated with diseases in systems other than the respiratory tract (Table 2). A disorder of the reproductive tract of female cattle, characterised by pustular lesions of the genitalia, had been recognised in central Europe during the late 19th and early 20th centuries and the names given to this syndrome included vesicular venereal disease, vesicular vaginitis, coital exanthema, coital vesicular exanthema and blaschenausschlag (Witte, 1933). A similar disorder had occasionally been reported in North America where it was termed infectious pustular valvovaginitis (IPV) by Kendrick and others (1958).

During the studies of IBR and IPV, Kendrick and others (1958) found out that serum from a calf which had recovered from IBR neutralised IPV virus. This suggestion of similarity led to comparative studies of the 2 viruses, which were subsequently shown to be indistinguishable from each other.

Bouters, Vandeplassche, Florent, Leunen and Devos (1960) described a condition in Dutch cattle characterised by balanoposthitis and orchitis. This condition was then termed infectious pustular balanoposthitis (IPB). The virus isolated from affected cases caused IPV in heifers. Huck, Miller, Evans, Stables and Ross (1971) reported an outbreak of penoposthitis in bulls at an artificial insemination centre in Britain and found that the condition was associated with IBR/IPV virus infection. Clinically, there was haemorrhage and a mucopurulent discharge from the preputial orifice. On penial extrusion, lesions were found to range from localised papular roughened appearance to distinct red spots or frank haemorrhages. The distinct red spots progressed to ulcers but libido did not appear to be decreased. In advanced cases adhesions prevented extrusion of the penis.

There has been considerable conjecture with regard to the role of IBR virus as a cause of infertility. However, it has been well established that severe cases of IPB may result in temporary or permanent impairment of a bull's ability to mate (Huck and others, 1971), and that BHV 1 contaminated semen may cause endometritis (Bouters and others, 1964; Kendrick and McEntee, 1967; Parsonson and Snowdon, 1975) and IPV (Conradi, Hubrig and Wohanka, 1960). On the other hand, Huck and others (1971) and Parsonson (1964) reported that BHV 1 had no effect on the semen quality and claimed that there was no reduction in conception rate following the use of BHV 1 contaminated semen. Nonetheless, a number

of investigators have reported a lowered conception rate in herds after BHV 1 contaminated semen had been used (Kendrick and McEntee, 1967; White and Snowdon, 1973; Loretu, Marinov, Genov and Bohnel, 1974; Parsonson and Snowdon, 1975), although Allan, Dennett and Johnson (1975) and Parsonson and Snowdon (1975) reported that in general BHV 1 did not appear to interfere with fertility in natural breeding. In South Africa and Kenya, there is no doubt that a genital disease known as "Epivag" (epididymitis vaginitis), and first described by Daubney, Hudson and Anderson (1938), results in infertility. Recently this condition has been shown to be caused by BHV 1 (Kaminjolo, Nyaga, Omuse and Mutiga, 1975).

Conjunctivitis has been reported to occur alone without any accompanying upper respiratory tract lesions (Abinanti and Plumer, 1961; St. George, 1965; Timoney and O'Connor, 1971). The syndrome was characterised by profuse serous to purulent lachrymal discharge, intense swelling of the palpebral conjunctiva which was occasionally covered by fine petechial haemorrhages. Protrusion of the conjunctiva from below the third eyelid had been observed as well as injection of the bulbar conjunctival vessels. In some outbreaks, numerous granular lesions were seen on both the bulbar and palpebral conjunctiva. Although BHV 1 has been isolated from 47 per cent of 32 ocular carcinomas (Taylor and Hanks, 1969), it is unlikely to be the primary aetiological agent (Russell, Wynne and Loguvam, 1956).

Johnston, Simmons and McGavin (1962) in Australia described an outbreak of encephalitis in a group of calves, which had been purchased for veal production, and the virus isolate from this outbreak was serologically indistinguishable from IBR. Clinically affected animals showed slight incoordination, which later progressed to ataxia. In the terminal stages affected animals were so ataxic that they stumbled and fell on attempting to walk. Clonic spasms of the legs, neck and lumbar muscles as well as blindness were features of this form of the disease. Some animals recovered but remained blind.

Profuse diarrhoea, which may be of a haemorrhagic nature, has been observed to be a feature of BHV 1 infection in young calves in which mortalities up to 100 per cent were experienced (V n Kruiningen and Bartholomew 1964; Curtis and others 1966; Lomba, Wellemans, Bienfet and Leunen, 1973; Reed, Bicknell and Bury, 1973; Morin, Lariviere and Lallier, 1976). Lomba and others (1973) reported that, not only did

IBR cause high mortalities in neonates, but also postpartum complications in cows; both syndromes were responsible for major economic losses.

There has been only one report of chronic dermatitis associated with BHV 1 infection. This affected the perineal skin of bulls and BHV 1 was isolated from both the semen and skin scrapings of the bulls (Bwangamoi and Kaminjolo, 1971).

Following the discovery that the viruses responsible for causing IBR and IPV were identical (Kendrick and others, 1959) McKercher and Theilen (1963) offered an explanation of how the newer syndrome (IBR) could have developed. Imports of live cattle into the United States from Europe ceased in 1930 and so the virus must have been imported before then, presumably in cattle which had recovered from IPV. It was suggested, therefore, that the virus then had become adapted to the respiratory tract of feedlot cattle since they are sexually inactive. The large numbers of susceptible animals being fattened in close proximity resulted in the development of a new severe respiratory syndrome.

Since its initial reports in North America, the disease has been reported from virtually every country in which large numbers of cattle are reared (Table 3). When first recognised in Europe, IBR was reported as being a relatively mild upper respiratory tract disorder (Dawson and others, 1962; Straub, 1978a) compared to the disease reported originally from North America (Miller, 1955). The great difference in clinical severity was probably due to the fact that much smaller numbers of cattle were at risk on the European farms. However, the whole disease scene changed dramatically in 1974 when a particularly severe upper respiratory tract condition swept across Belgium, France, Germany and Netherlands (Van Nieuwstadt, Straver and Holzhauer, 1977; Straub, 1978). The clinical and epidemiological features of this new syndrome resembled severe IBR and on further investigation, this was confirmed. In 1977, similar incidents of a severe upper respiratory tract disease were recognised in Britain and this too was subsequently confirmed as being severe IBR. Since then, many outbreaks have been reported from almost every area of this country.

A detailed investigation of fifteen such incidents of IBR provides the basis for this thesis.

TABLE 1. The major clinical signs which have been reported from field incidents of infection of the respiratory tract with bovine herpesvirus 1 (infectious bovine rhinotracheitis).

MAJOR CLINICAL SIGNS OF INFECTIOUS BOVINE RHINOTRACHEITIS
Dullness. Reduced appetite - anorexia in severe cases. Pyrexia (104-108°F).
Bilateral serous to mucopurulent nasal discharge - blood-tinged in severe cases.
Halitosis. Congestion and diphtheritic plaques on nasal mucosa.
Frequent coughing - occasionally, with the production of tracheal casts.
Whistling or wheezing respiratory sounds. Tachypnoea (Respiratory Rate = 30-60/minute).
Hyperpnoea - dyspnoea with mouth-breathing in severe cases.
Profuse serous lachrymation resulting in matting of the hair on the cheeks.
Slight to intense bilateral conjunctivitis with oedema and punctate haemorrhages in severe cases.
Excessive frothy or ropy salivation.
A sudden reduction in milk yield in lactating animals - agalactia in severe cases.
Sub-cutaneous emphysema posterior to the withers, along the belly and on the thighs.
Diarrhoea - haemorrhagic in severe cases.
A degree of hyperexcitability.
Marked loss of body weight in protracted cases.

After Curtis and others (1966)
Dawson and others (1962)
McKercher and others (1957)
Miller (1955)
Shroeder and Moys (1954)

Table 2. The major clinical signs of syndromes, other than infectious bovine rhinotracheitis, which have been reported from field incidents of infection with bovine herpesvirus 1.

MAJOR CLINICAL SIGNS
(1) REPRODUCTIVE TRACT INFECTION
(a) Infectious pustular vulvovaginitis
Slight dullness. Reduced appetite - anorexia in severe cases. Pyrexia (103-104.5°F).
Frequent micturition. Back arching. Tail swishing. Mucoid to mucopurulent vaginal discharge.
Oedematous and congested vulva and posterior vagina with numerous pustules (1-2mm diameter).
Anterior vaginitis and cervicitis.
Slight reduction in milk yield in lactating animals.
Abortion - commonly from 4th to 7th month of gestation. Retention of placenta may then occur.
Reduced conception rates in infected herds.
(b) Infectious pustular balanoposthitis
Mucopurulent preputial discharge - blood-tinged in severe cases. Oedematous prepuce.
Localised roughened lesions at penile-preputial junction and all over prepuce.
In severe cases - widespread ulceration of epithelium resulting in exposure of sub-epithelial tissues.
In complicated cases - adhesions of penis can occur which may result in penile distortion and loss of libido.

After Allan and others (1975)
Bouters and others (1960)
Crane and others (1964)
Daubney and others (1938)
Huck and others (1971)
Kaminjolo and others (1975)
Kendrick and others (1958)
Loretu and others (1974)
Parsonson and Snowdon (1975)
Kendrick and Straub (1967)

Table 2. (Cont'd)

MAJOR CLINICAL SIGNS
(2) OCULAR INFECTION
Profuse bilateral serous to purulent lachrymation. Matting of hair on cheeks.
Protrusion of conjunctiva below third eyelid. Injection of bulbar conjunctival vessels.
Oedema and fine petechial haemorrhages on palpebral conjunctiva.
Conjunctival oedema with numerous granular lesions.
Slight nasal discharge. Coughing. Pyrexia (103-104.5°F).
After Abinanti and Plumer (1961) Quin (1961) St. George (1965) Timoney and O'Connor (1971)
(3) CENTRAL NERVOUS SYSTEM INFECTION
Circling. Head pressing. Grinding of teeth. Pyrexia (104-107°F).
Slight incoordination progressing to ataxia.
Clonic spasms of legs, neck and lumbar muscles.
Recovered animals may remain blind. Death occurs in 4-5 days.
After Bartha and others (1969) Gough and James (1975) Johnstone and others (1962) Reed and others (1973)

Table 2. (Cont'd)

MAJOR CLINICAL SIGNS
(4) DIGESTIVE SYSTEM INFECTION
Diarrhoea - haemorrhagic in severely affected young calves. Often accompanied by conjunctivitis and signs of respiratory tract infection. Frequently fatal in young calves.
After Curtis and others (1966) Lomba and others (1973) Van Kruiningen and Bartholomew (1964)
(5) INTEGUMENTARY SYSTEM INFECTION
Chronic dermatitis of perineal skin of bulls standing at artificial insemination centre.
After Bwangamoi and Kaminjolo (1971)

Table 3. The dates on which infectious bovine rhinotracheitis was first confirmed in each country.

YEAR	COUNTRY	AUTHORS
1954	U.S.A.	Shroeder and Moys.
1955	U.S.A.	Miller.
1959	New Zealand	Webster and Manktelow.
1960	West Germany	Grunder and others.
1961	Canada	Studdert and others.
1962	United Kingdom	Dawson and others.
1964	Australia	Snowdon.
	Chad and Cameroon	Provost and others.
	Rumania	Coman.
	Italy	Moretti and others.
	Holland	Straver and others.
1967	France	Faye and others.
	Hungary	Csontos and Maczko.
	Yugoslavia	Bratanovic and others.
1968	Greece	Stouraitis and Cardassis.
1969	Czechoslovakia	Mensik and Rozkosny.
1970	East Germany	Hahnefeld and others.
1971	Tanzania	Rweyemamu and Staak.
1972	Japan	Nishimado and others.
1973	Belgium	Lomba and others.
	Korea	Park and others.
1974	Mexico	Martell and others.
	Poland	Baczynski and others.
	Egypt	Hafez.
	Iran	Bagdadi and Martin.
1975	Cyprus	Gibbs and others.

After Gibbs and Rweyemamu (1977)

CHAPTER 2

A DETAILED INVESTIGATION OF 15 INCIDENTS OF INFECTIOUS BOVINE RHINOTRACHEITIS

INTRODUCTION

Infectious bovine rhinotracheitis was first described in Britain by Dawson and others (1962), although the same incident had been reported previously by Darbyshire and others (1962). The outbreak occurred during autumn in store and fattening bullocks which had been bought from various sources in England. Clinical signs developed 4 weeks after the final group of purchased animals had arrived and had been put into covered straw yards. The presenting features were profuse lachrymation, severe conjunctivitis with marked oedema and punctate haemorrhages. There was a bilateral serous to mucopurulent nasal discharge with congestion of the nasal mucosa and focal areas of shallow ulceration. Moist coughing was heard occasionally and several animals had slight diarrhoea. Within a week of the first case being noticed, about 80 per cent of the cattle had developed clinical evidence of disease although none died. Since this outbreak occurred in Oxfordshire, the virus isolate was called the "Oxford-strain" of IBR virus.

Darbyshire and Shanks (1963) reported another outbreak of IBR in which a suckled cow and her six month old calf were affected while at pasture in the autumn. The farmer complained of profuse serous lachrymation which was so copious in the cow that it had caused scalding of the face. On close examination, conjunctivitis with oedema of the eyelids was found. Both animals also had a slight purulent nasal discharge although coughing was not heard. Only these two animals out of 45 homebred cows and their calves were affected initially although one further calf developed conjunctivitis with lachrymation five weeks later. The clinical signs persisted for 3-4 weeks and since this outbreak occurred in Aberdeenshire, the virus isolate was called the "Aberdeen strain" of IBR virus.

In addition to respiratory disease, cattle infected with bóvine herpes virus 1 can develop reproductive, nervous and enteric disease (Gibbs and Rweyemamu, 1977), although it is unusual for more than one system to be infected at the same time. Nevertheless, Collings, Gibbs and Stafford (1972) reported concurrent respiratory and genital disease in a herd of 140 dairy animals in Somerset. Most of the cattle had been bought-in as maiden heifers or in-calf cows from various parts of Britain and Eire. Two to three weeks after the cows had been housed together for the first time, many were heard to be

coughing. Some of these were also seen to have profuse lachrymation with extensive soiling on their cheeks, congestion as well as oedema of the conjunctiva. There was a bilateral mucopurulent nasal discharge and congestion of the nasal mucosa. Approximately 30 per cent of the cows developed respiratory and genital signs. All these cows had been served by one young bull which developed a blood-stained preputial discharge, while the penis remained normal.

Prior to the above incident, Huck and others (1971) had reported the characteristic features of infectious balanoposthitis in bulls. The outbreaks described by Huck and others (1971) and by Collings and others (1972) occurred during the months of October to December and, from both, a virus serologically indistinguishable from IBR was isolated.

When first observed, infectious bovine rhinotracheitis in Britain was a mild syndrome characterised by serous lachrymation, conjunctivitis, nasal discharge and occasional coughing (Dawson and others 1962; Darbyshire and Shanks 1963; Collings and others 1972). However, this situation changed dramatically in 1977 and during the past two winter housing periods (1977-78 and 1978-79), there has been a marked increase nationwide in both the number of incidents of IBR and in their severity.

The clinical, epidemiological, microbiological and pathological features of 15 outbreaks of the "new" severe form of IBR will be presented in this chapter. One incident will be described in detail while relevant features of the remaining 14 outbreaks have been summarised and are presented in Appendix 1.

MATERIALS AND METHODS

(1) Disease incidents

Possible outbreaks of IBR were referred to us by practising veterinary surgeons and a farm visit was arranged within 48 hours of receiving this information. Interesting cases for further clinical, pathological and microbiological investigations were usually purchased at this time. Nasal and/or ocular swabs were taken for virus isolation and the bleeding of animals for serum antibody assay was also arranged.

(2) Clinical examination

Individual animals with signs of respiratory disease were examined on the farm and, when any were purchased for further study, they were examined again on admission to the Veterinary School. It is the findings from this second examination that are recorded in the individual case histories presented in Appendix 1.

The following clinical terminology has been used:-

(a) Presenting signs. These include the clinical abnormalities detected on the farm at the first veterinary clinical examination and also the farmer's reasons for seeking veterinary advice.

(b) Respiratory rate. The resting respiratory rate of a healthy bovine animal in a cool environment is less than 30 respirations per minute. Tachypnoea was considered to be present when the resting respiratory rate was equal to or greater than 30 respirations per minute.

(c) Respiratory depth. During the respiratory cycle in a healthy bovine animal, the movement of the abdominal muscles on the right side is almost imperceptible. Hyperpnoea was considered to be present when it was obvious that the abdominal muscles were being used at rest to assist respiration. Animals were considered to be slightly hyperpnoeic when their abdominal respiratory effort was just noticeable, to be moderately hyperpnoeic when there was an obvious increase in their abdominal effort and to be grossly hyperpnoeic when a marked abdominal effort was involved and the individual was seen to rock antero-posteriorly while breathing.

(d) Dyspnoea. The presence of respiratory distress in cattle can only be assessed objectively. An animal with a marked abdominal

respiratory effort and respirations that could be heard without the use of a stethoscope was considered to be dyspnoeic. When an individual was seen to be mouth-breathing with its tongue protruding and was heard to be grunting on expiration, it was considered to be severely dyspnoeic.

(e) Auscultation. The boundaries of the area of pulmonary auscultation extend from the point of the elbow dorsally to the posterior tip of the scapula then posteriorly to the eleventh inter-costal space and finally antero-ventrally to the point of the elbow.

(f) Adventitious lung sounds. Two main types of adventitious sounds are recognised in cattle with pulmonary disease; crackles which term is self-explanatory and rhonchi which include both low and high pitched squeaks.

(g) Rectal temperature. The normal rectal temperature range in adult cattle is from 100.5°F to 102.5°F and an animal was considered to be pyrexic when its rectal temperature was equal to or greater than 103°F.

(3) Pathological examination

All the animals were slaughtered humanely and exsanguinated. Samples of tissue for light microscopy were taken from nasal conchae, nasopharynx, trachea, bronchi, lung parenchyma, liver, abomasum, kidney and brain. The samples were collected in 10 per cent formol saline, corrosive formol and Carnoy's fixatives. They were then dehydrated and cleared in a double embedding series before embedding in paraffin wax under vacuum. Sections of 4-5 microns thick were cut with a rotary microtome and stained with haematoxylin and eosin. When specific features were to be demonstrated, the following stains were also used: alcian blue/periodic acid-schiff at pH 2.6 (mucus), martius scarlet blue (fibrin and globule leukocytes) and phosphotungstic acid haematoxylin (inclusion bodies).

(4) Ultrastructural examination

Electron microscopic examination of stained sections made from tracheal mucous membrane was carried out using an AEI 6B. Small pieces of tracheal mucous membrane, which were excised from each animal within 5-10 minutes of its being killed, were fixed in a mixture

of 1.3 per cent paraformaldehyde and 1.6 per cent glutaraldehyde according to Karnovsky (1965). The samples were fixed for from 4 to 6 hours, washed in a 0.067 M cacodylate rinsing solution for up to 12 hours and post-fixed in 1 per cent osmium tetroxide (Millonig's phosphate pH 7.2) for 1 hour. They were dehydrated in 70 per cent, 90 per cent and 100 per cent ethyl alcohol for 3 periods of 5 minutes, 2 periods of 5 minutes and 4 periods of 15 minutes respectively, then rinsed in propylene oxide for 3 periods of 10 minutes. Individual samples were embedded in gelatin capsules in an araldite mixture consisting of equal amounts of resin (CY212) and hardener (HY 964) plus 1 per cent accelerator (HY 960) (Polaron Equipment Ltd., Hertfordshire) according to the following protocol: a period of 1 hour in a 50/50 mixture of propylene oxide and araldite, an overnight soak in an 80 per cent:20 per cent mixture of propylene oxide and araldite, transferred to fresh araldite which was then polymerised by heating at 60°C for 48 hours.

Tissue culture cells showing typical BHV1 cytopathic effect were removed from the glass surface by means of rubber policeman and spun at 672 g for 5 minutes in a GF-6 MSE refrigerated centrifuge. The resultant pellet was cut into small pieces which were then fixed by the method described above for the tissue samples.

Ultrathin sections of approximately 500 A° were cut using an LKB-ultratome and sections were mounted on copper grids. The sections were stained in 20 per cent uranyl acetate for 1 minute (Watson, 1958), rinsed in methanol and then stained in lead citrate for 1 minute (Reynolds, 1963) before being finally rinsed in 0.02N sodium hydroxide.

(5) Microbiological examination

(a) Bacterial isolation and identification

Samples of tissues and exudates were collected in sterile phosphate buffer saline (PBS) (pH 7.3). An aliquot from each sample was inoculated onto 2 plates of 10 per cent horse blood agar and a single plate of MacConkey's agar. One horse blood and one chocolate blood agar plate were incubated microaerophilically and the rest incubated aerobically, all at 37°C. After 24-48 hours incubation, estimates were made of individual colony numbers. Individual species of bacteria were identified according to the methods of Cowan and Steel (1965).

(b) Mycoplasmal isolation and identification

Duplicate samples of tissue (0.5-1.0cm^3) were collected into 1.8ml of liquid media for the detection of glucose fermenting mycoplasmas. These samples were lightly chopped and incubated at 37°C for 30-45 minutes. Serial ten-fold dilutions of each sample were made by transferring 0.2ml into 1.8ml of the appropriate broth, each being diluted to 10^{-6} of the original tissue sample. The inoculated broths were incubated at 37°C and subcultured onto solid media as soon as a colour change in the broth was apparent. Even if no colour change had occurred, they were subcultured at day 3 and day 7 post-inoculation. All solid media were incubated in an atmosphere of 5 per cent carbon dioxide in air.

Glucose fermenting mycoplasmas were isolated in glucose serum broth (Gourlay and Leach, 1970) and identified by immunofluorescence. This was based on the indirect method of Rosendal and Black (1972), slightly modified in that firstly, the agar blocks were fixed to a glass slide with a wax/vaseline mixture and secondly, the background agar autofluorescence was reduced by counterstaining with 0.1 per cent Evan's blue. Specific mycoplasma antisera were supplied by the WHO/FAO Reference Laboratory (Aarhus, Sweden) and used with a goat antirabbit serum conjugated with fluorescein isothiocyanate.

Urea-splitting mycoplasmas were detected in U3 broth which differed from U2 broth of Howard and Gourlay (1973) by the addition of 0.25 mg per ml of magnesium sulphate. Ureaplasma species were identified as described by Gourlay (1968).

Arginine-splitting mycoplasmas were grown in a modified medium used for the detection of Ureaplasma species (Taylor-Robinson, Haig and Williams, 1967). This broth contained 0.5 per cent yeast extract (Difco, Difco Laboratories, Michigan, U.S.A.), 0.2 per cent arginine, and 0.025 per cent thalium acetate (pH 7.0). *Mycoplasma arginini* was identified by immunofluorescence using the method already described for the identification of glucose fermenting mycoplasmas.

(c) Viral isolation and identification

Cell cultures were prepared for virus isolation from bovine embryonic kidney (BEK), calf kidney (CK) and calf testis (CT) by the following methods.

Primary BEK and CK cultures were prepared from bovine embryos, obtained from cows mainly in the last trimester of pregnancy, and from 1-4 week old calves respectively. Kidneys from both the dead embryos and calves were collected asceptically into a sterile beaker and the cortices finely minced. The resulting tissue fragments were washed with phosphate buffer saline (pH 7.3) until they appeared free of blood. They were then washed once with 0.25 per cent trypsin (Gibco, Biocult, Glasgow, UK) and the supernatant discarded. Fresh 0.25 per cent trypsin was added and then incubated at 37°C and the supernatant was again discarded. A further quantity of fresh 0.25 per cent trypsin was added and the tissues trypsinised at 37°C for 25 minutes. The resulting supernatant containing the cells was collected in a sterile Erlenmeyer flask and stored at 4°C after 1-2 ml of foetal bovine serum (FBS) had been added. The above process was repeated until sufficient cells had been collected. The resultant cell suspension was filtered through a double layer of gauze, put into graduated 15 ml centrifuge tubes and the cells sedimented by centrifugation at 146.6g for 15 minutes using a refrigerated centrifuge. The packed cell volume (PCV) was read and the cells were suspended in 100 times their volume in medium M199 containing 1 per cent non-essential amino acids, 5.6 per cent sodium bicarbonate, 1 per cent 3 molar magnesium chloride, tylosin 50 µg/ml (Tylan; Elanco Products Ltd., Hants, UK), penicillin 100 units/ml and dihydrostreptomycin 100 µg/ml (Streptopen; Glaxovet Ltd., Middlesex, UK) and 10 per cent FBS. Twenty ml of cell suspension were inoculated into 8 ounce prescription bottles and incubated at 37°C. Complete monolayers were generally formed within 7 days.

Secondary cultures were prepared by trypsinising monolayers grown in 8 ounce prescription bottles. The cell sheet was washed twice with 20ml of pre-warmed (37°C) PBS free of calcium and magnesium ions but containing 0.02 per cent sodium versenate. One ml of the same solution containing 0.25 per cent trypsin was inoculated into each of the 8 ounce prescription bottles which were left in the "hot-room" at 37°C until all the cells had detached. The cells were then resuspended in M199 medium as described above and re-seeded into 8 ounce bottles or tubes.

Primary CT cultures were prepared from testes obtained by a sterile surgical technique from 1-4 week old calves. The testicular pulp was exposed by cutting through the tunica albuginea. The initial procedure for obtaining the cells was identical to that already described for the primary BEK and CK cultures. After sedimentation, the cells were suspended in a known volume of M199 medium. A 1:10 dilution in 1 per cent trypan blue was counted in a Neubauer haemocytometer. The cells were finally seeded at 3×10^5/ml concentration and formed complete monolayers in 4-5 days.

Secondary cultures were prepared in the same manner as described above for the secondary BEK and CK cultures.

Cold overnight trypsinisation was also used on a few occasions as an alternative method of preparing primary BEK, CK and CT cultures. After the appropriate tissues (see above) had been collected, the mincing, washing, filtration and sedimentation of the cells was carried out as has already been described. The tissue fragments were trypsinised at room temperature for 45 minutes with the aid of a magnetic stirrer, after which time the cloudy suspension thus produced was discarded and replaced with fresh 0.25 per cent trypsin solution. Digestion was continued over-night at $4^{\circ}C$ prior to harvesting the cells.

Samples of tissue were collected asceptically, at necropsy, for virus isolation from the nasal conchae, nasopharynx, trachea, lung parenchyma and small bronchi. The samples were collected in sterile virus transport medium (VTM) which consisted of M199 medium, 5 per cent FBS, 1 per cent amphotericin B (Fungizone; Gibco Bio-Cult, Glasgow, UK) and penicillin-streptomycin of 100 units/ml and 100 µg/ml respectively. Virus isolation was carried out in tube cultures of secondary CK and CT cells. A 10 per cent suspension of each of the samples from affected animals was prepared in VTM and about 5 ml of this suspension was homogenised for three 30 second periods in a Colworth stomacher 80 Lab-Blender. The homogenate was filtered through sterile gauze and 0.5ml of this was used to inoculate each of the four tube cultures which were then incubated for 1 hour at $37^{\circ}C$ to allow virus adsorption. The tubes were checked daily up to four days for evidence of cytopathogenicity. Whole cultures were passaged 3 times before being finally discarded as negative. The nasal swabs were

treated in the same way after removal of the cotton-wool from the applicator sticks.

The virus isolates were identified by the features of their cytopathic effect (CPE), their ultrastructure and their neutralisation by hyperimmune serum. In addition, CK or BEK coverslip cultures were prepared in tubes, some being infected with the virus isolate and others left uninfected as controls. Infected and control cultures were fixed in Bouin's fluid, stained by haematoxylin and eosin and examined microscopically for cytological changes for 7 consecutive days. Fluid collected from a culture with an extensive CPE was transferred to copper grids, negatively stained with 2 per cent phosphotungstic acid and examined with an electron microscope at an instrumental magnification of times 20,000.

Growth of the stock virus isolate was achieved by inoculating 1ml of fluid from tubes showing an extensive CPE into 8 ounce prescription bottle cultures, which were left at 37°C for 1 hour for virus adsorption. Twenty ml of maintenance medium (M199 medium + 5% FBS) was then added and the cultures incubated at 37°C. The virus was harvested when approximately 90 per cent of the monolayer showed a CPE; this was usually on the second or third day post-inoculation. The harvested virus was kept at -70°C overnight and allowed to thaw at room temperature the following day. The iceblock was used to scrape the cells, which were still attached to the glass wall, by shaking the bottle up and down. The resulting fluid was centrifuged at 168g for 15 minutes in a refrigerated GF-6 MSE centrifuge. The supernatant (virus suspension) was put into 0.5ml ampoules and stored at -70°C until required.

For the serological identification of the virus-isolate, neutralisation tests were carried out with rabbit antiserum to IBR virus supplied by the Department of Microbiology, Moredun Institute, Edinburgh. Two-fold dilutions of serum were mixed with a constant amount of virus and held at 37°C for 1 hour to allow neutralisation. Four tube cultures of CK cells were then inoculated with each mixture so that each received 100 $TCID_{50}$ of virus. The tubes were incubated at 37°C and examined up to the fifth day post-inoculation for any evidence of cytopathic effect.

(6) Serological examination

Serum neutralising antibody titres were determined by means of a microtitre system in which each well received 30 $TCID_{50}$ of virus. The full details of this test are given in Chapter 3.

RESULTS

(1) Details of one incident of infectious bovine rhinotracheitis

History of incident

Two groups of cattle arrived on an Aberdeenshire farm during February, 1978; 39 bullocks, which had been bought in the south of Scotland, arrived on February 7 and 49 weaned, single-suckled calves arrived on February 13 from the west coast via Aberdeen market. Group 1 was made up of the 39 bullocks together with the 3 biggest suckled calves; the remaining 46 calves comprised Group 2. These groups were housed adjacent to each other in a large shed, in which there already were two groups (Group 3 and Group 4) of 40, nearly fat bullocks, 18 to 24 months of age (Figure 1).

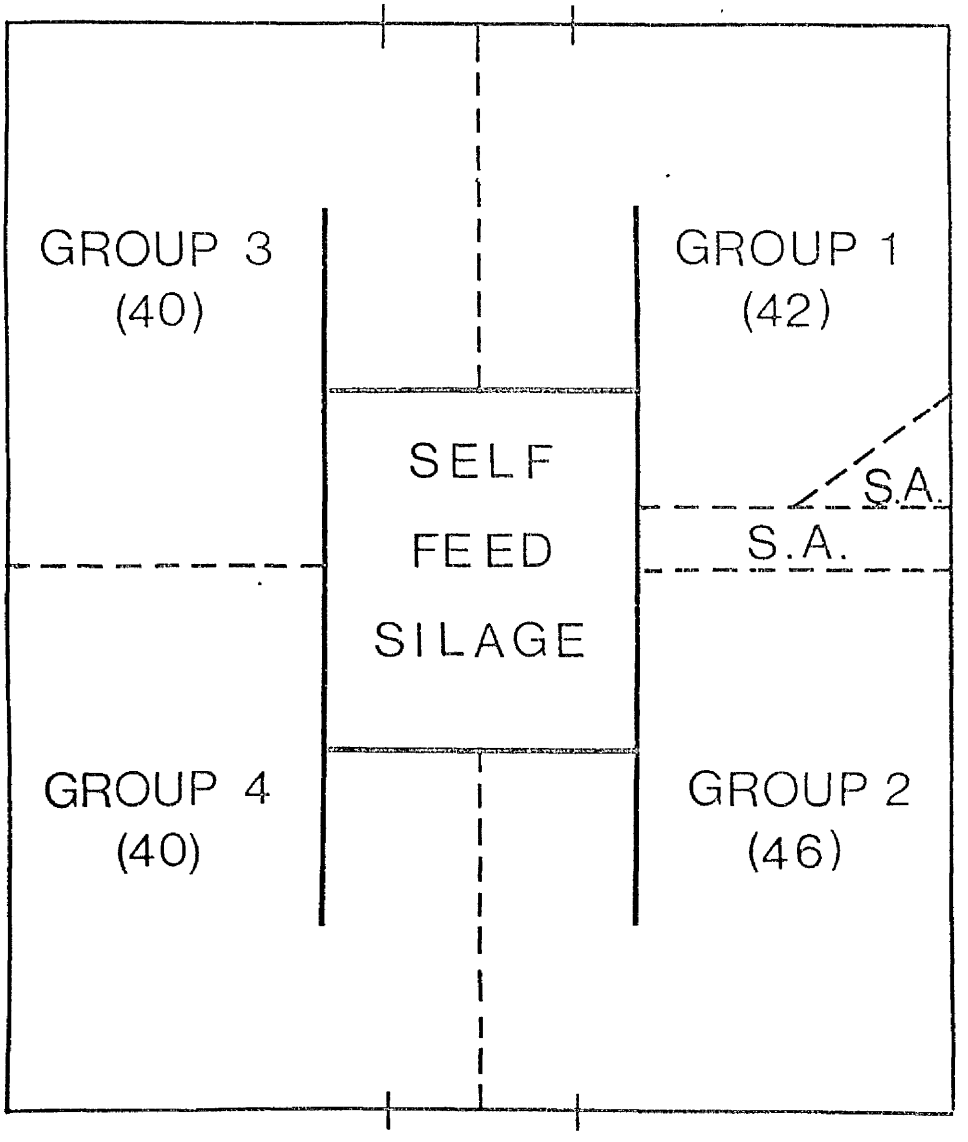
Clinical features

In the morning of February 19 a bullock in Group 1 was seen to be very dull and to have respiratory distress. On clinical examination, pyrexia ($105^{o}F$) was detected and fluid sounds as well as rhonchi were heard anteroventrally on both sides of the chest. A diagnosis of transit fever was made and 1 gram oxytetracycline (Terramycin; Pfizer Ltd., Kent, UK) and 6 milligrams betamethasone (Betsolan; Glaxo Vet Ltd., Middlesex, UK) were given intramuscularly. Antibiotic therapy was given daily for 10 days but despite this its condition deteriorated for the next 3 days and then slowly improved during the following 2 weeks.

In the evening of February 19, case 98 and another calf in Group 2 were seen standing alone and not eating. On closer inspection they were seen to be drooling saliva and to have seromucoid nasal and ocular discharges (Figure 2). Although both cases were pyrexic ($105^{o}F$; $107^{o}F$), neither animal was tachypnoeic and adventitious lung sounds were not heard on auscultation. Again 1 gram oxytetracycline and 6 milligrams bétamethasone were given intramuscularly.

During the week February 20-25 the condition of case 98 deteriorated and it became severely dyspnoeic in spite of the following regime of treatment: 1 gram oxytetracycline for 1 day, 4 grams tylosin (Tylan 200; Elanco Ltd., Hampshire, UK) for 1 day, 5 grams penicillin and dihydrostreptomycin (Streptopen; Glaxovet Ltd., Middlesex, UK) for 3 days. On every subsequent occasion the antibiotic treatment consisted of 5 grams penicillin and dihydrostreptomycin given intramuscularly.

FIGURE 1. The position of the calves in Group 2 relative to the bullocks in the other 3 groups sharing the same accommodation.



( ) = No. of ANIMALS

S.A. = SICK ANIMALS

FIGURE 2. A close up of Case 99 from outbreak 1 showing a marked bilateral copious mucopurulent nasal discharge and profuse salivation. Note, the animal is dull.

The second calf improved slowly although its temperature remained elevated for several days. On February 25, 14 milligrams of betamethasone were given in addition to the antibiotic treatment and both calves had improved considerably by the next day. However, on February 27, case 98 was again mouth-breathing although the second calf had maintained its clinical progress. At this time, a third animal was slightly dull with pyrexia ($103^{\circ}F$); it was drooling saliva, had conjunctivitis with profuse serous lachrymation and a seromucoid nasal discharge. Adventitious lung sounds were not heard. All three animals were given antibiotic treatment.

On February 28 another 3 calves in Group 2 were seen to be ill by the farmer and a further 6 were found to be similarly affected when the rest of the group was examined. The common presenting signs were profuse serous lachrymation, seromucoid nasal discharge, slight dullness and frequent coughing. On clinical examination, there was severe conjunctivitis, congestion of the nasal mucosa, pyrexia and tachypnoea (Respiratory Rate = 40 to 80 per minute). Adventitious lung sounds were not heard although the respirations were harsh. Eight of the nine calves were treated with antibiotics while the ninth (case 99) was not. At this time 2 animals (cases 98 and 99) were transported to the University of Glasgow Veterinary School. A detailed clinical examination was carried out on both cases and the results are summarised in Table 4. Blood samples and nasal swabs were taken for further investigation.

Several animals in Group 1 had a seromucoid nasal discharge and a few others had obvious serous lachrymation. Two of these had one eye half-shut with swollen eye-lids and, since the farmer had already seen corneal opacities in both, infectious bovine keratoconjunctivitis was diagnosed. None of the older bullocks in Group 3 and 4 were ever seen to have ocular or nasal discharges either during this period or subsequently.

On March 1, the 34 calves in Group 2 which had not been treated were examined individually: 31 calves (91%) had a nasal discharge (24 seromucoid, 7 mucopurulent), 14 calves (41%) were pyrexic, 13 calves (38%) had lachrymation (10 serous, 3 purulent) with conjunctivitis (7 unilateral, 6 bilateral) and 11 calves (32%) had congested oral mucous membranes. Sporadic coughing was also heard. Each pyrexic calf was given daily antibiotic therapy until its temperature returned to normal. Only 4 calves were treated more than twice and the final treatment was given on March 6. No new cases arose after this date and

Table 4. Summary of clinical signs and haematological findings of cases 98 and 99 on admission to the veterinary school.

CLINICAL SIGNS	CASE 98	CASE 99
Demeanour	Very dull	Dull
Temperature	104.9°F	103.5°F
Lachrymation	Matted hair	Slight serous
Conjunctivitis	Yes	Yes
Keratitis	No	No
Nasal discharge	Purulent	Mucopurulent
Nasal mucous membranes	Congested	Congested
Diphtheritic plaques	Yes	No
Halitosis	Yes	Yes
Respiratory rate per min	30	40 to 50
Respiratory depth	Inspiratory dyspnoea	Gross hyperpnoea
Coughing	No	Occasional, single
Tracheal sounds	Loud "rattle"	No
Adventitious lung sounds	Occasional rhonchus/ crackles A/V on R	Rhonchus 1/3 up on left
Thoracic pain	No	No
Oral mucous membranes	Congested	Congested
Drooling saliva	Yes - frothy	No
Duration of illness prior to slaughter	10 days	1 day
Treated	Yes	No
PCV per cent	34	28
Hbg per 100 ml	11.0	9.1
RBC 10^6 cu mm	7.79	5.74
WBC cu mm	10,400	7000
Neutrophils cu mm	7176	4550
Lymphocytes cu mm	3224	2450
Eosinophils cu mm	0	0

all the calves appeared to have recovered fully by March 14.

An acute severe outbreak of IBR was diagnosed. Of the 46 calves at risk, 44 had clinical signs of infection and 12 were severely ill. The morbidity rate was virtually 100 per cent and, although no fatalities occurred on the farm, case 98 would almost certainly have died.

On April 11, 7 weeks after the first case was seen, the calves in Group 2 were re-examined and blood samples taken.

Post-mortem examination

Case 98. There was a severe acute necrotising rhinitis and pharyngitis (Figure 3). The mucous membranes of the nasal conchae and the nasopharynx were congested and a moderate number of petechial haemorrhages were also seen. A large amount of mucopurulent exudate was present in the nasal passages and there was necrosis of the mucous membrane of the conchae in the rostral part of the nose with necrotic debris adhering to the mucous membrane.

There was a severe obstructive necrotising laryngotracheobronchitis (Figure 4). The larynx was obstructed by piled up necrotic debris. Most of the luminal surface of the trachea, throughout its length, was covered by a yellowish soft pseudomembrane, which had a rough surface. On areas of the trachea and bronchi not covered by necrotic debris, congestion and petechial haemorrhages were seen.

There was an extensive acute exudative interstitial pneumonia with fibrinous pleurisy. The cranial parts of both lungs were affected. A moderate degree of interstitial emphysema was present in the non-pneumonic areas of the cranial, middle and caudal lobes of the lungs.

There were early degenerative changes in the liver which contained 10 to 12 liver flukes and one microscopic area of necrosis resembled a fluke tract. In the abomasum there was a moderate number of circular haemorrhagic ulcers 1 to 2 cm in diameter. A considerable number of infarcts were found in both kidneys.

On microscopic examination an acute necrotising rhinitis with hyperplasia and dysplasia of the epithelium, which in many places was infiltrated by neutrophils, was detected. Subepithelially, there was a considerable number of lymphocytes and infiltrating neutrophils; some of the lymphocytes had formed follicular accumulations. Plasma cells surrounded the submucosal glands many of which were hyperplastic and dilated with secretion. Similar changes were seen in the mucous membrane

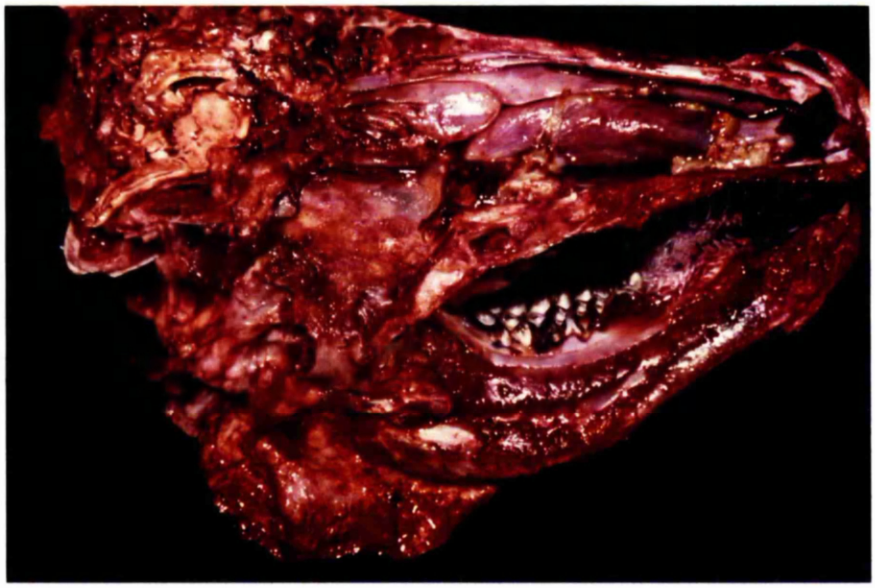


FIGURE 3. A sagital section of the head of Case 98 from outbreak 1; a considerable amount of necrotic debris is lying in the nasal passages.

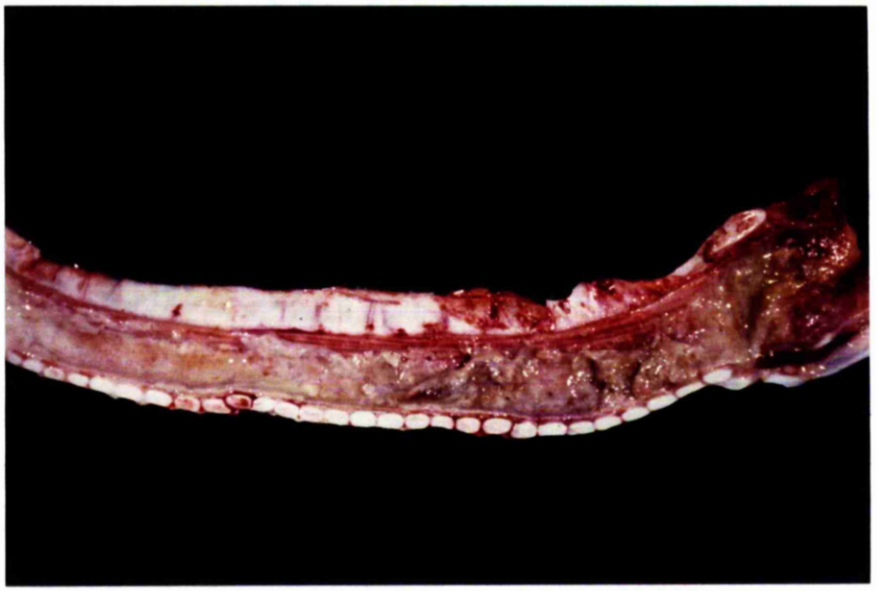


FIGURE 4. Larynx and trachea of Case 98 from outbreak 1. The mucous membrane is covered by a thick diphtheritic exudate throughout its length; note the swelling and necrotic debris in the larynx.

of the pharynx. No inclusion bodies were found in the epithelial cells of the upper respiratory tract.

There was a severe, acute, necrotising, tracheitis with extensive destruction of the ciliated epithelium (Figure 5). Intranuclear inclusion bodies were not seen in the isolated fragments of the tracheal epithelium that remained. Below the necrotic layer, the lamine propria was congested with focal haemorrhages and patches of fibrin deposition. Neutrophils had infiltrated the lamina propria as had lymphocytes, which formed follicular aggregates in some areas. The tracheal submucosal glands were increased in size, many had dilated ducts and tubules lined by flattened epithelial cells; the lumena were usually filled with secretion. Plasma cells surrounded many glands. Pools of mucus, which were seen in the upper lamina propria, appeared to have been produced by leakage from obstructed or damaged gland ducts. Inclusion bodies were not seen in the epithelial cells of the glands.

Areas of necrosis were seen in the pneumonic areas and there was evidence of early organisation. Thrombosed branches of pulmonary veins were also found.

Case 99. This animal had an acute necrotising rhinitis, pharyngitis and laryngotracheobronchitis almost identical macroscopically and microscopically to that in case 98. The larynx, however, was not obstructed to the same degree by necrotic debris. A purulent pneumonia was present in the cranial and middle lobes of the lungs with interstitial emphysema in the adjacent non-pneumonic tissue (Figure 6). There was a considerable amount of purulent exudate in the bronchi related to the pneumonic area. In small bronchi and large bronchioles within the lesion there was focal necrosis of the epithelium. Several peribronchiolar lymphocytic accumulations were present. Microvesicles infiltrated by neutrophils were seen in the epithelium of some small bronchi. Intranuclear inclusion bodies in epithelial cells were not found at any level of the respiratory tract. In this case, globule leukocytes were present in moderate numbers in the epithelium of the nasal conchae, the pharynx and the bronchi.

The liver was pale with early degenerative changes and there were a few globule leukocytes in the bile ducts. There was a moderately severe ostertagiasis; parasites were seen in sections of abomasal mucosa and there were many globule leukocytes.

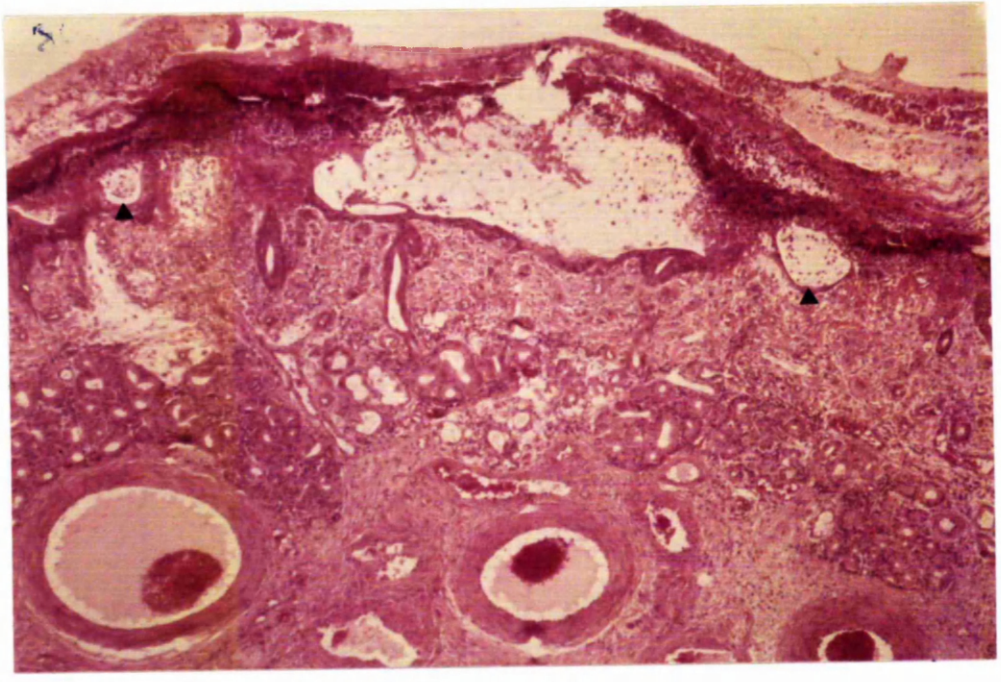


FIGURE 5. Trachea from Case 98 from outbreak 1 with excess mucous secretion from dilated glands producing pools of mucus subepithelially (▲). Note also the severe necrosis of the epithelial layer.

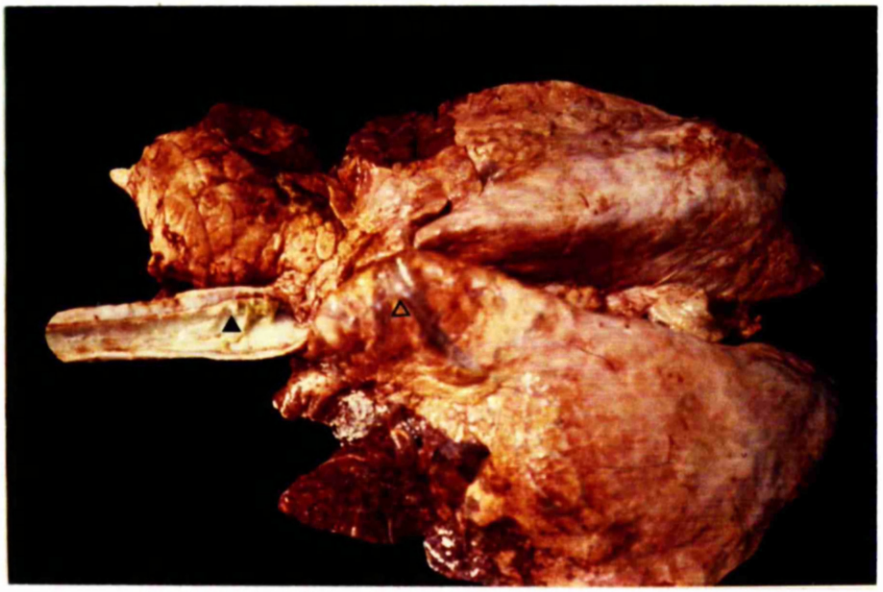


FIGURE 6. Trachea and lung from Case 99 from outbreak 1. There is a moderately severe pneumonia affecting the cranial area of the lungs and there is also interstitial emphysema (△). Necrotic exudate due to tracheitis can be seen on the mucosal surface of the trachea (▲) in its terminal position.

In both cases, free virus particles morphologically indistinguishable from herpesvirus, were recognised by electron microscopical examination of samples of necrotic debris from the tracheal mucous membrane.

Microbiological examination

The results are given in Table 5. Infectious bovine rhinotracheitis virus was isolated from all tissues sampled. A CPE appeared within 24 hours of inoculation of the tissue culture (Figure 7) and had involved 60 to 80 per cent of the cells by 48 hours post-infection (PI) (Figure 8). Initially, it consisted of small foci of round, refractile cells but spread of infection occurred so rapidly that, by 36 to 48 hours PI, its focal origin was no longer apparent. Infected cells varied in size according to the degree of ballooning present, but a minority were considered larger than the rest and, on staining with haematoxylin and eosin, were found to contain up to four nuclei. Intranuclear inclusions were not found in any cell. As the CPE progressed, the ballooned cells became shrunken and were frequently linked by long, straight strands of cytoplasm. During a second passage in CK cells, the infectivity titre reached $10^{7.7}$ TCID 50 per ml.

Electron microscopic examination of negatively stained fluid collected from a culture with an extensive CPE revealed the presence of small numbers of typical herpesvirus virions, both naked and enveloped.

In the neutralisation test the highest serum dilution used, 1 in 128, neutralised the virus inoculated into all four tubes thus confirming that the isolate was a bovine herpesvirus 1. The titres of antibody detected by the microtitre neutralisation test are given in Table 6. Neutralising antibodies were detected in 23 of the 44 sera examined.

Mycoplasma bovis was isolated in large numbers from both cases (Table 5). The organism was recovered from the trachea and bronchus of case 98 and the nasal concha and bronchus of case 99. No other mycoplasmas were isolated.

A profuse growth of a variety of bacteria resulted from culture of all tissues examined (Table 5). Streptococcus pneumoniae was the only recognised pathogen cultured and was limited to the trachea of case 98.

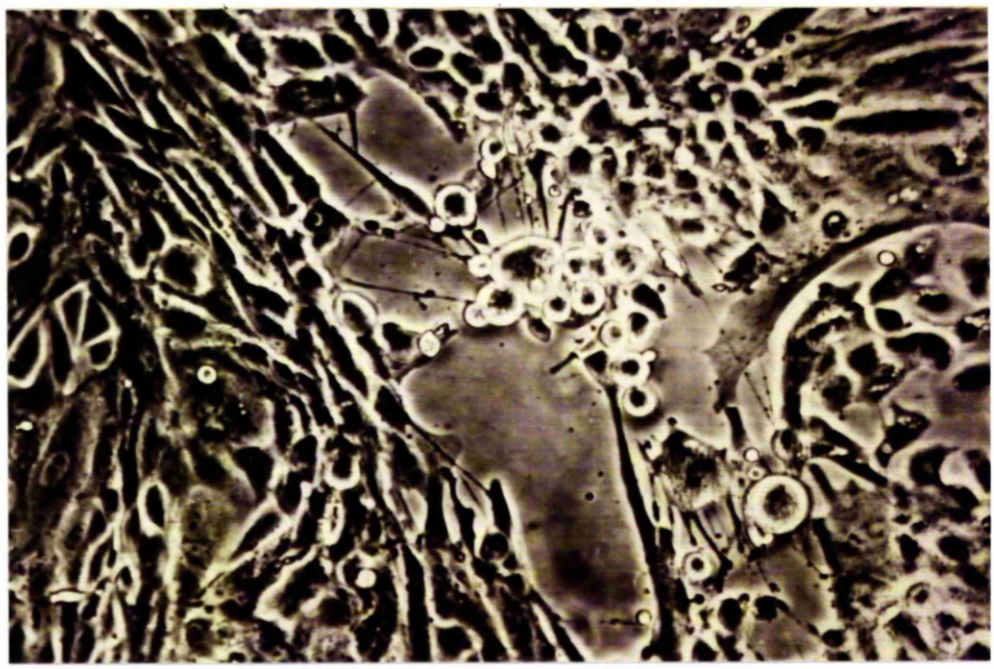


FIGURE 7. Early focal cytopathic effect produced by bovine herpesvirus 1, 24 hours post-infection. Retraction of cells has resulted in the formation of small spaces. Phase contrast X300.

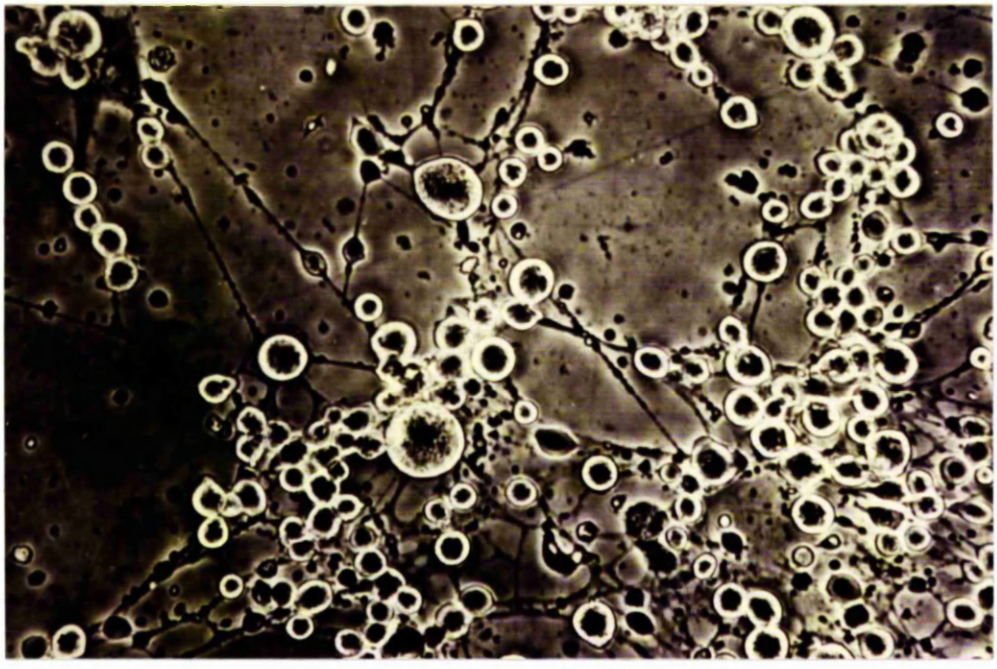


FIGURE 8. Advanced cytopathic effect due to bovine herpesvirus 1, 48 hours post-infection; note the straight strands of cytoplasm and the great variation in the size of the rounded cells. Phase contrast X300.

TABLE 5. The results of the microbiological examination of two calves with infectious bovine rhinotracheitis.

CASE	TISSUE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
98	Nasal swab	IBR	ND	ND
	Nasal concha	IBR	ND	ND
	Nasopharynx	IBR	Alcaligenes faecalis Enterobacter aerogenes	-
	Trachea	IBR	Proteus sp. Strep pneumoniae	M.bovis
	Bronchus	IBR	Aerococcus viridans	M.bovis
99	Nasal swab	IBR	ND	ND
	Nasal concha	IBR	Enterobacter aerogenes Flavobacterium sp.	M.bovis
	Nasopharynx	IBR	ND	ND
	Trachea	IBR	Proteus sp. Flavobacterium sp.	-
	Bronchus	IBR	Flavobacterium sp.	M.bovis

ND = Not done

TABLE 6. The results of the serum neutralisation test for examination of antibodies from the first incident of infectious bovine rhinotracheitis.

Titre*	No. of animals with the titre
32	1
24	3
16	1
12	6
8	3
6	3
4	6
4	21

* Serum dilution reciprocals

(2) Detailed results of 15 incidents of infectious bovine rhinotracheitis

Farmer's observations

The clinical abnormalities as observed by the farmers are summarised in Table 7. The most common, single sign of illness was a reduction in appetite which was observed in 9 incidents; in 5 outbreaks (2,5,7,12,15) affected individuals were said to be anorexic and, in incident 6, affected animals refused to eat hay while continuing to eat concentrates. Combined serous ocular and nasal discharges were observed in 8 incidents and, in a further 5 either nasal discharge (3 incidents) or ocular discharge (2 incidents) was noticed. Therefore in only outbreaks 10 and 11 were these signs not noticed by the farmer. In 3 of the 7 incidents in which coughing was heard (4,8,14), affected individuals were said to cough frequently. Other signs of respiratory disease included tachypnoea (5 incidents) and hyperpnoea (2 incidents). A few of the severely affected animals in incident 10 were said to have been "snoring".

In 6 incidents, affected individuals were said to be dull; generally the animals were considered to be slightly dull, although in incidents 2 and 15 they were moderately to severely dull. The drooling of saliva which was noticed in 4 incidents was stated to be profuse in incidents 2 and 12. A sudden drop in milk yield was reported in 2 of the 4 outbreaks involving dairy cattle and in incident 15 agalactia was a feature.

Clinical signs

Pyrexia was detected in every sick animal subjected to a clinical examination in each incident. The values ranged from 103-107.5^{o}F.

At the time of the farm visit, it was established that, in every incident, the food consumption of affected animals had decreased (Table 8). In 5 outbreaks (2,3,9,12,15), the animals were anorexic for 2-3 days at the beginning of the illness. In outbreaks 1,6,7,8 and 14 only roughage was refused while concentrates continued to be eaten, albeit more slowly. In incident 13, it was said that concentrates were left whereas the hay was eaten.

In 8 incidents, affected animals were considered to be slightly dull whereas in the other 7, a moderate to severe degree of dullness was observed.

TABLE 7 The initial clinical abnormalities recognised by the farmers in 15 outbreaks of infectious bovine rhinotracheitis.

FARMER'S COMPLAINT	OUTBREAK NUMBER															TOTAL	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	NO.	%
REDUCED APPETITE	+	+			+	+	+			+		+		+	+	9	60
SEROUS OCULAR AND NASAL DISCHARGE	+	+			+	+	+						+	+	+	8	53
COUGHING		+	+	+				+			+		+	+		7	47
DULLNESS	+	+					+		+			+			+	6	40
TACHYPNOEA		+	+		+					+			+			5	33
DROOLING SALIVA	+		+							+		+				4	27
NASAL DISCHARGE			+					+	+							3	20
OCULAR DISCHARGE				+								+				2	13
HYPERPNOEA										+	+					2	13
REDUCED MILK YIELD								+							+	2	13
BLOOD ON TAIL									+							1	7

TABLE 8. Summary of the clinical signs based on observations during the farm visits, observations by the practicing veterinary surgeons and on sequential examination of severely affected individuals purchased from incidents of infectious bovine rhinotracheitis.

CLINICAL PARAMETER	OUTBREAK NUMBER		
	1	2	3
Appetite	Reduced for silage	Anorexia	Anorexia
Dullness	Slight	Slight to severe	Moderate
Eyes - Discharge	Profuse serous	Serous to purulent	NO EYE INVOLVEMENT
- Conjunctivitis	Moderate to severe	Moderate to severe	
- IBK	Nil	Present	Several cases
Nose - Discharge	Seromucoid Congestion. Diphtheritic plaques.	Seromucoid-mucopurulent Congestion. Diphtheritic plaques.	Seromucoid-mucopurulent Congestion. Diphtheritic plaques.
Coughing	Frequent	Frequent	Occasional. Halitosis.
Tachypnoea	Moderate to severe	Moderate	Slight - severe Respiratory stertor
Hyperpnoea	Slight	Slight	Slight - severe
Dyspnoea	Present	Present	Present
Pneumonia	In severe cases	In severe cases	In severe cases
Drooling saliva	Common	Common	Common. Profuse.
Diarrhoea	Nil	Nil	A few severe cases
Milk yield	-	-	-
Weight-loss	4 weeks	6-8 weeks	4-6 weeks
Temperature	103-107°F	104.5-106°F	103-105.1°F

TABLE 8. (Cont'd)

CLINICAL PARAMETER	OUTBREAK NUMBER		
	4	5	6
Appetite	Reduced by 50% for 3-5 days	Reduced	Reduced for hay and silage
Dullness	Slight	Slight - severe	Slight
Eyes - Discharge	Serous - mucoid	Mucoid - purulent	Mucopurulent
- Conjunctivitis	Slight - severe. Granular lesions	Mild - moderate	Mild - moderate
- IBK	Present	Present	Nil
Nose - Discharge	Serous - mucoid.	Profuse serous	Mucoid - mucopurulent
- Lesions	Diphtheritic plaques	Nil	Nil
Coughing	Frequent	Occasional	Frequent
Tachypnoea	Slight - moderate Respiratory stertor	Slight - moderate	Slight. Respiratory stertor
Hyperpnoea	Slight	Slight	Slight
Dyspnoea	Present	Nil	Nil
Pneumonia	In severe cases	In severe cases	Nil
Drooling saliva	Common	Present	Nil
Diarrhoea	In one animal	Nil	Nil
Milk yield	-	Reduced by 3-4 gallons	-
Weight-loss	4-6 weeks	-	1-2 weeks;
Temperature	104-106°F	104.5-106°F	103-107°F

TABLE 8. (Cont'd)

CLINICAL PARAMETER	OUTBREAK NUMBER		
	7	8	9
Appetite	Reduced for hay	Reduced for silage	Anorexia
Dullness	Slight - moderate	Slight	Slight
Eyes - Discharge	Serous - mucoid	Serous	Serous
- Conjunctivitis	Moderate - severe with oedema	Moderate - severe	Slight - moderate
- IBK	Present	Nil	Present
Nose - Discharge	Serous - mucoid	Serous	Serous - mucopurulent
Lesions	Nil	Congestion. Diphtheritic plaques. Haemorrhages.	Congestion. Diphtheritic plaques. Few haemorrhages.
Coughing	Frequent	Frequent	Frequent
Tachypnoea	Nil	Slight - moderate	Nil
Hyperpnoea	Nil	Slight	Nil
Dyspnoea	Present	Nil	Nil
Pneumonia	Present	Nil	Nil
Drooling saliva	Common	Present	Nil
Diarrhoea	Nil	In 2 animals	Nil
Milk yield	-	Reduced	-
Weight-loss	4 weeks	Nil	Suckled calves 4-6 weeks
Temperature	103-105°F	103-106°F	103-104.5°F

TABLE 8. (Cont'd)

CLINICAL PARAMETER	OUTBREAK NUMBER		
	10	11	12
Appetite	Reduced - anorexia	Reduced - anorexia	Anorexia
Dullness	Slight - severe	Slight	Slight - moderate
Eyes - Discharge	Serous - mucoid	NO EYE INVOLVEMENT	Serous
- Conjunctivitis	Slight - moderate		Slight - moderate
- IBK	Present	Present	Nil
Nose - Discharge	Mucoid - mucopurulent	Serous	Serous - mucopurulent
- Lesions	Congestion. Diphtheritic plaques.	Nil	Nil
Coughing	Frequent	Frequent. Productive	Occasional
Tachypnoea	Slight - moderate	Slight	Slight - moderate
Hyperpnoea	Slight - moderate	Slight - moderate	Slight - moderate. Respiratory stertor.
Dyspnoea	Nil	Nil	Nil
Pneumonia	In severe cases	Nil	Nil
Drooling saliva	Common	Present	Common
Diarrhoea	In one animal	In a few cases	Nil
Milk yield	-	Reduced	-
Weight loss	Marked, 4-6 weeks	In 6-7 severely affected cases	1 week
Temperature	103-106°F	103-107.5°F	103.107°F

TABLE 8. (Cont'd)

CLINICAL PARAMETER	OUTBREAK NUMBERS		
	13	14	15
Appetite	Reduced for concentrates	Reduced for hay	Anorexia
Dullness	Slight	Slight	Moderate - severe
Eyes - Discharge - Conjunctivitis - IBK	Serous - mucopurulent Moderate - severe Present	Serous Moderate Present	Serous Moderate - severe Present
Nose - Discharge - Lesions	Mucoid - mucopurulent Congestion. Diphtheritic plaques.	Serous - mucopurulent Congestion. Diphtheritic plaques.	Mucoid - mucopurulent Nil
Coughing	Frequent	Frequent	Frequent. Halitosis
Tachypnoea	Slight - moderate	Slight - moderate	Slight - moderate
Hyperpnoea	Slight - moderate	Nil	Slight - moderate
Dyspnoea	Nil	Nil	Nil
Pneumonia	In severe cases	Nil	In severe cases
Drooling saliva	Present	Present	Present
Diarrhoea	Nil	Nil	Nil
Milk yield	-	-	Agalactia
Weight-loss	Marked, 8-12 weeks	Marked, 2-3 weeks	Nil
Temperature	103-106°F	103-106°F	103-107°F

Ocular discharge, which was usually bilateral, was a common finding in 13 incidents and in 6 of these it was so profuse that the hair on the cheeks of affected individuals became matted. Initially, the discharge was serous but, subsequently, it became mucoid or mucopurulent in character. On close examination, individuals with bilateral conjunctivitis were seen in each of the 13 outbreaks although the severity and the number affected varied markedly. There was marked congestion of the conjunctiva with oedema and protrusion of the nictating membrane in severe cases (Figure 9). There was also granular lesions on both the bulbar and palpebral conjunctiva as well as injection of the scleral vessels in a few animals in incident 4 (Figure 10). Punctate haemorrhages were not observed in either mildly or severely affected individuals.

Infectious bovine keratoconjunctivitis (IBK) was diagnosed in 11 farms and, retrospectively, it was established that there had been a marked increase in the incidence of this condition on farm 13 and a slight increase on farms 3 and 6. In contrast, in outbreak 7 the incidence was said to have been slightly less than in previous years. The number of cases which developed on the other 7 farms was unchanged.

A bilateral nasal discharge was present in a large proportion of the animals in every outbreak. Initially this discharge was serous (Figure 11) but later became mucoid or mucopurulent in advanced cases. In severely affected individuals in 9 incidents, there was marked congestion of the nasal mucosae and yellowish-brown, diphtheritic plaques were seen in either one or both nares. Haemorrhages were seen in the nasal mucosae of single animals admitted from outbreaks 8 and 9.

A degree of foetid halitosis was detected in every animal in which diphtheritic plaques were seen, but was particularly marked in incidents 3 and 15. A sweetish odour was detected in case 98 admitted from incident 1.

A sudden increase in the frequency of coughing was present at the beginning of every incident. Although, in 12 outbreaks, severely affected individuals coughed frequently, paroxysms of coughing were never observed. Productive coughing was only confirmed in incident 11 in which tied, dairy cattle were affected. Blood-streaked mucus was seen on the byre wall soon after the animals became ill.

Slight to moderate tachypnoea and hyperpnoea were observed in several animals in 13 incidents. Respiratory stertor developed in severely affected individuals from incidents 3,4,6 and 12, while dyspnoea

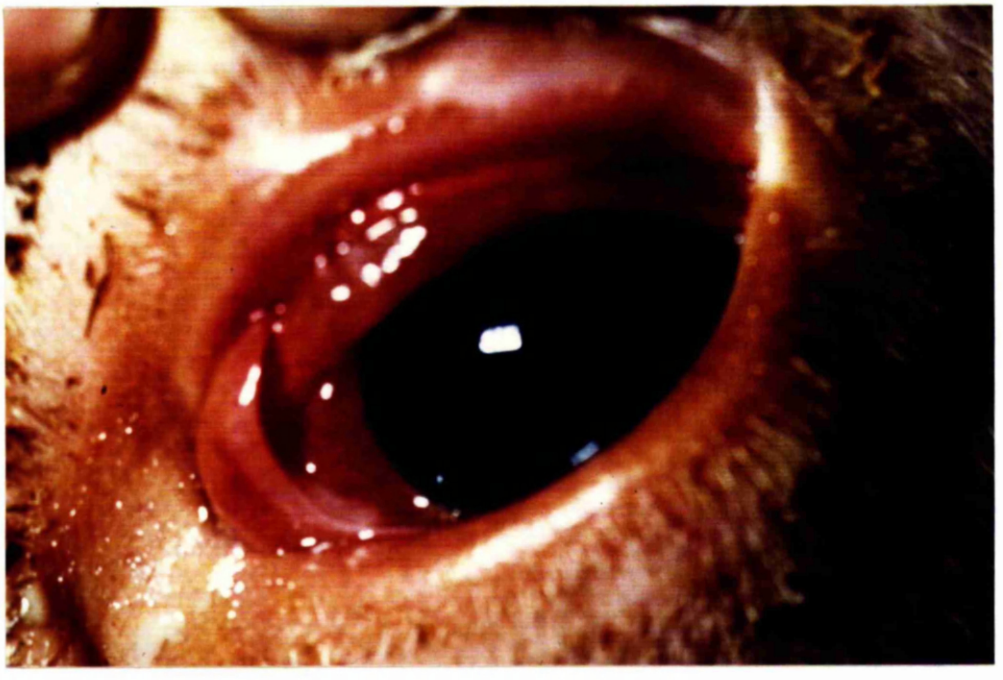


FIGURE 9. A case from outbreak 4. Severe conjunctivitis with obvious conjunctival oedema and mucoid ocular discharge can be seen in the medial canthus; note there is no keratitis.

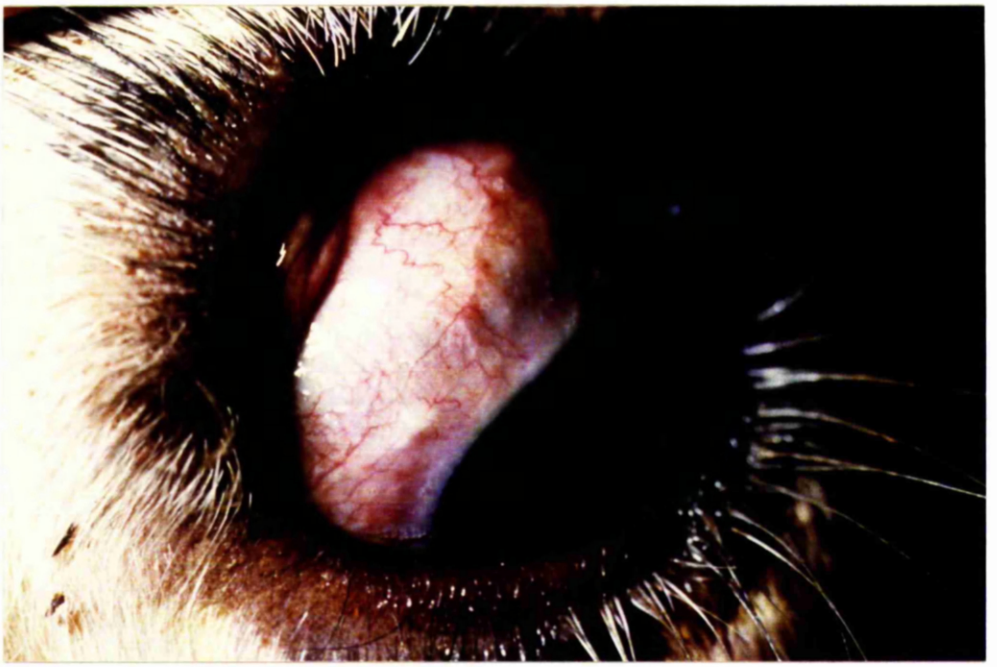


FIGURE 10. Another case from outbreak 4 with scleral oedema, injected blood vessels and a few granular lesions.

FIGURE 11. A Hereford heifer from outbreak 3 showing a copious bilateral mucoid nasal discharge, as well as frothy saliva at the commissures of the mouth.

(Figure 12) was observed in particularly severe cases from incidents 1, 2,3,4 and 7. Adventitious lung sounds were only heard in the two cases from outbreak 1.

Drooling of saliva (Figure 2) was a feature in 13 incidents but only in 7 was it a common finding. Profuse frothy salivation was a characteristic feature of incidents 3 and 12. On oral examination, the only clinical abnormality detected was slight congestion of the mucous membranes.

During the course of the disease, fattening beef cattle did not put on weight for a period of from 1-8 weeks and severely affected individuals lost up to 30 kilograms body-weight. The milk yield of lactating dairy cows decreased by 1-4 gallons depending on their level of production; in some cases there was agalactia.

Diarrhoea which was observed in a few individual animals in 6 incidents was not a feature of any of the cases admitted to the Veterinary School. The only animal, which was seen to have diarrhoea and which was examined at necropsy, had died from mucosal disease.

Abortions which were not due to Brucella abortus infection occurred during outbreaks 4 (6 cows), 5 (1 heifer), 7 (1 heifer) and 8 (2 heifers). A dramatic reduction in conception rate to 50 per cent also occurred in herd 5 while in herd 8 a few heifers returned to the bull 6 weeks after the clinical signs of IBR were first noticed. Pustular lesions were present on the posterior aspect of the udder of one of the cows from outbreak 8 (Figure 13).

The duration of clinical signs, i.e. from the time the first case was seen to be ill until clinical signs were no longer observed, ranged from 1 week in outbreak 12 to 8 weeks in outbreak 10 (Figure 14).

The drug therapy which was administered in 14 incidents (Table 9) consisted of parenteral antibiotics for at least 3 days. Oxytetracycline was given alone in 7 incidents and together with penicillin and dihydrostreptomycin in another 4 outbreaks. Betamethasone was also given to several animals in 2 outbreaks (1,3) and etamphylline camsylate (Millophylline; Dales Pharmaceuticals Ltd., Yorkshire, UK) was used in outbreak 7. The clinical response as determined by the farmer and general practitioner was said to have been variable (5 incidents), poor (4 incidents), good (3 incidents) and fair (2 incidents).

FIGURE 12. A close up of a case from outbreak 3 just prior to death, with severe respiratory distress. Note the amount of froth in the gutter.

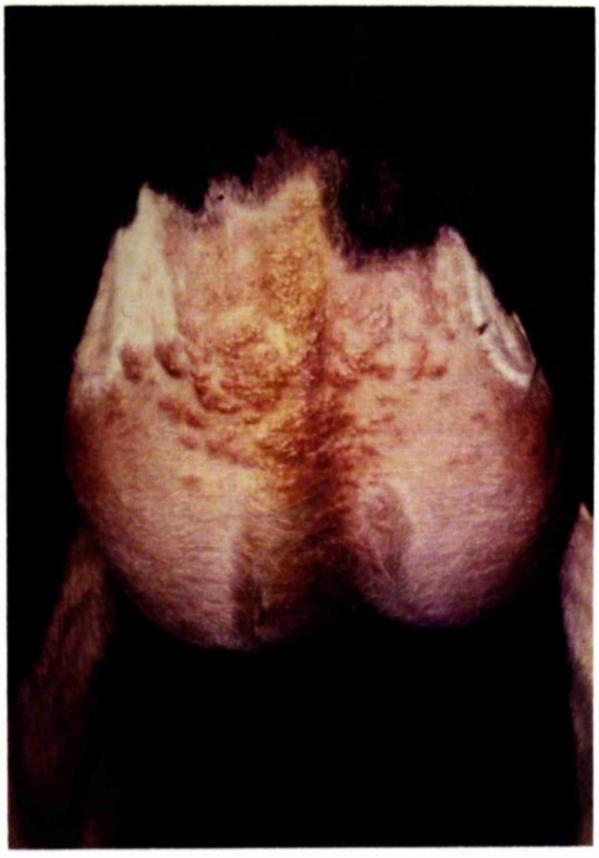


FIGURE 13. The posterior aspect of the udder of a cow from outbreak 8 showing pustular lesions.

FIGURE 14. The duration of the clinical signs in the 15 incidents of infectious bovine rhinotracheitis.

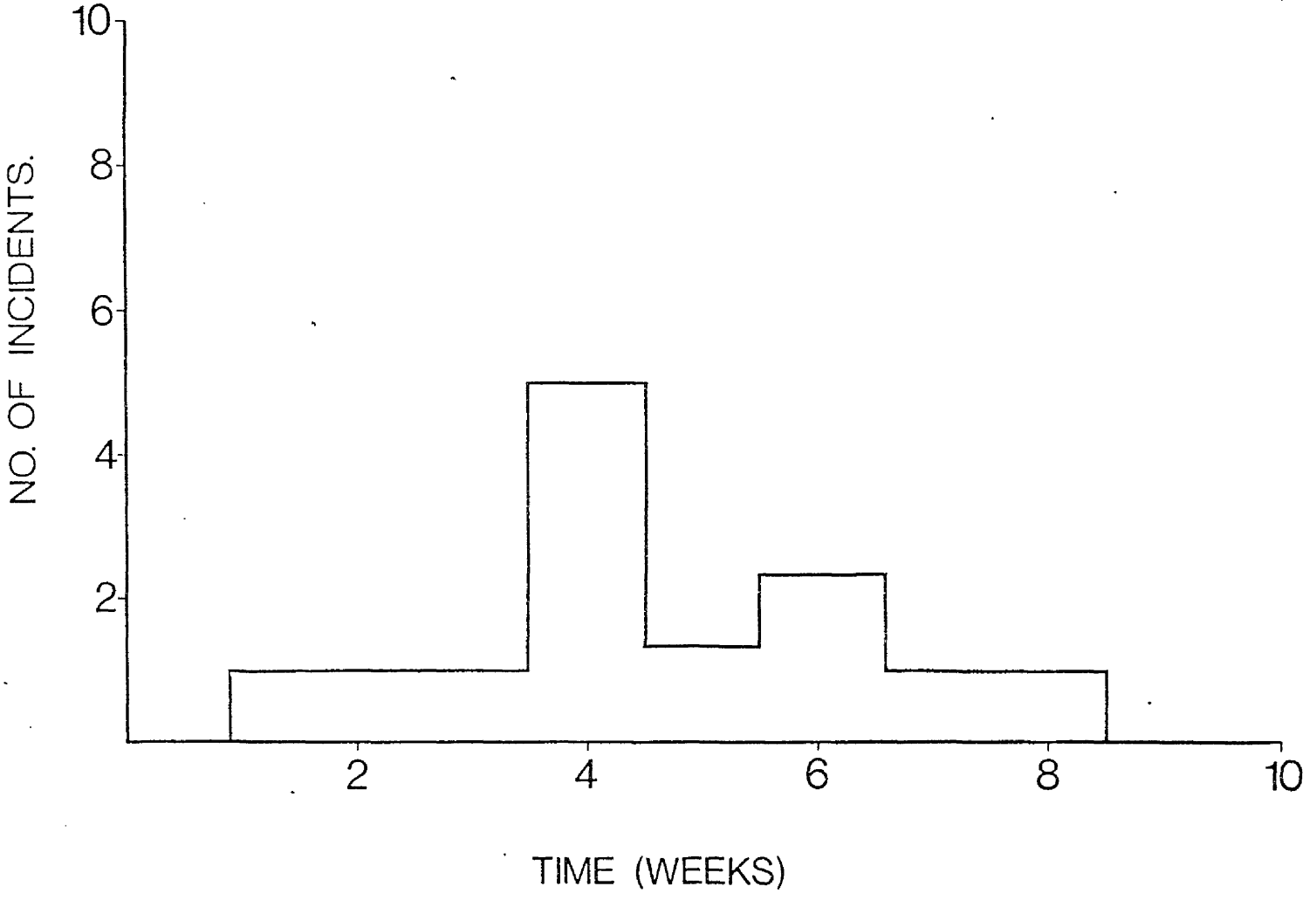


TABLE 9. The regime of drug therapy and the clinical response in 15 outbreaks of infectious bovine rhinotracheitis.

OUTBREAK NUMBER	DRUG THERAPY		CLINICAL RESPONSE
	ANTIBIOTIC	OTHER DRUGS	
1	oxytetracycline penicillin and dihydrostreptomycin	betamethasone	variable
2	oxytetracycline penicillin and dihydrostreptomycin		poor
3	oxytetracycline penicillin and dihydrostreptomycin	betamethasone	variable
4	oxytetracycline penicillin and dihydrostreptomycin		poor
5	oxytetracycline penicillin		good
6	oxytetracycline		fair
7	oxytetracycline	etamiphylline camsylate	variable
8	oxytetracycline		poor
9	none given		
10	oxytetracycline		fair
11	oxytetracycline		good
12	chlortetracycline		good
13	oxytetracycline		variable
14	oxytetracycline		variable
15	penicillin		poor

betamethasone - Betsolan : Glaxovet, Middlesex.
chlortetracycline - Chloromycetin : Parke Davis, Gwent.
etamiphylline camsylate - Millophylline : Dales Pharmaceuticals, Yorkshire.
oxytetracycline - Terramycin : Pfizer, Kent.
penicillin
penicillin and dihydrostreptomycin - Streptopen: Glaxovet, Middlesex.

Morbidity and mortality rates

There was considerable variation in the morbidity rate, from 10 per cent in outbreak 9 to 100 per cent in outbreaks 1,3 and 7 (Figure 15). In 9 incidents more than 90 per cent of the animals at risk were seen to be affected.

The mortality rate varied from zero in incidents 5,6,9,11 to 8 per cent in incident 7 (Figure 16). In 6 of the 11 incidents, only a single animal died but, in incident 2, 19 of the 280 animals at risk died or were slaughtered in extremis.

Financial loss

A breakdown of the estimated cost sustained in each outbreak is presented in Table 10. These losses were considered under 3 headings: firstly, the market value of fatal and culled cases, secondly, the feeding costs for the period when fattening beef cattle did not put on weight, and thirdly, the remaining costs which included treatment and the value of lost milk production. On 8 of the 10 beef units, total losses exceeded £1,000 with those on farms 2 and 3 being particularly dramatic, £20,000 and £10,000 respectively. The cost per beef animal at risk also varied markedly ranging from £71 on farm 2 to £6 on farm 12. The average cost per beef fattening unit was £5,600 or £36 per animal at risk. In contrast the losses on the dairy farms were much less averaging only £800 per incident or £6 per animal at risk.

The overall cost of feeding the beef cattle for several additional weeks represented 67 per cent of the total cost of IBR on these units, while the value of animals lost was only 23 per cent of the total. None of the dairy farmers apportioned any cost to the need for extra feeding and their losses were divided almost equally between deaths and culls (53%) and other costs (47%). However, on farms 5,8 and 11, the other costs including the value of milk unavailable for sale were more than twice those from animal losses.

Epidemiological features

Clinical signs developed in 13 of these incidents within 4 weeks of animals being purchased. In the remaining 2 incidents, a bull and single suckled calves (incident 9) had been bought-in several months before the onset of clinical signs.

Eleven of the 15 incidents occurred in beef animals and 4 in dairy cattle (Table 11). Ten of the 11 incidents in beef cattle involved

FIGURE 15. The morbidity rates in the 15 incidents of infectious bovine rhinotracheitis.

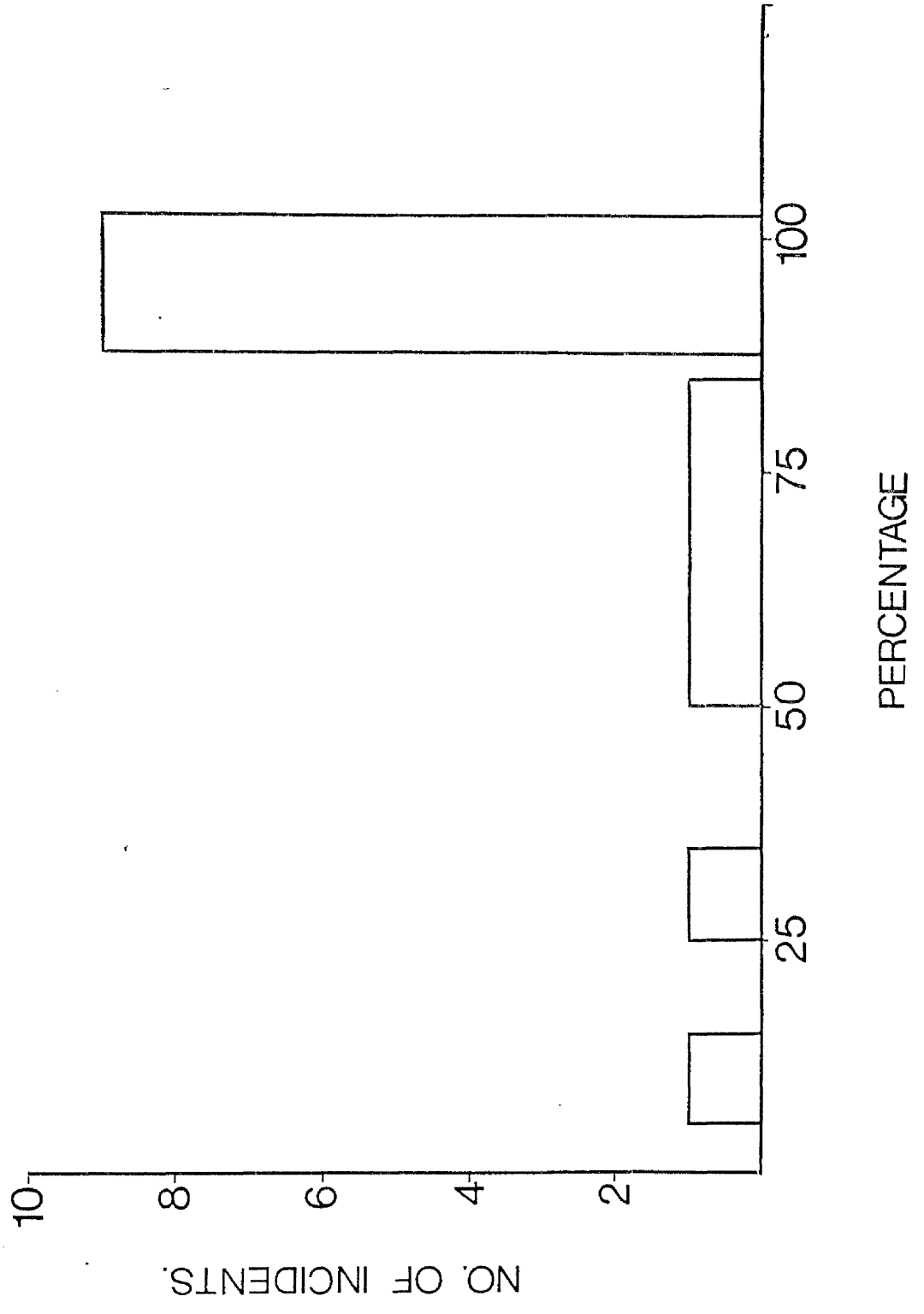


FIGURE 16. The mortality rates in the 15 incidents of infectious bovine rhinotracheitis.

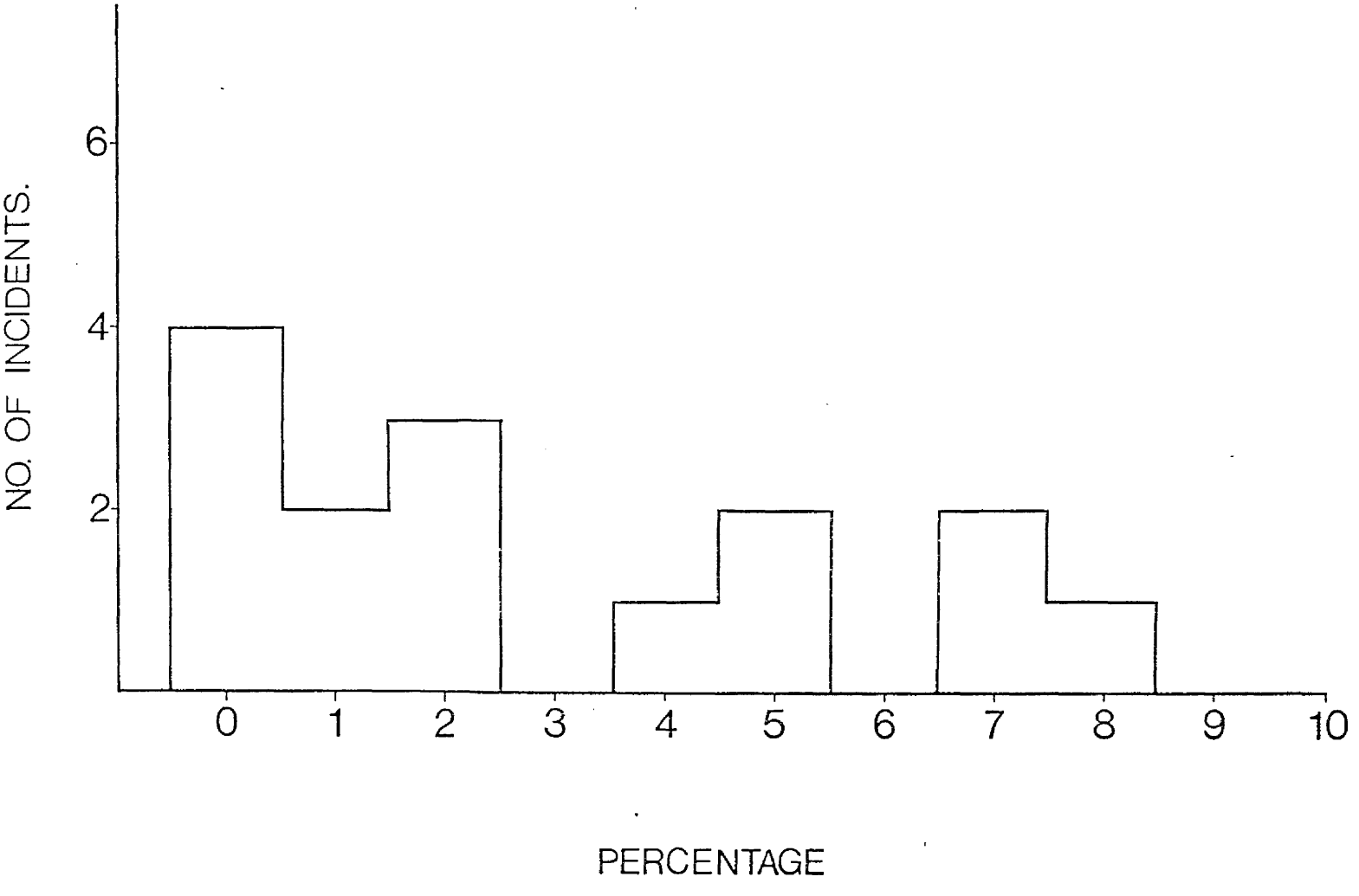


TABLE 10. A breakdown of the estimated cost of 15 incidents of infectious bovine rhinotracheitis.

OUTBREAK	ENTERPRISE	BREAKDOWN OF COSTS (£)			TOTAL COST (£)	NO. CATTLE AT RISK	COST PER ANIMAL (£)
		DEATHS/ CULLS	EXTRA FEEDING	OTHER COSTS			
1	FATTENING	225	1,100	250	1,575	46	34
2	FATTENING	4,800	13,500	1,700	20,000	280	71
3	FATTENING	2,500	5,000	2,500	10,000	150	67
4	FATTENING	200	6,000	-	6,200	400	16
5	DAIRY	-	-	1,200	1,200	300	4
6	FATTENING	-	700	300	1,000	100	10
7	FATTENING	200	250	-	450	13	35
8	DAIRY	700	-	150	850	150	6
9	SUCKLERS	200	-	200	400	86	5
10	FATTENING	3,025	4,500	500	8,025	300	27
11	DAIRY	-	-	100	100	53	2
12	FATTENING	250	-	150	400	64	6
13	FATTENING	1,400	5,600	120	7,120	130	55
14	FATTENING	250	750	200	1,200	92	13
15	DAIRY	1,000	-	50	1,050	43	24
TOTAL		14,750	37,400	7,420	59,570	2,207	27

TABLE 11 The type of cattle affected and the system of husbandry in 15 outbreaks of infectious bovine rhinotracheitis.

TYPE OF CATTLE AFFECTED		OUTBREAK NUMBER															TOTAL
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
BEEF	FATTENING	+	+	+	+		+	+			+		+	+	+		10
	STORES				+												1
	COWS AND CALVES									+							1
DAIRY	LACTATING					+			+			+				+	4
	IN-CALF HEIFERS					+			+								2
	CALVES					+											1
	BULLS								+							+	2
SYSTEM OF HUSBANDRY																	
LOOSE-HOUSED	SLATTED FLOOR		+		+									+	+		4
	STRAW YARD	+		+			+	+			+		+				6
	CUBICLES					+			+								2
TIED												+				+	2
GRAZING										+							1

fattening animals which were loose-housed either in straw yard (6 incidents) or on slats (4 incidents) (Figure 17). These animals were fed hay and/or silage together with a rolled barley-based concentrate mixture. Hammer-milled barley was the basis of the ration given to the animals in incidents 2 and 13. The beef cow affected in incident 9 was at grass. Of the 4 incidents involving dairy cattle, 2 occurred in animals tied by the neck in byres and, in the other 2, the animals were loose-housed in cubicles. The cows were fed hay and/or silage as well as a commercial high protein concentrate.

Fourteen outbreaks occurred during the winter-housing period while the fifteenth occurred at the beginning of June (Figure 18). The geographical distribution of these incidents is presented in Figure 19. The outbreaks were widely distributed throughout Scotland (Highland region - 1 outbreak, Grampian region - 2 outbreaks, Central region - 2 outbreaks, Strathclyde region - 6 outbreaks, Dumfries and Galloway - 2 outbreaks) and northern England (Cumbria - 1 outbreak, Yorkshire - 1 outbreak).

Virus isolation

At the time of the first farm visit, nasal and/or ocular swabs were taken from several animals in 11 incidents (Table 12). The virus was isolated from nasal swabs from at least one animal in every incident and from 42 of the 56 nasal swabs examined (75%). The virus was isolated from only 3 of the 5 incidents from which ocular swabs were taken and, in total, from 5 of the 9 swabs examined (56%).

Virus was isolated from nasal and/or ocular swabs taken from individual cases admitted from 2 of the 4 incidents from which they were not obtained at the time of the farm visit.

Serological examination

Paired sera were examined from incidents 6,10,11 and 12 only (Table 13). A total of 113 paired sera were examined from incidents 6, 11 and 12. At a first examination, antibodies were present in only 18 samples (16%) whereas, at a second examination, the number of positive sera had increased to 96 (85%). This difference was statistically very highly significant ($p < 0.001$). Since the titres of the paired sera from outbreak 10 were higher at the first examination (acute phase) than at the second (convalescent phase), they were not included within Table 13.

FIGURE 17. The form of husbandry and type of animal under which infectious bovine rhinotracheitis has commonly been encountered.

FIGURE 18. The seasonal occurrence of the 15 incidents of infectious bovine rhinotracheitis.

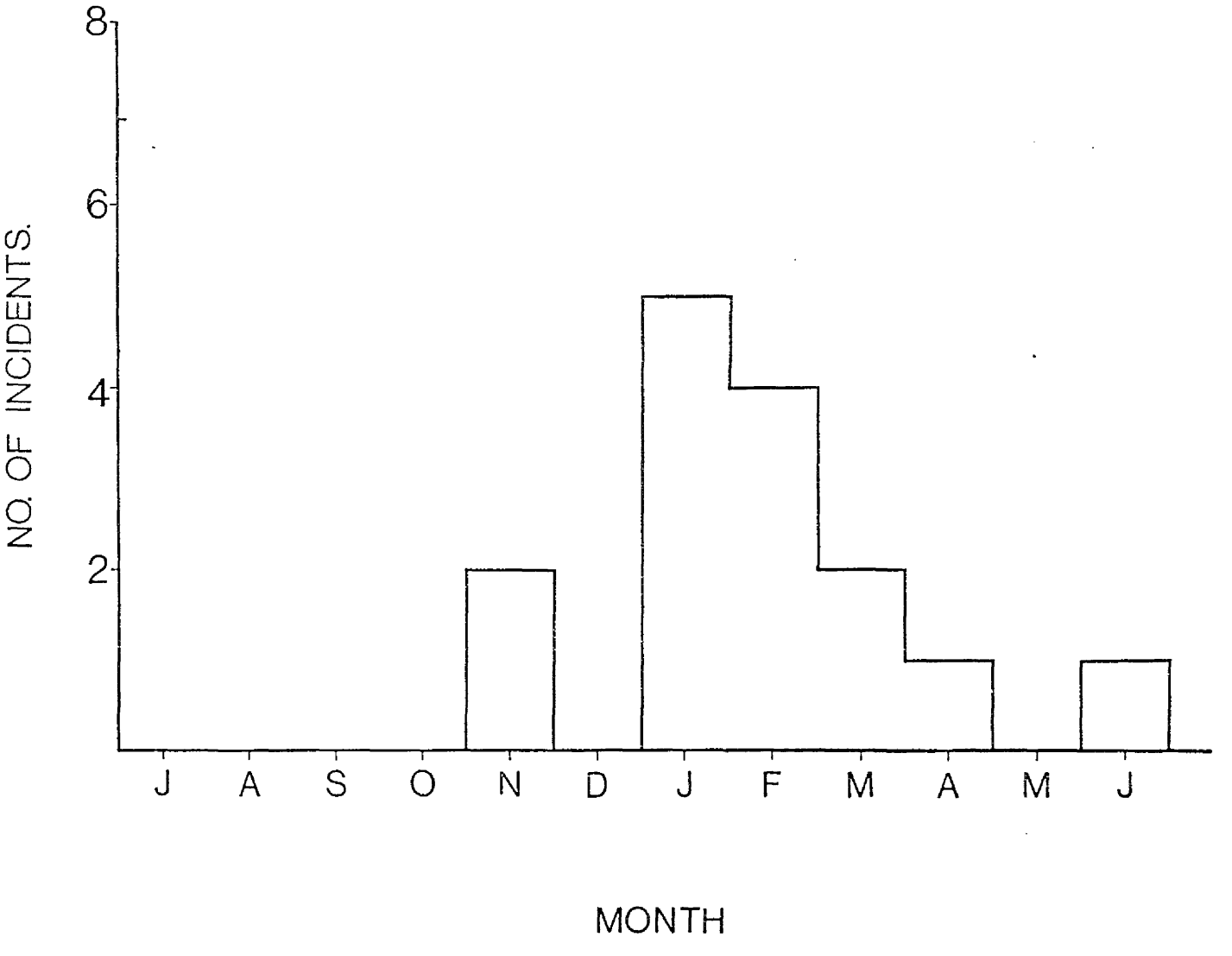


FIGURE 19. The geographical distribution of the 15 incidents of infectious bovine rhinotracheitis.

TABLE 12 The results of the examination of swabs for virus from 15 incidents of infectious bovine rhinotracheitis.

STUDY	SWABS TAKEN		OUTBREAK NUMBERS															TOTAL	(%)
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
HERD	NASAL	NO.	ND	8	5	5	9	5	ND	ND	ND	5	ND	6	5	5	3	56	
		POS.		7	5	5	6	3				1		5	4	5	1	42	(75)
	OCULAR	NO.	ND	2	ND	2	1	ND	ND	ND	ND	ND	ND	2	ND	2	ND	9	
		POS.		2		2	1							-		-		5	(56)
INDIVIDUAL	NASAL		+	+	+	-			+	ND	+	+		ND	ND		+		
ANIMAL	OCULAR		ND	ND	ND	-			ND	ND	+	ND		ND	ND		+		

TABLE 13. The results of the serum neutralisation test for the examination of antibodies in paired sera from 4 of the 15 incidents of infectious bovine rhinotracheitis.

OUTBREAK NO.	FIRST EXAMINATION		SECOND EXAMINATION	
	No. Sampled	No. Positive	(%)	No. Positive	(%)
6	16	4		14	
11	51	6		42	
12	46	8		40	
TOTAL	113	18	(16)	96	(85)
10	3	34	(100)	3	(100)

Sera from animals selected at random during the convalescent phase were available from 13 incidents (Table 14). There was a wide range in the proportion of animals in which antibodies were present, from 7 per cent in outbreak 8 to 100 per cent in outbreaks 3 and 10. Antibodies were present in 236 of the 484 samples examined (49%). Serum antibody titres were estimated in 12 incidents and in every one the mean titre was equal to or greater than 10.0; the highest was 26.4 in outbreak 9. The mean titre of the 236 positive sera was 15.2. The distribution of the titres of the individual positive sera is shown in Figure 20.

Post-mortem examination

One or more animals from 9 of the 15 incidents were available for post-mortem examination and the relevant findings are summarised in Table 15. When more than a single animal was examined from an outbreak, the significant findings from each case have been included in the table.

Numerous petechial haemorrhages were found in the nasal mucosae (Figure 21) and in other organs (heart, liver, spleen, kidneys, bladder) of both animals admitted from incident 8. A few haemorrhages were also present in the turbinates and/or nasal pharynx of animals from incidents 1,3 and 13.

A moderate to severe rhinitis was present in cases from 8 of the 10 incidents. This was characterised by necrosis of nasal mucosae which was covered by a thick yellowish-brown, diphtheritic pseudomembrane.

A moderately severe pharyngitis and severe laryngitis were present in 6 and 7 incidents respectively. In 5 incidents (1,2,3,4,7), there was a severe obstructive necrotising laryngotracheobronchitis (Figure 22). The mucous membranes of the larynx, epiglottis, trachea and major bronchi were covered by a thick yellow diphtheritic exudate. Beneath this exudate, the surface appeared congested, haemorrhagic and even granular in some cases. Widespread petechial and echymotic haemorrhages were seen in the tracheal mucosa of both animals from incident 8 (Figure 23). The trachea of recovering severe clinical cases from incident 10 showed marked oedema and congestion without evidence of necrotic material, although some frothy exudate was evident (Figure 24).

A moderate to severe exudative pneumonia was present in the anterior and ventral segments of both lungs in all 9 outbreaks. Fibrinous pleurisy was present in 4 incidents (1,3,10,13) and, in incidents 3 and 10, large thrombi were seen in the major pulmonary vessels (Figure 25).

TABLE 14. The results of the serum neutralisation test for the examination of antibodies in 15 incidents of infectious bovine rhinotracheitis.

	OUTBREAK NUMBER															TOTALS	(%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
NO. CATTLE AT RISK	46	280	150	400	300	100	13	150	86	300	53	64	ND	ND	43	1985	
NO. SAMPLED	44	42	17	25	36	16	12	83	86	4	51	46	ND	ND	22	484	(24)
NO. POSITIVE	23	21	17	20	19	14	5	6	9	4	42	40	ND	ND	16	236	(49)
MEAN TITRE	11.2	14.8	20.5	12.1	10.0	20.9	16.8	10.0	26.4	13.0	ND	14.7	ND	ND	11.8	15.2	

TABLE 15. A summary of the significant postmortem examination findings of animals from 9 of the 15 incidents of infectious bovine rhinotracheitis.

SITE	OUTBREAK NUMBERS				
	1	2	3	4	5
Turbinates	Congestion. Rhinitis. Necrosis. Haemorrhages.	Congestion. Rhinitis.	Congestion. Rhinitis. Haemorrhages.-	Normal	ND
Nasopharynx	Severe pharyngitis.	Severe pharyngitis.	Pharyngitis. Haemorrhages.	Normal	ND
Larynx	Severe obstructive laryngitis.	Severe obstructive laryngitis.	Severe obstructive laryngitis.	Necrotising laryngitis.	ND
Trachea	Severe tracheitis covered with yellowish necrotic material.	Severe tracheitis.	Severe tracheitis. Widespread haemorrhages. Necrotic material covering surface.	Congestion. Tracheitis.	ND
Lungs	Acute exudative interstitial pneumonia with fibrinous pleurisy. Interstitial emphysema.	Exudative pneumonia consolidation. Interstitial emphysema.	Consolidation. Fibrinous pleurisy. Thrombi in large vessels.	Consolidation. Oedema. Haemorrhages. Interstitial emphysema.	ND
Abomasum	Haemorrhagic ulcers.	Haemorrhagic ulcers.	Congestion. Oedema.	Normal	ND
Kidneys	Infarcts.	Infarcts.	Several infarcts.	Normal	ND
CNS	Normal.	Normal.	Normal.	Normal	ND

Table 15. (Cont'd)

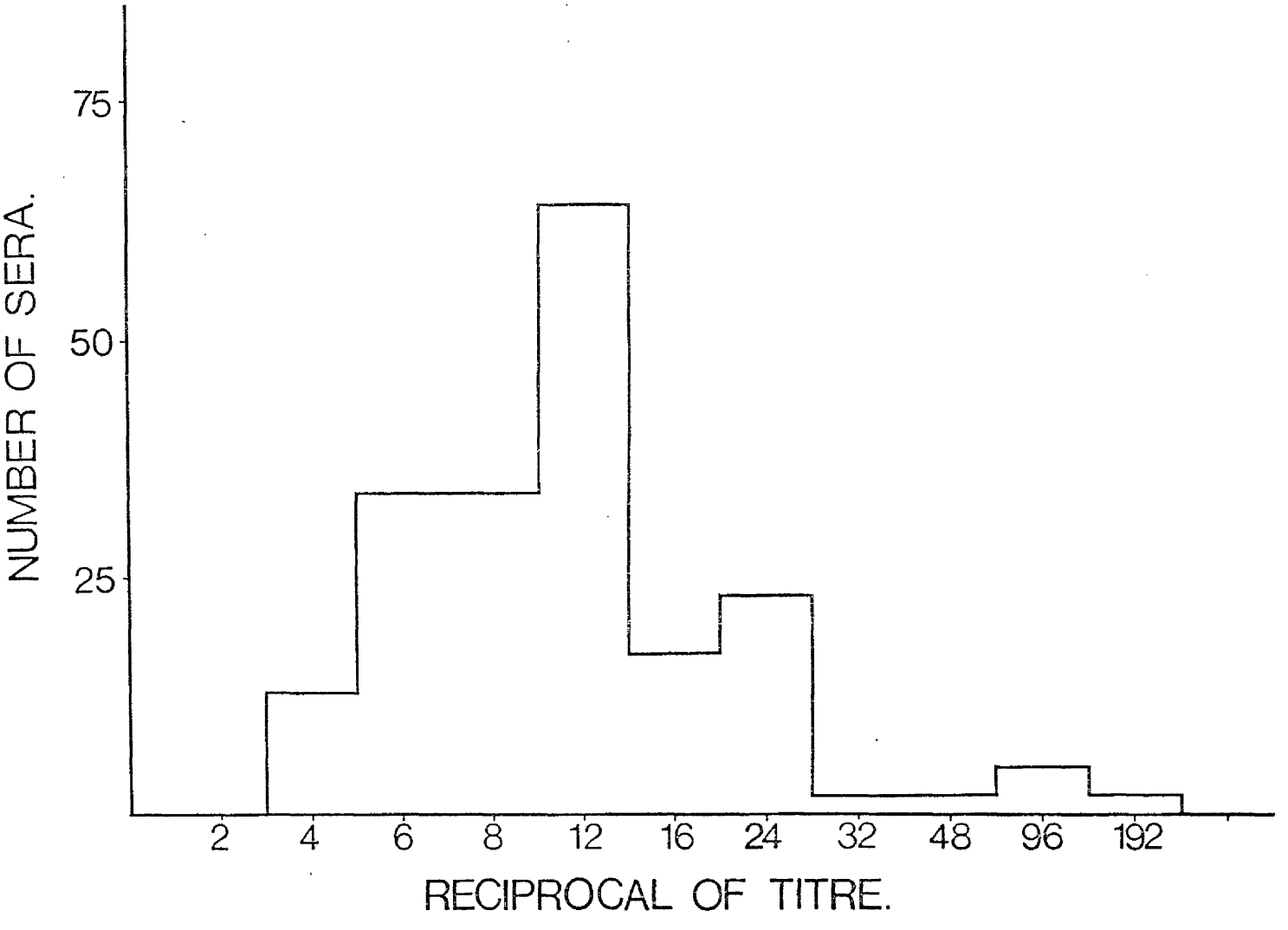
SITE	OUTBREAK NUMBERS				
	6	7	8	9	10
Turbinates	ND	Rhinitis.	Rhinitis. Widespread haemorrhages.	ND	Rhinitis. Necrosis.
Nasopharynx	ND	Pharyngitis.	Congestion. Haemorrhages.	ND	Congestion. Mucopus present.
Larynx	ND	Severe obstructive laryngitis.	Congestion. Haemorrhages.	ND	Congestion. Mucopus present.
Trachea	ND	Severe tracheitis. Haemorrhages.	Congestion. Haemorrhages.	ND	Congestion. Mucopus present.
Lungs	ND	Consolidation of the cranial lobes.	Congestion. Numerous haemorrhages.	ND	Severe pneumonia with widespread adhesions to thoracic wall and diaphragm. Suppuration in L. lung. Coagulative necrosis. Thrombi in large vessels. Interstitial emphysema.
Abomasum	ND	Normal.	Congestion. Numerous haemorrhages.	ND	Severe Ostertagiasis.
Kidneys	ND	Normal.	Enlarged. Congested. Petechial haemorrhages.	ND	Infarcts.
CNS	ND	Normal.	Normal.	ND	Normal.

Table 15. (Cont'd)

SITE	OUTBREAK NUMBERS				
	11	12	13	14	15
Turbinates	ND	Rhinitis.	Rhinitis. Necrosis.	ND	ND
Nasopharynx	ND	Severe pharyngitis.	Pharyngitis. Necrosis. Haemorrhages.	ND	ND
Larynx	ND	Severe laryngitis.	Severe laryngitis. Haemorrhages.	ND	ND
Trachea	ND	Severe tracheitis.	Tracheitis. Haemorrhages.	ND	ND
Lungs	ND	Extensive pneumonia. Interstitial pneumonia. Haemorrhages.	Interstitial pneumonia. Pleurisy. Emphysema.	ND	ND
Abomasum	ND	Normal.	Normal.	ND	ND
Kidneys	ND	Infarcts.	Infarcts.	ND	ND
CNS	ND	Normal.	Normal.	ND	ND

ND = Not done

FIGURE 20. The distribution of the titres of the individual positive sera from the 15 incidents of infectious bovine rhinotracheitis.



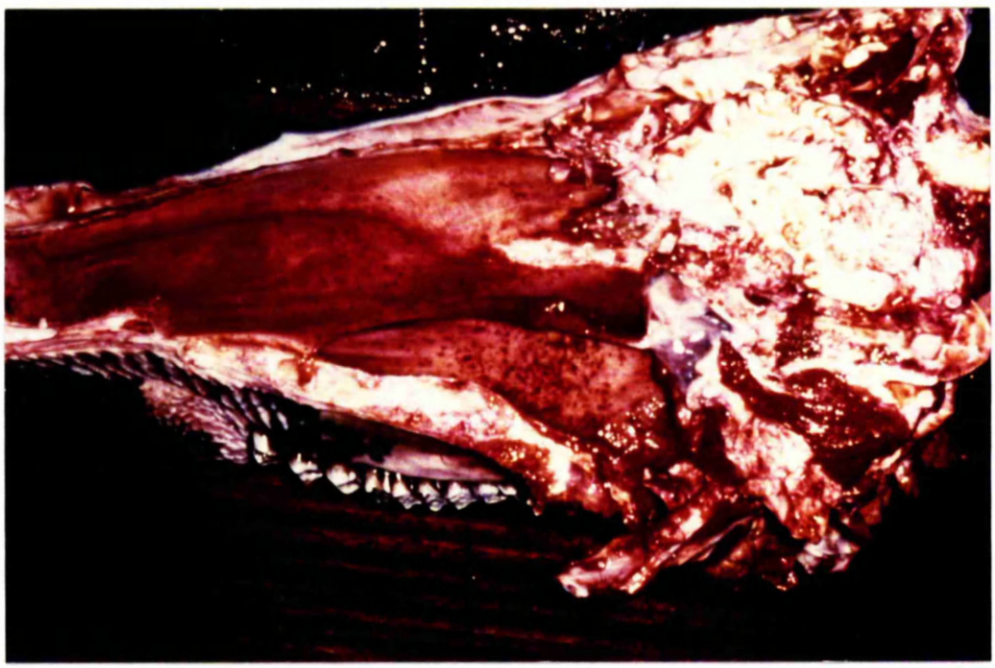


FIGURE 21. Sagital section of a dairy cow head from outbreak 8. There is marked congestion of conchae and nasopharynx with widespread petechial haemorrhages.

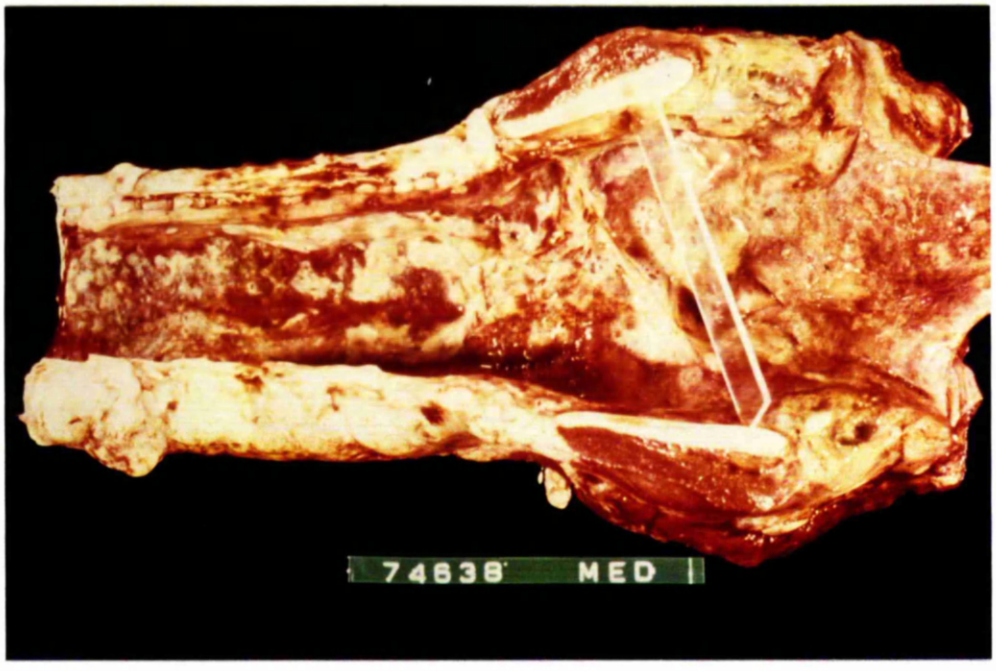


FIGURE 22. Larynx and anterior portion of the trachea from outbreak 3. The mucous membranes of the larynx and anterior portion of the trachea are covered by necrotic debris which is contributing to the obstruction of the larynx.

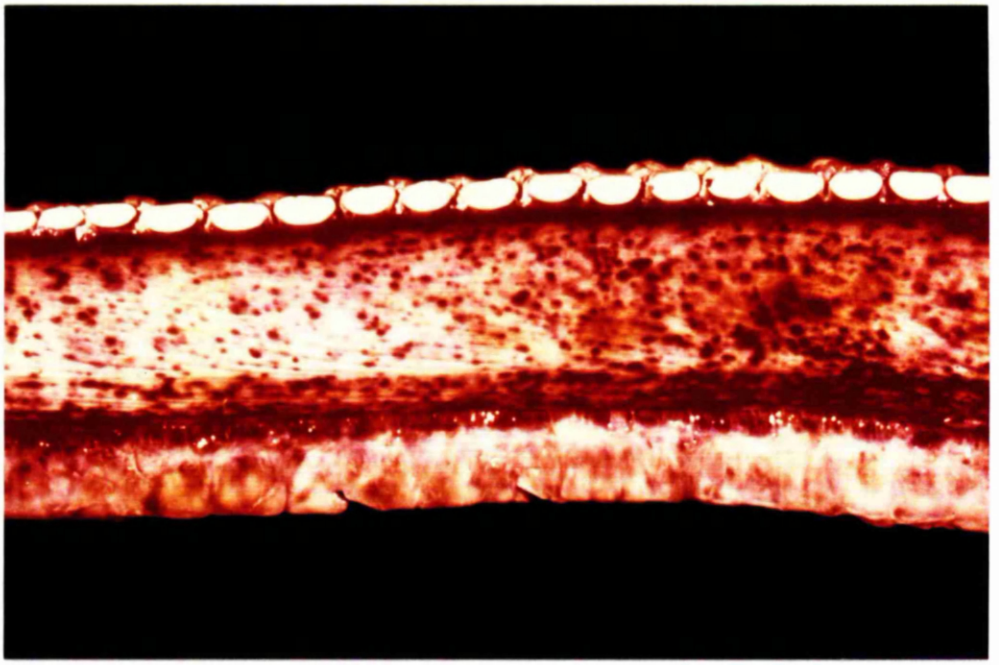


FIGURE 23. A section of trachea from a cow in outbreak 8. Marked widespread petechial and ecchymotic haemorrhages can be seen.

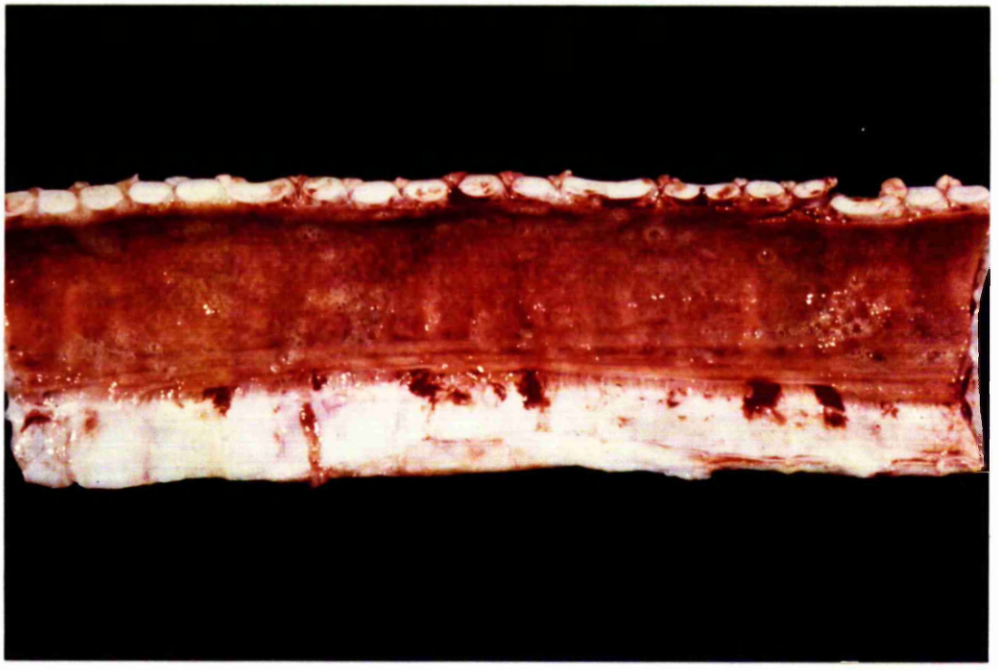


FIGURE 24. Trachea from a recovering severe clinical case from outbreak 10. Note the mucous membrane is severely oedematous and congested without evidence of necrotic material, although some frothy mucous exudate is present.

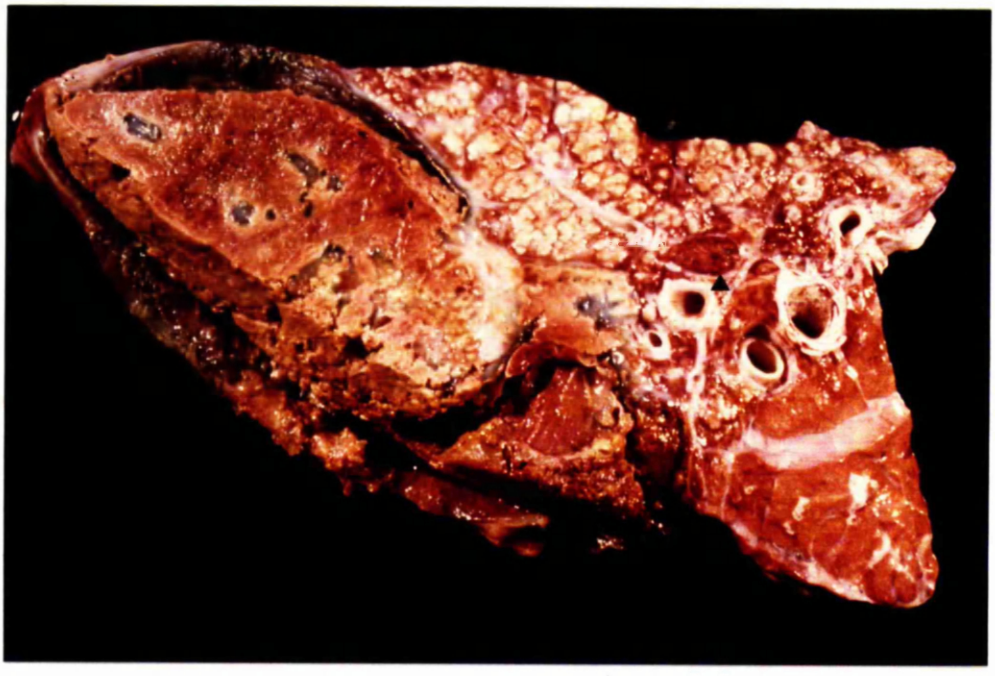


FIGURE 25. Lungs from outbreak 10. A cross-section of the anterior segment of the caudal lobe of the right lung with a severe suppurative pneumonia and large mass of necrotic pulmonary tissue probably resulting from infarction; note the thrombus (▲) in a large pulmonary vein.

Extensive renal infarction was present in incidents 1,2,3,10,12 and 13 (Figure 26). Haemorrhagic ulcers were seen in the abomasum of animals originating from 4 incidents (1,2,3,4). Abnormalities were not detected in the brain.

On microscopic examination, in virtually every case there was an acute necrotising rhinitis with hyperplasia and necrosis of the epithelium which was infiltrated by neutrophils (Figure 27). Subepithelially, in the nasal mucosa there were numerous lymphocytes and neutrophils. The pharynx was infiltrated by lymphocytes which formed discrete, germinal centre-like areas subepithelially (Figure 28). Severe laryngitis and tracheitis with congestion and neutrophilic infiltration of the lamina propria was a feature of most of these cases (Figure 29). The submucosal glands were markedly dilated with disruption causing pools of mucus to accumulate in the lamina propria (Figures 29 and 30). An acute exudative interstitial pneumonia characterised by extensive interstitial dilatation with fibrin plugs and cellular infiltration was observed in incidents 1 and 2 (Figure 31). In most areas, there was much airway plugging by necrotic debris, some of which appeared to be organising. There was bronchitis, bronchiolitis and alveolitis with macrophages and neutrophils as the dominant cell types. The alveolar walls were thickened as a result of oedema and cellular infiltrate.

Generally, the kidneys and liver were congested and infiltrated by neutrophils.

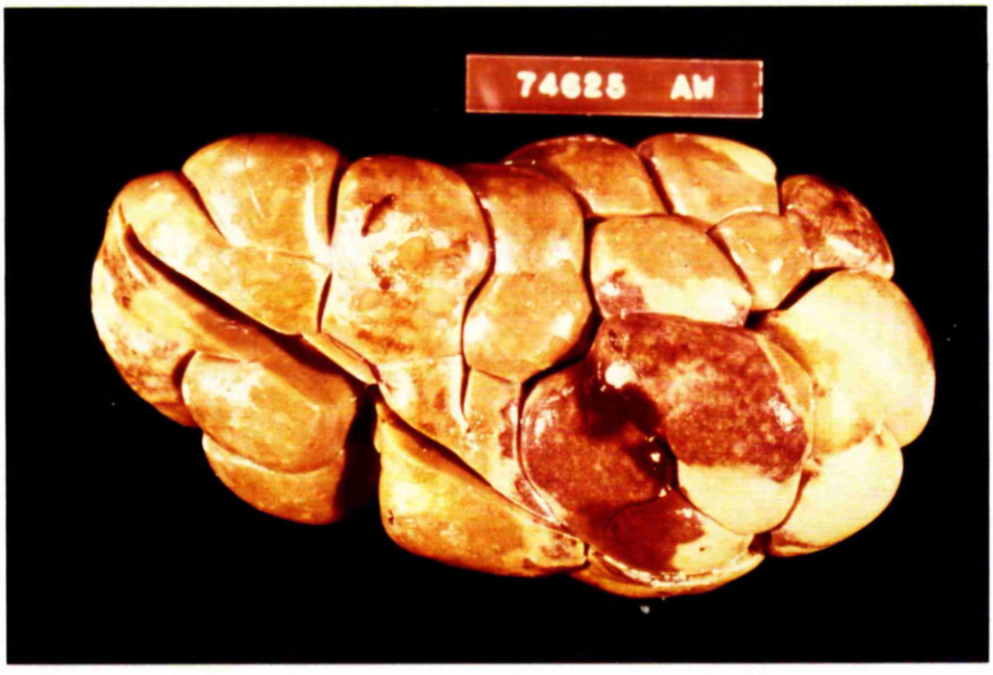


FIGURE 26. Kidney from outbreak 2. Pale infarcts are present in some lobules as well as more recent red infarcts.

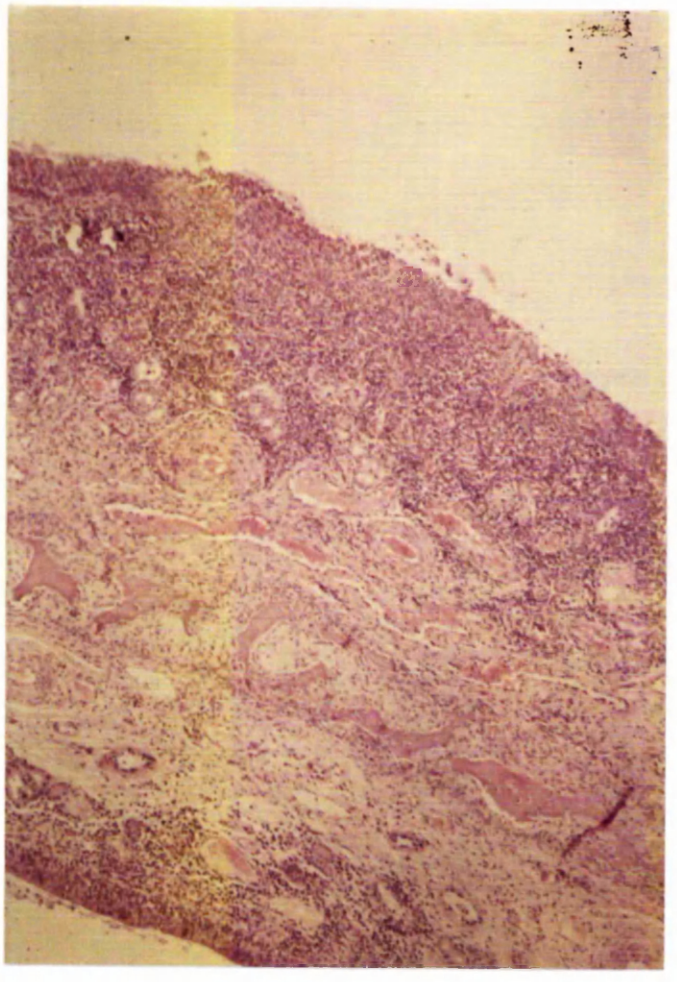


FIGURE 27. Nasal conchae of a case from outbreak 1. Areas of necrosis in the epithelium as well as infiltration by neutrophils and lymphocytes can be seen. HE X40

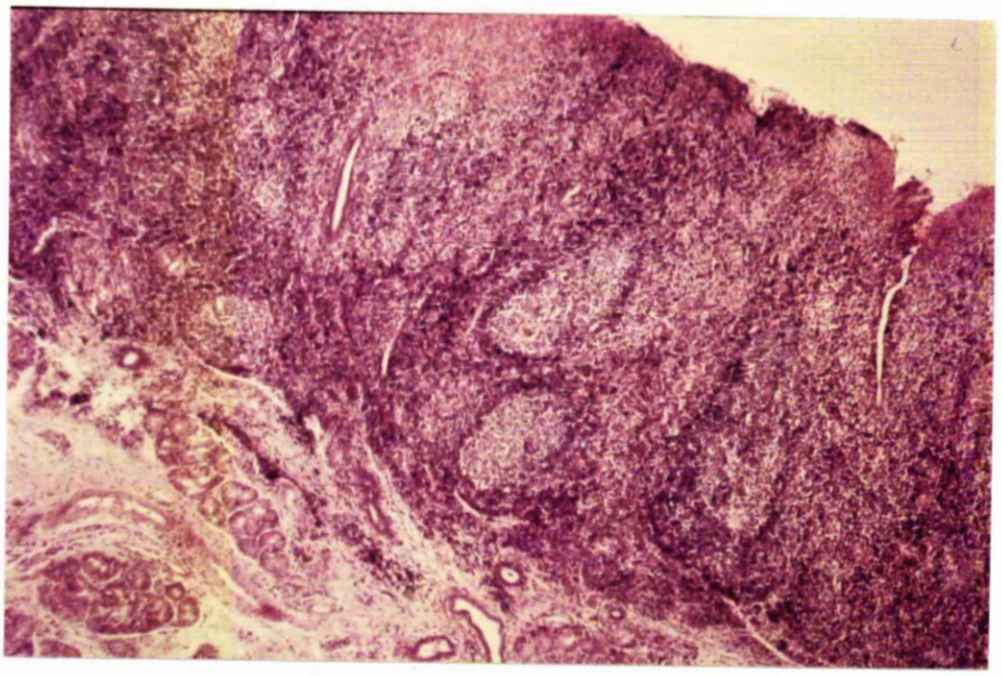


FIGURE 28. A section of pharynx from a case in outbreak I. There is marked accumulation of lymphocytes subepithelially forming discrete germinal centre-like areas and loss of epithelium from most of the surface. HE X40

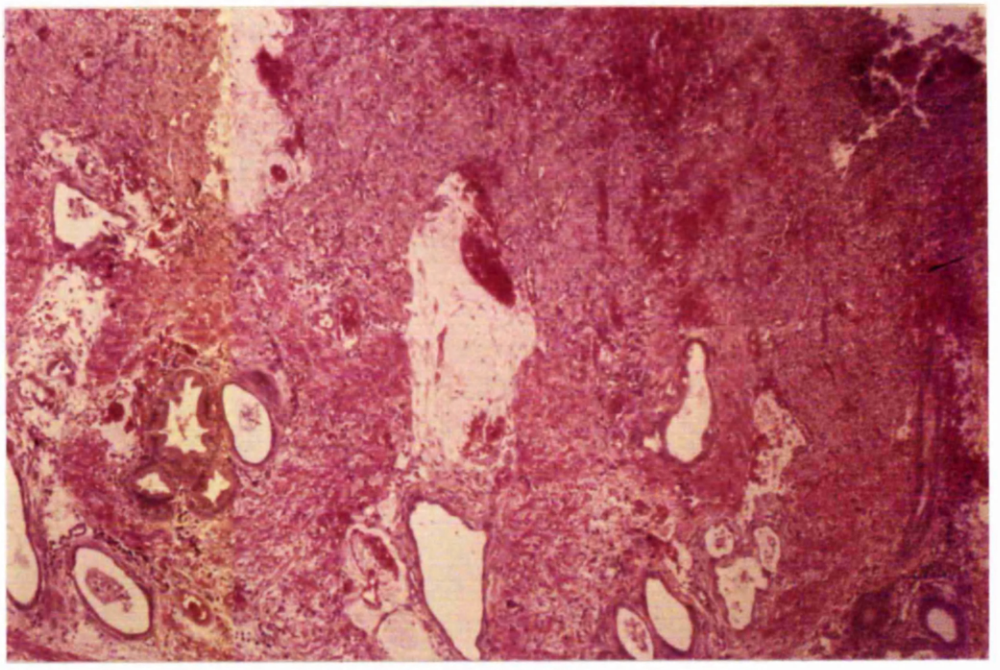


FIGURE 29. Trachea from Case 98 from outbreak 1 with severe tracheitis, congestion and neutrophilic infiltration of the lamina propria. The submucosal glands are severely dilated with disruption causing pools of mucus to accumulate in the lamina propria. HE X 40

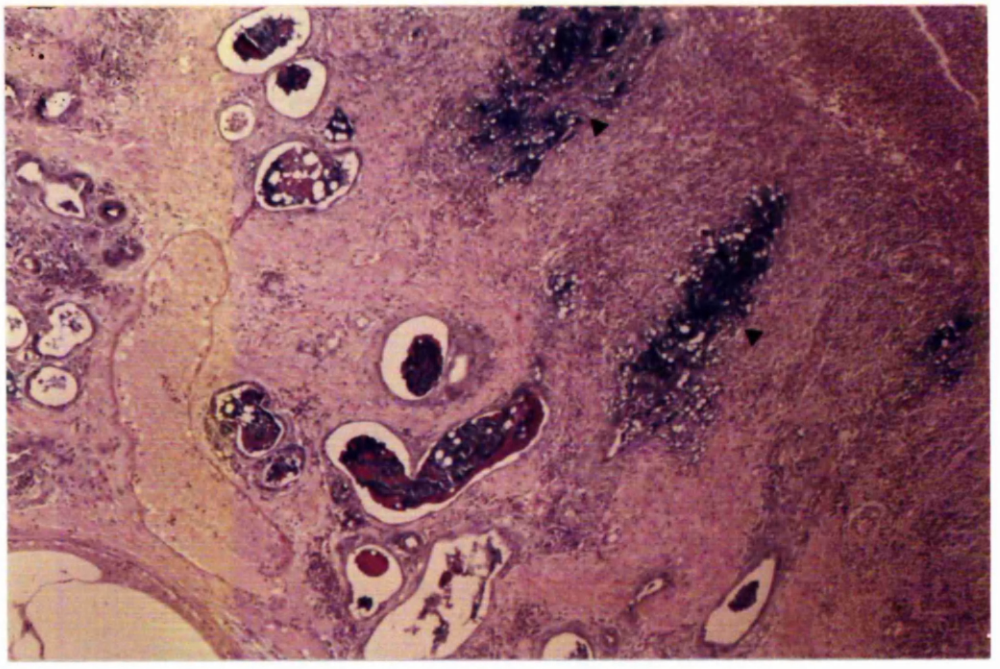


FIGURE 30. Section of the trachea of Case 98 from outbreak 1 specifically stained for mucus glycoproteins. The submucosal glands are severely dilated, filled with mucus and pools (▲) are recognised in the lamina propria. Alcian blue/Periodic Acid-Schiff X 30.

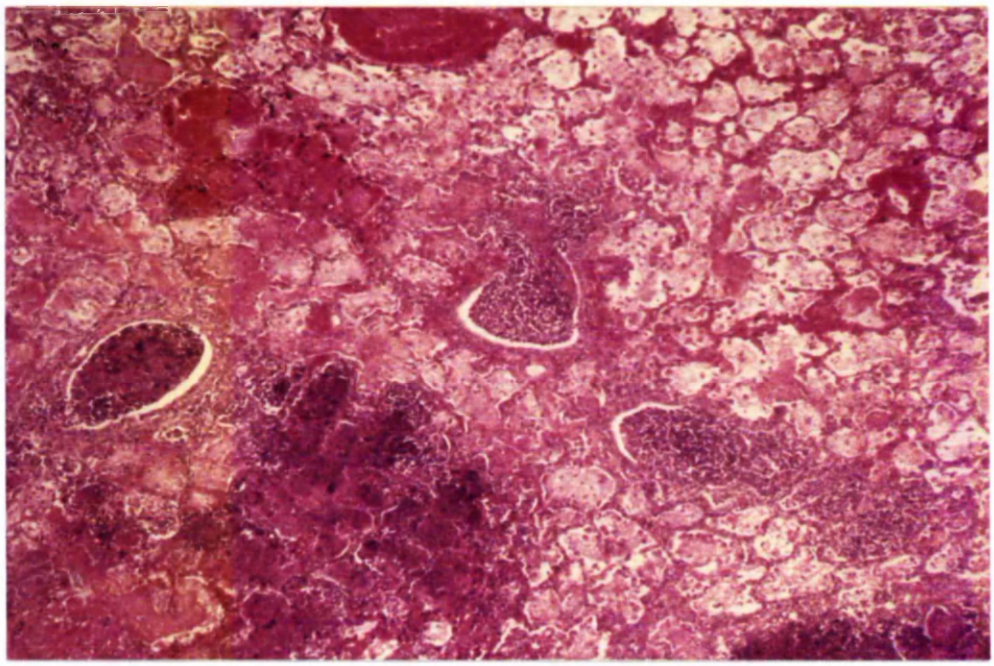


FIGURE 31. Lung from Case 98 from outbreak 1 with severe exudative pneumonia. There is congestion and oedema in the alveoli with plugging of bronchioles by inflammatory cells and their debris. HE X40

DISCUSSION

Prior to the development of severe IBR, respiratory disorders were not commonly encountered on any of these 15 farms although pneumonia in young housed calves and transit fever in beef cattle had been diagnosed sporadically on a few farms in previous years. Transit fever is the name given to an acute respiratory disease which affects recently purchased fattening cattle in the autumn and winter (Hepburn, 1925; Anderson, 1939; Pickering, 1939). Since many incidents of severe IBR involved recently purchased cattle, it was not unexpected that the term "transit fever" was used to describe this syndrome which was recognised with increasing frequency during the winter of 1977-78. However, it soon became obvious that transit fever and the "new" respiratory syndrome were different; the clinical signs of transit fever were those of a severe exudative pneumonia whereas those of the "new respiratory syndrome" were confined mainly to the upper respiratory tract and eyes. The difference between the two conditions was particularly obvious in incident 1, which has been described in detail. A single bullock became very dull, anorexic, pyrexic, tachypnoeic with adventitious lung sounds. A diagnosis of transit fever was made and there was a slow, but definite, clinical response to antibiotic therapy. In marked contrast, the condition which arose later in another group of recently purchased cattle initially affected several of those at risk; they were slightly dull, had a reduced appetite, profuse ocular and nasal discharge but no evidence of pneumonia.

A bilateral serous nasal discharge was common in every incident during the initial stages of the disease, but it was difficult to appreciate fully the number of animals affected since many affected individuals were seen to be constantly licking their noses. However, as the disease progressed the discharge became mucoid and, in severely affected or protracted cases, a bilateral mucopurulent discharge was often seen. Cracking and ulceration of the muzzle as was reported in the original descriptions of "red nose" in the United States of America (Miller, 1955) were never observed even in protracted cases. On close examination of the nares however, there was usually obvious congestion of the nasal mucosa and yellowish-brown, diphtheritic plaques were often seen on the ventral and lateral aspects of the nasal passages. In such cases, the foetid smell characteristically associated with a necrotising secondary infection was often present. However, in one of the two cases admitted from outbreak 1 a sweetish unpleasant odour was detected. In this case, it is possible that the peculiar smell had resulted from the

profuse growth of Alcaligenes faecalis which has been stated to produce a "strawberry-like smell" (Holding and Shewan, 1974).

An increase in the frequency of coughing was one of the commonest early signs of disease. The type of coughing was said by many farmers to suggest that affected cattle "were trying to clear their throats". Although coughing was frequent, only single coughs were heard; paroxysms of coughing such as occur in parasitic bronchitis or severe calf pneumonia, were not reported.

Clinical signs indicative of pneumonia were not observed in the early stages of any of these outbreaks but in severe or prolonged cases admitted to the Glasgow Veterinary School, pneumonia was invariably present at necropsy. Although rhonchi and crackles were heard cranio-ventrally in 2 animals, the respirations were so harsh as to make it very difficult to decide whether the noises were in fact adventitious lung sounds or were referred from the trachea.

Another common presenting sign and one which was observed in 13 of the 15 incidents was a profuse, often bilateral, serous ocular discharge. In some animals this persisted for several days resulting in the hair of the cheeks becoming matted and remaining so for a considerable time afterwards. The cause of the lachrymation was a moderate to severe conjunctivitis and in a few of the severely affected cases, there was obvious conjunctival oedema. Neither spontaneous eversion of the oedematous conjunctiva nor punctate haemorrhages as described by Dawson and his colleagues (1962) were observed. While conjunctivitis was not the only sign of infection in any of these outbreaks, Imray (1979a) has reported that in a few incidents in the present epidemic conjunctivitis was the only clinical abnormality detected. This agrees with previous observations (Abinanti and Plumer, 1961; Timoney and O'Connor, 1971).

One or two severely affected animals on two farms had marked congestion of their scleral blood vessels and also small 1-2mm subepithelial granular lesions. Two calves experimentally infected with IBR also developed similar lesions (Chapter 5).

Only on 1 of the 11 farms on which IBK was present was there said to have been a sudden increase in the number of new cases coincidental with the development of IBR. Previous workers have also noted an association between IBR and IBK (Hughes and others, 1964; St. George, 1965) and evidence of a synergistic effect between this virus and Moroxella bovis has been demonstrated experimentally (Pugh, Hughes and Packer, 1970).

Drooling of saliva was also observed in 13 incidents and this appeared to be most common in animals which became ill at the beginning of an incident. This usually consisted of fairly constant dribbling which caused the hairs on the lower jaw to become wet. However, the salivation was so profuse in outbreak 12 that the practitioner who first examined these cases considered seriously the possibility of foot and mouth disease. During the initial stages of the disease, the water in the troughs was often seen to be covered with copious amounts of saliva and nasal discharge. Excessive salivation, as this is usually termed, has previously been observed in field cases in North America (Miller, 1955; McKercher, 1959; Curtis and others, 1966) and also following experimental infection with this strain (Strichen, Chapter 5) as well as other strains of virus. Although there may very well have been an increased secretion of saliva from the submucosal pharyngeal glands, almost all cases examined at necropsy had a severe necrotising pharyngitis. Consequently, the drooling of saliva probably resulted more from an unwillingness or inability to swallow the normal production of saliva since it is secreted continually in cattle (Coats, Denton, Goding and Wright, 1956). This deduction would appear to be substantiated by the observation that week-old calves suffering from IBR had difficulty in swallowing since milk was observed coming down their nostrils while feeding (Baker and others, 1960). Their breathing also became noisy, apparently due to oedema in the laryngeal region. In the current epidemic, severely affected individuals which were frequently admitted as chronic or terminal cases, were mouth-breathing with large amounts of froth around their mouths. In these cases, dyspnoea was due to large amounts of necrotic debris which had caused a considerable degree of obstruction of the larynx and nasal passages. The character of mouth-breathing was peculiar in that affected animals breathed in through their mouths but out through their noses; this too confirmed the almost total obstruction of the upper respiratory tract.

Although oral lesions were not found either clinically or at necropsy, a reduction in appetite was apparent in every incident. On 4 farms, the animals rejected hay and/or silage while continuing to eat concentrates. This would suggest that roughage was painful to chew and/or swallow. On the other hand, the owner of farm 13 said that his cattle ate their hay but left their concentrates.

In outbreaks 2 and 13, the farmers reported that several animals had been found dead after being obviously only ill for 2 or 3 days. Unfortunately, none of these so-called "sudden deaths" were examined at

the Veterinary School. However, the case admitted from outbreak 7 died as it was being unloaded and, at necropsy, its larynx was found to be almost totally occluded with necrotic debris. Therefore, it is highly likely that many, if not all, of these individuals which were found dead after a short illness, had died as a result of widespread severe obstruction of the upper respiratory tract. Support for this hypothesis was apparent when dyspnoeic animals were observed; such cases often had inspiratory dyspnoea.

On the 4 farms on which abortions occurred, it was never confirmed that IBR had been responsible. Nevertheless, IBR virus infection was blamed for 6 cows aborting on farm 4 even though clinical signs of respiratory disease were never seen in any of the in-calf cows and the virus was not isolated from any of the 6 foetuses. In outbreak 8, it was noticed that several calved heifers returned to the bull a few weeks after their first service. This problem had not occurred before the IBR incident and has not recurred since and so the farmer is of the opinion that this temporary decrease in conception rate was a manifestation of the IBR infection. The dramatic reduction in conception rate which occurred in outbreak 5 immediately the clinical signs of IBR had subsided, was later confirmed as being due to Vibrio fetus infection.

An increase in the mortality rate of young calves as has been reported by Van Kruiningen and others, 1964 and by Lomba and others, 1973, did not occur on any of the 5 farms during the time when IBR was prevalent in the cows. However, in outbreak 5, there was said to have been an increase in the frequency of coughing in the calves and also in the number treated for "virus pneumonia". Infectious bovine tracheitis virus was isolated from nasal and ocular swabs taken from 2 calves with typical clinical signs. Unfortunately, when one died after allegedly becoming blind, a post-mortem examination was not undertaken. It is possible that the last mentioned calf had died following an IBR-induced encephalitis. This syndrome has yet to be confirmed in Britain although it has been well documented in Australia (Johnstone and others, 1962) and in North America (Reed and others, 1973).

Diarrhoea, which has been reported in previous incidents (Miller, 1955; Van Kruiningen, 1964) was observed in a few individuals but in none of these was there definite evidence that this was any more than a coincidence. Abomasal ulceration may have been partially responsible for the diarrhoea although the only animal examined at necropsy which had significant abomasal lesions was not diarrhoeic.

There was no confirmatory evidence that dermatitis had resulted from infection with IBR virus although peculiar circular skin lesions were seen on the posterior aspect of the udder of one cow. The exact cause of these lesions was never determined and attempts to isolate the IBR virus from skin scrapings were not successful.

In common with the vast majority of other workers, IPV were not observed in any of the animals clinically affected with IBR. In addition, there was no history of any female having shown typical clinical signs of IPV prior to, or following, our farm visits. However, this syndrome had been confirmed 4 to 5 years previously on farm 5 and, at that time, clinical evidence of respiratory involvement was not observed.

A course of antibiotic therapy was given to sick animals in 14 outbreaks but, overall, the clinical response was variable. This was not entirely unexpected since antibiotics do not reduce the severity of a primary viral infection. However, it has been stated that, in many instances, severe clinical signs are the result of secondary bacterial infections (McKercher and others, 1957; Curtis and others, 1966). Therefore, the apparent failure or success of antibiotic treatment in an individual animal is dependent not only on the efficacy of the specific drug used, but also on the stage of the disease at which the treatment is given.

Corticosteroids are frequently given to young cattle when a diagnosis of "virus pneumonia" has been made. It is possible that the administration of betamethasone, which was given to several animals in 2 outbreaks, exacerbated the disease because it is well known that drugs with glucocorticoid activity inhibit the development of immunity, particularly cell-mediated immunity. Therefore, in cattle infected with IBR virus, the use of such drugs is contraindicated because cell-mediated immunity is considered to be more important than humoral immunity in herpesvirus infections.

Clinical signs were first observed on all bar 2 of the farms less than 4 weeks after the introduction of animals purchased from a market. In one incident, affected individuals had recovered after only 1 week whilst, in others, new cases continued to arise for about 8 weeks. The average time for the disease to spread throughout a unit was from 4-5 weeks by which time almost all the cattle at risk had been obviously affected. The disease was most severe in those herds in which there was high turnover of animals; these were usually beef fattening units.

Undoubtedly, the more cattle there were housed together, especially on slatted floors, the more severe was the clinical syndrome.

On the 2 farms where the mortality rate was highest (incidents 3, 13), a hammer-milled barley-based concentrate ration was being fed and, although molasses had been included to minimise the dustiness, it was noticeable that many animals had their muzzles and all round their mouths covered in finely powdered concentrate. On close examination of several animals, particles of food could be seen adhering to the nasal mucosa. There can be little doubt that this had contributed to the severity of the clinical syndrome and probably also to the high mortality rate on these 2 units.

The large cattle fattening units are busiest during the winter housing period, when their cattle turnover is greatest, and it is also at this time that IBR has been found to be most prevalent and most severe. While animals undoubtedly develop clinical signs of IBR when kept outside during the winter, the clinical signs are relatively mild. The same is true in grazing animals during the summer as was seen in outbreak 9.

The established relationship between the fattening of purchased cattle and the likelihood of their contracting IBR explains the geographical distribution of not only the incidents described here but also those reported by Cuthbertson and Wood (1979) and by Imray (1979_a). Furthermore, this finding also explains why IBR has so far not been confirmed in Wales, the Inner and Outer Hebrides, Orkney or Shetland since young cattle are exported to be fattened elsewhere and virtually no young animals are imported.

The virus of IBR was isolated from nasal and ocular swabs taken from affected animals in 13 incidents. The frequency of virus isolation was greater from nasal than from ocular swabs, therefore, the former should be recommended for the purposes of virus isolation from field outbreaks. In addition swabs should be taken from several affected animals since excretion of virus occurs for only 12-14 days post-infection (Chapter 5). Consequently this is the reason why the virus was not isolated from several protracted cases admitted to the Veterinary School. The virus isolates induced rounding and ballooning of bovine tissue culture, but inclusion bodies were not seen. The classical isolates of IBR virus (Colorado and Oxford) not only produced a similar cytopathogenic effect but also large Cowdry type A intranuclear inclusion bodies (Dawson and others, 1962). Although intranuclear inclusion bodies are a frequent

feature of BHV1 infections, they are not a constant finding since some North American respiratory isolates have also failed to show this characteristic (Madin and others, 1956; York and others, 1957).

Paired serum samples were available from 4 incidents and in 3 of these a significant number of animals seroconverted. The prevalence of neutralising antibodies overall was 49 per cent and was much higher in fattening beef cattle (75%). These values are considerably higher than the average found recently in the serological survey (Chapter 3). Consequently, for routine diagnostic purposes the sampling of a representative number of animals during the convalescent phase should be sufficient to confirm recent IBR infection.

In the animals examined at necropsy, the range of lesions and their distribution were basically similar although obviously there were differences of degree. Inflammation of the upper respiratory tract as well as diffuse diphtheritic pseudomembrane formation were characteristic of most of the outbreaks. The yellowish necrotic debris was present in sufficient quantities to produce partial to total obstruction of the nasal passages and of the larynx. Its presence during respiration was almost certainly the cause of the tracheal rattles which were occasionally heard on auscultation. Although haemorrhages were seen on the epithelium in several animals, they were present throughout the body in both animals from outbreak 8. Their widespread nature suggests that a secondary septicaemic agent, such as leptospira species, had caused these lesions although attempts to isolate such an agent were unsuccessful.

The exudative pneumonia, which was present in every animal examined at necropsy, involved the cranial lobes and the cranio-ventral segments of the caudal lobes. Interstitial emphysema was present in 5 animals and this had probably occurred as a result of increased airway resistance produced by the severe obstructive laryngotracheobronchitis. These findings are in agreement with the observations of McKercher and others (1957) and Curtis and others (1966). A feature of particularly severely affected individuals was thrombosis of the large pulmonary vessels which does not appear to have been reported previously.

In addition to the respiratory tract lesions, renal infarction was commonly encountered and probably had resulted from emboli from the thrombosed pulmonary veins.

The most striking feature of the microscopic changes was necrosis of the epithelium; in severe cases virtually the whole of the

epithelium of the upper respiratory tract had been eroded and had been replaced by a yellowish-green or yellowish-brownish diphtheritic pseudomembrane. These lesions had almost certainly caused blockage of the opening of the mucosal gland ducts since pools of mucus were seen in the lamina propria. Furthermore, there was obvious severe dilatation of many of the tracheal sub-mucosal gland tubules and ducts. In none of these animals were intranuclear inclusion bodies recognised in the tissues examined histologically. This was not unexpected since they developed soon after infection and can only be seen for a relatively short period of approximately up to 60 hours (Crandell and others, 1959).

Encephalitis, liver necrosis and enteritis, which have all been attributed to IBR virus infection (Gibbs and Rweyemamu, 1977), were not detected.

The recovery of Mycoplasma bovis from 5 outbreaks may be significant in view of the fairly extensive pneumonia present. This organism has already been isolated from outbreaks of pneumonia in housed calves in Britain (Thomas and others, 1975; Allan, Obi, Wiseman, Cornwall, Selman, Msolla and Pirie, 1978). The susceptibility of these animals to M. bovis infection may very well have been increased as the result of infection with IBR virus. The only other recognised pathogens isolated were Pasteurella haemolytica and Streptococcus pneumoniae. It is tempting to speculate that the bacteria and mycoplasma had significantly influenced the severity of the disease. However, it is perhaps more significant, that recognised pathogens were not isolated from the other animals.

This severe form of IBR seriously affected the level of productivity of affected animals. It was interesting to ascertain that, despite the relatively high number of casualties including both dead animals and culls, this represented only 23 per cent of the total financial loss from these incidents. In the beef fattening animals, the cost of extra feeding represented 67 per cent of the financial loss; this was by far the most expensive, single cost. The other expenses, which included the cost of drugs, treatment and the value of the milk lost, represented only 13 per cent of the total amount. Even though these figures can only be regarded as estimates, no allowance has been made for the amount of nervous energy expended by individual farmers living through an incident of severe IBR. Indeed, one of these farmers had to seek medical advice since he was on the verge of a nervous breakdown. The astronomically high average cost per incident (£3,840) and the very high cost per animal

at risk (£240) illustrates in the clearest possible manner the need for an effective control programme for this disease.

The clinical, epidemiological and pathological features of these 15 outbreaks of IBR have confirmed that the disease is virtually identical to that first reported in the United States of America during the 1950s (Miller, 1955; McKercher and others, 1957).

CHAPTER 3

A SEROLOGICAL SURVEY OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS INFECTION IN SCOTTISH HERDS

INTRODUCTION

Following the recognition of clinical IBR (Dawson and others, 1962; Darbyshire and Shanks, 1963), a serological survey was undertaken to determine the occurrence and distribution of antibodies to the virus in the United Kingdom (Dawson and Darbyshire, 1964). Of 2,000 sera obtained from abattoirs (1000) and from suspected incidents of mucosal disease (1000), serum neutralising antibodies to IBR virus were detected in only 42 (2.1%) samples. These workers concluded that infection was wide-spread throughout the country although outbreaks of clinical disease had not been reported frequently.

More recently Kirby, Martin and Waring (1978) examined 2368 sera taken during the brucellosis eradication scheme from dairy herds in Berkshire, Buckinghamshire and Oxfordshire. Using the indirect haemagglutination test, they found antibodies in 162 (6.8%) sera. Despite considerable variation in the prevalence of antibodies between these counties, again infection was not known to be associated with clinical disease. It can be deduced, therefore, from these and other reports, that IBR was a relatively mild and economically unimportant respiratory disease, although infection was present in many different parts of the country.

During the winter-housing period of 1977-78, a severe upper respiratory disease appeared in Britain; this was later confirmed as being IBR (Wiseman, Msolla, Selman, Allan, Cornwell, Pirie and Imray, 1978). Many such incidents were confirmed in the Grampian region of Scotland (Cuthbertson and Wood, 1979; Imray, 1979a) although the disease has also occurred in most of the other regions (Wiseman, Selman, Msolla, Pirie and Allan, 1979). Since the nature of the disease had changed dramatically, it was decided to investigate, in detail, the prevalence of antibodies to IBR virus in Scotland.

MATERIALS AND METHODS

Herd description

A "self-contained" herd has been defined as a herd into which purchased animals had not been introduced for several years. A "bought-in" herd has been defined as a herd into which purchased animals had been introduced recently.

Serum samples

Blood samples were taken from 10 adult animals, selected at random, from a number of herds in Scotland. The blood was collected from the caudal vein into vacutainers with no additive (Becton-Dickinson, UK. Ltd., England). The samples were stored overnight at $4^{o}C$ and, after being spun at 1300g for 30 minutes, the serum was removed, put in a waterbath at $56^{o}C$ for 30 minutes, left to cool and then stored at $-20^{o}C$ until required.

Infectious bovine rhinotracheitis virus

The virus which was isolated from Outbreak 1 was grown as described in Chapter 2. In the serum neutralisation test, this virus was in its third passage in secondary CK cultures.

Tissue cultures

The secondary CT cultures, which were prepared as described in Chapter 2, were used in the serum neutralisation test. The secondary CT monolayers were dispersed with a mixture of 0.25 per cent trypsin (2ml of 2.5% trypsin in versene) and a 0.02 per cent versene in PBS. The cells were then suspended in M199 medium, containing 10 per cent FBS, at a concentration of 2×10^{5} cells/ml.

Serum-virus neutralisation tests

The serum neutralisation tests were carried out in flat-bottomed, tissue culture-grade microtitre plates (Linbro-Cooke-96, Flow Laboratories, Scotland). The serum samples were diluted initially to 1:4 with sterile PBS.

Using a dropper pipette, 0.025ml of M199 medium was dropped into each well except for those in the first column. An equal volume of a 1:4 dilution of serum was placed in the first and second wells of the

first two columns and in each well of the last column. Two rows were used for each test serum sample. Using a 0.025ml hand multi-diluter and starting with the second column, serial two-fold dilutions were made.

To each well, except those of the serum control column, there was added 0.025ml of virus suspension containing $30TCID_{50}$/ml. This had previously been determined using the microtitre system and was titrated each time the test was performed to check the titre.

The Plates were then sealed with sterile aluminium foil and incubated at 37°C for 2 hours to allow neutralisation. At the end of this period, 0.1ml of the secondary CT cell suspension was added to each well. The plates were then incubated at 37°C in an atmosphere of 5 per cent carbon dioxide.

The test was read on the third day by examining the cell sheet in the wells for evidence of CPE using an inverted microscope. The titre of the test serum was expressed as the reciprocal of the final dilution of the serum present in the serum/virus mixture at the 50 per cent end-point estimated according to the method of Karber (1931). The plates were used widthwise so that only dilutions from 1:4 to 1:256 were possible; the last column was the serum control.

RESULTS

The detailed results of the prevalence of serum neutralising antibodies to IBR virus in the individual herds are given in Apppendix 2. These herds have been classified according to the geographical location of the farm, the vast majority of which were situated in the Grampian and Strathclyde regions, Figures 32 and 33 respectively.

A total of 1152 serum samples from 114 herds were examined. Antibodies, at a dilution of 1:4 or greater, were demonstrated in 140 (12%) samples and in 58 (51%) herds. Of the 1039 dairy cattle sampled, 117 (11%) had antibodies compared to 23 (20%) of the 113 beef animals sampled. In 9 out of 11 (82%) of beef herds, there was at least 1 animal with antibodies whereas only 49 out of 103 dairy herds (48%) were similarly involved. Clinical signs suggestive of IBR had been observed in previous years in 7 herds and the proportion of positive sera in these herds was greater (5/7:71%) than in the others (53/107:50%).

The prevalence of antibodies in dairy cattle in the various regions is given in Table 16. There was no significant difference in the prevalence between the total number of positive sera in 2 main regions, Grampian (47/409:12%) and Strathclyde (62/560:11%). A small number of samples only were examined from the Central and Dumfries and Galloway regions; in the former region 8 (20%) of the 40 samples were positive, but antibodies were not detected from any of the 30 sera from cattle in the latter region.

The prevalence of antibodies was significantly higher ($p<0.05$) in cows (110/877:13%) than in heifers (7/162:4%). In the Grampian region, the proportion of cows (43/373:12%) and heifers (4/36:11%) with antibodies was similar. However, in Strathclyde, considerably more cows (59/464:13%) than heifers (3/96:3%) had antibodies; this difference was statistically significant ($p<0.05$).

There was very little difference between the prevalence of antibodies in the self-contained herds (103/928:11%) and in the bought-in herds (14/111:13%). In Grampian, considerably more samples from self-contained dairy herds were positive (44/348:13%) than from the bought-in dairy herds (3/61:5%). The effect of the presence of Holstein cattle on the prevalence of antibodies in samples from Grampian region can be seen in Table 17. Twenty-three (40%) of the 58 samples from "Holstein"

herds were positive compared to only 24 (7%) of the 351 sera from the other herds in the region. Only Holstein cattle had been added to these herds and so they have been classified as "self-contained". If, for the purposes of this particular study, the herds to which Holstein cattle had been added were considered as "bought-in" herds, then the prevalence of neutralising antibodies was significantly higher ($p<0.01$) in the bought-in than in the self-contained herds (Table 17). In the Strathclyde region 51 (10%) of the 510 samples from self-contained dairy herds were positive compared with 11 (22%) of the 50 samples from bought-in herds.

The herds were divided into 3 groups according to their size (up to 100 cows, from 101 to 200 cows, more than 201 cows). In the Grampian region, there were seropositive animals in 7 of the 19 herds (37%) with less than 100 cattle, in 8 of the 16 herds (50%) with 101 to 200 cattle and in 4 of the 5 herds (80%) with more than 201 animals. A single animal with antibodies was present in 6 herds (86%) with less than 100 animals, in 4 herds (50%) with 101 to 200 animals, but in none of the 4 herds with more than 201 animals. In the Strathclyde region there were seropositive animals in 19 of the 41 herds (46%) with less than 100 cows and 7 of the 15 herds (46%) with 101 to 200 cows. There was only a single positive animal in 8 of the 19 herds (42.1%) and 3 of the 7 herds (42.9%) in either group respectively. Therefore, there appeared to be a positive relation between the prevalence of antibodies and herd size in the dairy cattle in the Grampian region, but not in Strathclyde.

When the mean herd size of these 3 large groups detailed above, was calculated, it was found that there was an obvious positive relationship between the mean herd size and the percentage of seropositive animals overall and also in the Grampian region (Figure 34).

The effect of region, herd size and type of herd on the prevalence of serum neutralising antibodies is presented in Table 18. The mean titre of all positive samples was 9.6 (Table 19). Although the geometrical mean titre of antibodies in the dairy animals (9.6) was greater than that of the beef cattle (9.3), this difference was not statistically significant. The mean antibody titre of cattle from the Grampian region was significantly less ($p<0.05$) than that in the animals from Strathclyde (10.6). Within the Grampian area, although the herds

in which there were Holsteins had a lower mean titre than the others, 7.8 compared to 9.3, this difference was not statistically significant. The mean titre of the samples from the heifers was 11.7 while the mean titre of the samples from the cows was 9.3; this difference was not statistically significant. The mean titre of the Grampian heifers was 18.3 compared to 13.9 of those from Strathclyde. There was no significant difference between the mean titres of the animals in the self-contained and bought-in herds. However, the mean titre (8.5) of those self-contained herds from the Grampian region was significantly lower ($p<0.05$) than that of the self-contained herds in the Strathclyde region (11.0).

FIGURE 32. The geographical distribution and the number of herds sampled in the districts of the Grampian region.

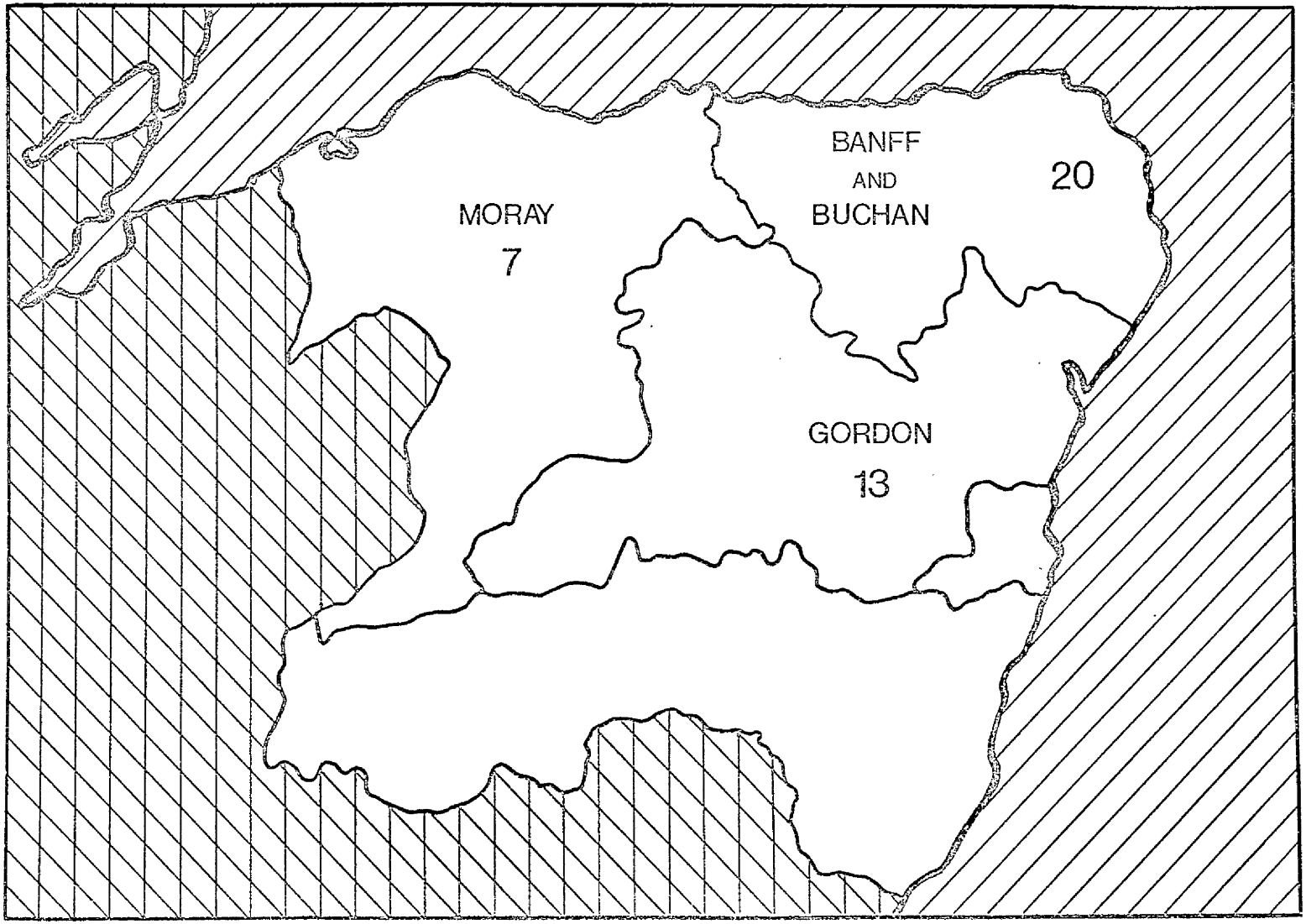


FIGURE 33. The geographical distribution and the number of herds sampled in the districts of the Strathclyde region.

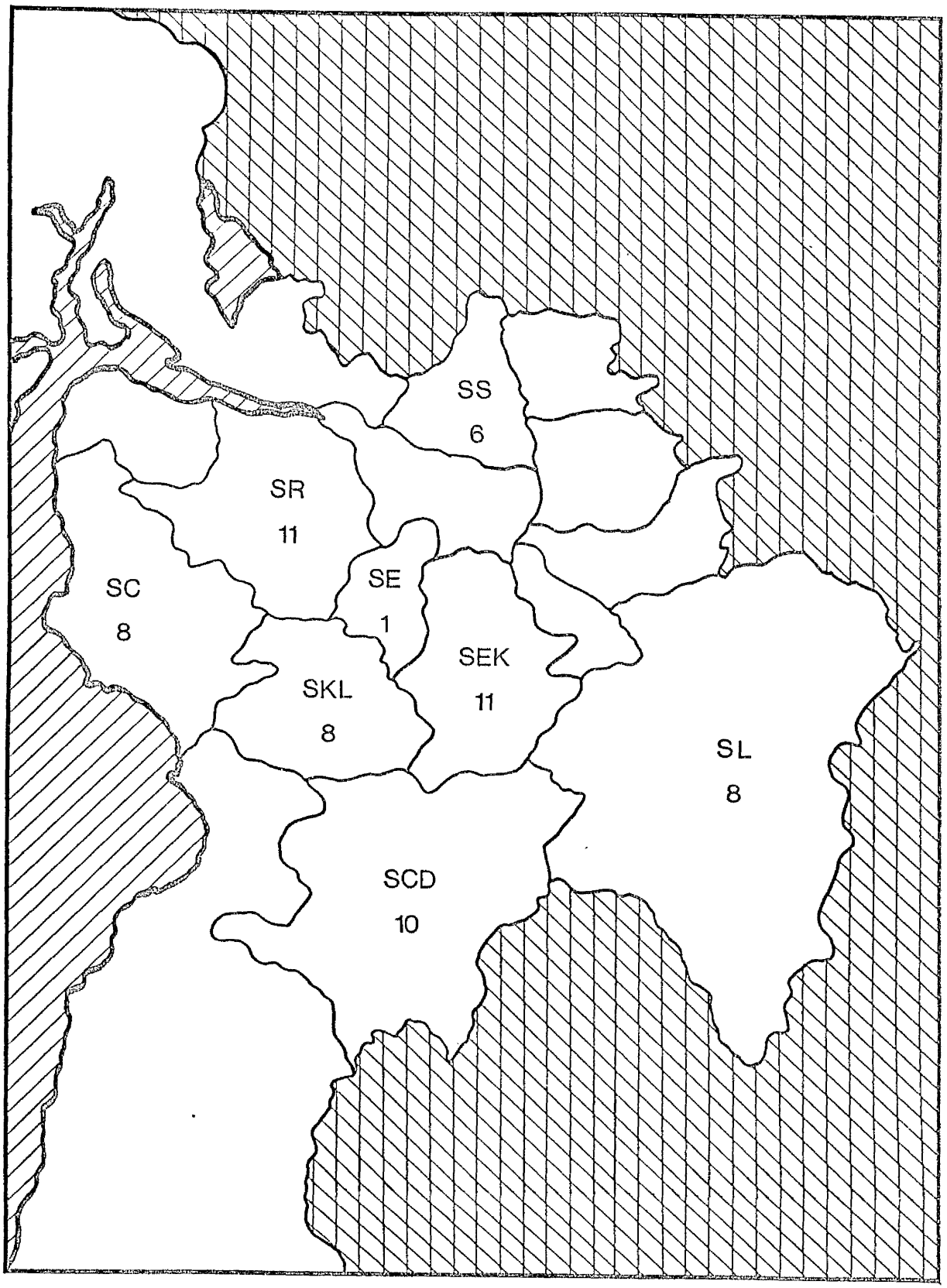


TABLE 16. The effect of the age of the dairy cattle sampled and the type of herd on the prevalence of serum neutralising antibodies to IBR virus.

REGION	TOTAL NUMBER			NO. OF COWS			NO. OF HEIFERS			TYPE OF HERD					
										SELF-CONTAINED			BOUGHT-IN		
	SAMPLED	POSITIVE	(%)	SAMPLED	POSITIVE	(%)	SAMPLED	POSITIVE	(%)	SAMPLED	POSITIVE	(%)	SAMPLED	POSITIVE	(%)
GRAMPIAN	409	47	(12)	373	43	(12)	36	4	(11)	348	44	(13)	61	3	(5)
STRATHCLYDE	560	62	(11)	464	59	(13)	96	3	(3)	510	51	(10)	50	11	(22)
CENTRAL	40	8	(20)	40	8	(20)	-	-	-	40	8	(20)	-	-	-
DUMFRIES AND GALLOWAY	30	0	(0)	-	-	-	30	0	(0)	30	-		-	-	
TOTAL	1039	117	(11)	877	110	(13)	162	7	(4)	928	103	(11)	111	14	(13)

TABLE 17. The effect of the presence of Holstein cattle in the prevalence of serum neutralising antibodies to IBR virus in dairy herds in the Grampian region.

TYPE OF HERD	NUMBER OF CATTLE			NUMBER OF HERDS		
	SAMPLED	POSITIVE	(%)	SAMPLED	POSITIVE	(%)
HOLSTEINS	58	23	(40)	5	4	(80)
OTHERS	351	24	(7)	35	20	(57)
TOTAL	409	47	(12)	40	24	(60)

FIGURE 34. The relationship between mean herd size and the proportion of animals with antibodies to infectious bovine rhinotracheitis virus.

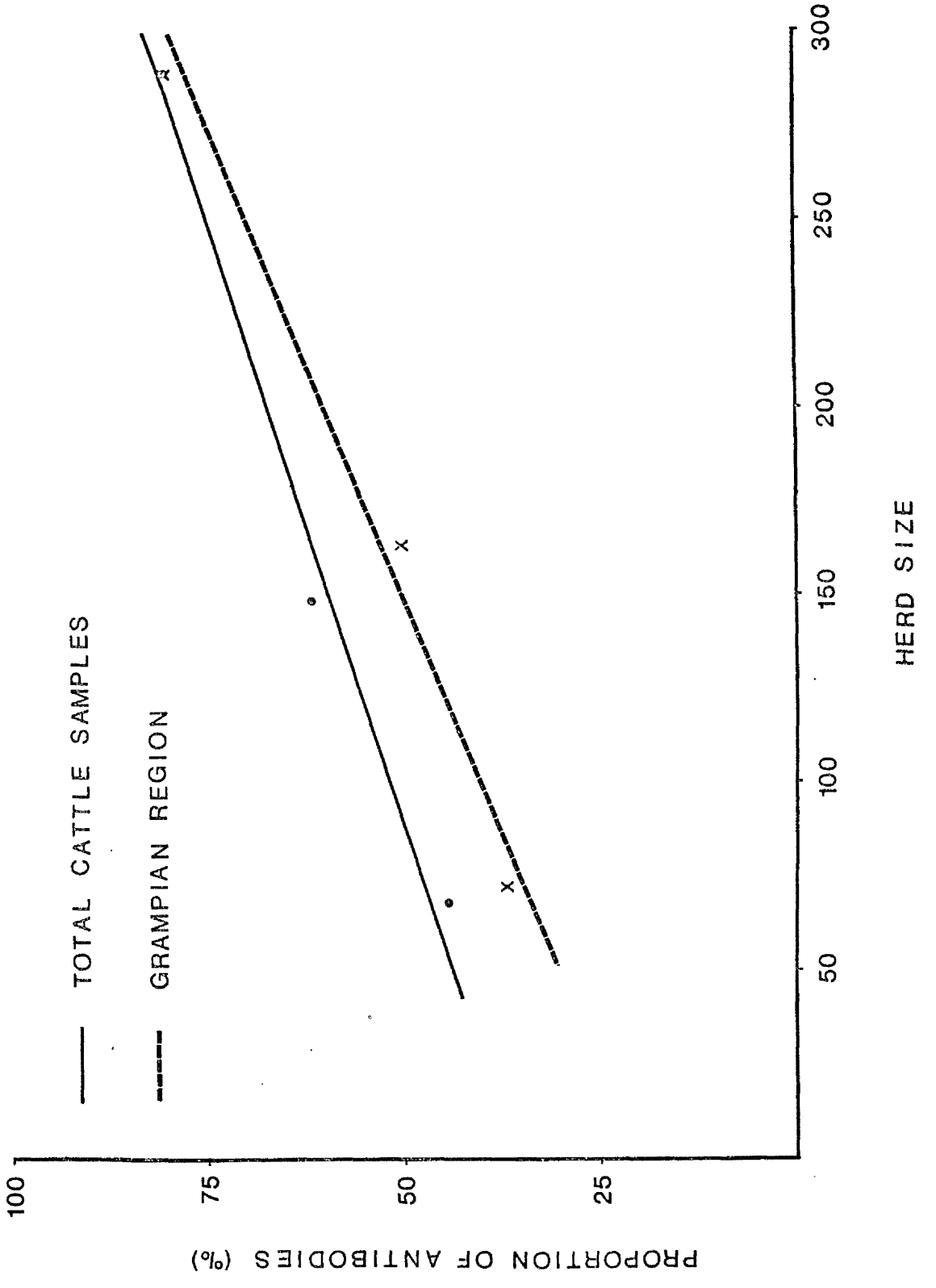


TABLE 18. The effect of herd size on the prevalence of serum neutralising antibodies to infectious bovine rhinotracheitis virus.

REGION	HERD SIZE 0 - 100					HERD SIZE 101 - 200					HERD SIZE 201 +				
	No. Herd	No. Pos. (%)	Average Herd size	No. Sampled	No. Pos. (%)	No. Herd	No. Pos. (%)	Average Herd size	No. Sampled	No. Pos. (%)	No. Herd	No. Pos. (%)	Average Herd size	No. Sampled	No. Pos. (%)
TOTAL	76	49 (65)	68	764	83 (11)	34	16 (47)	148	340	39 (12)	4	4 (100)	289	48	18 (6)
GRAMPIAN	19	18 (42)	76	191	8 (4)	17	8 (47)	163	170	21 (12)	4	4 (100)	289	48	18 (6)
STRATHCLYDE	41	19 (46)	72	410	47 (12)	15	7 (47)	136	150	15 (10)	-	-	-	-	-
BEEF	9	8 (89)	34	93	20 (22)	2	1 (50)	116	20	3 (15)	-	-	-	-	-

TABLE 19. The effect of the region, age of dairy animals and the type of herd on the mean titres of antibodies to infectious bovine rhinotracheitis virus.

Reciprocal titre	Total No. of Animals	Type of Cattle		DAIRY ANIMALS ONLY						
				Region			Age of Animal		Type of herd	
		Beef	Dairy	Grampian	Strathclyde	Central	Cows	Heifers	Self-contained	Bought-in
4	19	1	18	9	8	1	17	1	15	3
6	26	7	19	10	8	1	19	-	17	2
8	21	7	14	8	4	2	14	-	12	2
12	48	5	43	12	28	3	40	3	39	4
16	11	1	10	5	5	-	9	1	7	3
24	12	1	11	2	8	1	10	1	11	-
32	2	-	2	1	1	-	1	1	2	-
64	-	-	-	-	-	-	-	-	-	-
96	1	1	-	-	-	-	-	-	-	-
TOTAL	140	23	117	47	62	8	110	7	103	14
GMT	9.6	9.3	9.6	8.5	10.6	9.5	9.4	11.7	9.8	8.6

DISCUSSION

The overall incidence of serum neutralising antibody to IBR virus in adult cattle from several areas in Scotland was 12 per cent. This figure is considerably greater than those found in previous serological surveys in Britain; Dawson and Darbyshire (1964) and Kirby and others (1978) found only 2.1 per cent and 6.8 per cent respectively, of the samples they examined to be positive. This difference may have resulted from an increase in the incidence of infection with IBR virus, or from the use of more sensitive antibody assay systems or from both of these. Clinical signs suggestive of IBR had been observed in previous years in 7 of the 114 herds surveyed and the proportion of antibodies was greater in these herds (71%) than in the others (50%).

The severe form of IBR has mainly affected beef cattle and the prevalence of antibodies in the beef herds sampled was greater than in the dairy herds. This finding was to be expected. The infection in these incidents had usually been introduced following the purchase of a number of animals since, in general, the turnover in beef herds is greater than in dairy herds. Consequently, it might have been expected that the incidence in the self-contained herds would be less than that in herds in which several animals had been recently bought-in. However, in the dairy animals, no difference was found between self-contained herds and the others. There was an insufficient number of beef animals sampled to allow a valid comparison to be made.

The recent severe incidents have been particularly common in the Grampian region which is a major cattle importing area and yet the prevalence of antibodies in dairy cattle was virtually identical to that of the Strathclyde region. But IBR is mainly a disease of beef cattle and these will have almost no contact with dairy stock. On the other hand, the prevalence of antibodies was significantly greater in the Grampian heifers than in those from the Strathclyde region. This finding strongly suggests that there had been a large increase in the recent infections with this virus in the Grampian area. This is further substantiated by the relatively high mean titre of the Grampian heifers.

That there was a positive relationship between the proportion of seropositive animals and herd-size, is in contrast with the findings of Kirby and others (1978) who reported that herd size had no influence on the prevalence of antibodies. Overall, the incidence of antibodies

was much higher in dairy cows than in dairy heifers; this suggests that the infection was endemic or had been introduced by the use of contaminated semen following artificial insemination. This method of introducing BHV 1 infection into a self-contained herd has been suggested by Kirby and others (1978) and may be correct since, in a survey of all artificial insemination centres in the United Kingdom, neutralising antibodies were found in 21 per cent of the bulls sampled (Report, 1970). However, it is virtually impossible to confirm or to refute this suggestion without detailed service records because both artificial insemination and natural service are used in virtually every dairy herd.

Holstein animals had been brought onto 5 of the farms in the Grampian area and significantly more of the animals sampled had antibodies in these 5 herds (40%) than in the others (7%). The only herd in which antibodies were not detected had imported a Holstein bull. These results support the findings of French workers who found serum neutralising antibodies to IBR virus in almost 50 per cent of imported, Holstein cattle sampled (Dannacher and Fedida, 1978). In contrast, less than 10 per cent of native French cattle were seropositive. From this it can be inferred that Holstein cattle had probably introduced the "new" IBR virus.

It has been stated that the serum neutralisation test which was used in this present survey is comparatively insensitive and, consequently, other methods of detecting serum antibodies such as plaque inhibition and indirect haemagglutination (IHA) tests have been studied. The IHA test was stated to be approximately 10 times more sensitive than the serum neutralisation test in detecting and assaying antibodies to IBR virus (Shimuzu, Isayama, Kawakami, Murase, Kawano 1972; Kirby, Martin, Ostler, 1974). Despite this, Kirby and others (1978) found only 6.8 per cent of sera to be positive. Therefore it would appear that had the serum neutralisation test been used by those workers the prevalence of antibodies to IBR virus would have been much less. It is also possible that, so far the more virulent form of infection is limited to the northern half of Britain and is slowly spreading southwards. Potgieter and Mare (1974) have shown that neutralisation of Herpesviruses was markedly enhanced by guinea pig complement and thus had the complement been used in this neutralisation test, the proportion of animals with antibodies and the mean antibody titres might well have been much higher. Such a finding would strongly support the hypothesis

that a more severe and rapidly spreading form of IBR was recently introduced into the north of Britain.

The mean titre of the positive sera in this study was 9.6 which is similar to the mean titre (11.6) of the samples examined by Dawson and Darbyshire (1964). Since complement was not used in either survey then the sensitivity of the serum neutralisation tests used in the 2 surveys was probably similar. Even allowing for the fact that the IHA test is more sensitive than the serum neutralisation test, the mean titre obtained by Kirby and others was 1:470. This is more than 30 times the mean titre (15.2) of the sera obtained from 15 confirmed field incidents of the disease (Chapter 2). It is interesting to note that there was no significant difference between the mean titre in this survey and those of the field incidents. This finding was not entirely unexpected since it has been reported that the antigenic response to herpesviruses is poor (Martin, 1976) and that it may take as long as 10 months until the peak titre is reached (McKercher, 1959).

A 10 per cent sample was considered adequate for the purposes of this survey in view of the fact that there was background information regarding the prevalence of antibodies nationwide (Dawson and Darbyshire, 1964; Kirby and others, 1978) and the numerous IBR incidents which had been reported during the previous winter housing period (Wiseman and others, 1978; Cuthbertson and Wood, 1979; Imray, 1979a). The widespread nature of antibodies to IBR virus represents a serious potential hazard, since it has been well established that "stressed" carrier animals can excrete the virus (Sheffy and Davies, 1972), which could, in turn, infect in-contact animals, the vast majority of which are likely to be susceptible.

CHAPTER 4

A COMPARISON OF SEVERAL PHYSICAL CHARACTERISTICS OF THE STRICHEN, COLORADO AND OXFORD STRAINS OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

INTRODUCTION

Bovine herpesvirus 1 infection in cattle has been associated with various clinical syndromes (Tables 1 and 2). In view of the varied clinical manifestations caused by or associated with BHV 1 infection, it was considered that antigenic variation might exist between strains or isolates. When Buening and Gratzek (1967) compared the neutralisation kinetics of 4 BHV 1 isolates from cases of IBR (Colorado, Los Angeles) and calf enteritis (ISU 1, ISU 2), they found that there were minor differences between enteritis ISU 1 and the other isolates. On the other hand, in a similar study of 7 BHV 1 isolates, Bowling, Goodheart and Plumer (1967) were unable to find any antigenic variation. More recently, House (1972) was able to show by neutralisation kinetics that minor antigenic differences did exist between isolates. Using the same method of comparison, Bagust (1972) found that isolates from cases of IBR and IPV/IPB did not show any differences in antigenic composition, although the encephalitis isolate (N569) was antigenically distinct. In similar studies, using different classes of antibody prepared in rabbits, Potgieter and Mare (1974) demonstrated slight antigenic differences between several isolates from the genital and respiratory tracts.

Since the Strichen isolate was associated with a much more severe respiratory tract disease than had been recognised previously in the United Kingdom, selected characteristics of this isolate were compared with those of 2 well established prototype respiratory disease strains of widely different virulence, namely Colorado and Oxford. The characteristics selected for comparison were plaque type, growth rate and envelope antigenicity. Plaque type was studied by observing plaque diameter and morphology; viral growth rates were defined by measuring the eclipse periods and rate of viral release; and antigenicity was examined by kinetic neutralisation. These characteristics were chosen because each has been associated with variability in virulence in other virus systems; for example, isolates of feline calicivirus (Ormerod and Jarrett, 1977), poliovirus (Sabin, 1957) and foot and mouth disease virus (Sellers, Burt, Cumming and Stewart, 1959) derived from small plaques were less virulent than those from large plaques. The growth rate was considered important as it might indicate the rapidity with which each strain might spread in the

infected animal. The antigenic differences might be expected to be related to changes in the viral surface structure which could influence the cytotropism of each virus (Buening and Gratzek, 1967) or be associated with variability in virulence owing to differences in the extent of the neutralisation reaction *in vivo*.

MATERIALS AND METHODS

Tissue cultures

Primary meningeal cultures were prepared from bovine embryos in their second trimester obtained from the abattoir. After the skull had been asceptically opened, the meninges were removed and placed in a sterile universal bottle and then finely minced using a fine pair of sterile scissors. The fragments were washed 3 times with PBS and finally inoculated into 8 ounce prescription bottles containing 20 ml of Eagle's medium. This medium consisted of 1 per cent glutamine, 20 per cent FBS (inactivated at 56°C for 30 minutes), 1 per cent non-essential amino acids, 50 Ug/ml tylosin, 100 units/ml penicillin and 100 Ug/ml dihydrostreptomycin. Complete monolayers were generally formed in 3-4 weeks and could be subcultured 35-40 times. This foetal bovine meningeal cell line was used in its 20th passage for the one-step growth curves as well as for ultrastructural studies. It was chosen for this purpose because, although the growth and morphogenesis of IBR virus have been studied in several types of bovine cell cultures, meningeal cells have never been used for work of this type.

A foetal bovine lung (FBL) semi-continuous cell line was used for the studies of neutralisation kinetics, plaque size and plaque morphology. This cell line which was obtained from the Central Veterinary Laboratory Ministry of Agriculture, Food and Fisheries, Weybridge, was used because it grew more quickly and formed more even and compact monolayers than CT or meningeal cultures and also because the plaques were more distinct.

Viruses

The virus isolated from outbreak 1 (Chapter 2) is here referred to as the "Strichen strain" of IBR virus. This strain was grown and stored as described in Chapter 2. It was used in its third passage at a titre of $10^{7.7}TCID_{50}$/ml.

The Colorado and Oxford strains were obtained from the Central Veterinary Laboratory, Weybridge, and were used in their 28th and 6th passages respectively. Both were used at a titre of $10^{8.1}TCID_{50}$/ml.

Rabbit antiserum production

Preparation of the antigen for immunising rabbits. Eight-ounce prescription bottles of well monolayered FBL cell cultures were each inoculated with 1ml of $10^2 TCID_{50}$/ml of virus and left to adsorb at 37°C for 2 hours, after which 20ml of maintenance medium was added to each bottle. These cultures were changed to a serum-free medium after 12 hours and then re-incubated at 37°C until about 90 per cent of the monolayers showed cytopathic effect.

The virus was then harvested, dispensed and stored as described under Chapter 2.

Production of hyperimmune serum. A 1:1 concentration of undiluted virus and complete Freund's adjuvant (Miles Laboratories Ltd., UK) was prepared. A 0.2ml volume of virus/adjuvant mixture was inoculated intradermally into each of the rear footpads of every rabbit and 0.1ml virus/adjuvant mixture into 8 sites on the back as well as 8 sites on the abdomen of every rabbit.

Three weeks later, the rabbits were given similar inoculations intradermally of a 1:1 concentration of virus and incomplete Freund's adjuvant into the back and abdomen only.

After a further 3 weeks, the rabbits were given 1ml of the antigen intravenously.

Ten days after the intravenous challenge the rabbits were bled. The serum obtained will be referred to as "late serum". The bovine Strichen antiserum used was obtained from a calf which had been challenged with the virus intranasally and developed typical clinical signs of IBR. The serum was inactivated at 56°C for 30 minutes, dispensed and stored at -20°C.

Plaque size and morphology

Plaque size and morphology were compared in the semicontinuous FBL cell line. Cell suspensions were prepared as described in detail in Chapter 3.

Monolayers were grown in disposable 30mm diameter, tissue culture, petri dishes (Flow Laboratories, Scotland) by inoculating 2ml of cell suspension. The plates were incubated at 37°C in an atmosphere of 5 per cent carbon dioxide until monolayers had formed. The 3 strains

of IBR virus (Stricken, Colorado, Oxford) were diluted to contain 100 PFU and 0.5ml was inoculated into each of the 4 plates. Adsorption was allowed to take place at 37°C in an atmosphere of 5 per cent carbon dioxide for 2 hours, after which the virus suspension was removed using a vacuum pump. The monolayers were washed in medium before application of the overlay. Into each of the plates 2ml of a 0.6 per cent agar overlay (equal amounts of double concentration M199 and 1.2% Difco agar) were inoculated. After the overlay had solidified, the plates were incubated at 37°C in an atmosphere of 5 per cent carbon dioxide.

On day 4 post inoculation, the plates were examined microscopically and then fixed and stained for 24 hours in a mixture of 0.5 per cent crystal violet and 10 per cent formalin. The agar overlay was then removed and plates were left to dry in air. Measurement of plaque size was achieved by randomly taking 100 plaques and measuring them with a diameter measuring template (Life Science Instruments, Miles Laboratories, Indiana, USA). The plaque morphology was examined using an inverted microscope.

One-step growth curve and ultrastructure studies

In order that at least 95 per cent of the cells would be infected with virus, an input multiplicity of infection of 3 was used. Meningeal cell monolayers were dispersed with a mixture of 0.25 per cent trypsin (2ml of 2.5% trypsin in 18ml versene) and a 0.01 per cent versene in PBS.

For the Stricken virus, 18.5×10^6 cells were mixed with 1.2ml of virus (titre = $10^{7.7}$ $TCID_{50}$/ml) and the volume made up to 40ml with Eagle's growth medium. For the Colorado and Oxford viruses 11×10^6 cells were mixed with 2.2ml of each of the viruses (titre = $10^{8.1}$ $TCID_{50}$/ml) and the volumes made up to 22ml with Eagle's growth medium.

Each mixture was agitated with a magnetic stirrer at 4°C for 2 hours after which the relevant number of tubes were each inoculated with 1ml of virus-cell suspension (0.5×10^6 cells) mixture and incubated at 37°C. Samples for the one step growth-curve were collected at 2, 4, 6, 8, 12, 16, 18, 20, 24, 36 and 48 hours post inoculation. The cell (intracellular virus) and the fluid (extracellular virus) fractions were separated and the latter fraction was spun in a GF-6 MSE refrigerated centrifuge at 1600 rpm for 5 minutes to sediment the cells, which were then

resuspended in 1ml of Eagle's maintenance medium together with the original cell fraction. Both the cell and the fluid fractions were immediately stored at -70°C until required for titration which was carried out by the quantal method using 5 culture tubes per 10-fold dilution.

Samples for the electron microscopic studies were taken at 0.00, 0.15, 1, 2, 3, 4, 6, 8, 13, 16, 18, 20, 24, 36 and 48 hours post inoculation. This was undertaken with the Strichen strain only. After collecting the sample, the cells were removed from the glass surface of the test tubes using a rubber policeman. The cells were then spun in a GF-6 MSE refrigerated centrifuge at 336g for 5 minutes to form pellets, which were processed for electron microscopic examinations as described in detail in Chapter 2.

Neutralisation kinetics

Kinetic neutralisation of the 3 virus strains was carried out in a water-bath at 37°C. Eagle's medium containing 1 per cent FBS and 1 per cent glutamine was used throughout the experiments as a diluent, while freeze dried, guinea pig complement (Gibco-Biocult, Scotland) at a 1:3 dilution was used as a source of complement. The viruses were diluted to contain 2×10^4 PFU/ml while antisera were diluted so that 0.5ml neutralised approximately 90 per cent of a 0.5ml preparation of the homologous virus in about 30 minutes. Prior to use, all reagents were chilled to 4°C.

The test was carried out by rapidly mixing 0.5ml of the virus, 0.5ml of rabbit antiserum and 0.1ml of complement. A 0.1ml volume of the mixture was immediately diluted 100-fold by blowing it into 9.9ml of chilled diluent and the samples were then stored at 4°C in a tray of wet ice. This was considered to be the zero minute sample. The rest of the virus-antiserum mixture was incubated in a waterbath at 37°C. Subsequent samples were taken at every 5 minutes up to 30 minutes and, at each time, diluted 100-fold immediately.

The amount of virus present in each diluted sample was determined by the plaque method. Four plates of FBL cells were used for each of the samples and every plate received 0.5ml of the diluted sample. The rest of the procedure was similar to that described in detail above under "plaque size and morphology".

Plaques were counted under a cold-light illuminator and a statistical estimate of the rate of virus neutralisation was calculated by linear regression and by applying "Student's" t test. The neutralisation rate constant (K) for a virus and a particular antiserum was calculated by McBride's (1959) formula; $K = D/t\ 2.3 \log_{10} Vo/Vt$ in which:

D = Dilution of antiserum
t = Time (minutes)
Vo = Initial amount of virus at time zero
Vt = Residual virus at time "t"

For the purposes of comparison, a normalised constant (NK) was obtained by assigning a value of 100 to the homologous system and calculating values for the heterologous system.

RESULTS

Plaque size and morphology

Previous work had shown that the maximum plaque size was achieved on day 3 post inoculation. Therefore, in these experiments plaques were fixed and stained on day 3 post inoculation.

On day 1 post inoculation, small microscopic foci were evident and they became macroscopic by day 2 post inoculation. On day 3 the plaques had grown to their maximal size. By this time both small and large plaques were observed and the corresponding size of the plaques of the 3 virus strains is presented in Table 20. The mean plaque sizes of the 3 strains were Strichen (1.88 ± 0.05), Colorado (1.91 ± 0.05) and Oxford (1.61 ± 0.05). There was no significant difference between the mean plaque size of the Strichen and the Colorado strains but the mean plaque size of both was highly significantly ($p < 0.001$) greater than those of the Oxford strain of IBR virus.

Morphologically, the plaques produced by each virus were similar, being circular with deeply staining cells forming a peripheral ring. Some of the plaques had a clear centre while others had a few strands of infected cells protruding into the interior giving a slightly ragged appearance.

One step growth curve and ultrastructure studies

The results of the infectivity titrations are shown in Table 21. Both the cell associated and extracellular virus from all the 3 strains first appeared at 8 hours post inoculation and continued to rise logarithmically until 18 hours post inoculation (Figures 35, 36, 37). There was a slight drop in the intracellular as well as extracellular virus from 18 hours to 24 hours which continued at a faster rate until 48 hours post inoculation. The pattern of virus release in all the strains was similar with the virus being rapidly released from the cell although the Colorado strain seemed to be released faster than the other 2 strains (Figures 36 and 38).

The amount of extracellular and intracellular virus at 18 and 48 hours post inoculation is shown in Table 22. The amount of virus produced per cell was highest with the Strichen virus and least with the Oxford virus; this difference was not statistically significant. The difference in the amounts of extracellular and intracellular virus at 48 hours post inoculation was not significant either.

Electron microscopic studies revealed intranuclear, intracytoplasmic and extracellular virus particles. In the intranuclear site, the virus particles appeared circular or elliptical with either a clear core or with an electron dense nucleoid or with homogenously electron dense material filling the particles (Figure 39). Intracytoplasmic virus particles were seen in transport vacuoles which in turn were surrounded by a dense matrix (Figure 40). The extracellular virus particles which had acquired additional membranes during their passage to the cell surface had spiked outer membranes (Figure 41).

The virus was first seen intranuclearly at 8 hours post inoculation (Figure 42). At 12 hours post inoculation groups of virus particles were seen intranuclearly, a few particles were seen in the cytoplasm in transport vacuoles (Figure 43) and extracellular virus particles were seen infrequently (Figure 44). At 16 hours post inoculation, virus particles were seen in transport vacuoles in the cytoplasm and in groups intranuclearly (Figures 40 and 45 respectively). It was also at this time (16 hours) that extracellular virus particles were seen in considerable numbers (Figure 46) and large numbers of virus particles were released at 18 hours (Figure 47). Although the amount of virus being released by each cell decreased between 24 and 48 hours, there was still appreciable numbers of particles being released at 24 and 36 hours and even by 48 hours post inoculation, small numbers of virus particles were still evident in the nucleus.

Neutralisation kinetics

The neutralisation curves of the 3 virus strains by the 4 antisera (rabbit anti-Strichen, bovine anti-Strichen, rabbit anti-Colorado and rabbit anti-Oxford) are presented in Figures 48, 49, 50 and 51. The neutralisation rate constant (K) and normalised constant (NK) values for the 3 viruses are presented in Table 23, while the detailed results of plaque counts are presented in Appendix 3.

All 3 viruses were neutralised by each antiserum. In each case the rate of the reaction increased with time until the inactivation curve was a straight line, indicating one-hit kinetics. All the 3 viruses formed a shoulder and this initial shoulder was most marked with the Strichen strain regardless of the serum used. Both the rabbit (Figure 48) and bovine (Figure 49) Strichen antisera neutralised the heterologous Colorado (Figure 50) and Oxford (Figure 51) strains

at a faster rate than the homologous virus ($p < 0.05$). The neutralisation rates of the 3 viruses by the Oxford antiserum were not significantly different, while the Colorado antiserum neutralised the homologous virus significantly faster than the heterologous viruses.

TABLE 20. The results of comparison of plaque sizes of the 3 strains of infectious bovine rhinotracheitis virus.

IBR-STRICHEN STRAIN	IBR-COLORADO STRAIN	IBR-OXFORD STRAIN
1.5, 1.7, 2.0, 2.2,	2.5, 2.0, 2.5, 2.6,	1.5, 1.0, 1.2, 1.1,
2.5, 1.8, 3.0, 3.0,	3.0, 2.0, 2.2, 2.0,	1.8, 1.5, 1.4, 1.0,
1.5, 1.5, 1.5, 1.7,	2.1, 1.7, 1.8, 2.0,	1.2, 1.4, 1.8, 1.2,
2.0, 1.8, 1.8, 1.8,	2.0, 2.1, 3.5, 3.0,	2.1, 2.3, 2.2, 2.0,
2.1, 2.0, 2.0, 1.6,	2.0, 2.0, 1.1, 1.2,	2.0, 1.8, 2.8, 3.0,
1.9, 1.4, 1.3, 1.2,	3.0, 1.8, 1.5, 1.2,	2.6, 2.2, 1.5, 1.1,
1.1, 2.0, 2.2, 2.0,	2.2, 2.7, 2.2, 1.8,	1.7, 2.2, 1.5, 1.2,
2.0, 2.0, 1.3, 3.2,	2.0, 1.6, 1.2, 1.4,	1.1, 2.0, 1.2, 1.0,
2.5, 2.0, 1.8, 3.0,	2.0, 1.8, 1.6, 2.0,	1.8, 1.5, 1.7, 1.2,
1.8, 1.8, 1.8, 1.1,	2.1, 2.5, 3.0, 2.4,	1.3, 1.7, 2.0, 1.2,
1.2, 1.4, 2.0, 1.7,	1.8, 1.5, 1.8, 2.0,	1.4, 1.7, 1.8, 1.8,
1.8, 1.5, 2.4, 1.9,	2.3, 2.2, 2.5, 1.8,	1.2, 1.2, 1.1, 1.0,
1.6, 1.2, 1.0, 1.2,	1.8, 1.4, 1.5, 1.3,	1.0, 2.0, 1.4, 1.8,
1.3, 1.5, 2.0, 2.0,	2.0, 1.2, 1.2, 1.3,	1.4, 1.1, 1.5, 1.5,
2.0, 1.7, 3.0, 1.5,	1.1, 1.1, 2.0, 1.5,	1.6, 1.6, 2.0, 2.1,
1.5, 1.5, 1.6, 1.4,	1.8, 1.4, 1.2, 1.6,	1.2, 1.0, 2.0, 1.1,
3.5, 2.0, 2.0, 2.1,	2.0, 1.8, 2.0, 1.5,	1.0, 1.5, 2.0, 1.2,
2.0, 2.0, 1.8, 1.5,	1.5, 1.0, 1.2, 1.4,	1.5, 1.6, 1.8, 1.7,
1.5, 1.5, 1.7, 1.7,	1.4, 1.7, 1.6, 1.5,	1.2, 2.0, 2.0, 2.1,
1.8, 1.2, 1.5, 1.5,	1.2, 2.0, 1.8, 1.8,	2.3, 1.5, 1.8, 0.8,
2.0, 2.0, 2.0, 3.0,	1.8, 2.0, 1.5, 1.5,	1.1, 1.2, 1.4, 1.7,
2.3, 1.5, 1.8, 1.5,	2.0, 2.0, 2.0, 2.0,	1.8, 2.2, 2.0, 1.6,
2.4, 1.5, 2.0, 2.0,	1.5, 1.6, 3.4, 3.2,	1.5, 1.2, 2.0, 1.8,
2.1, 2.2, 1.5, 2.8,	2.8, 1.3, 2.2, 2.6,	2.5, 1.1, 1.5, 1.2,
3.0, 3.2, 1.5, 1.8.	2.0, 2.2, 3.0, 2.5.	1.2, 2.0, 2.1, 2.0.
Range. 1.0-3.5mm	Range. 1.0-3.5mm	Range. 0.8-3.0mm
Mean. $1.88^{\pm}0.05$	Mean. $1.91^{\pm}0.05$	Mean. $1.61^{\pm}0.05$

TABLE 21. The infectivity assay results of the Strichen, Colorado and Oxford strains of infectious bovine rhinotracheitis virus (Log_{10} $TCID_{50}$)

SAMPLE TIME	STRICHEN STRAIN		COLORADO STRAIN		OXFORD STRAIN	
	Extracellular	Intracellular	Extracellular	Intracellular	Extracellular	Intracellular
2 hours	5.5	5.3	5.5	4.9	-	-
4 hours	5.1	4.7	5.3	4.7	5.1	4.5
6 hours	5.3	4.5	5.5	4.5	5.7	4.7
8 hours	5.7	6.1	6.3	5.3	6.3	5.3
12 hours	6.7	7.3	7.1	6.7	6.9	6.7
16 hours	8.3	7.7	7.9	7.3	7.5	7.7
18 hours	8.5	8.1	8.3	7.9	8.1	8.3
20 hours	8.1	7.9	8.1	7.7	7.9	8.1
24 hours	7.7	7.5	7.9	7.5	7.7	7.9
36 hours	7.5	7.3	7.7	7.3	7.5	7.5
48 hours	7.3	7.3	7.3	6.9	7.3	7.1

TABLE 22. Comparison of the amount of extracellular and intracellular virus of the 3 strains at 18 and 48 hours post inoculation.

VIRUS STRAIN	STRICHEN		COLORADO		OXFORD	
Sample time	Extracellular	Intracellular	Extracellular	intracellular	Extracellular	Intracellular
18 hours	$10^{8.5}$	$10^{8.1}$	$10^{8.3}$	$10^{7.9}$	$10^{8.1}$	$10^{8.3}$
48 hours	$10^{7.3}$	$10^{7.3}$	$10^{6.9}$	$10^{7.3}$	$10^{7.3}$	$10^{7.1}$

FIGURE 35. The one-step growth curve of the Strichen strain of infectious bovine rhinotracheitis virus.

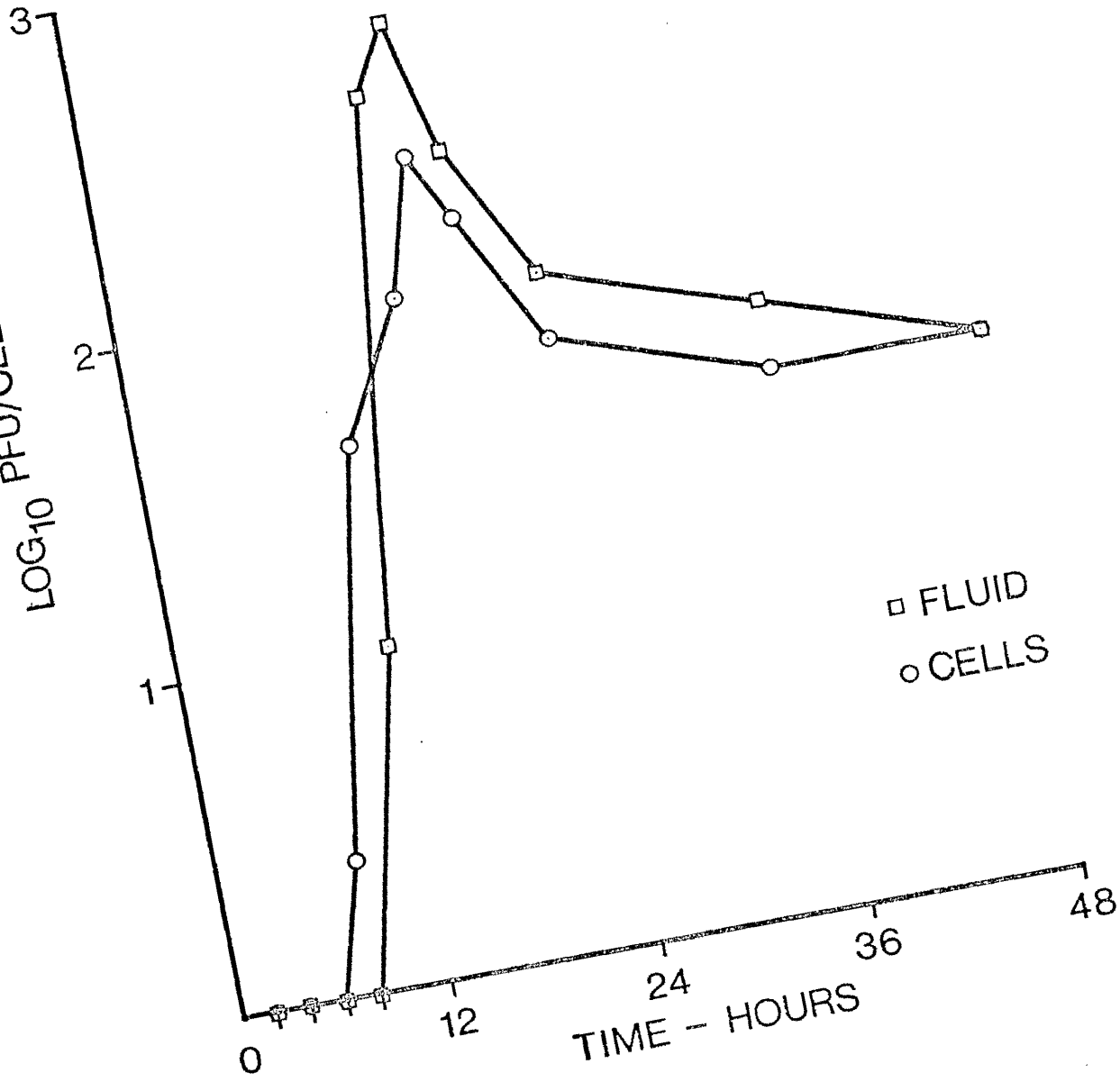


FIGURE 36. The one-step growth curve of the Colorado strain of infectious bovine rhinotracheitis virus.

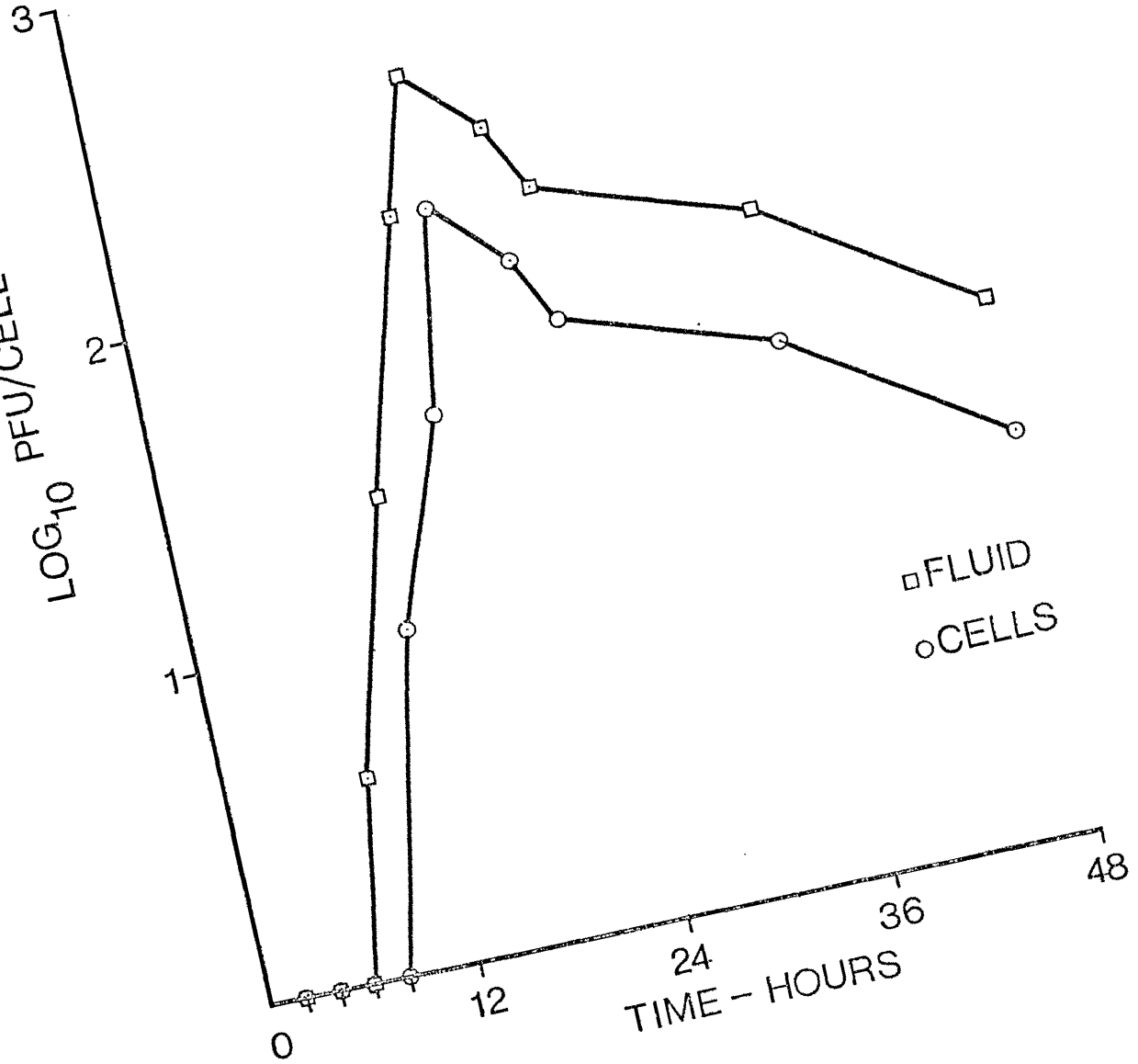


FIGURE 37. The one-step growth curve of the Oxford strain of infectious bovine rhinotracheitis virus.

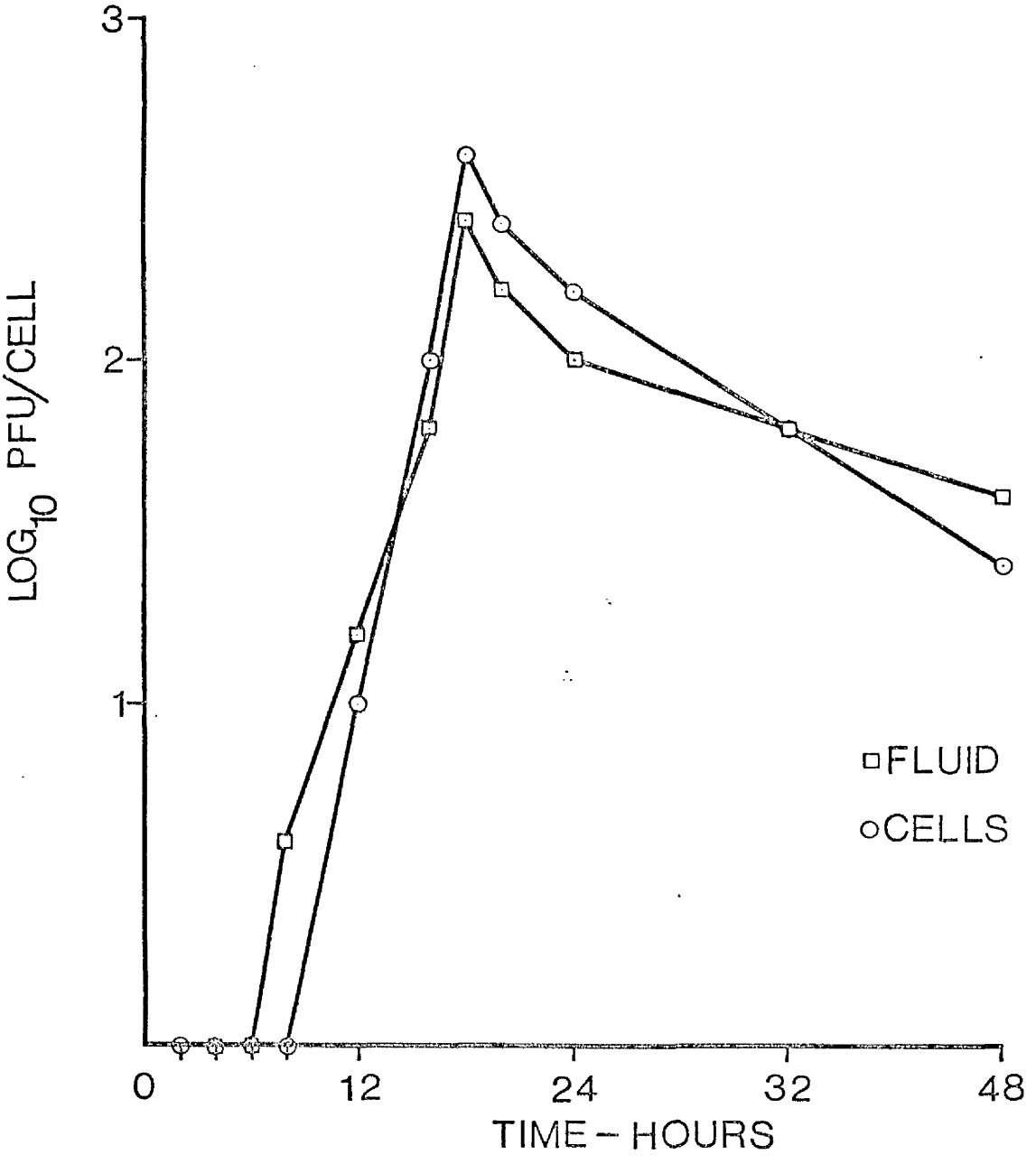
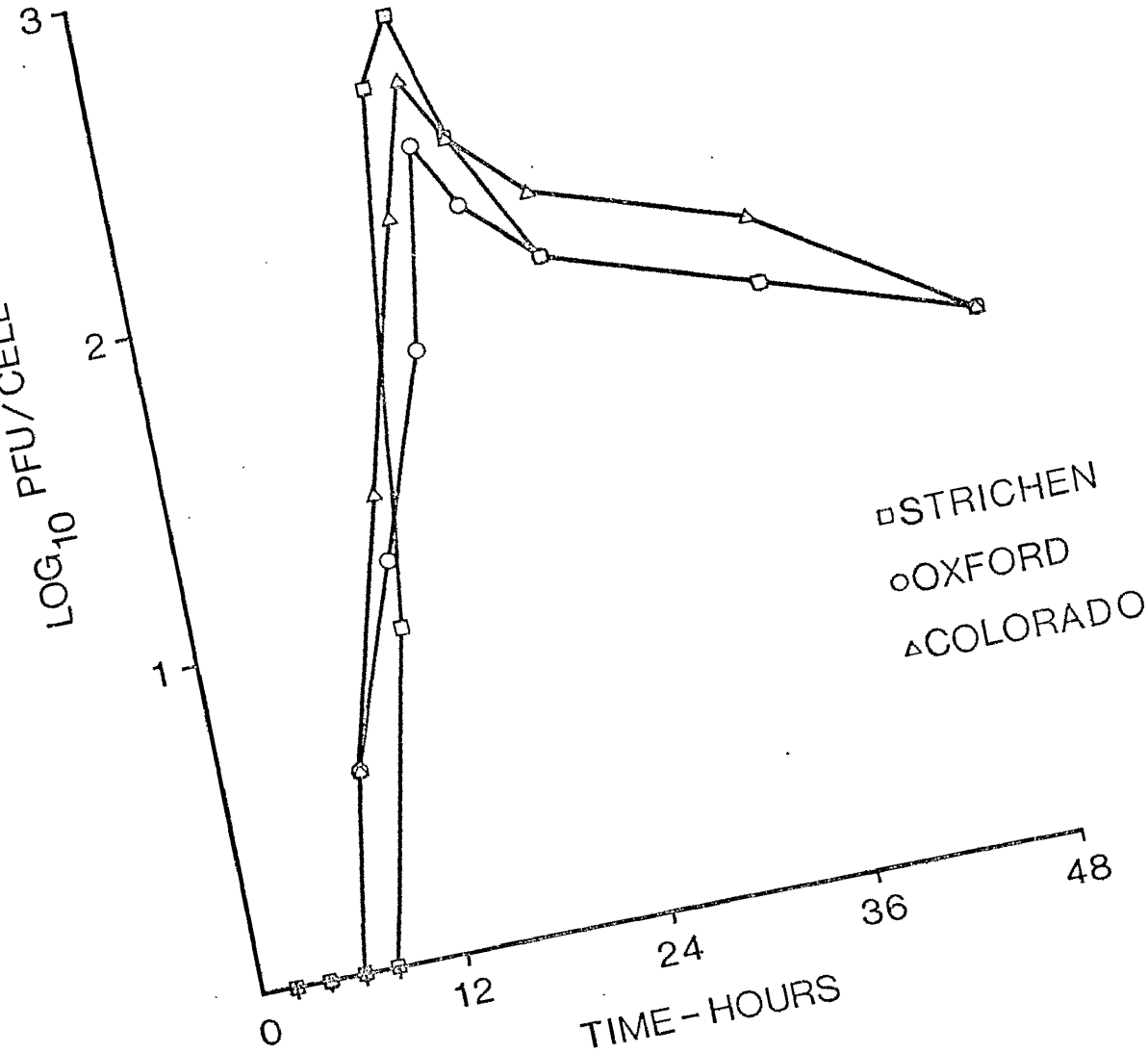


FIGURE 38. The one-step growth curve of infectious bovine rhinotracheitis viruses. The total quantity of virus released.



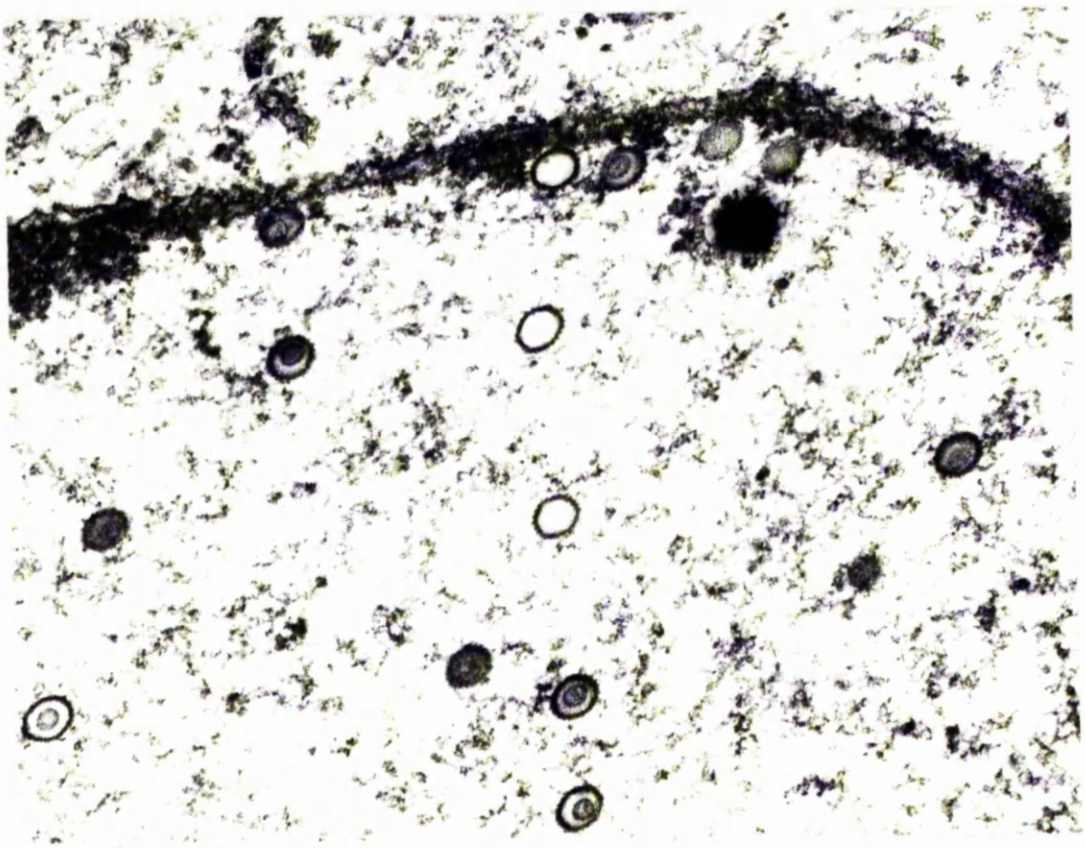


FIGURE 39. Unenveloped virus particles are seen in the nucleus. X 60,000.

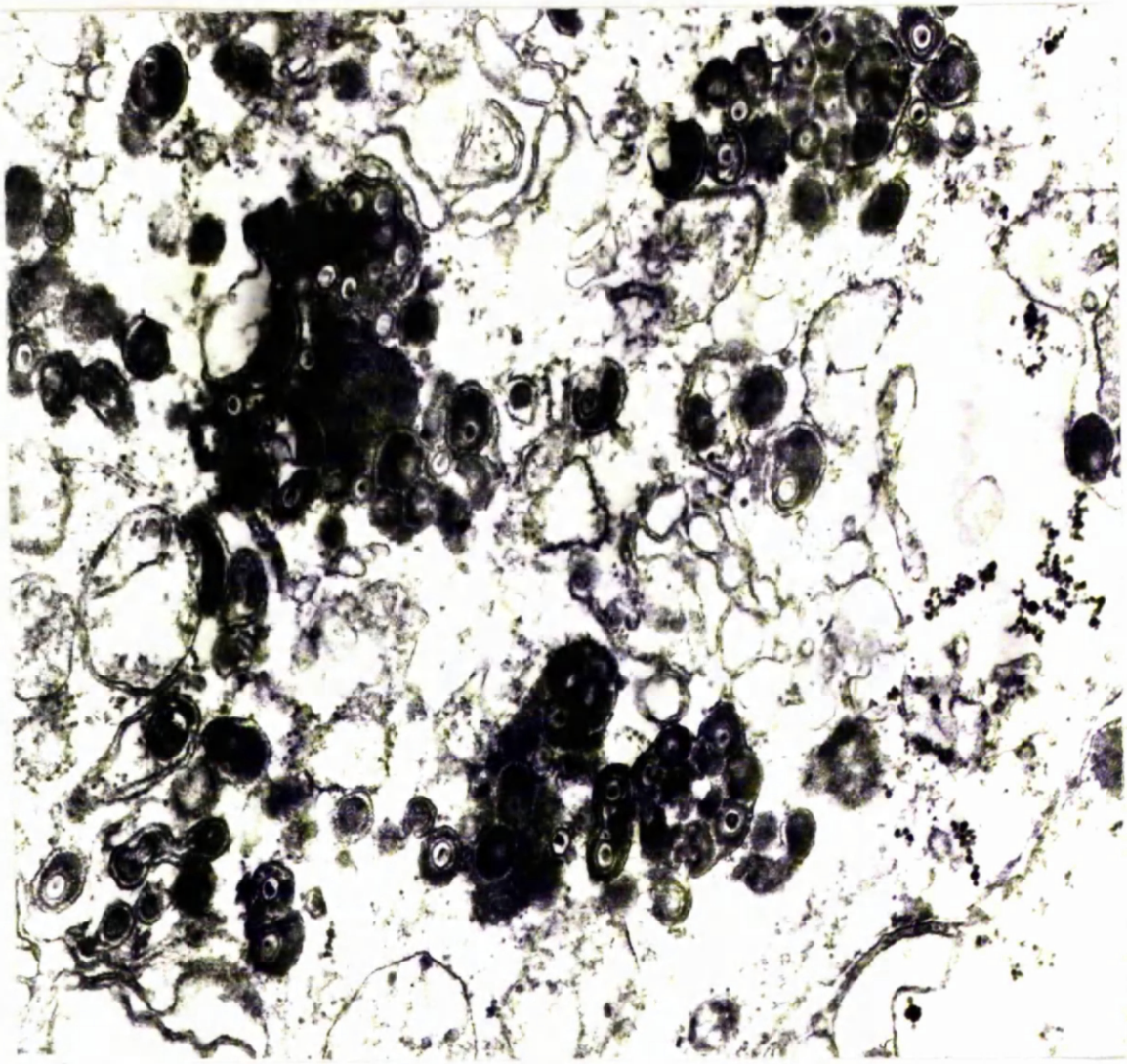


FIGURE 40. In the cytoplasm, many enveloped virus particles are seen in groups in electron dense matrices. X 32,000

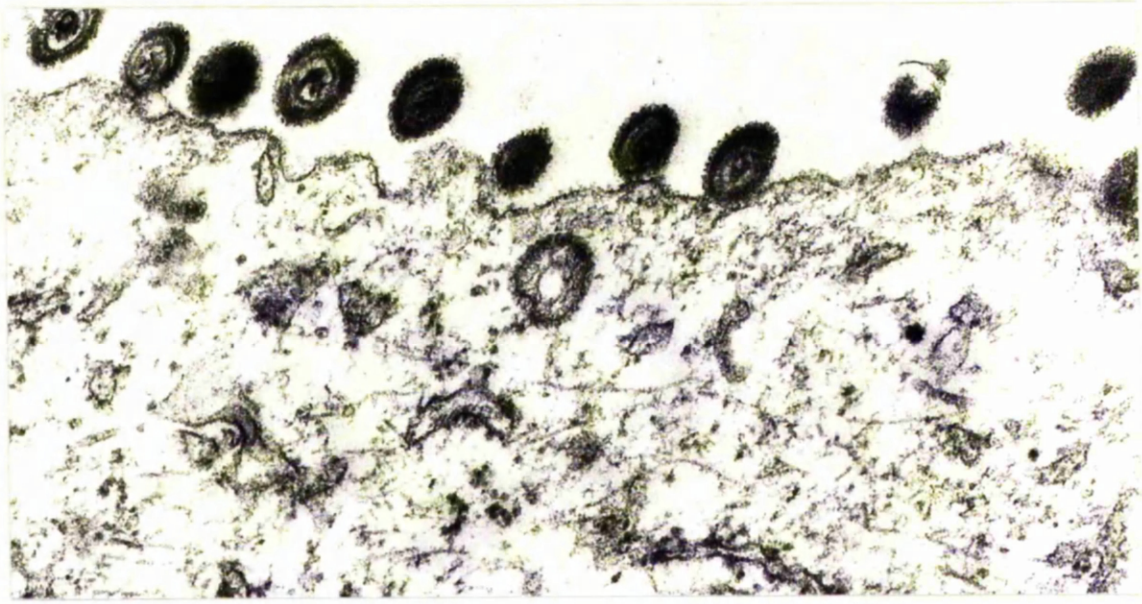


FIGURE 41. Extracellular virus particles are seen along the cell surface membrane. X 60,000

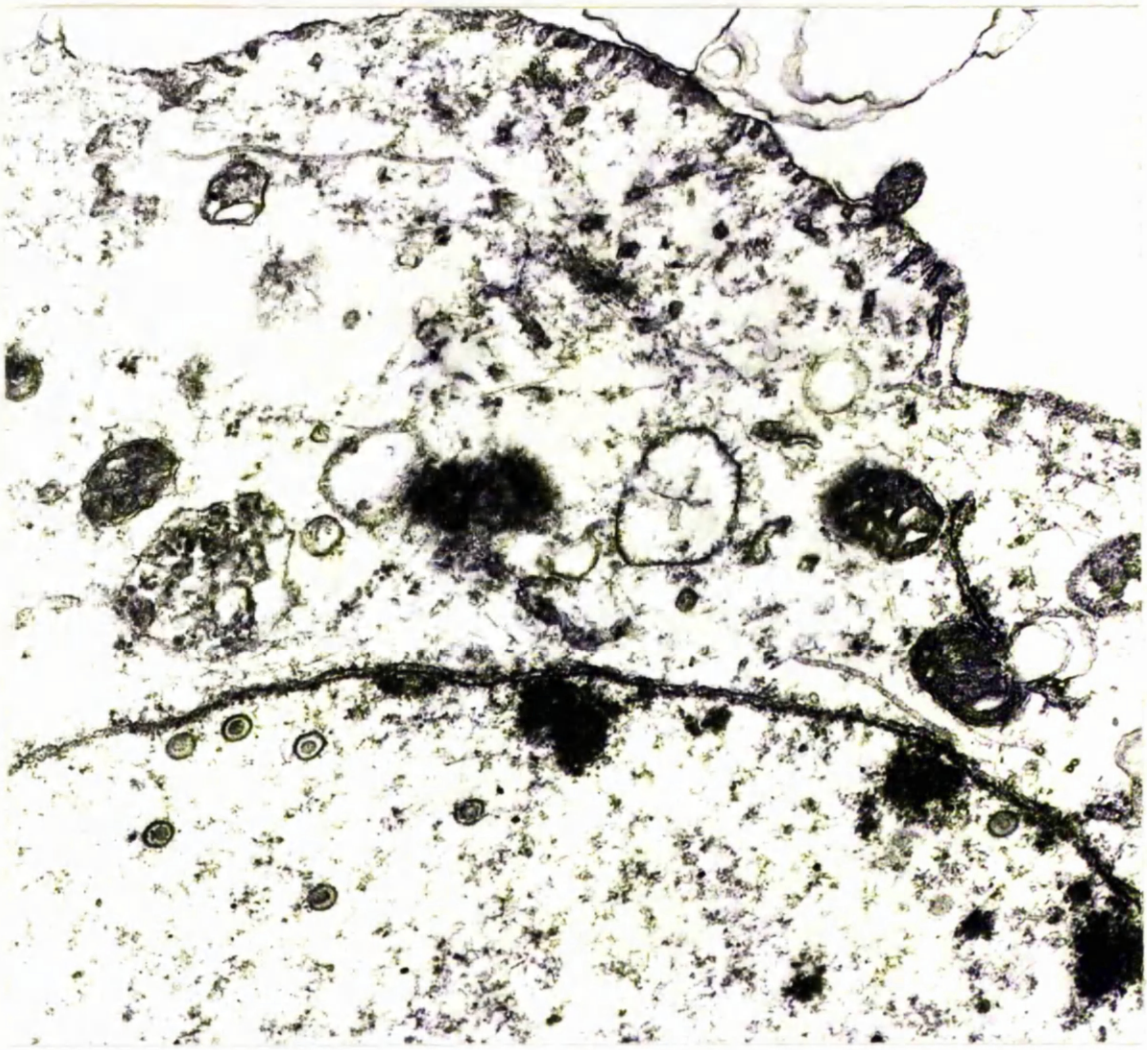


FIGURE 42. Unenveloped virus particles are seen in the nucleus. X 40,000.

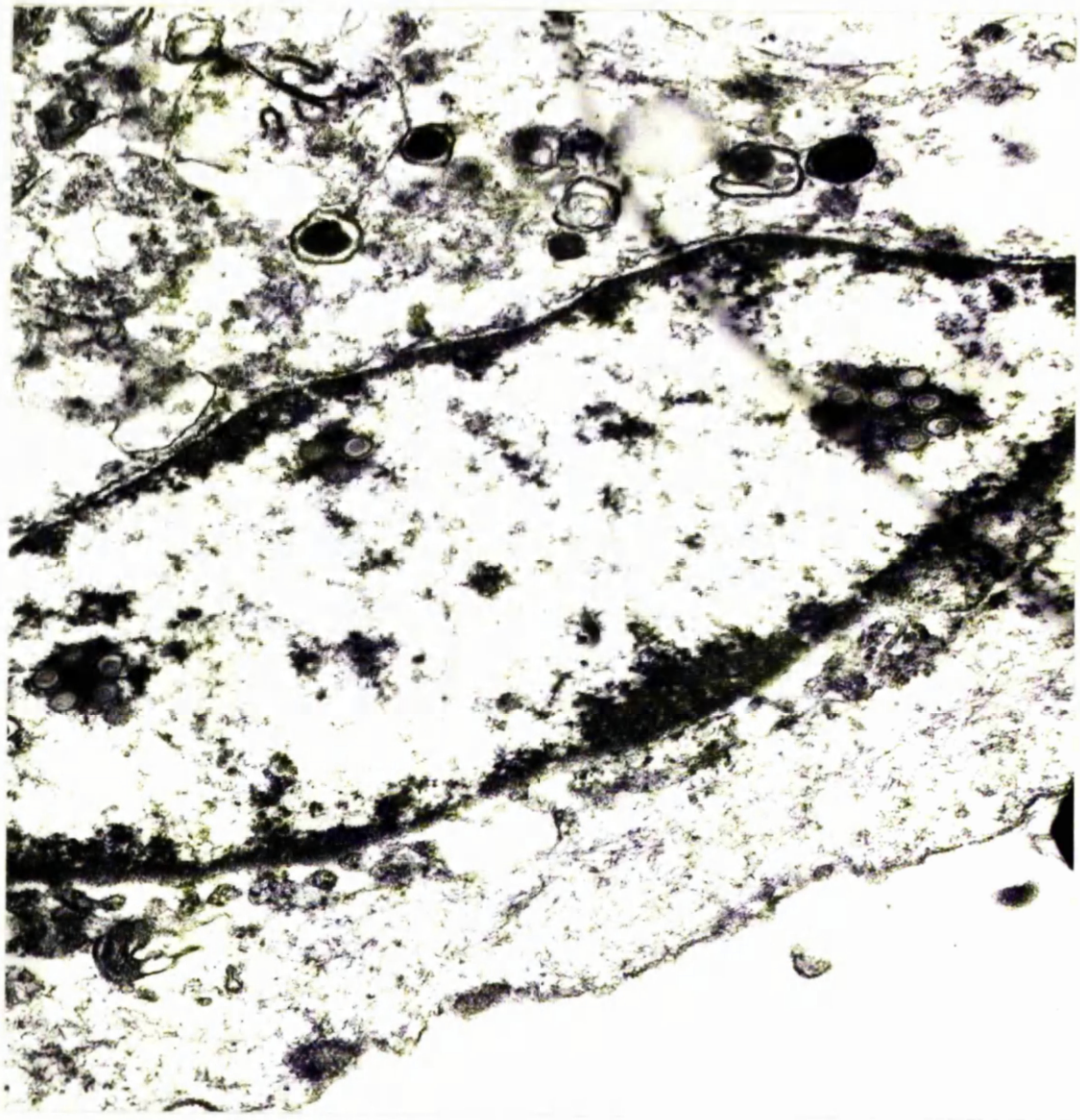


FIGURE 43. Three groups of virus particles are seen in electron dense matrices. Virus particles are also seen in cytoplasmic vacuoles. X 40,000.

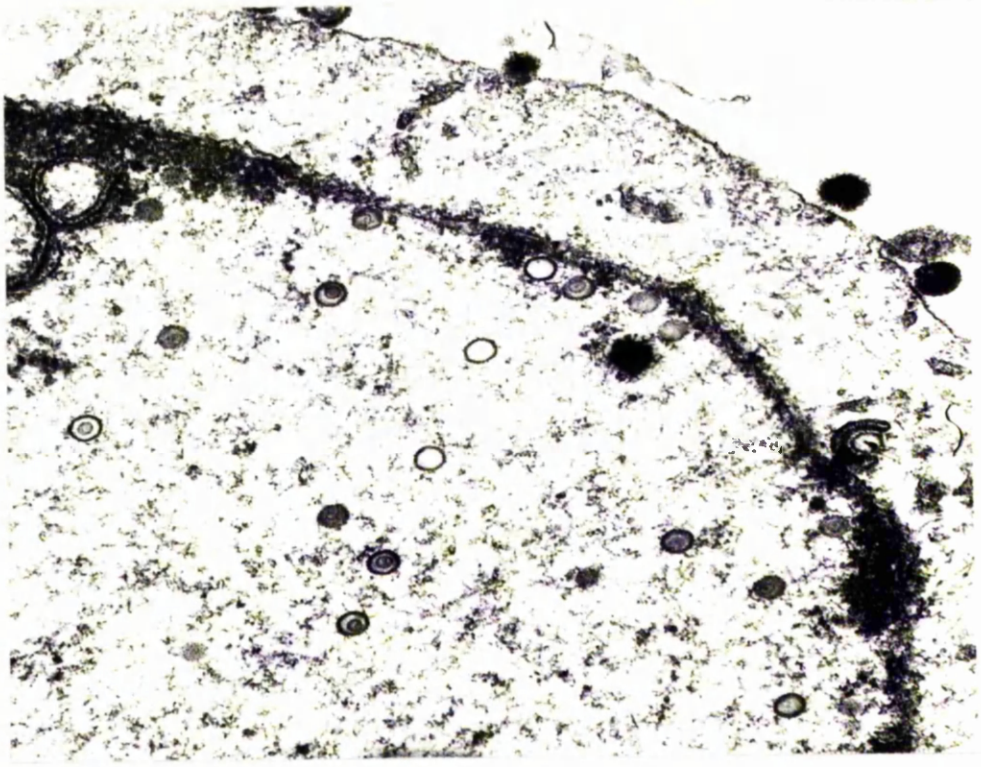


FIGURE 44. Virus particles are seen intranuclearly and, extracellularly, at the cell surface membrane. X 40,000.

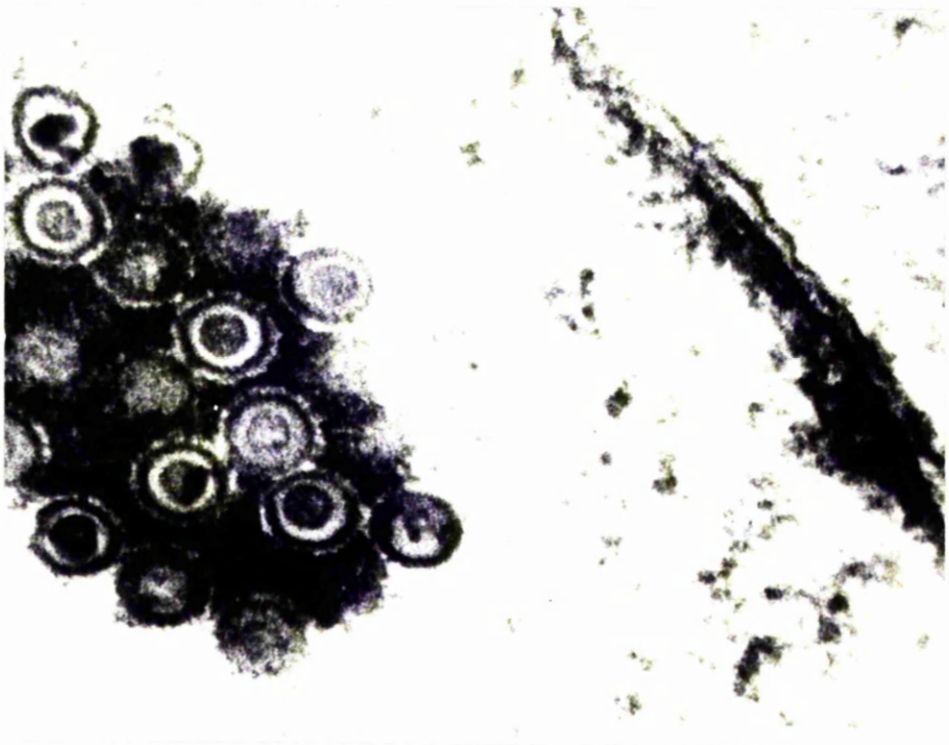


FIGURE 45. Intranuclear virus particles, with dense cores, are seen in an electron dense. X 120,000.

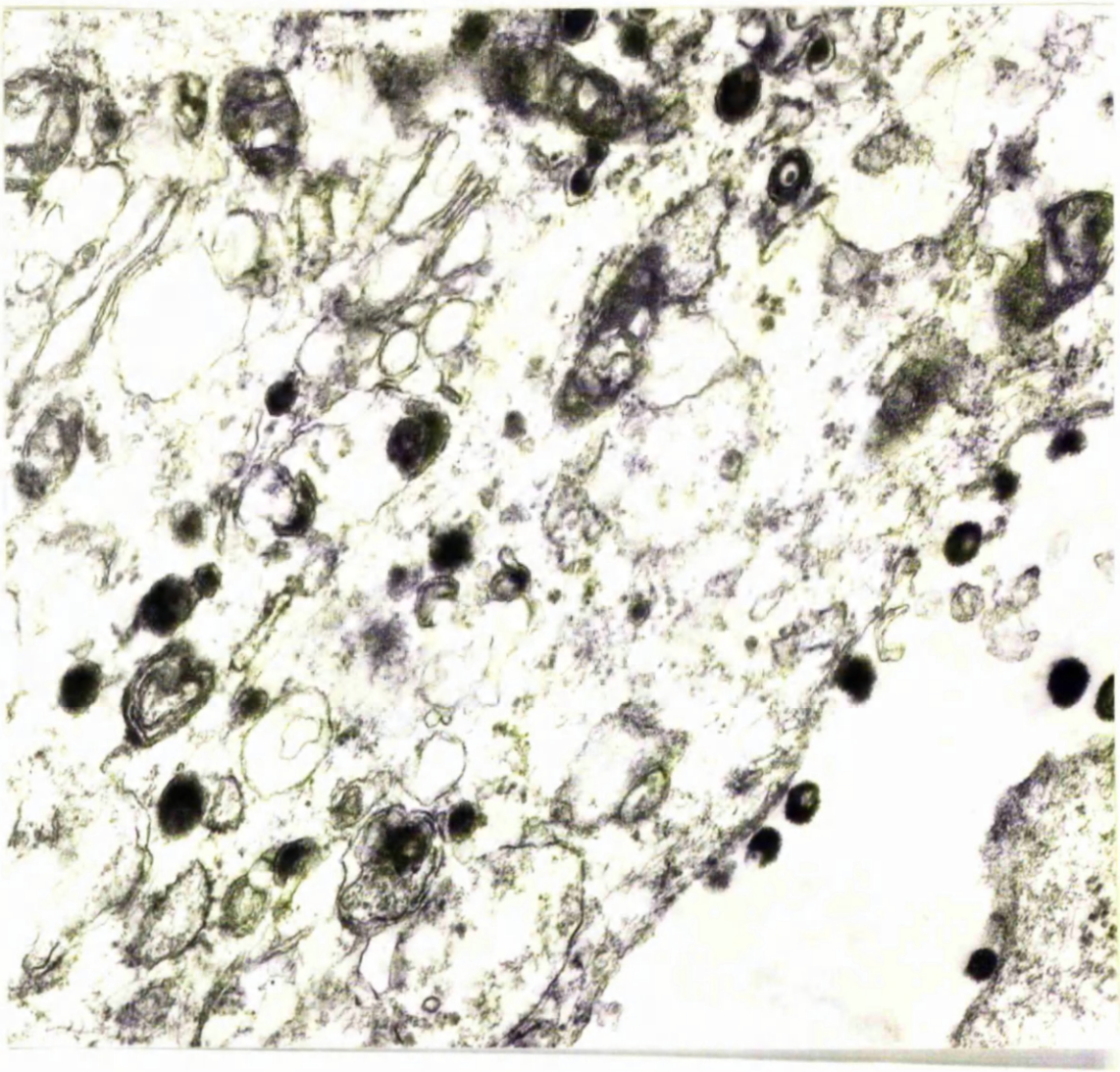


FIGURE 46. Extracellular virus particles are seen along the cell surface membrane and intracytoplasmic virus particles are seen in vacuoles. X 40,000.

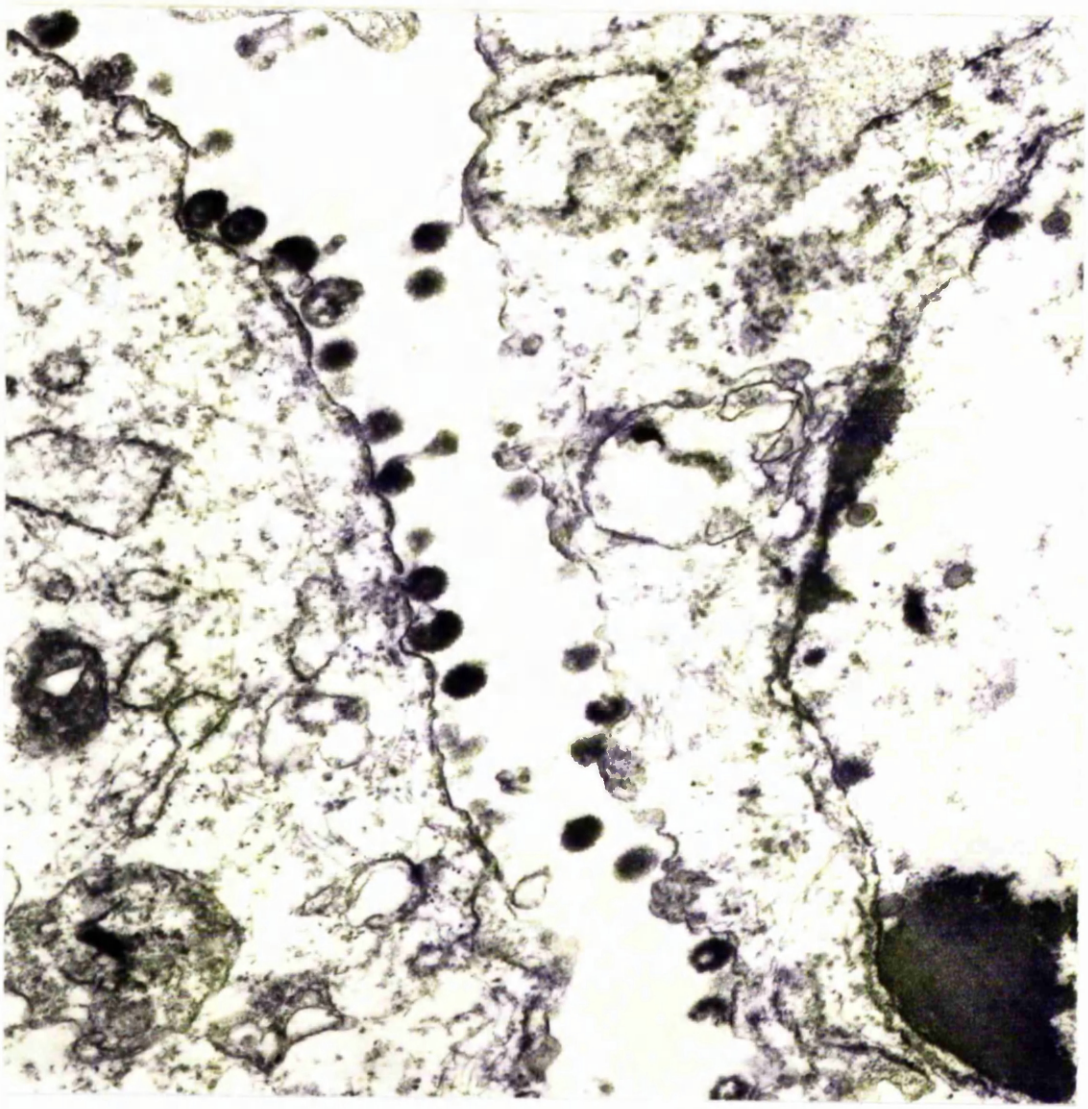


FIGURE 47. Virus particles are seen at the surface of 2 cells and within the nucleus of the cell on the right. X 40,000.

TABLE 23. The results of comparison of reciprocal neutralisation rates of 3 strains of infectious bovine rhinotracheitis virus.

VIRUS STRAIN	SERUM							
	ANTI-STRICHEN (rabbit)		ANTI-COLORADO (rabbit)		ANTI-OXFORD (rabbit)		ANTI-STRICHEN (bovine)	
	K	NK	K	NK	K	NK	K	NK
STRICHEN	2.98	100	6.44	52	8.42	141	0.96	100
COLORADO	4.47	150	12.42	100	8.42	141	2.15	224
OXFORD	5.46	183	7.82	63	5.97	100	1.39	145

FIGURE 48. The results of the neutralisation of the rabbit antiserum against the Strichen strain of infectious bovine rhinotracheitis virus with the Strichen, Oxford and Colorado strains.

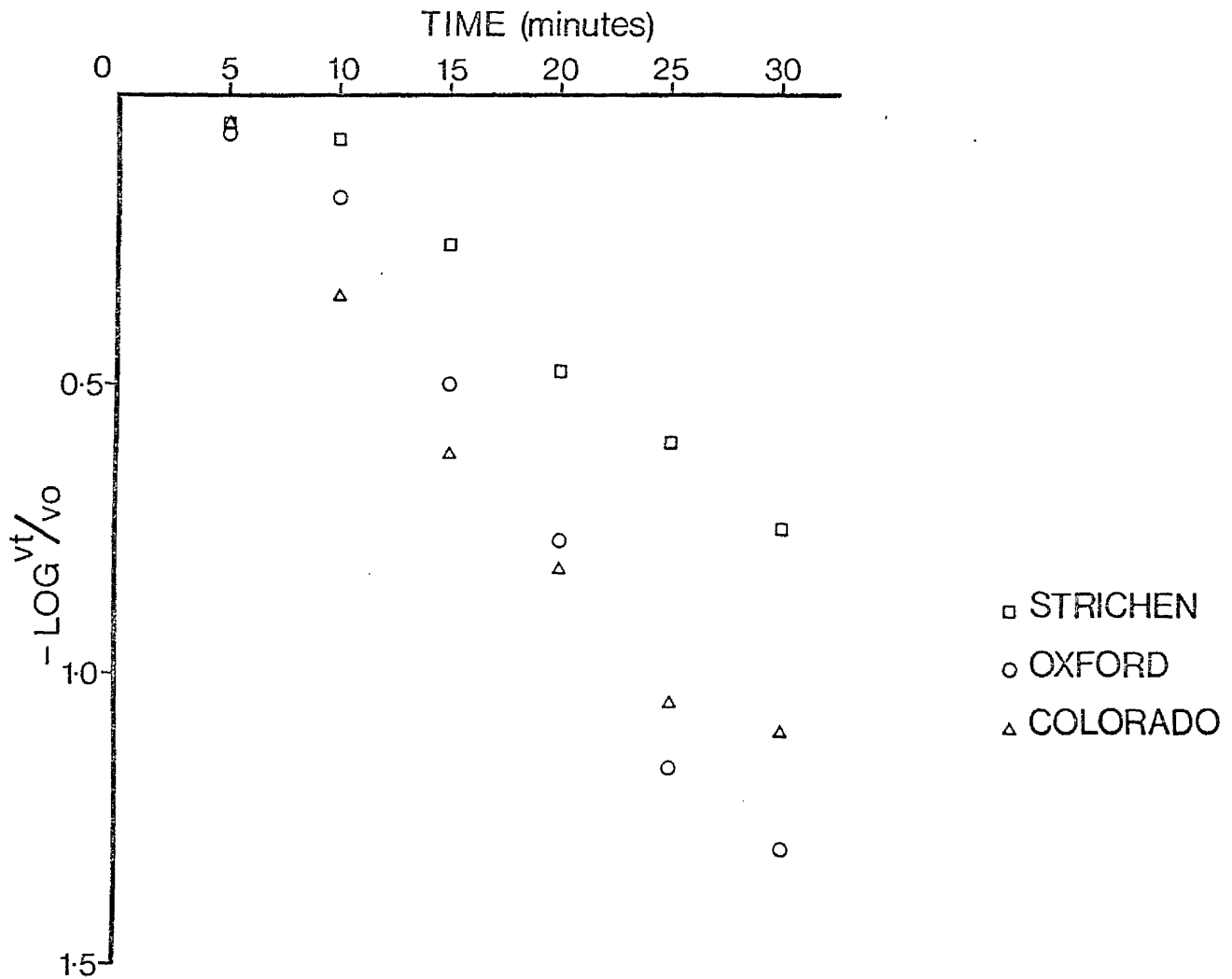


FIGURE 49. The results of the neutralisation of the bovine antiserum against the Strichen strain of infectious bovine rhinotracheitis virus with the Strichen, Oxford and Colorado strains.

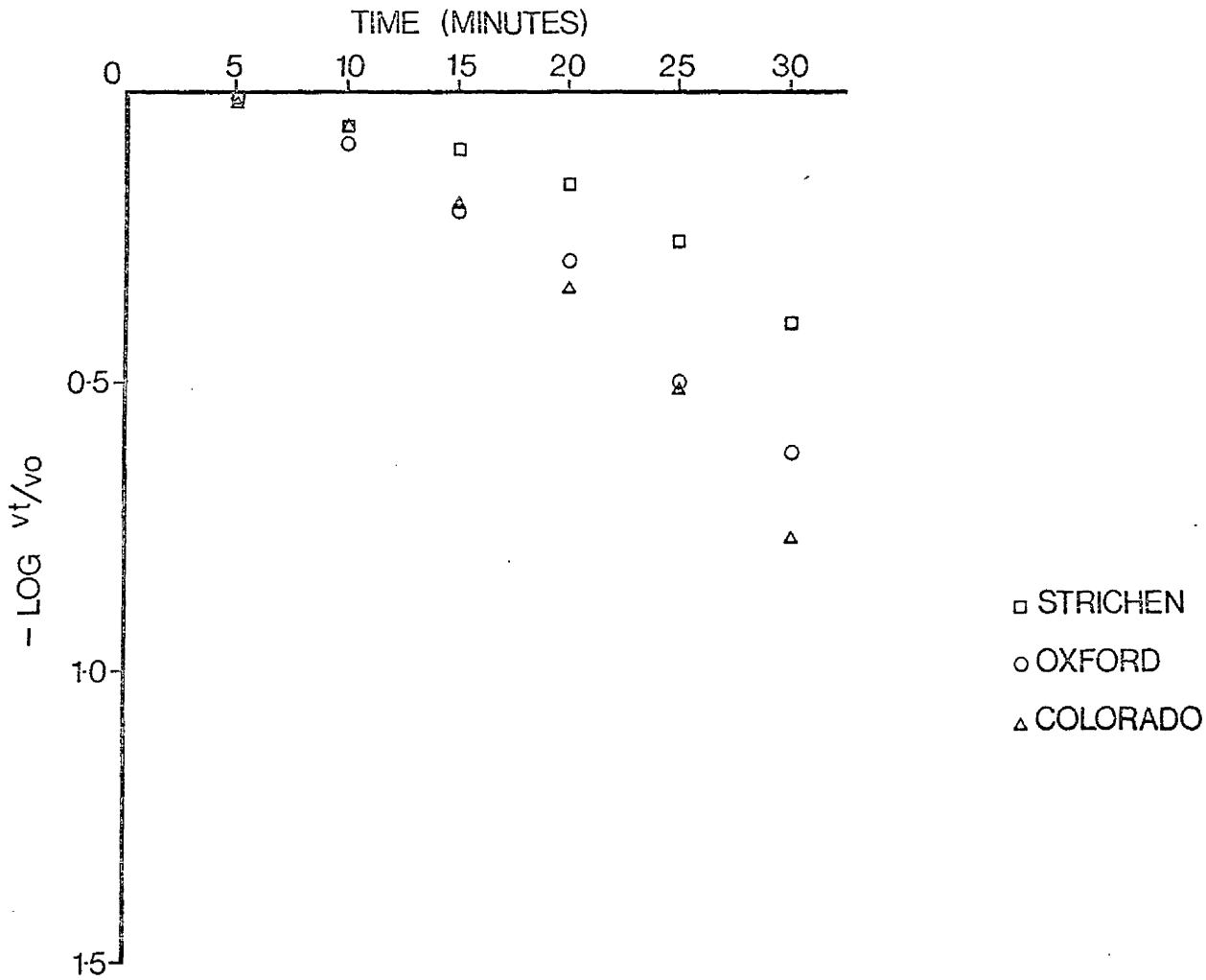


FIGURE 50. The results of the neutralisation of the rabbit antiserum against the Colorado strain of infectious bovine rhinotracheitis virus with the Strichen, Oxford and Colorado strains.

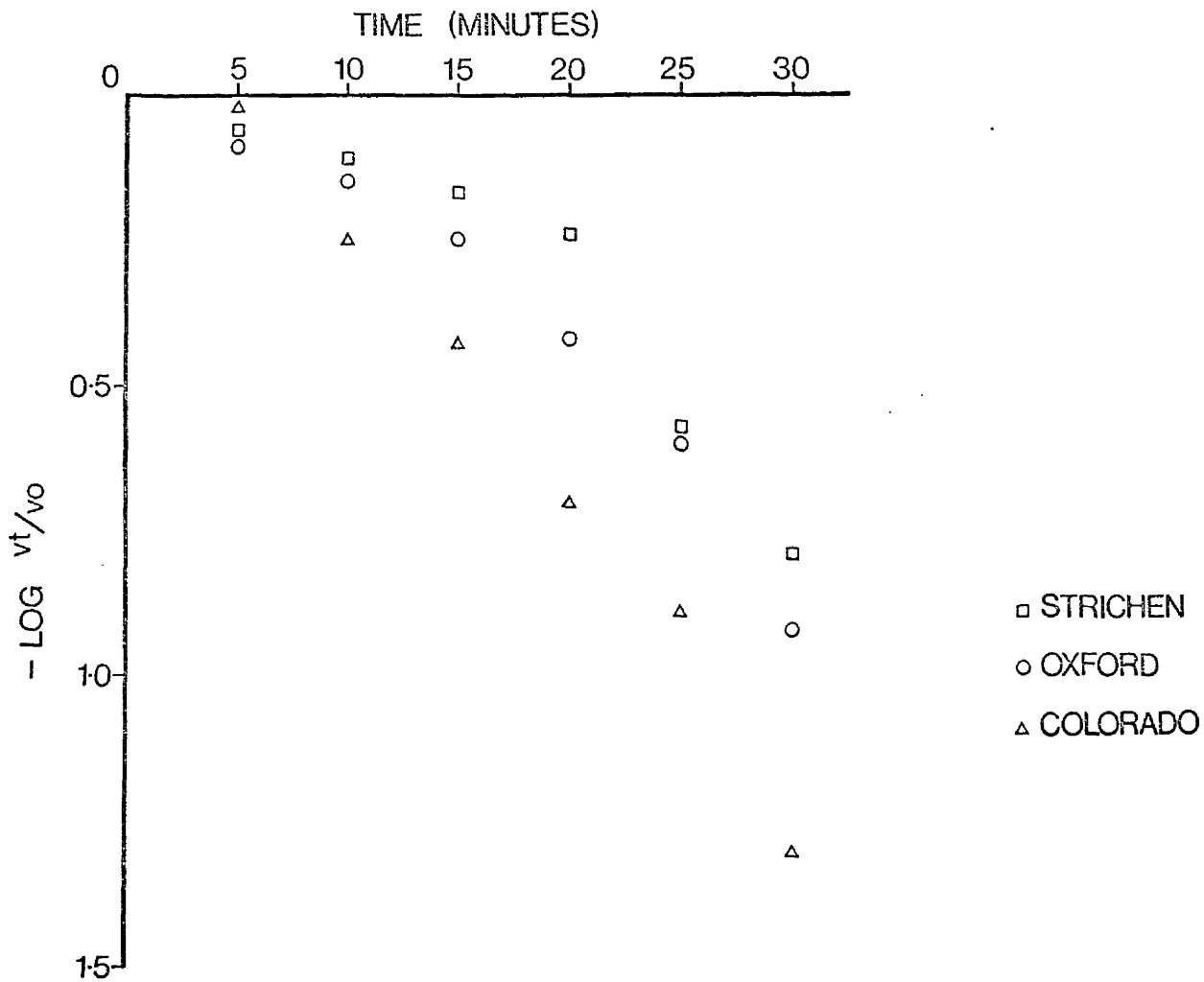
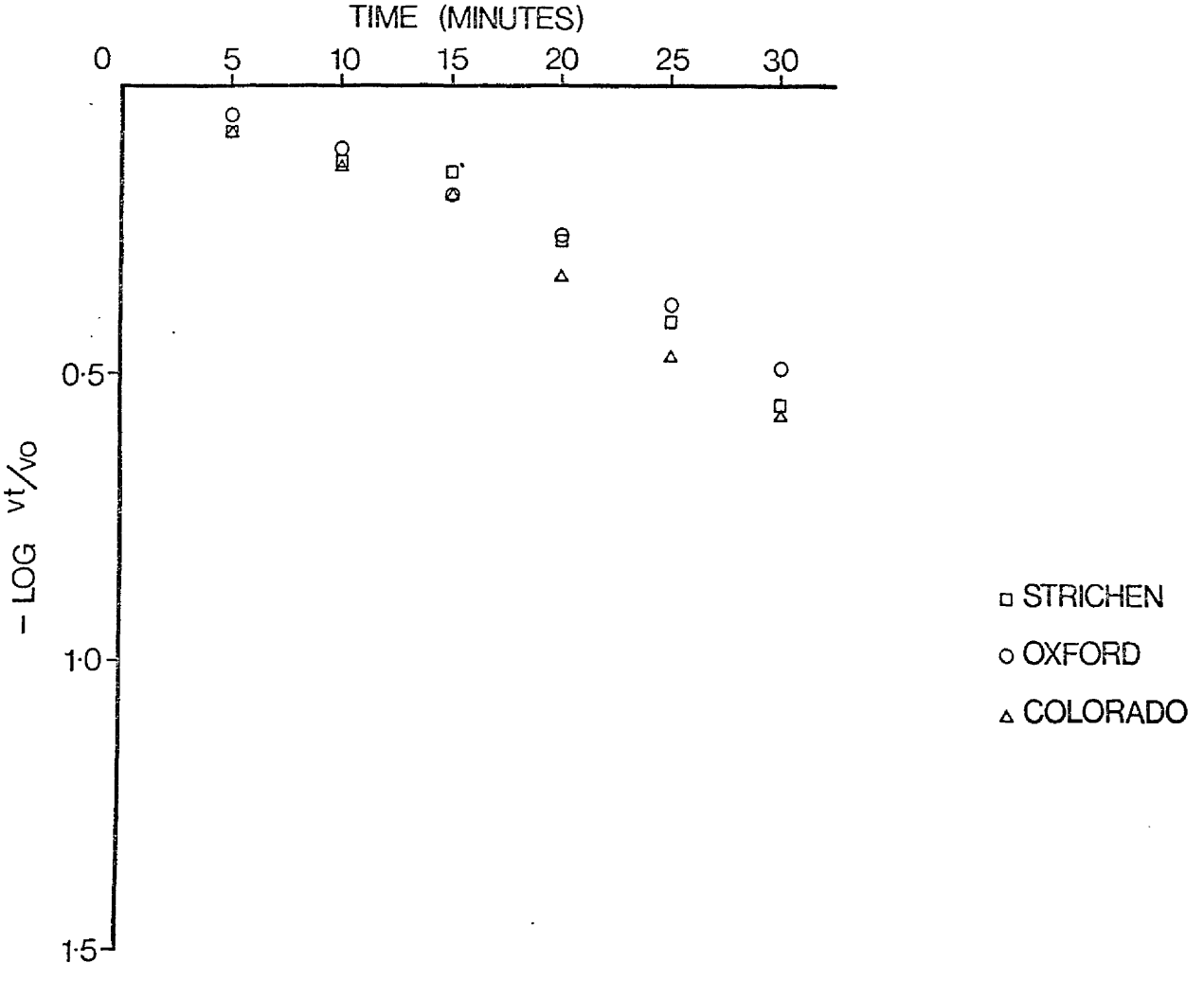


FIGURE 51. The results of the neutralisation of the rabbit antiserum against the Oxford strain of infectious bovine rhinotracheitis virus with the Strichen, Oxford and Colorado strains.



DISCUSSION

The morphological features of the plaques produced by the Strichen, Colorado and Oxford strains of IBR virus were identical. On average the plaques produced by the Oxford strain of IBR virus were significantly smaller than those of the Strichen and Colorado strains whereas the mean size of the Colorado and Strichen plaques were similar. These results appear to support the in vivo pathogenicity experimental findings (Chapter 5) in which it was found that the Oxford strain was the least pathogenic of the 3 strains. This is in keeping with the findings of Ormerod and Jarrett (1977) and Rapp (1963) found that small plaque variants of feline calicivirus and herpes simplex virus respectively were less pathogenic than variants isolated from larger plaques. On the other hand, Holmes, Kemen and Joubert (1979) found that plaques formed by the modified live, virus vaccine derived from the R-ACH equine herpesvirus 1 were comparatively larger than several field strains.

The rate of virus multiplication and release may also be related to the virulence of the virus (Buening and Gratzek, 1967) as these may indicate the rapidity with which the virus is able to spread within the animal. In these studies, both the extracellular and intracellular viruses appeared at 8 hours post inoculation. This observation was further confirmed in the electron microscopic studies in which the virus was first seen in the nucleus at 8 hours post infection as was also observed by Jasty and Chang (1972). The viral yield per cell was highest with the Strichen virus followed by the Colorado virus and Oxford virus produced the least amount of virus per cell. This again is consistent with the finding that the Oxford virus was found to be the least pathogenic of the 3 viruses.

Results of the virus neutralisation studies of the 3 strains indicated that they are related since all were neutralised by the different antisera. That the Strichen rabbit antiserum neutralised the heterologous viruses much faster than the homologous virus is unlikely to be coincidental, but rather a property of the Strichen virus itself since antiserum produced in the bovine produced identical results even when repeated several times. This observation is similar to that of Buening and Gratzek (1967) who found that an antiserum to the ISU-IBR-1 virus neutralised the heterologous virus at a faster rate than the homologous virus. With each of the 3 virus strains, a shoulder was

present in the neutralisation curve, suggesting that neutralisation results from a multi-hit process. Evidence for this has now been obtained for a number of viruses, not only for a study of neutralisation kinetics alone (Westaway, 1965; Della-porta and Westaway, 1977) but also quantitative precipitation experiments indicated that more than one antibody molecule attaches before neutralisation takes place (Rappaport, 1970). Studies with radiolabeled antiviral immunoglobulins also suggested that more than one antibody attaches before neutralisation is detected (Rowlands, 1967; Oldstone, Cooper and Larson, 1974). However with the Strichen virus, the shoulder was more pronounced than with the other 2 strains. This would suggest that the Strichen virus possesses more critical sites with which antibodies can react before neutralisation is complete. Another possibility is that several successive but different events may be necessary for the neutralisation of IBR virus; some strains may be neutralised more efficiently than others in the absence of complement. For example, neutralisation of the Strichen virus may be more complement dependant than that of the other strains used in these studies. If so, this is one possible explanation for the shoulder in the curve. This hypothesis remains to be examined experimentally.

That the Strichen virus is neutralised less efficiently than the other 2 viruses by anti-Strichen antiserum might be very important in the epidemiology of IBR virus. It is conceivable that because of this, the Strichen virus is able to spread rapidly in the affected animal to infect large numbers of cells and to produce a disease which is more severe and more prolonged than that associated with either the Colorado or Oxford strains.

These studies have confirmed that the neutralisation constants of the Strichen strain differed from those of the Colorado and Oxford strains. This distinct biological characteristic would indicate that the Strichen strain differs from the other 2 strains in minor antigenic components which may be related to the severity of both field and the experimentally produced disease.

STUDIES ON SEVERE INFECTIOUS BOVINE RHINOTRACHEITIS IN BRITAIN

TWO VOLUMES

VOLUME 2

by

PETER M. MSOLLA, B.V.M., D.T.V.M.

Thesis submitted for the degree of Doctor of Philosophy in the Faculty of Veterinary Medicine, Department of Veterinary Medicine, University of Glasgow.

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CHAPTER 5

EXPERIMENTAL STUDIES WITH THE VIRUS OF INFECTIOUS BOVINE RHINOTRACHEITIS

INTRODUCTION

The fulfilment of Koch's postulates (1884) provides confirmation that a micro-organism, which has been isolated from a sick animal suffering from a hitherto unrecognised syndrome, is indeed the causal agent. Calves exposed to BHV 1, under controlled conditions, have developed typical clinical and characteristic pathological features (McKercher and others, 1955; Webster and Manktelow, 1959; Abinanti and Plumer, 1961; Hughes and others, 1964; Markson and Darbyshire, 1966). However, the severity of the clinical response and the extent of the pathological lesions were much less severe in the experimental than in the field disease. The major clinical and pathological features of the experimental disease have been summarised in Table 24.

In addition to challenging cattle with the IBR virus by natural routes of infection, such as the intranasal and intra-ocular routes, experimental studies have also revealed that this virus will also produce IPV when inoculated intravaginally (McKercher and others, 1959). However, respiratory tract isolates appear to produce less severe signs than vaginal isolates when inoculated intravaginally. Similarly, vaginal isolates tend to produce milder respiratory disease than respiratory isolates when given intranasally (McKercher and others, 1959). It was deduced therefore that isolates varied in their affinity for different tissues.

In common with many other herpes viruses, a state of latency can develop following a primary infection with BHV 1 (Gibbs and Rweyemamu, 1977). When recovered cattle are stressed, the virus can be reactivated and it has been shown to be excreted intermittently for up to 578 days post infection (p.i.) (Snowdon, 1965). The reactivated virus may then give rise to the acute clinical syndrome in susceptible incontact animals. This phenomenon of virus reactivation has also been produced artificially following several successive daily treatments with corticosteroids (Sheffy and Davies, 1972; Sheffy and Rodman, 1973). Under field conditions natural stress factors which have been confirmed as inducing virus reactivation and excretion include pregnancy (Snowdon, 1965) and caesarian section (Lomba and others, 1976). Another example was demonstrated by Mensik, Pospisil, Suchankora, Cepica, Rozkosny and Machatkova (1976) who challenged recovered cases of IBR with bovine parainfluenza type 3 virus. The calves developed typical signs of acute rhinotracheitis 3 to 5 days post-challenge. Since infestation with the lungworm, Dictyocaulus viviparus, is very common in Britain, it was decided

to assault recovered cases of IBR with this parasite in order to determine whether or not this form of natural challenge could be an important factor in the dissemination of infection.

In North America where IBR is endemic, effective prevention of disease depends upon a regime of regular vaccination. It has been confirmed that, modified live virus (MLV) vaccines are more efficient than inactivated vaccines in protecting against natural challenge (Bartholomew, 1973). At present in the United Kingdom, inactivated virus vaccines only can be used in cattle. As a result of the ever increasing demands of both farmers and general practitioners regarding advice on the control of IBR, it was decided to test the efficacy of the only vaccine which contains IBR antigens and which is currently available in this country (Pneumovac Plus; C-Vet., Bury St. Edmunds, England). This is a pentavalent vaccine which also contains antigens derived from bovine parainfluenza type 3 virus, bovine adenovirus 3, bovine reovirus 1 and bovine viral diarrhoea virus.

The aims of this experimental investigation were:-

1) To study the pathogenicity of the most recent IBR virus isolate (Strichen strain).
2) To compare the pathogenicity of the Colorado and Oxford IBR virus strains.
3) To ascertain whether the Strichen strain of IBR virus could produce infectious pustular vulvovaginitis.
4) To study the effect of infection with Dictyocaulus viviparus on animals which had recovered from experimental infection with the Strichen strain of IBR virus.
5) To test the efficacy of the vaccine, Pneumovac Plus, against challenge with the Strichen strain of IBR virus.

TABLE 24. A summary of the main details in experimental studies with infectious bovine rhinotracheitis virus.

Author	Experimental Animals	Method of Challenge	Clinical Response	Serological Response	Pathological findings
McKercher and others (1955)	3 Guernsey 1 Hereford 6-8 months old calves	Intranasal	Dullness. Anorexia. Pyrexia (105.5-107.5°F) Bilateral serous nasal discharge. Diphtheresis. Tachypnoea. Respiratory stertor. Dyspnoea. Loss of weight.	ND	Rhinitis. Diphtheritic lesions in nasal mucosa. Pharyngitis with petechial haemorrhages. Laryngitis. Tracheitis. Acute bronchopneumonia. Local lymphnode enlargement.
Webster and Manktelow (1959)	3 one-week old calves. 3 yearlings 2 adult animals	Intranasal Intratracheal Intraocular (one drop) Intravenous	*Most severe disease in young calves. Dullness. Anorexia. Pyrexia (105.5°F). Conjunctivitis with serous ocular discharge. Bilateral mucopurulent nasal discharge. Coughing. Respiratory stertor. Dyspnoea. Enlargement of superficial lymphnodes. **Milder reaction in yearlings. ***In the two cows very mild clinical response without any temperature reaction.	ND	Rhinitis. Diphtheritic lesions. Laryngitis. Tracheitis. Small areas of lung-consolidation. Local lymphnode enlargement.

TABLE 24. (Cont'd)

Author	Experimental Animals	Method of Challenge	Clinical Response	Serological Response	Pathological findings
Abinanti and Plumer (1961)	4, 3-6 week old calves	Interpalpebral	Slight dullness. Pyrexia (104°F). Profuse serous ocular discharge. Conjunctival oedema with fine haemorrhages. Sero-mucoid nasal discharge.	Neutralising antibodies detected in 3 calves only.	ND
Hughes and others (1964)	6, 4-6 months old calves	Instilled into the conjunctival sac	Dullness. Pyrexia (103-104.2°F). Conjunctivitis with serous ocular discharge. Severe kerato conjunctivitis. Bilateral serous nasal discharge.	Neutralising antibodies detected in 5 calves only.	ND
Markson and Darbyshire (1966)	14, 2 months old calves	Intranasal and Intraconjunctival	Dullness. Pyrexia (105.4°F). Conjunctivitis with serous ocular discharge. Slight bilateral serous to mucopurulent nasal discharge. Respiratory stertor. Slight to moderate diarrhoea. Pneumonia.	No evidence of neutralising antibodies by day 14 p.i. when the last calf was killed.	Conjunctivitis. Hyperaemia of nasal mucosa. Pneumonitis.

SECTION I

THE EFFECT ON THE BOVINE RESPIRATORY TRACT OF INFECTION WITH THE STRICHEN STRAIN OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

MATERIALS AND METHODS

Experimental animals

Four different age groups (A, B, C and D) of cattle each comprising of 4 animals were used in these experiments. Thus in Group A, 4 (A1, A2, A3 and A4) 18 month old Friesian cross bullocks were used, while in Group B, 4 (B1, B2, B3 and B4) 6 month old Friesian crosses were used. In Group C, again 4 (C1, C2, C3 and C4) 5 week old Ayrshire cross calves and in Group D, 4 (D1, D2, D3 and D4) 2 week old Ayrshire cross calves were used.

In addition to the 4 stirks in Group B two more (B5 and B6) 6 month old Friesian crosses were used as incontact animals.

Housing

During the time of this experiment, the animals were housed in well-ventilated loose-boxes which measured 15' x 15' x 9'. In addition to the straw bedding, calves of less than 3 weeks of age (Group D) had their boxes heated with infra-red lamps during the severe cold winter.

Feeding

The calves for these experiments were bought into the veterinary school when they were approximately 1 week old. On arrival, they were fed approximately 8 pints of fresh pre-warmed milk twice daily (morning and evening) for up to 2 weeks. From the age of 3 weeks, the calves received 12 pints of milk a day and had ad libitum access to calf pencils (BOCM-Silcocks, England). This regime was followed until the animals were 8 weeks of age, after which they were given concentrates (2-3 lbs. per day) and hay ad libitum. Older bullocks received 4 lbs of low protein dairy concentrate cake (BOCM-Silcocks, England) once daily and hay ad libitum.

Challenge procedure

Prior to challenge the animals were left to become acclimatised in the boxes for a minimum period of 7 days. The virus used was the

Strichen isolate, i.e. the isolate from field outbreak 1 which had been grown and stored as described in detail in Chapter 2. Each animal was challenged intranasally with a tissue culture suspension (titre = $10^{7.7}TCID_{50}/ml$). A 6 inch long sterile plastic canulla was fitted to the tip of a dropper syringe and 1.0ml of tissue culture suspension was inoculated into each nostril. Afterwards the calf's head was held in an upward position for roughly 1 minute to ensure maximal viral retention.

In experiment 2 the incontact animals, (B5 and B6), were put into the same box as animals B2 and B3 on day 6 p.i..

Clinical examination

The animals were subjected to a thorough clinical examination twice daily for 7 days prior to challenge. This examination, which was done in the morning (9.00am) and afternoon (4.00pm), included the checking of demeanour, appetite, rectal temperature, pulse rate, respiratory rate, auscultation of the thorax and evidence of ocular and nasal lesions.

After challenge, the animals were subjected to a routine clinical examination twice daily as described above for a period of 14 days and thereafter, every other day for up to 21 days p.i..

Haematological examination

During the week prior to challenge, blood was collected from the jugular vein into vacutainers containing potassium diamine tetra-acetic acid (EDTA) on 3 occasions.

After challenge, blood was collected in a similar manner daily for 14 days. The packed cell volume (PCV) was established by the micro-haematocrit technique (Fisher, 1962), while the total number of leucocytes was found by using a model D-Coulter Counter (Coulter Electronics Ltd., Dunstable, Beds). The differential white cell counts were made on Leishman-stained blood films and 200 cells were counted.

Pathological examination

At various intervals after challenge, as will be shown in detail in the results, animals in each group, apart from Group A, were slaughtered for pathological and virological examinations.

The details of the routine for the pathological examination was similar to that already described in Chapter 2. Specimens from the

trachea of Group B animals were taken for detailed studies using scanning electron microscope (SEM). The tissues were fixed in 3 per cent glutaraldehyde, dehydrated in increasing concentrations of acetone and transferred to liquid Co_2 for critical point drying. The dried specimens were mounted on aluminium stubs, sputter coated with gold and examined with a Phillips 501B scanning electron microscope.

Serological examination

Blood for serology was collected into vacutainers not containing any additive in the same manner and at the same time as the samples for haematological examination. They were stored overnight at $4^{o}C$ and, after being spun at 1300g for 30 minutes, the serum was separated from the blood clot using a rubber teat and pasteur pipette. The serum was placed in a water bath at $56^{o}C$ for 30 minutes, left to cool and thereafter stored at $-20^{o}C$ until required. Specific antibody screening for IBR virus was undertaken by the constant virus-varying serum dilutions, serum neutralisation test using flat-bottomed microtitration plates, as has already been described in detail in Chapter 3.

During the week prior to challenge, serum from every animal was screened for antibodies to IBR virus. After challenge, blood samples were collected daily from day 6 to 15 p.i. and, thereafter, at weekly intervals.

Virus isolation

Prior to challenge, nasal and ocular swabs were taken using a cotton swab stick-applicator (Exogen Ltd., Dumbarton Road, Glasgow, UK) on 2 occasions at 2-3 day intervals to check for the presence of IBR virus. After they had been taken the swabs were broken off into virus transport medium.

After challenge, nasal and ocular swabs were collected daily from day 4 to day 15 p.i.. Both swabs and tissue specimens derived from tissue of experimental calves taken at necropsy were treated in a similar manner as has already been described in detail in Chapter 2. Four secondary CK tubes were used for every specimen for virus isolation. Inoculated CK tubes were examined daily for 5 days for evidence of cytopathogenicity before a second passage was made. Passage was achieved by using whole cultures after freezing ($-70^{o}C$) and thawing. Samples were discarded as negative if a cytopathogenic effect had not developed by the fifth day p.i. of third passage tubes.

RESULTS

Experiment 1. Group A bullocks.

Clinical features. The detailed clinical findings are summarised in Appendix 4, Table 1.

On day 3 p.i., animals A1, A2 and A4 appeared dull and there was a slight drop in the amount of food eaten by the group. There was moderate pyrexia (103-105°F) as well as a slight serous ocular discharge as a result of a moderately severe conjunctivitis. There was slight bilateral serous nasal discharge. By day 4 p.i., a bilateral seromucoid nasal discharge and diphtheritic lesions had developed in the nasal mucosa of all 3 animals. In A2 numerous small haemorrhages were also evident. The 3 animals were tachypnoeic (RR = 36-60/min) and frequent, single non-productive coughs were observed. All 3 animals were seen to be drooling saliva. The temperatures were back to within the normal range on day 5 p.i. while most of the other clinical signs had more or less cleared by day 9 p.i.. Drooling of saliva was observed from day 3 to 6 p.i..

Bullock A3 was found to be seropositive with a titre of 1:12 prior to challenge and the only clinical response in this animal was a slight serous nasal discharge and congestion of the nasal mucosa. During this time, the temperature remained within the normal range.

On haematological examination neutropenia developed in every animal following injection. The decrease in the number of neutrophils was smallest in A3 which was seropositive when exposed. The mean total white blood cell and neutrophil numbers on specific days following challenge of the 3 seronegative bullocks are presented in Table 25 During the first 8 days post exposure the decrease in the mean number of neutrophils in these 3 animals (Figure 52) was statistically significant ($p < 0.01$). However, there was no correlation between the decrease in the numbers of neutrophils in the 3 seronegative individuals and the severity of the clinical signs.

Virus isolation

These results are shown in Table 26. Virus was recovered regularly from day 3 until day 13 p.i. and from day 3 until 8 p.i. from nasal and ocular discharges respectively.

Serological examination

Neutralising antibodies were demonstrated in A1 and A4 on day 7 p.i. while in A2 they were detected on day 10 p.i.; the titres reached the maximum of 1:128, 3 weeks after challenge. Animal A3, which had an antibody titre of 1:12 prior to challenge responded in a less dramatic way reaching a maximum titre of only 1:32 after 4 weeks.

Pathological examination

None of the animals in this group were slaughtered.

TABLE 25. The mean total white blood cell and neutrophil numbers on specific days following the challenge of different age groups of cattle with the Strichen strain of infectious bovine rhinotracheitis virus.

	GROUP A		GROUP B		GROUP C	
DAY	TOTAL WHITE CELLS	NEUTROPHILS	TOTAL WHITE CELLS	NEUTROPHILS	TOTAL WHITE CELLS	NEUTROPHILS
0	10133 ± 2136	6020 ± 866	8350 ± 1782	3115 ± 615	8200 ± 2538	4885 ± 1737
2	ND	ND	9225 ± 954	4017 ± 811	7675 ± 2546	3600 ± 760
3	9033 ± 3134	5200 ± 3299	9075 ± 1582	4207 ± 992	8375 ± 2360	4580 ± 886
4	9500 ± 2193	4750 ± 1457	10750 ± 625	4665 ± 1315	10475 ± 4496	5998 ± 2791
6	9266 ± 2701	4938 ± 1603	9350 ± 2198	4123 ± 1019	7700 ± 2828	5400 ± 2234
8	6800 ± 2488	2476 ± 999	6300 ± 2970	2650 ± 1485	7050 ± 1202	4940 ± 1725
10	7767 ± 404	3260 ± 180	6050 ± 2051	2680 ± 141	7350 ± 1900	3900 ± 1867
12	ND	ND	7850 ± 1485	3230 ± 42	7250 ± 1061	4397 ± 2266
14	ND	ND	9200 ± 707	4180 ± 127	5750 ± 636	3120 ± 382
18	ND	ND	7600 ± 1273	3460 ± 1640	6850 ± 495	4210 ± 255
20	ND	ND	5600 ± 3536	2590 ± 3007	5900 ± 1838	3100 ± 806

TABLE 26. The results of examination of swabs and tissues for virus from the experimentally infected animals with the virus of infectious bovine rhinotracheitis.

SOURCE OF VIRUS ISOLATION	A1	A2	A3	A4	B1	B2	B3	B4
Nasal swab	+ (12)	+ (13)	+ (13)	+ (12)	+ (6)	+ (13)	+ (13)	+ (6)
Ocular swab	+ (6)	+ (7)	+ (8)	+ (8)	+ (6)	+ (10)	+ (9)	+ (6)
Brain	ND	ND	ND	ND	+ (6)	NA	NA	-
Turbinates	ND	ND	ND	ND	+ (6)	NA	NA	+ (6)
Nasopharynx	ND	ND	ND	ND	+ (6)	NA	NA	+ (6)
Larynx	ND	ND	ND	ND	+ (6)	NA	NA	+ (6)
Upper trachea	ND	ND	ND	ND	+ (6)	NA	NA	+ (6)
Lower trachea	ND	ND	ND	ND	+ (6)	NA	NA	+ (6)
Lungs	ND	ND	ND	ND	-	NA	NA	-
Retropharyngeal LN	ND	ND	ND	ND	+ (6)	NA	NA	+ (6)
Bronchial LN	ND	ND	ND	ND	+ (6)	NA	NA	+ (6)
Mediastinal LN	ND	ND	ND	ND	+ (6)	NA	NA	+ (6)
Liver	ND	ND	ND	ND	-	NA	NA	-
Gall bladder	ND	ND	ND	ND	ND	NA	NA	ND
Spleen	ND	ND	ND	ND	-	NA	NA	-
Kidneys	ND	ND	ND	ND	-	NA	NA	+ (6)
Vaginal swab	NA	NA	NA	NA	NA	NA	NA	NA
Vaginal mucosa	NA	NA	NA	NA	NA	NA	NA	NA
Uterus	NA	NA	NA	NA	NA	NA	NA	NA

TABLE 26. cont'd

SOURCE OF VIRUS ISOLATION	C1	C2	C3	C4	D2	D3	D4
Nasal swab	+ (13)	+ (4)	+ (12)	+ (6)	+ (12)	+ (5)	+ (12)
Ocular swab	+ (7)	+ (4)	+ 10	+ (6)	+ (10)	+ (4)	+ (10)
Brain	- (33)	NA	NA	- (33)	NA	-	NA
Turbinates	-	NA	NA	-	NA	+ (5)	NA
Nasopharynx	-	NA	NA	-	NA	+ (5)	NA
Larynx	-	NA	NA	-	NA	+ (5)	NA
Upper trachea	-	NA	NA	-	NA	+ (5)	NA
Lower trachea	-	NA	NA	-	NA	+ (5)	NA
Lungs	-	NA	NA	-	NA	+ (5)	NA
Retropharyngeal LN	-	NA	NA	-	NA	+ (5)	NA
Bronchial LN	-	NA	NA	-	NA	-	NA
Mediastinal LN	-	NA	NA	-	NA	+ (5)	NA
Liver	-	NA	NA	-	NA	-	NA
Gall bladder	-	NA	NA	-	NA	-	NA
Spleen	-	NA	NA	-	NA	-	NA
Kidneys	-	NA	NA	-	NA	-	NA
Vaginal swab	NA	NA	NA	NA	NA	NA	NA
Vaginal mucosa	NA	NA	NA	NA	NA	NA	NA
Uterus	NA	NA	NA	NA	NA	NA	NA

TABLE 26. cont'd

SOURCE OF VIRUS ISOLATION	E1	E2	E3	E4	F1	F2	F3	F4
Nasal swab	+ (4)	+ (6)	+ (12)	+ (12)	+ (6)	+ (11)	+ (4)	+ (9)
Ocular swab	-	+ (6)	+ (9)	+ (10)	+ (5)	-	-	+ (10)
Brain	+ (4)	-	NA	NA	-	NA	-	NA
Turbinates	+ (4)	+ (6)	NA	NA	+ (6)	NA	+ (4)	NA
Nasopharynx	+ (4)	+ (6)	NA	NA	+ (6)	NA	+ (4)	NA
Larynx	+ (4)	+ (6)	NA	NA	+ (6)	NA	+ (4)	NA
Upper trachea	+ (4)	+ (6)	NA	NA	+ (6)	NA	+ (4)	NA
Lower trachea	+ (4)	+ (6)	NA	NA	+ (6)	NA	+ (4)	NA
Lungs	-	-	NA	NA	-	NA	+ (4)	NA
Retropharyngeal LN	+ (4)	+ (6)	NA	NA	+ (6)	NA	+ (4)	NA
Bronchial LN	-	+ (6)	NA	NA	+ (6)	NA	-	NA
Mediastinal LN	+ (4)	-	NA	NA	+ (6)	NA	-	NA
Liver	-	-	NA	NA	-	NA	-	NA
Gall bladder	-	-	NA	NA	-	NA	-	NA
Spleen	-	-	NA	NA	-	NA	-	NA
Kidneys	+ (4)	-	NA	NA	-	NA	-	NA
Vaginal swab	NA	NA	NA	NA	NA	NA	NA	NA
Vaginal mucosa	NA	NA	NA	NA	NA	NA	NA	NA
Uterus	NA	NA	NA	NA	NA	NA	NA	NA

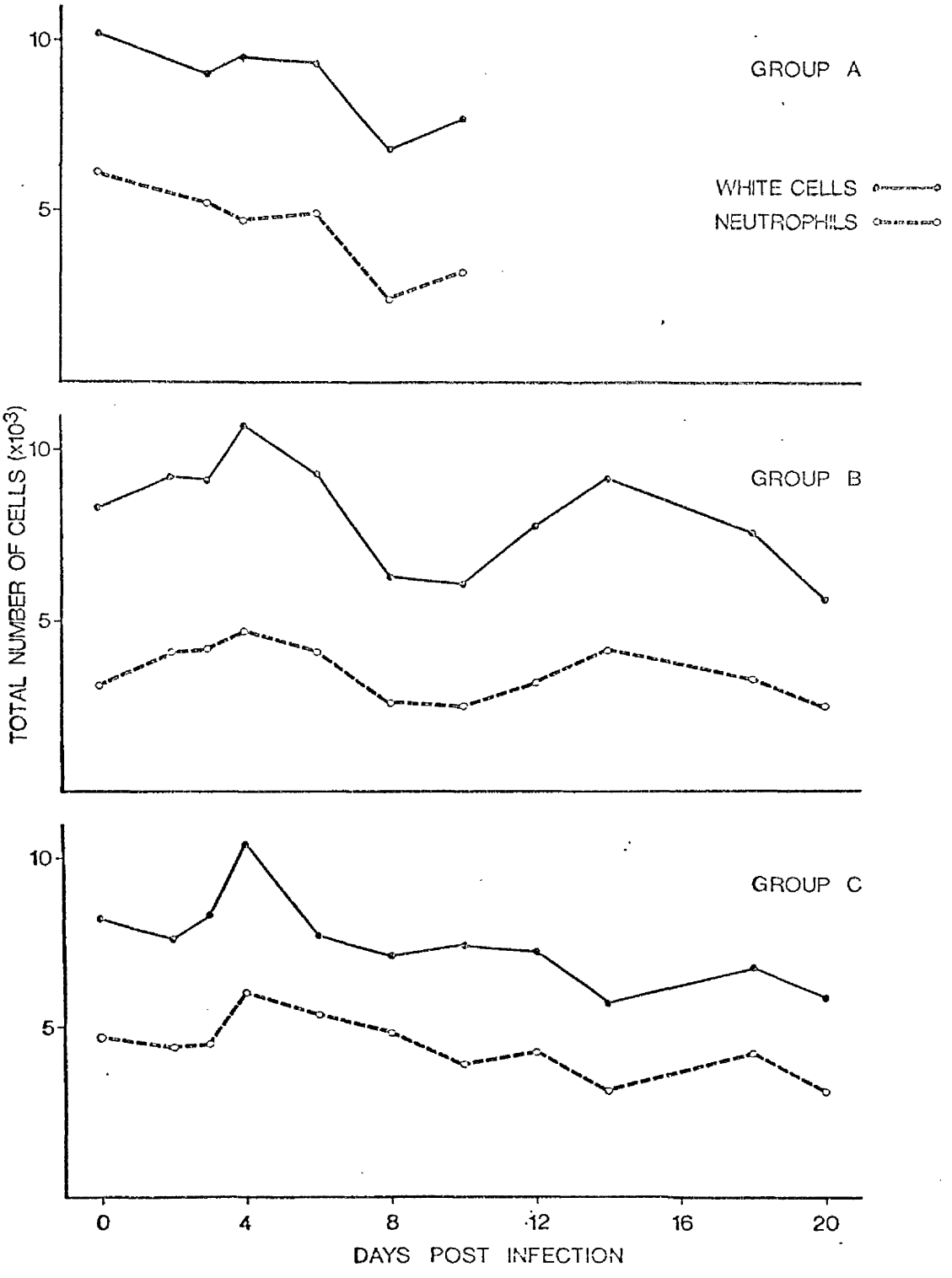
TABLE 26. cont'd

SOURCE OF VIRUS ISOLATION	G1	G2	G3	G4	H1	H2	H3	H4
Nasal swab	-	-	-	-	+ (8)	+ (18)	+ (21)	+ (15)
Ocular swab	-	-	-	-	-	-	-	-
Brain	ND	ND	ND	ND	-	NA	NA	-
Turbinates	ND	ND	ND	ND	+ (15)	NA	NA	+ (17)
Nasopharynx	ND	ND	ND	ND	-	NA	NA	-
Larynx	ND	ND	ND	ND	-	NA	NA	-
Upper trachea	ND	ND	ND	ND	+ (15)	NA	NA	-
Lower trachea	ND	ND	ND	ND	-	NA	NA	-
Lungs	ND	ND	ND	ND	-	NA	NA	-
Retropharyngeal LN	ND	ND	ND	ND	-	NA	NA	-
Bronchial LN	ND	ND	ND	ND	-	NA	NA	-
Mediastinal LN	ND	ND	ND	ND	-	NA	NA	-
Liver	ND	ND	ND	ND	-	NA	NA	-
Gall bladder	ND	ND	ND	ND	-	NA	NA	-
Spleen	ND	ND	ND	ND	-	NA	NA	-
Kidneys	ND	ND	ND	ND	-	NA	NA	-
Vaginal swab	+ (4)	+ (21)	+ (19)	+ (4)	NA	NA	NA	NA
Vaginal mucosa	+ (4)	NA	NA	+ (4)	NA	NA	NA	NA
Uterus	+ (4)	NA	NA	+ (4)	NA	NA	NA	NA

- = Negative for virus
+ = Positive for virus

NA = Not applicable
ND = Not done
( ) = Last day of virus recovery

FIGURE 52. The mean total white blood cell and neutrophil numbers on specific days following challenge of 3 groups of cattle with the Strichen strain of infectious bovine rhinotracheitis virus.



Experiment 2. Group B stirks.

Clinical features. The detailed clinical findings are summarised in Appendix 4, Table 2.

On day 3 p.i., all 4 stirks were dull, had slight serous nasal discharge and a moderate pyrexia (103-104.5°F). On the 4th and 5th days p.i., the above clinical signs became more severe and the most persistent presenting signs were seromucoid to mucopurulent nasal discharge, sporadic single, dry, non-productive coughing, pyrexia (104-107.5°F), congestion of the nasal mucosa as well as diphtheritic lesions. On close examination, pin-point haemorrhages were evident on the nasal mucosa which bled easily. Moderate conjunctivitis accompanied by serous lachrymation was a feature common to every stirk. In B2, conjunctivitis was of a granular nature. All 4 stirks were tachypnoeic (RR = 40-75/min) and respirations were harsh. Drooling of saliva was observed from day 4 to 6 p.i..

The calves were severely ill from day 3 p.i. and from day 4 to day 6 p.i. every animal was severely dyspnoeic and anorexic. B1 and B4 were slaughtered on day 6 p.i.. From day 8 onwards, the remaining 2 stirks B2 and B3 gradually recovered.

The mean total white blood cell and neutrophil numbers on specific days following challenge are presented in Table 25. During the first 4 days post exposure (Figure 52), the mean total numbers of white cells and of neutrophils increased; from day 4 to day 10 post exposure the mean numbers decreased; from day 10 to day 14 the mean numbers increased again and from day 14 to day 20 they decreased. The decrease in the mean numbers of total white cell from day 4 to day 10 was statistically significant ($p < 0.01$) as was the overall decrease from day 4 to day 20 ($p < 0.05$).

The 2 incontact stirks, B5 and B6, which were introduced on day 6 p.i. became dull, unwilling to eat, moderately pyrexic (103-104.5°F) and tachypnoeic (RR = 36-40/min) 5 days after introduction. Their nasal mucosa became congested and a bilateral, seromucoid, nasal discharge was evident. Animal B6 developed a bilateral conjunctivitis which was accompanied by purulent ocular discharge. On day 6 post contact, the above clinical signs became more overt with B5 and B6 becoming much duller and easy to handle. They were anorexic and their temperatures went up to 106.5°F. There was a bilateral seromucoid to mucopurulent

nasal discharge and diphtheritic lesions were observed on the nasal mucosa. At this stage, they were seen to be more tachypnoeic (RR = 50-70/min) and dyspnoeic. Sporadic non-productive coughing was heard. The conjunctivitis which had been seen on day 5 post contact only in B6 now became evident in B5, although in B6, it was more severe, granular in nature and accompanied by a copious mucopurulent ocular discharge which heavily soiled the hair on the cheeks (Figure 53). The clinical signs persisted for 7 days (12 days post contact) before they began to regress.

Virus isolation

These results are shown in Table 26. Virus was regularly recovered from day 3 to day 13 and day 3 to 10 p.i. from nasal and ocular discharges respectively.

Serological examination

Neutralising antibodies were demonstrated in the 2 surviving stirks (B2 and B3) on day 12 p.i.; their maximum titres were 1:32, 3 weeks p.i.. In the incontact animals (B5 and B) neutralising antibodies were first demonstrated on day 14 p.i. and had attained a maximum titre of 1:24 in 3 weeks post contact.

Pathological examination

The 2 stirks, B1 and B4 were killed on day 6 p.i.. There was congestion of the nasal mucosa and the nasal sinuses were filled with mucopurulent exudate (Figure 54). Mild rhinitis and laryngitis characterised by excessive mucous secretions were observed in both stirks. Small diphtheritic lesions were also evident in the nasopharynx of both animals. There was a moderately severe tracheitis involving the whole length of the trachea. Many haemorrhages, approximately 2-3mm in diameter, were observed in the pharynx, in the trachea and also in the major bronchi (Figure 55). A slight degree of pneumonia was observed in the right middle lobe of A1. The local lymph nodes (retropharyngeal, bronchial and mediastinal) were enlarged, oedematous and congested. The liver, spleen, kidneys and brain were macroscopically normal.

Microscopically, the nasal sinuses were markedly congested, oedematous and there was a mild rhinitis. The epithelium was infiltrated by globule leucocytes and the submucosal glands were

surrounded by aggregations of lymphocytes. The submucosal glands appeared active with many plasma cells in the surrounding tissues. The number of goblet cells appeared to be significantly reduced. There was a moderately severe pharyngitis characterised by oedema, congestion and lymphocytic cell infiltration and a severe laryngitis with loss of epithelium and extensive lymphocytic and plasma cell infiltration.

There was a mild tracheitis with neutrophil and globule leucocyte infiltration. The bare patches of tracheal epithelium (Figure 56) which were seen when examined with the scanning electron microscope, were almost certainly due to virus infection of the ciliated cells resulting in destruction of cilia. Mucus produced in excess was seen on the surface of trachea coming from the submucosal gland duct (Figure 57). The submucosal glands appeared active and dilated (Figure 58). A few lymphocytes and plasma cells were present in the lamina propria. There was mild bronchitis with globule leucocyte and neutrophil infiltration. There was peribronchiolar lymphoid accumulations. The alveoli walls were thickened and contained neutrophils.

There was a slight congestion of the splenic matrix and capsule while the red-pulp was extensively infiltrated with neutrophils. In the white pulp there was germinal centre formation and these were surrounded by aggregates of plasma cells. In the retropharyngeal and bronchial lymph nodes a moderate congestion with massive neutrophilic infiltration was observed. There was extensive germinal centre formation with many plasma cells recognised in the medullary sinuses. In the kidneys, there was a mild reaction characterised by neutrophilic infiltration in the glomeruli and a few small lymphocytic follicles were observed in the proximal convuluted tubules.

The liver appeared normal although small number of lymphocytes and plasma cells were recognised around some blood vessels. The cerebellum and cerebral cortex appeared to be normal.

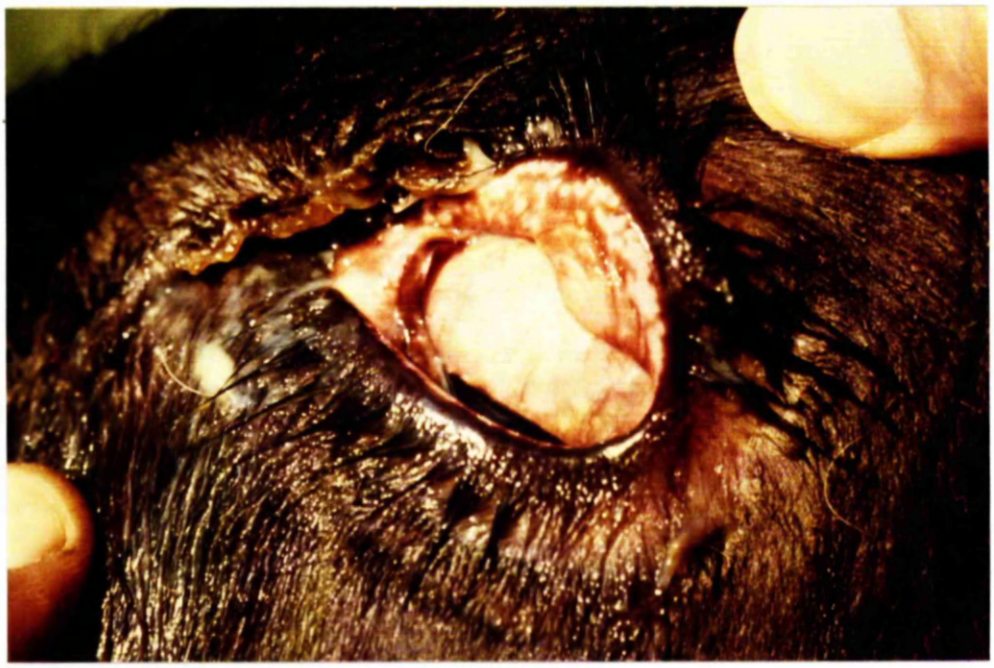


FIGURE 53. Experimental in-contact Case No. B6 with marked widespread granular lesions, to be seen in the conjunctiva and Sclera. There is also mucopurulent ocular discharge.

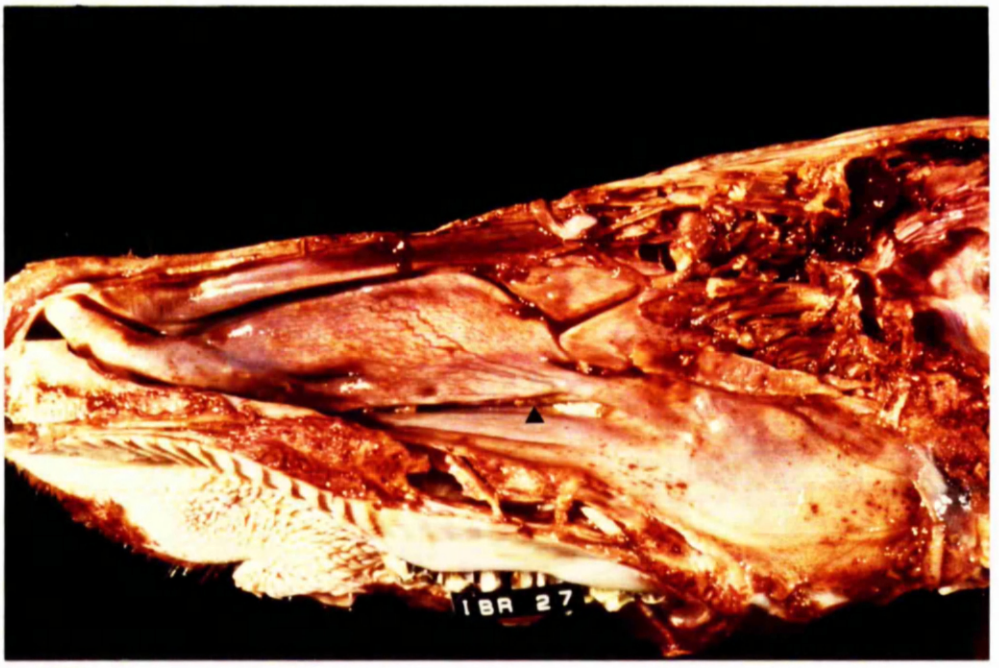


FIGURE 54. Sagittal section of experimental case B4 infected with IBR-Strichen strain and killed on day 6 post infection. There is necrosis of the ventral concha with debri (▲) lying in the nasal passages. Many petechial haemorrhages can be recognised throughout the nasopharynx.

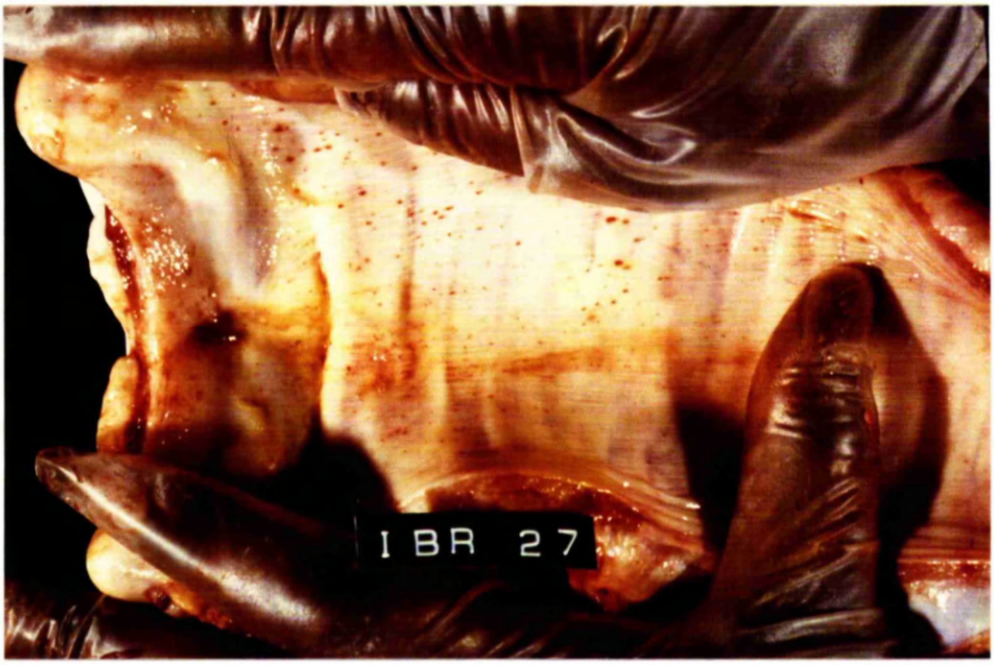


FIGURE 55. Larynx and trachea from experimental Case No. B4 killed on Day 6. There is oedema and many small petechial haemorrhages.

FIGURE 56. Scanning electron micrograph of the trachea of Case No. B4. The cilia in the centre have been destroyed by the virus exposing the ducts of the submucosal glands. X 1,250.

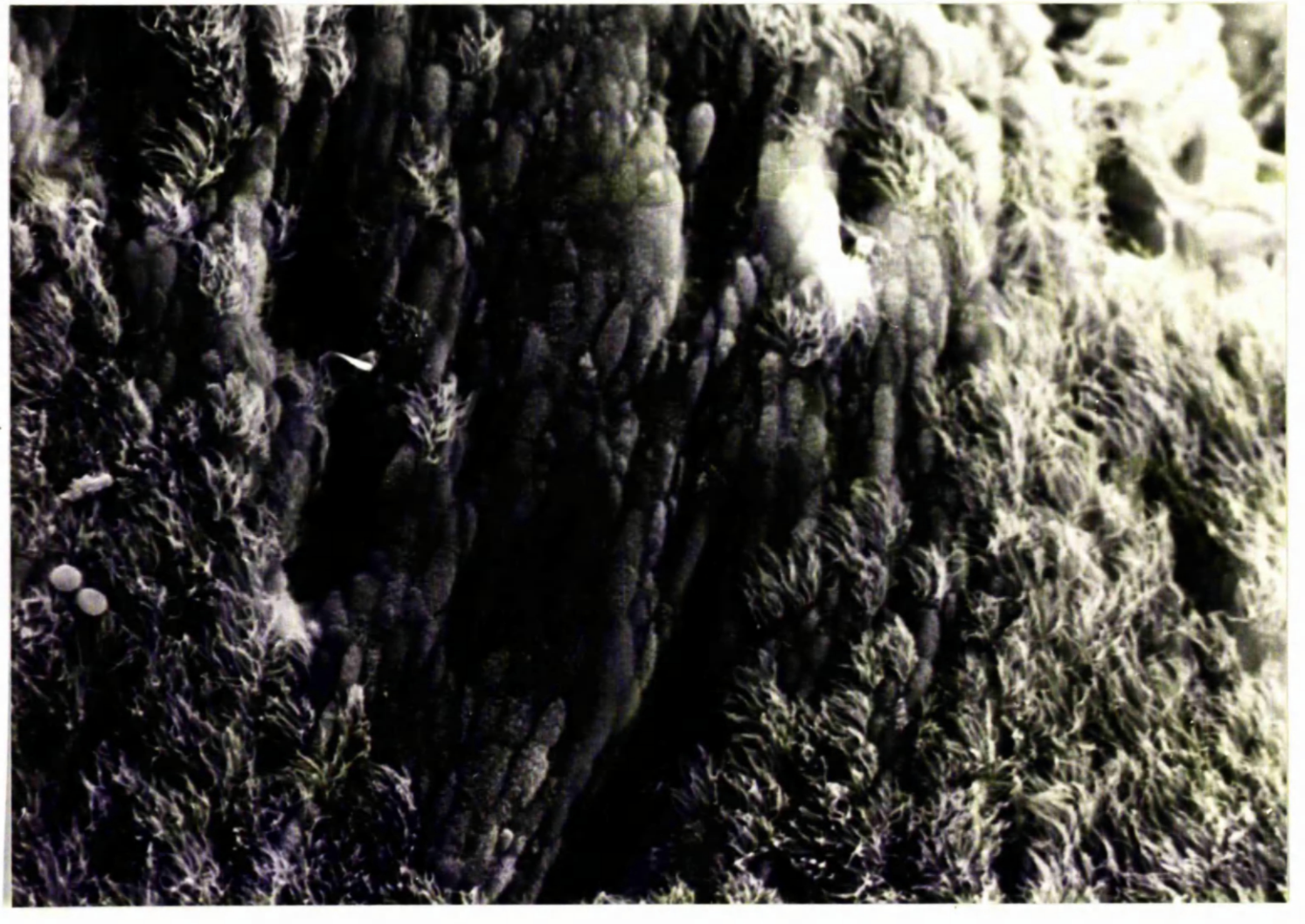


FIGURE 57. Scanning electron micrograph of the trachea of experimental Case No. B4. Mucus can be seen coming out of the submucosal gland duct. X 5,000.

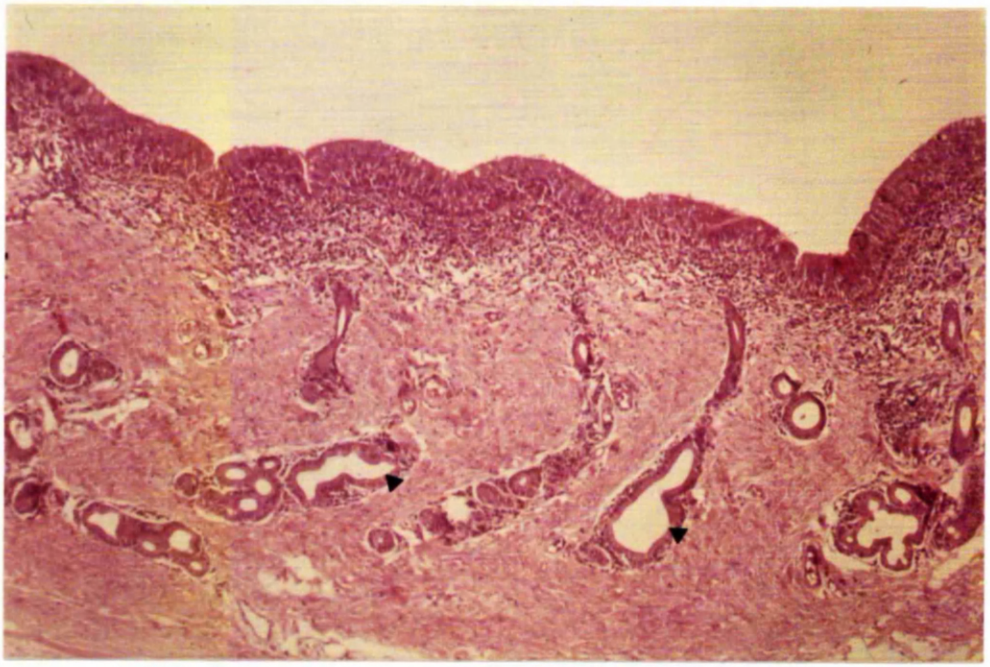


FIGURE 58. Tracheitis with neutrophilic infiltration of lamina propria and epithelium. Note the dilatation of most of the submucosal glands (▲). HE. X 110.

Experiment 3. Group C calves.

Clinical features. The detailed clinical findings are summarised in Appendix 4, Table 3.

At the end of day 2 p.i. the calves became dull and reluctant to feed. They all developed bilateral seromucoid nasal and serous ocular discharges.

On day 3 p.i. the above signs became more overt with body temperatures reaching 104°F. Congestion of nasal mucosae accompanied with copious serous to mucopurulent nasal discharge became more prominent. Close examination revealed scattered diphtheritic plaques on the nasal mucosa. Occasional single non-productive coughs were heard from all 4 calves and, on auscultation, harsh respirations could be heard especially over the cranio-ventral aspect of the lungfields. Slight serous to purulent ocular discharge as a result of conjunctivitis was a major clinical feature from as early as day 2 p.i. and by day 4 p.i. this had become worse when conjunctivitis, especially in calf C3, became granular in nature (Figure 59). The copious purulent ocular discharge poured out and matted the hair on the cheeks. The temperatures reached a peak of 106°F on day 4 p.i. and then returned to normal on day 7 p.i.. Calves C2 and C3 were killed on days 4 and 6 p.i. respectively. By day 8 p.i., calf C4 still had a profuse seromucoid nasal discharge and serous ocular discharge which had matted the hair on the animals face (Figure 60). Drooling of saliva was a feature on days 3 to 6 p.i..

The mean total white blood cell and neutrophil numbers on specific days following challenge are presented in Table 25. During the first 4 days post exposure (Figure 52) the mean total numbers of white cells and of neutrophils increased. From day 4 until day 20, the mean numbers decreased progressively.

Calves C1 and C4 were left for a total of 33 days after challenge. During this time they had persistent slight seromucoid nasal and serous ocular discharges, although their temperatures were within the normal range. They were miserable, they coughed persistently and were unwilling to eat. Pneumonia was diagnosed in both animals and they were killed on day 33 p.i..

Virus isolation

These results are shown in Table 26. Virus was regularly recovered from 3 to 13 and 3 to 10 days p.i. from nasal and ocular

discharges respectively.

Serological examination

Neutralising antibodies in the surviving 2 calves (C1, C4) were demonstrated on day 12 p.i. and reached a maximum titre of 1:16 in 3 weeks.

Pathological examination

The nasal conchae were congested and granular in appearance with a number of diphtheritic lesions. The sinuses were filled with whitish-yellow, thick mucous exudate (Figure 61) and this was common throughout the upper respiratory tract. There was rhinitis and a marked pharyngitis as well as numerous petechial haemorrhages. There was severe tracheitis with an accumulation of thick mucus in the area of the carina (Figure 62). There was marked pneumonia in the anterior left and right cardiac as well as the accessory lobes. The pneumonic lesions were smooth, purple in colour and contained many tiny haemorrhages. When observed the retropharyngeal lymph nodes were enlarged, oedematous and haemorrhagic.

Microscopically, the lesions were similar to those present in the 2, Group B stirks. There was a mild inflammatory reaction and slight hyperplasia of the epithelium of the nasal conchae (Figure 63) as well as laryngitis which was characterised by a moderately severe accumulation of lymphocytes forming discrete aggregates; and the glands slightly dilated (Figure 64). There was a moderately severe tracheitis with much inflammatory exudate in the lumen and the submucosal glands were dilated and surrounded by lymphocytes (Figure 65). The pneumonia was exudative and included a marked bronchitis. In general, the lesions were much more severe in this group than those of Group B.

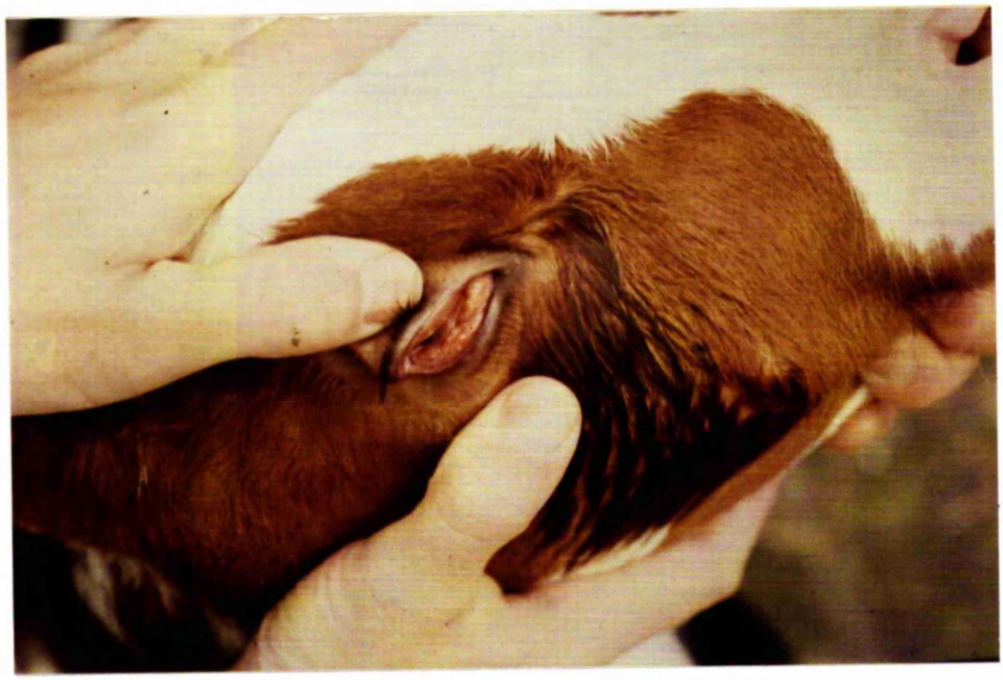


FIGURE 59. Case No. C3 at day 4 post infection with a profuse serous ocular discharge that lasted for many days. There is also conjunctivitis and conjunctival oedema, small granular lesions are also evident.

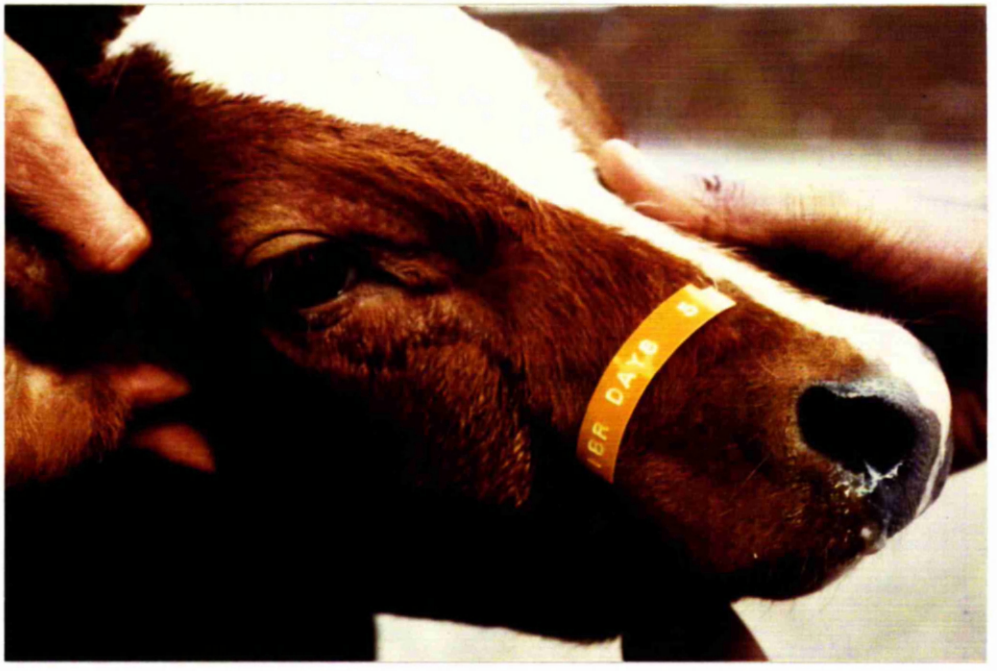


FIGURE 60. Experimental Case No. C4, 8 days post infection with IBR-Strichen strain. There is a profuse seromucoid nasal discharge and a serous ocular discharge which has matted the hair on the animal's face.

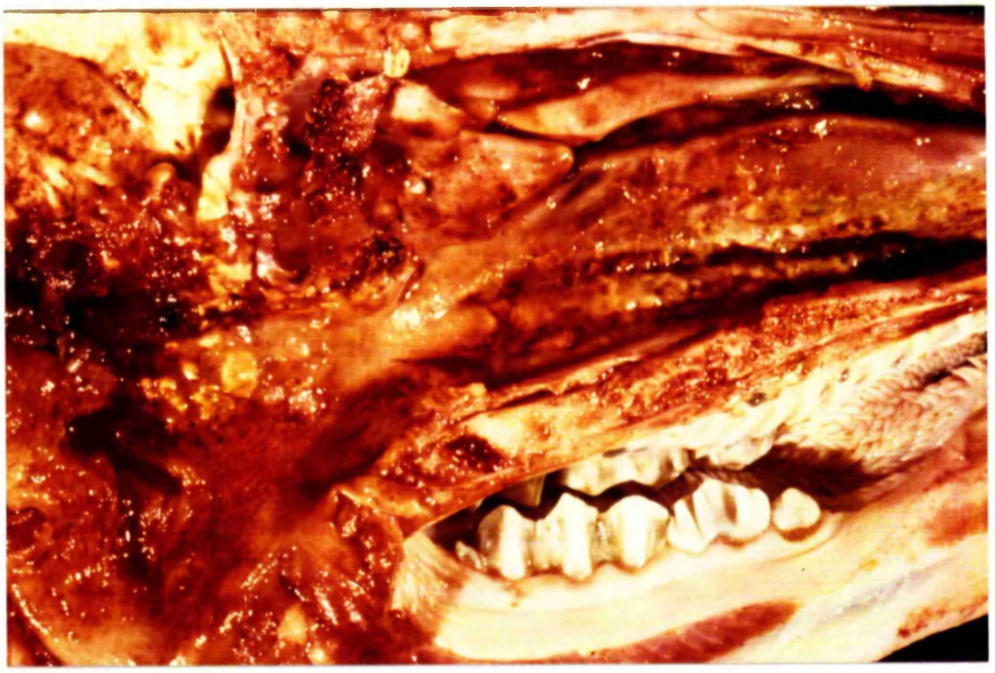


FIGURE 61. Sagital section of the head of Case No. C3 at Day 4 post infection with much necrotic debri lying in the nasal passages. There is mucosal oedema and severe congestion with a few petechial haemorrhages in the nasopharynx.

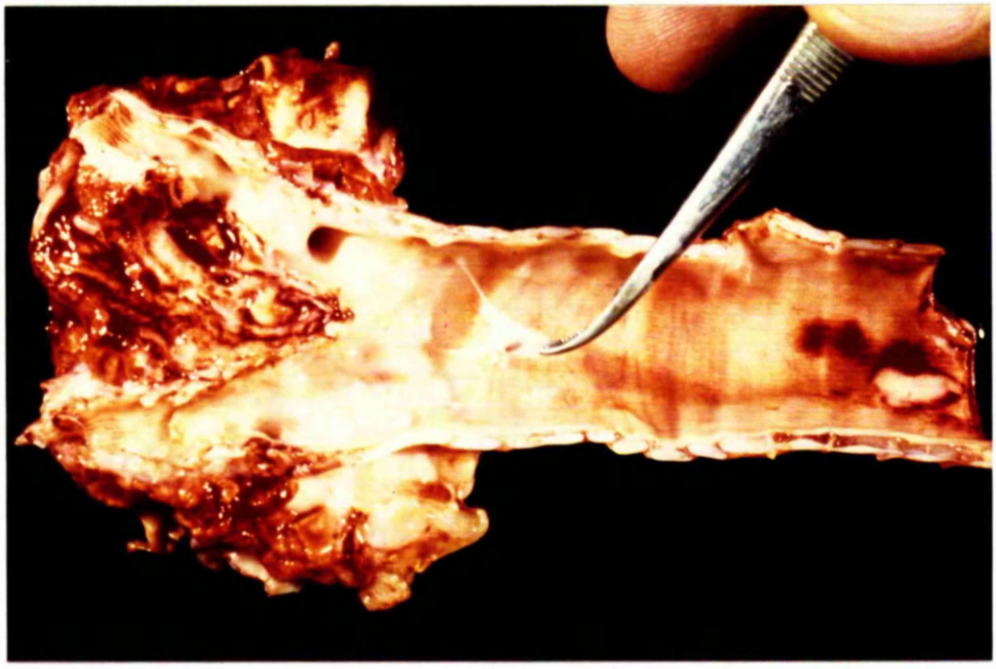


FIGURE 62. Case No. C3 at Day 4 post infection, with an accumulation of thick mucus in the area of carina.

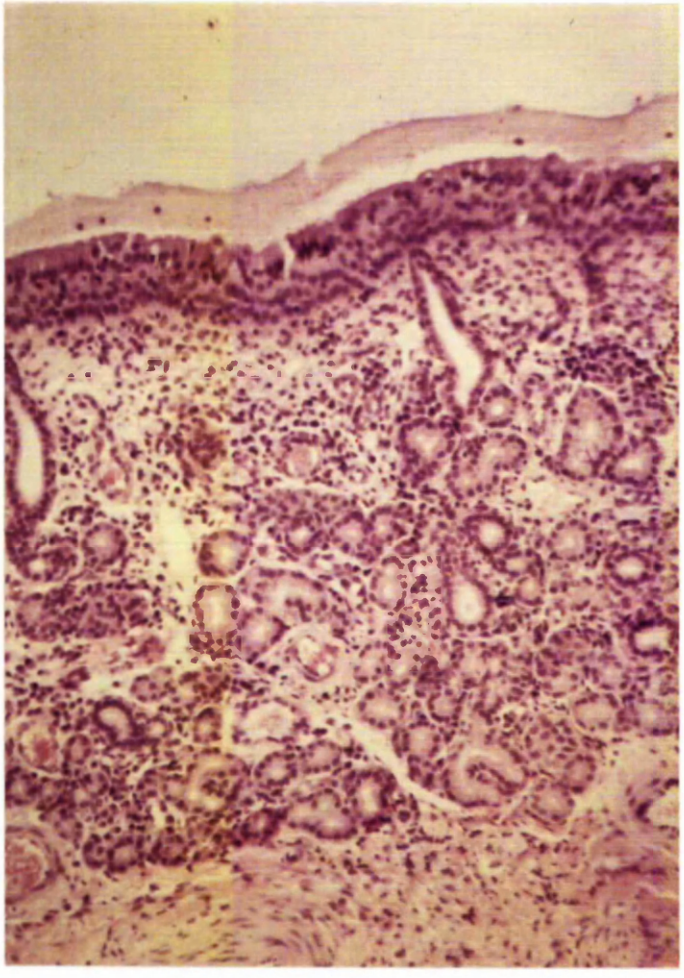


FIGURE 63. Case No. C3 at Day 4 post infection, with a mild inflammatory reaction and slight hyperplasia of the epithelium of the nasal conchae. HE X 250

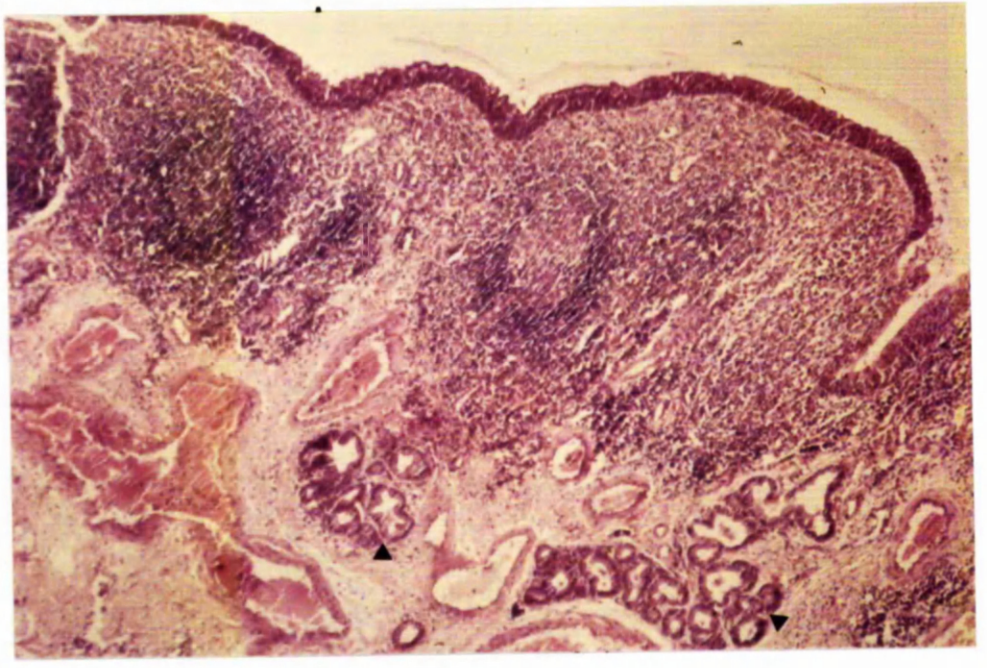


FIGURE 64. Case No. C3 at Day 4 post infection, with laryngitis characterised by moderately severe accumulation of lymphocytes forming discrete aggregates; the glands are slightly dilated (▲). HE. X 110.

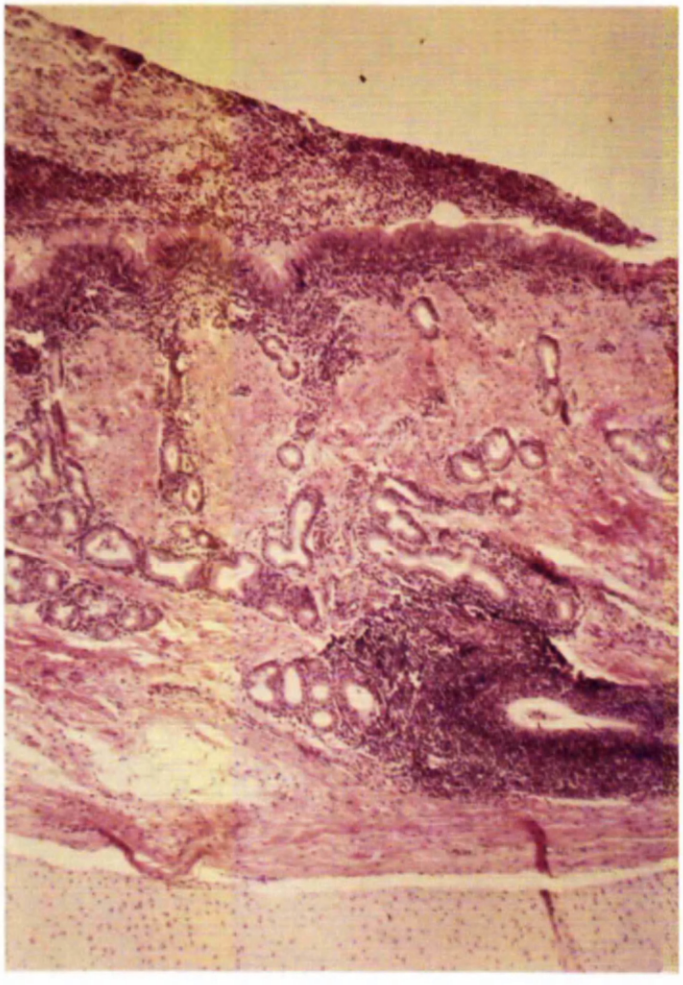


FIGURE 65. The trachea in Case No. C3. There is a moderately severe tracheitis with much inflammatory exudate in the lumen. The submucosal glands are dilated and some are surrounded by lymphocytes. HE X 110

Experiment 4. Group D calves.

Clinical features. The detailed clinical findings are summarised in Appendix 4, Table 4.

The calves in this group responded more quickly than those in any of the other groups; by day 2 p.i., all had become very dull with conjunctivitis and a purulent ocular discharge. The serous nasal discharge became copious and mucopurulent in nature. The diphtheritic lesions, which had been evident only on calf D1 on day 2 p.i., became obvious in the other 3 calves from day 3 to 6 p.i.. Respirations were harsh, coughing was frequent and all calves were pyrexic (104-106°F) from day 3 to 7 p.i.. The calves were tachypnoeic (RR = 40-60/min). Drooling of saliva was observed from day 3 to 6 p.i..

Calf D1, which was found dead on day 3 p.i., had been diarrhoeic but unfortunately a post-mortem examination was not carried out. Calf D3 was killed on day 5 p.i. for routine studies. Haematological examination was not undertaken in this group.

Virus isolation

These results are shown in Table 25. Virus was regularly recovered from days 3 to 12 and 3 to 10 p.i. from nasal and ocular discharges respectively.

Serological examination

Neutralising antibodies were demonstrated in the remaining 2 calves (D2 and D4) on day 12 p.i. and a maximum titre of 1:24 was reached by 3 weeks p.i..

Pathological examination

The nasal conchae were congested, oedematous and covered with whitish-yellow mucopus and many small diphtheritic lesions. There was a mild tracheitis and the lungs were congested, oedematous and strands of pus were seen in the airways. The retropharyngeal and bronchial lymph nodes were slightly enlarged and oedematous. The liver, kidney, spleen and brain appeared normal.

Microscopically, the nasal conchae were severely congested, oedematous with marked inflammation in the lamina propria and epithelium. The epithelium was hyperplastic, necrotic and it was covered by large

amount of inflammatory exudate. Sub-epithelially, there were many lymphocytic aggregates. The sub-mucosal glands appeared active with many lymphocytes, plasma cells and neutrophils in close association.

There was laryngitis with a slight mononuclear cellular infiltration sub-epithelially with a few areas of epithelial necrosis and neutrophilic cellular infiltration. A moderate tracheitis with epithelial hyperplasia and a few areas of metaplasia were observed. Lymphocytes had accumulated below the epithelium. The trachea was oedematous and contained numerous haemorrhages as well as slight neutrophilic infiltration into the epithelium. The submucosal glands appeared active. There was marked congestion and oedema of the interlobular septa as well as collapse of the alveoli. Neutrophils and macrophages were occasionally seen in these collapsed areas.

SECTION 2

(i) THE EFFECT ON THE BOVINE RESPIRATORY TRACT OF INFECTION WITH THE COLORADO STRAIN OF INFECTIOUS BOVINE RHINOTRACHEITIS

MATERIALS AND METHODS

Experimental animals

Four, 6 month old Ayrshire cross stirks, Group E (E1, E2, E3, E4), were used in this experiment.

Housing

This form of housing was identical to that described in detail in Section 1 of this Chapter.

Feeding

The feeding regime was identical to that already described in detail in Section 1 of this Chapter.

Challenge procedure

The virus used was the Colorado strain of IBR obtained from the Central Veterinary Laboratory - Ministry of Agriculture, Fisheries and Food (CVL-MAFF), Weybridge, England. Each animal was challenged intranasally with a 28th passage tissue culture suspension (titre = $10^{7.7}TCID_{50}$/ml) in a similar manner to that already described in detail in Section 1 of this Chapter.

The details of the clinical, pathological and serological examinations as well as the technique used for virus isolation were identical to those already described in Section 1 of this Chapter. Haematological examination was not undertaken in this experiment.

RESULTS

Experiment 5. Group E stirks.

Clinical response. The detailed clinical findings are summarised in Appendix 4, Table 5.

On day 2 p.i., all 4 calves were seen to be dull, unwilling to eat and they had a moderate pyrexia (103.0 - 105.2°F). There was serous nasal discharge with slight congestion of nasal mucosa. Single spasmodic dry coughs were heard and became more frequent and widespread when the animals were forced to move about in the box. The calves were tachypnoeic (RR = 40-60/minute) and, on auscultation, the respirations were harsh. By day 3 p.i. only E2 had a serous ocular discharge. On day 4 p.i., the above signs became much more severe with temperatures of up to 106.2°F being recorded; the congested nasal mucosal epithelium was now obviously eroded and covered with yellowish-brown diphtheritic plaques. The serous nasal discharge had increased in viscosity and amount becoming mucopurulent and copious, hanging 3-4 inches down from the nostrils (Figure 66). Coughing became much more frequent even when the animals were at rest. Conjunctivitis accompanied with serous ocular discharge was evident in all the stirks. There was profuse drooling of saliva without any accompanying evidence of stomatitis. The high temperatures lasted for 2 days only and the stirks had more or less recovered by day 8 p.i..

The 2 stirks E1 and E2 were slaughtered on day 4 and 6 p.i. respectively.

Virus isolation

These results are shown in Table 26. Virus was regularly recovered from day 3 to 12 and from day 3 to 10 p.i. from nasal and ocular discharges respectively.

Serological examination

Neutralising antibodies were detected in the remaining 2 stirks on day 12 p.i..

Pathological examination

The nasal conchae were severely congested in both cases and were covered by a thin fluid. There was a moderately severe tracheitis

(Figure 67). This inflammation was accompanied by haemorrhages as well as granular necrotic material in the laryngeal region (Figure 68). The retropharyngeal lymph nodes were severely congested, oedematous and haemorrhagic. There was no obvious lesion of pneumonia.

Microscopically, there was severe laryngitis and pharyngitis with massive lymphocytic accumulation subepithelially, forming discreet aggregates and germinal centres. Plasma cells as well as neutrophils, globule leucocytes and eosinophils were evident in the pharynx. In both the pharynx and larynx there was a marked epithelial hyperplasia with some necrosis. There was a moderately severe tracheitis with many small lymphoid aggregates directly below the epithelium. Globule leucocytes were not recognised, but neutrophils had infiltrated the epithelium. Plasma cells were abundant in the lamina propria and also around the glands which were hypertrophied.

The lungs were non-pneumonic apart from a few collapsed lobules. There were a few peribronchiolar lymphoid accumulations and, in addition, some eosinophils and globule leucocytes were seen in the bronchiolar walls. Germinal centre formation was prominent in the retropharyngeal, bronchial and mediastinal lymph nodes which were also congested and haemorrhagic. The mediastinal lymph nodes were infiltrated with large numbers of eosinophils. The spleen was severely congested, haemorrhagic with infiltration of large numbers of neutrophils. The kidney, liver, brain, gall bladder were normal.

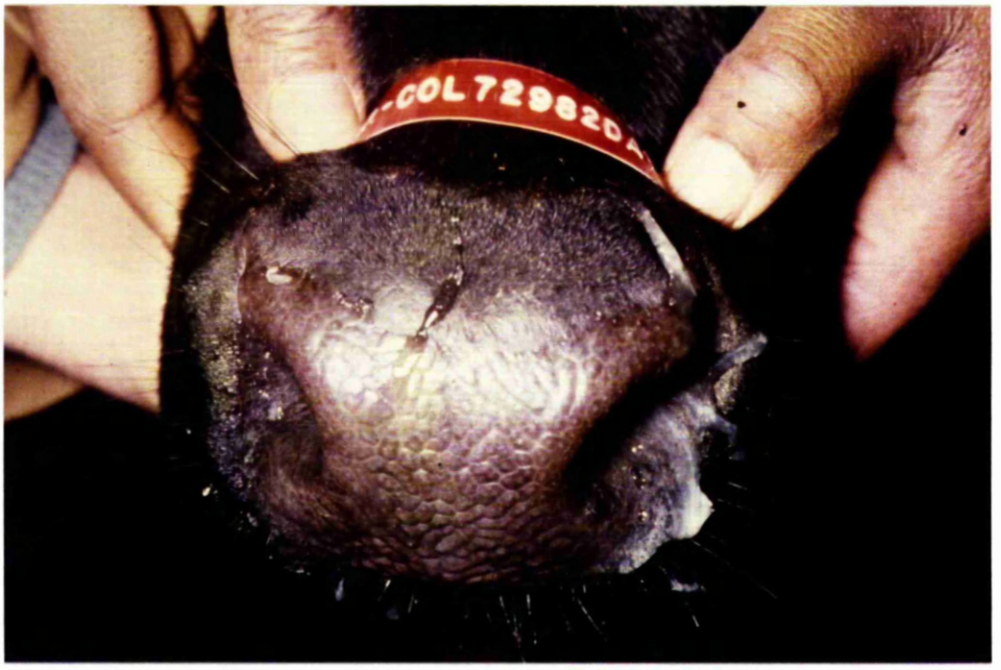


FIGURE 66. Case No. E1, 4 days post infection, with the Colorado strain of IBR; there is a mucopurulent nasal discharge which is mostly unilateral.

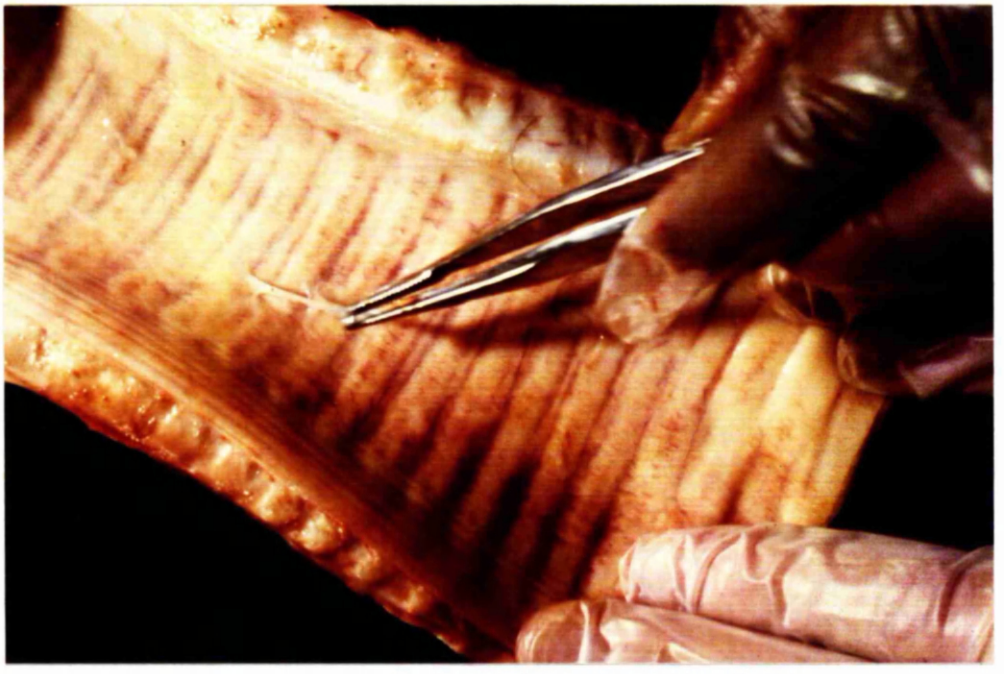


FIGURE 67. Case No. E1 at 4 days post infection with mucus exudate in the trachea.

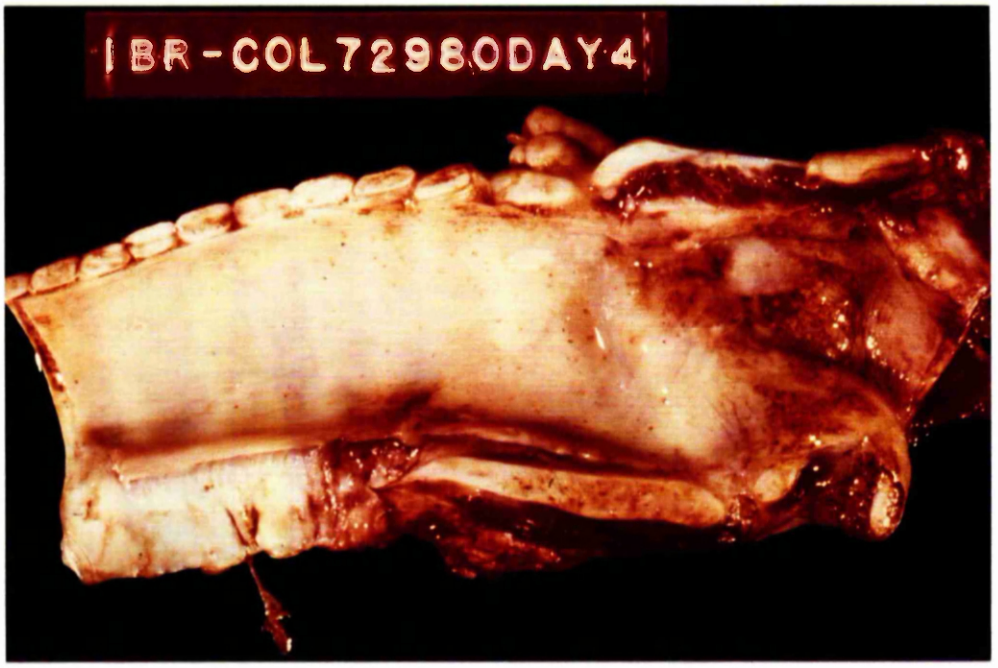


FIGURE 68. Case E1, 4 days post infection with the Colorado strain of IBR showing oedema and many petechial haemorrhages in the larynx and trachea.

(ii) THE EFFECT ON THE BOVINE RESPIRATORY TRACT OF INFECTION WITH THE OXFORD STRAIN OF INFECTIOUS BOVINE RHINOTRACHEITIS

MATERIALS AND METHODS

Experimental animals

Four, 6 month old Ayrshire cross stirks Group F (F1, F2, F3 and F4), were used.

Housing

This form of housing was identical to that described in detail in Section 1 of this Chapter.

Feeding

The feeding regime was identical to that described in detail in Section 1 of this Chapter.

Challenge procedure

The virus used was the Oxford strain of IBR obtained from CVL-MAFF. Each stirk was challenged intranasally with the 6th passage tissue culture suspension (titre = $10^{7.7}TCID_{50}$/ml) by the method described in detail in Section 1 of this Chapter.

The details of the clinical, pathological and serological examinations as well as the technique used for virus isolation were identical to those described in Section 1 of this Chapter. Haematological examination was not undertaken in this experiment.

RESULTS

Experiment 6. Group F stirks.

Clinical features. The detailed clinical findings are summarised in Appendix 4, Table 6.

On day 2 p.i., the calves became very slightly dull without any apparent loss of appetite. There was also a very slight increase in body temperature in 2 stirks (F1 and F2) reaching a peak of 103.0°F. Stirk F2 coughed sporadically and on auscultation, the respirations were slightly harsh. On day 3 p.i., all calves had a slight serous nasal discharge and they were slightly tachypnoeic (RR = 36-42/minute). The highest temperature recorded was 103.5°F and this only in stirk F1. There was slight congestion of the nasal mucosa in every animal without there being obvious lesions. On day 4 p.i., the picture was more or less the same although stirk F2, which had had the highest temperature, was seen to have a single diphtheritic lesion on the left nostril. The nasal mucosae of the other calves were severely congested. In spite of being dull, they did not go off their feed. By day 6 p.i., their temperatures were back to normal and they had virtually recovered from the infection. Stirks F3 and F1 were slaughtered on days 4 and 6 p.i. respectively.

Virus isolation

These results are shown in Table 25. Virus was regularly recovered from day 3 to 11 and from day 4 to 10 p.i. from nasal and ocular discharges respectively.

Serological examination

The remaining 2 calves developed detectable serum neutralising antibodies on day 12 p.i. and had a titre of 1:12.

Pathological examination

There was moderately severe congestion of the nasal conchae (Figure 69) without evidence of other lesions. There was a mild pharyngitis, mild laryngitis, mild tracheitis with numerous fine haemorrhages, and were confined to the anterior half of the trachea only. Sticky whitish mucous of glistening nature covered the whole length of the trachea (Figure 70). A few small areas of pneumonia were evident

in the anterior and posterior right cardiac lobes as well as the anterior left diaphragmatic lobe. These lesions were pale, grey-fawn and collapsed. The retropharyngeal lymphnodes were enlarged, oedematous and contained many haemorrhages. The liver, kidney, spleen and brain were macroscopically normal.

Microscopically, there was a mild rhinitis with epithelial hyperplasia and infiltration of the lamina propria with neutrophils, plasma cells and lymphocytes. There were many goblet cells in the epithelium. Mild pharyngitis and laryngitis were characterised by lymphocytic aggregates forming germinal centres. There was a very mild tracheitis with only occasional plasma cells and lymphocytes in the lamina propria while neutrophils had infiltrated the epithelium. There were also a few small haemorrhages and an increase in the number of goblet cells. The retropharyngeal lymphnodes were congested, haemorrhagic and had a few germinal centre formation. A marked congestion and neutrophil infiltration was evident in the spleen. The liver, kidney and brain appeared to be normal.

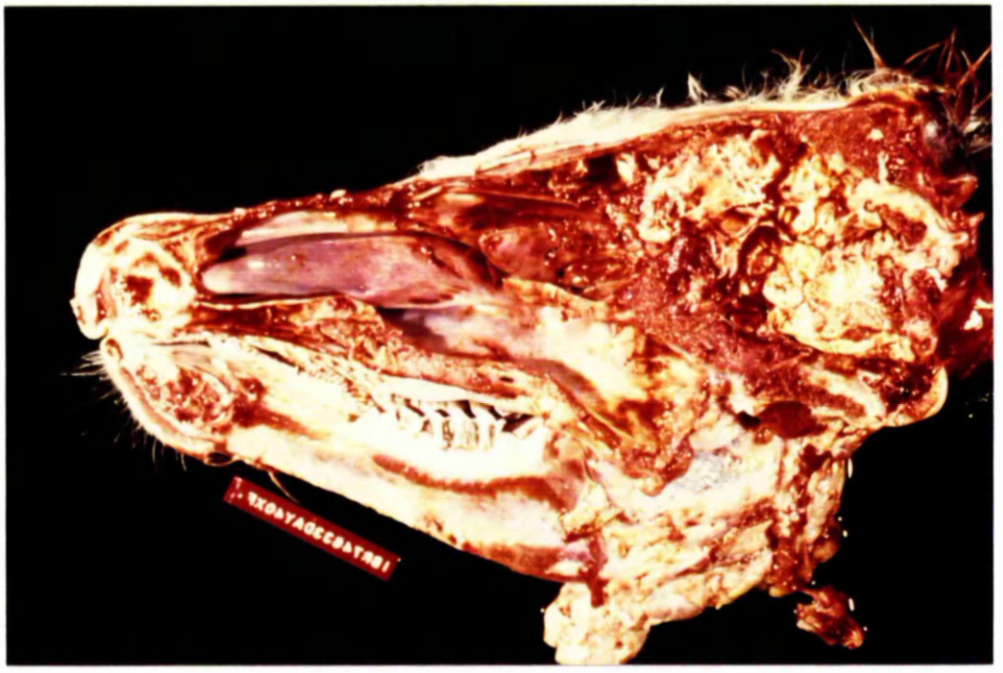


FIGURE 69. Sagital section of the head of Case No. F3,4 days post infection with Oxford strain of IBR; there are no visible macroscopic lesions.

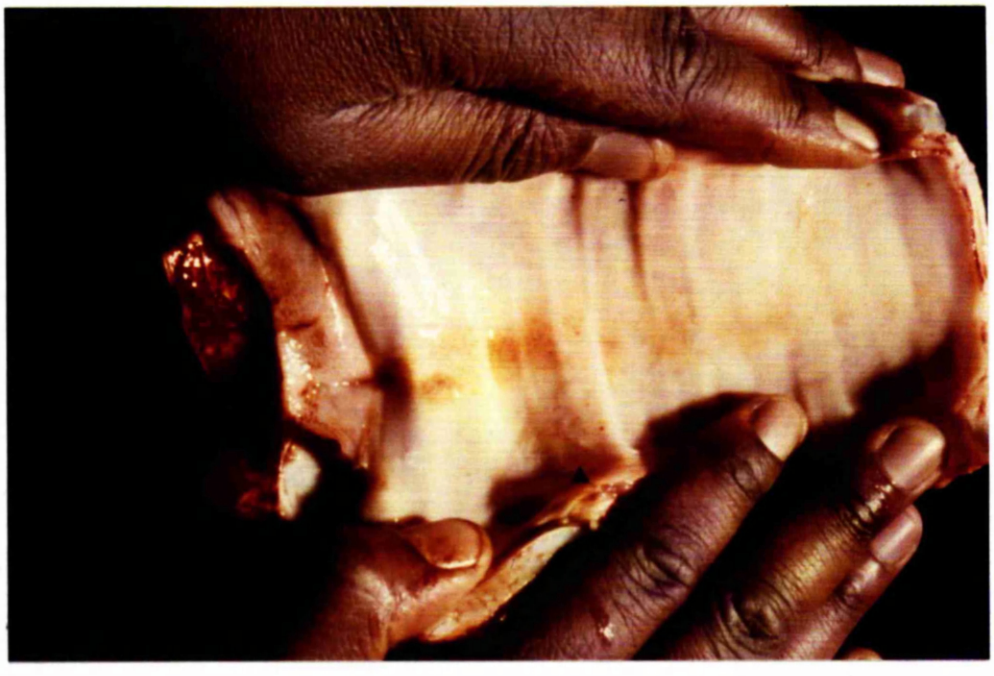


FIGURE 70. Larynx and trachea of Case No. F3 with a small number of pin-point haemorrhages (▲) in the anterior portion of the trachea.

SECTION 3

THE EFFECT ON THE BOVINE FEMALE REPRODUCTIVE TRACT OF INFECTION WITH THE STRICHEN STRAIN OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

MATERIALS AND METHODS

Experimental animals

Four, yearling Friesian cross heifers, Group G (G1, G2, G3, G4) were used.

Housing

This form of housing was identical to that described in detail in Section 1 of this Chapter.

Feeding

The feeding regime was identical to that described in detail in Section 1 of this Chapter.

Challenge procedure

The challenge virus was part of that batch of the virus whose preparation has been described in detail in Section 1 of this Chapter. Each animal was challenged by introducing 1.5ml of tissue culture virus suspension into the vagina, using a 6 inch long sterile plastic canulla fitted to the tip of a dropper syringe.

The details of the clinical, pathological and serological examinations as well as the technique used for virus isolation were identical to those described in Section 1 of this Chapter. However, haematological examinations were not undertaken and pathological specimens were only taken from the vagina and the uterus.

RESULTS

Experiment 7. Group G heifers.

Clinical features. The detailed clinical features are summarised in Appendix 4, Table 7.

On day 1 p.i., the vaginal mucosa was severely congested with numerous reddish vesicles. On day 2 p.i., these vesicles had progressed into pustules measuring 1-2mm in diameter (Figure 71) and slight mucoid discharge was observed on the floor of the vagina in 3 of the heifers. On day 3 p.i., the pustules had become more prominent and a few had sloughed leaving raw ulcerative lesions. A mucopurulent vaginal discharge (Figure 72) only developed in 3 heifers (G1, G2, G3). There was constant tail swishing in every animal and frequent micturation was also observed. Two heifers (G1, G4) were slaughtered on day 4 p.i. for postmortem examination. By day 5 p.i., the pustules in the other 2 heifers had sloughed. There was no systemic involvement and signs of the upper respiratory tract were not observed. By day 17 p.i., only a few lesions were still visible.

Virus isolation

These results are shown in Table 26. The virus was isolated regularly from vaginal swabs from day 4 to 9 p.i., and thereafter intermittently up to 21 days. The virus was recovered from the vaginal mucosae and uteri of G1 and G2 killed on day 4 p.i..

Serological examination

Antibodies were demonstrated on days 10 and 12 post challenge and both had a titre of 1:12.

Pathological examination

In both cases the pathological lesions were identical. There was congestion and numerous tiny pustules at the neck of the vagina. There was marked congestion with many haemorrhages at the neck of the cervix where there were also haemorrhagic erosions 1-2mm diameter. The cervix and the uterus also contained numerous pustules (0.5-1.0cm in diameter) although there was an absence of congestion or haemorrhage. Pulmonary lesions were not present.

Microscopically, there was a moderately severe vaginitis with mild neutrophilic and eosinophilic infiltration. Much of the reaction was a result of mononuclear cell infiltration, particularly of lymphocytes which had formed discrete aggregates subepithelially while numerous plasma cells were abundant intraepithelially. There were a few microvesicles containing inflammatory cells. The pustules and erosions of the vagina and neck of cervix appeared to be due to aggregates of lymphoid tissue which had infiltrated the epithelium and caused congestion. The cervix was generally normal and the pustules present appeared to have 2 forms: one was due to epithelial hyperplasia and dysplasia while others appeared as large fibrous accumulations infiltrated by a small number of lymphocytes.

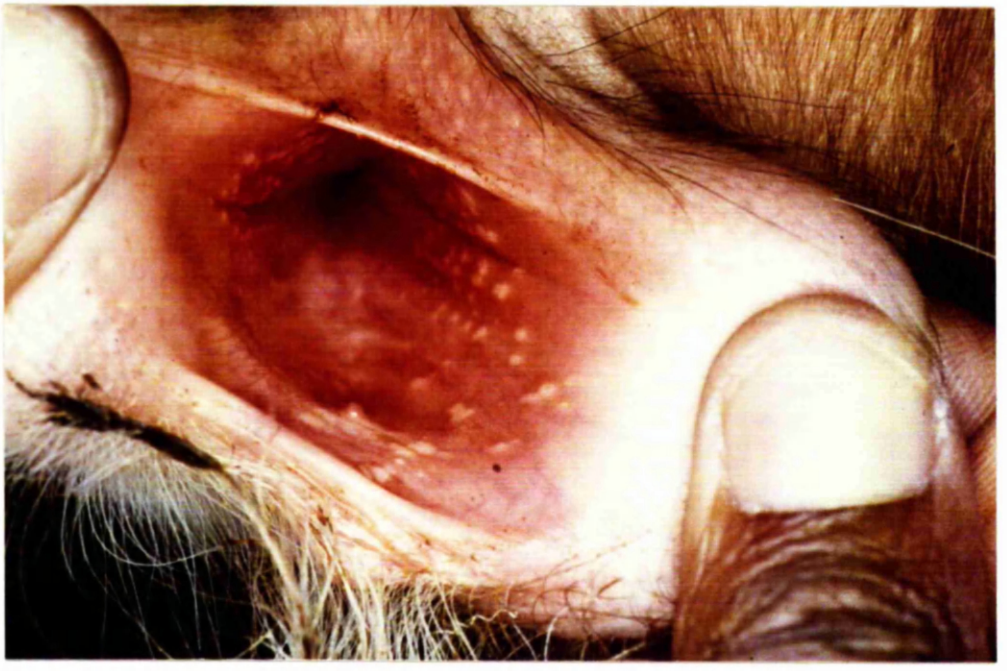


FIGURE 71. The posterior vagina of a heifer 2 days post infection by the intravaginal route with the Strichen strain of infectious bovine rhinotracheitis virus. There is congestion and numerous pustules on the vaginal mucosa.

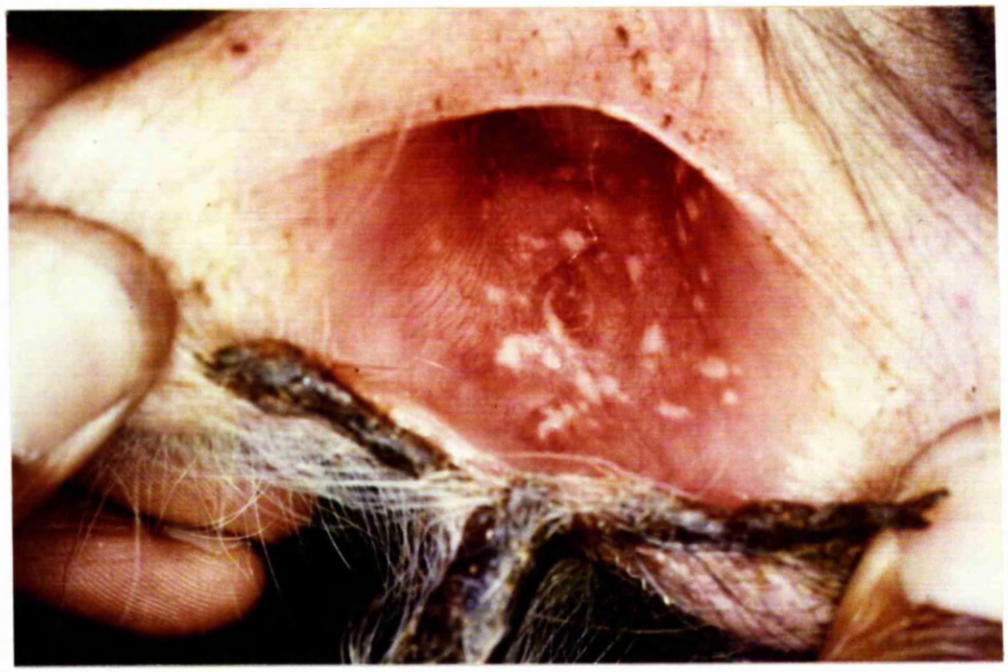


FIGURE 72. The posterior vagina of a heifer 3 days post infection by the intravaginal route with the Strichen strain of infectious bovine rhinotracheitis virus. There is congestion, numerous pustules (1-2mm in diameter) and a mucopurulent discharge on the floor of the vagina.

SECTION 4

THE EFFECT OF INFECTION WITH DICTYOCAULUS VIVIPARUS ON STIRKS WHICH HAD RECOVERED FROM AN EXPERIMENTAL INFECTION WITH THE STRICHEN STRAIN OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

MATERIALS AND METHODS

Experimental animals

Three groups (H1-H4; P1-P4; J1-J4) each consisting of 4 yearling stirks were used. These groups had recovered from an experimental infection with the Strichen strain of IBR 5 to 6 months prior to this experiment.

Housing

This form of housing was identical to that described in detail in Section 1 of this Chapter.

Feeding

The feeding regime was identical to that described in detail in Section 1 of this Chapter.

Challenge procedure

The Group H animals (H1, H2, H3, H4) were each challenged with 50 third stage lungworm larvae per Kg. bodyweight (Table 27); on average each animal was given 15,000 lungworm larvae.

The Group P animals (P1, P2, P3, P4) were each challenged with 1,000 third stage larvae; this represented an approximate dose of 3-4 lungworm larvae/Kg. bodyweight.

The Group J animals (J1, J2, J3, J4) were each vaccinated twice according to the manufacturers instructions, with an irradiated lungworm vaccine (Dictol; Glaxovet Ltd., Middlesex, UK). Each dose contained 1,000 irradiated lungworm larvae.

The details of the clinical (Groups H, P, J), pathological (Group H) and serological (Group H) examination as well as the technique used for virus isolation were identical to those already described in detail in Section 1 of this Chapter. Haematological examinations were not undertaken in this experiment.

RESULTS

Experiment 8. Group H, P and J animals.

Clinical features. The detailed clinical findings of Group H animals are summarised in Appendix 4, Table 8.

The first clinical evidence of disease in Group H animals was noticed on day 11 p.i. when single sporadic coughing was observed from all animals. On day 13 p.i., the 4 animals were all dull although they continued to eat. In addition to coughing, there was slight serous to seromucoid nasal discharge. On auscultation, the respirations were harsh. On day 14 p.i., the animals were tachypnoeic (RR = 60-100/min) and the seromucoid nasal discharges became more profuse. On auscultation, rhonchi was heard over the cranioventral aspect of the right side of the chest of H4. On day 15 p.i., the amount of food eaten by the group decreased and H2 was dyspnoeic. There was moderate pyrexia (103.2 to 105.6°F) which persisted for 6 days. Diphtheritic lesions were seen in nasal mucosa of H4. Two animals (H1 and H4) were slaughtered on days 15 and 17 p.i. respectively. In the 2 others, coughing continued for up to 4 weeks p.i.. In Group P, the only animal in which clinical signs were seen was P2 which had a very slight serous nasal discharge from day 12 to 16 p.i.. In Group J, there was only occasional coughing from days 8 to 10 p.i..

Virus isolation

These results are shown in Table 26. Virus was recovered irregularly from day 8 to 21 p.i. from nasal swabs. In the animals examined at necropsy, the virus was recovered from retropharyngeal lymph nodes, the upper trachea in H1 and the turbinates in H4, 15 and 17 days p.i. respectively.

Serological examination

The antibody titre rose from 1:12 to 1:24 (H2) and from 1:8 to 1:24 (H3) in 3 weeks.

Pathological examination

There was congestion of the nasal mucosa and severe pharyngitis with many haemorrhages and oedema (Figure 73). There was severe laryngitis and severe tracheitis with many haemorrhages (up to 2mm in diameter) in the anterior half of the trachea (Figure 74). The major

airways were filled with frothy white oedema fluid. The retropharyngeal, bronchial and mediastinal lymph nodes were oedematous, congested and contained a few haemorrhages. There were patches of pneumonia in the anterior and most distal regions of caudal lobes with a stringy, yellow pus present in the airways of the consolidated areas. There was marked emphysema in the anterior lobes. The right and left cardiac lobes were pneumonic with total consolidation and an appearance similar to that seen in cases of cuffing pneumonia such as collapsed fawn grey lesions. There were large areas of pneumonia in the diaphragmatic and middle lobes. Many lungworms were present in the bronchi of most lobes (Figure 75). The liver, brain, spleen, kidney and gall bladder were macroscopically normal.

Microscopically, there was congestion in the nasal conchae with marked epithelial and goblet cell hyperplasia as well as globule leucocyte infiltration. There was inflammatory exudate in the pharynx and nasopharynx with scattered small haemorrhages. A severe laryngitis with massive lymphoid infiltration resulting in germinal centre formation, were observed while plasma cells were abundant in the lamina propria. In many places, there was a loss of epithelium. There was a moderately severe tracheitis with epithelial hyperplasia and mucous gland hypertrophy although only a few gland tubules were surrounded by plasma cells. There were several small lymphoid follicles in the lamina propria which, in addition to congestion and haemorrhages, also contained many neutrophils.

Many lungworms were present in the consolidated parts of the diaphragmatic lobes and in areas eosinophils were abundant. There was a severe bronchitis with epithelial hyperplasia as well as dysplasia in some areas due to the inflammatory infiltrates. In the parenchymal tissue of these areas there was considerable oedema/mucus exudate mixed with macrophages and neutrophils. In several alveoli hyaline membrane formation was seen in the pneumonic areas in which there was no lungworm infestation, there was marked bronchitis, bronchiolitis and alveolitis. The submucosal glands were hyperplastic and dilated while the retropharyngeal, bronchial and mediastinal lymph nodes were slightly oedematous and congested. Many bronchioles were plugged with inflammatory exudate and there was extensive interlobular involvement in all pneumonic areas with oedema, fibrin and infiltration by lymphocytes, neutrophils and eosinophils. There was a marked congestion and neutrophilic infiltration of the spleen. The liver, kidney, gall-bladder and brain were normal.

TABLE 27. The amount of live and irradiated third stage lungworm larvae given to each individual animal.

EXPERIMENTAL CASE NO.	BODY WEIGHT (Kg)	AMOUNT OF LUNGWORM LARVAE DOSED
H1	209	10,450
H2	182	9,100
H3	364	18,200
H4	500	25,000
P1	300	1,000
P2	236	1,000
P3	240	1,000
P4	270	1,000
J1	280	All vaccinated with lungworm vaccine (Dictol) according to the manufacturers instructions. Each dose contained 1,000 irradiated lungworm larvae.
J2	305	
J3	258	
J4	275	

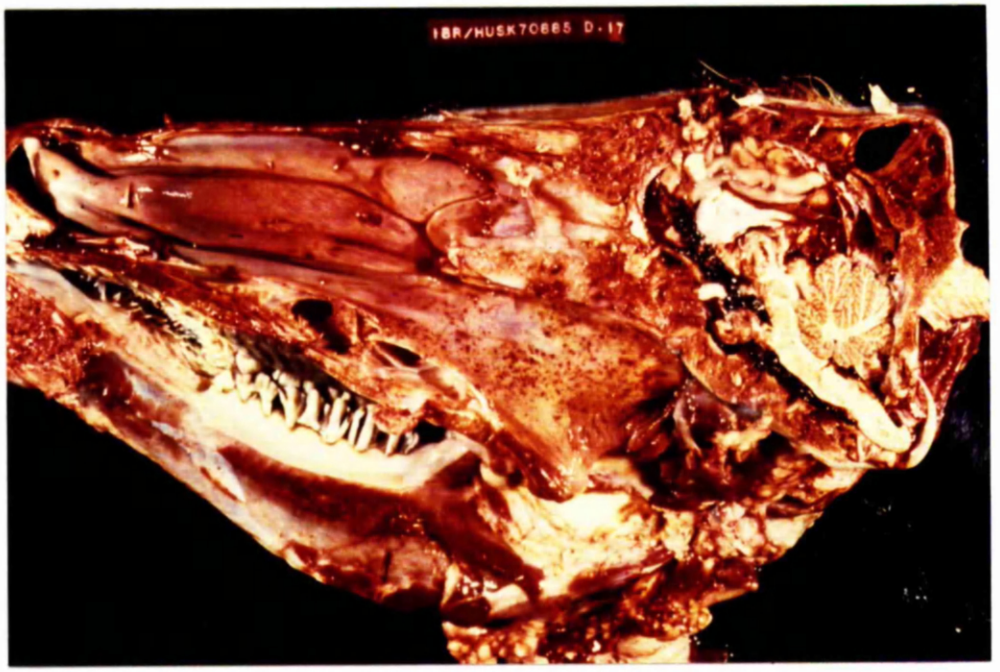


FIGURE 73. Case No. H4, a recovered IBR case, 17 days after challenge with Dictyocaulus viviparus larvae. Sagital section of the head with severe petechial haemorrhages throughout the nasal passages and pharynx.

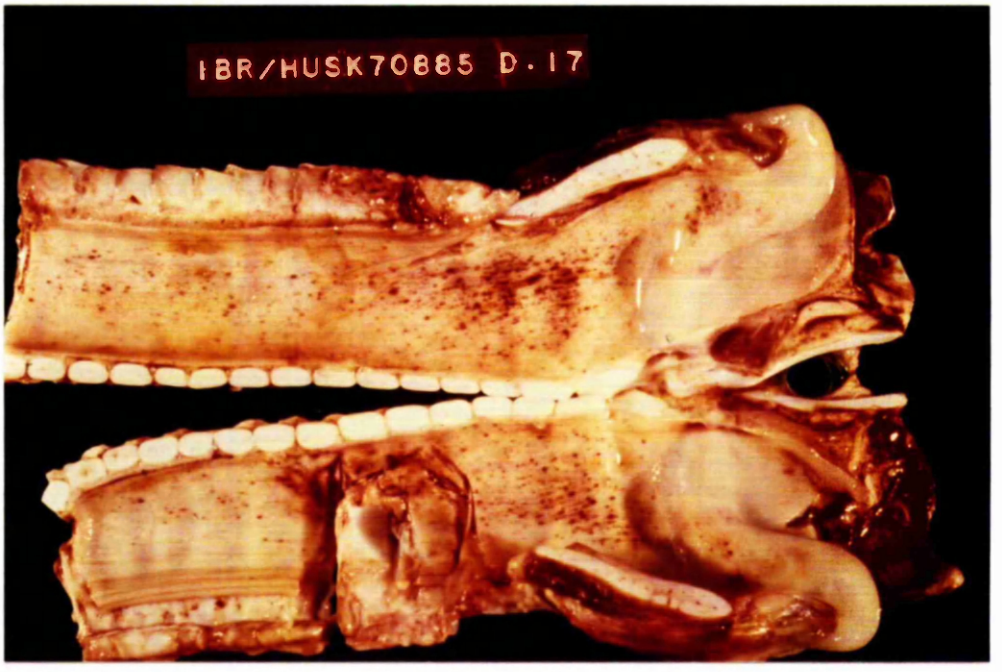


FIGURE 74. A recovered case of IBR No. H4, 17 days after challenge with Dictyocaulus viviparus larvae. The larynx and trachea have oedema in the mucous membrane and there is severe haemorrhage in the anterior portion of the trachea.

FIGURE 75. Case No. H4, 17 days after challenge with Dictyocaulus viviparus larvae; a large number of lungworms are lying within a major bronchus.

SECTION 5

THE EFFECT OF INTRANASAL CHALLENGE WITH THE STRICHEN STRAIN OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS ON "PNEUMOVAC PLUS" VACCINATED ANIMALS

MATERIALS AND METHODS

Experimental animals

Fifteen, 6 to 10 month old dairy cross bullocks were divided into 3 Groups (K, L, M) of 4 animals each and 1 Group (N) of 3 animals. The history and serological status of the steers at the start of the trial is shown in Table 28.

Housing

This form of housing was identical to that described in detail in Section 1 of this Chapter. Groups K and N were mixed together. Initially, all groups of animals were kept in strict isolation and attended by different stockmen although Groups K and N were subsequently housed together

Feeding

The feeding regime was identical to that described in detail in Section 1 of this Chapter.

Challenge procedure

The calves in Group K were vaccinated according to the manufacturers instructions, with an inactivated polyvalent vaccine containing IBR viral antigens (Pneumovac Plus, C-Vet, Bury St. Edmunds, England). Three weeks after the second vaccination, blood samples were taken to determine the serologic response to the IBR component in the vaccine. At 4 weeks after the second vaccination, the 12 steers in Groups K, L and M were challenged via the intranasal route in a manner similar to that already described in detail in Section 1 of this Chapter.

The Group N steers (N1, N2, N3) were housed together with Group K animals (K1, K2, K3, K4) on day 2 p.i. and the 7 animals remained together until the termination of the experiment. The Group N steers were used to monitor virus excretion by the vaccinates. The parameters used to check for the immunity of these steers were clinical signs (temperature, respiratory rate, ocular and nasal discharges as well as

lesions), virus excretion and the development of serum neutralising antibodies.

The details of the clinical and serological examinations as well as the technique used for virus isolation were identical to those described in Section 1 of this Chapter. None of these animals were slaughtered for postmortem examination.

RESULTS

Experiment 9. Groups K, L, M and N steers.

Clinical features. The detailed clinical findings of Group K steers are summarised in Appendix 4, Table 9.

On day 2 p.i., Group K (vaccinates) steers became dull and had a slight serous nasal discharge and a moderate pyrexia (103-104.2°F). On days 3 and 4 p.i. the 4 steers in Group K were very dull, anorexic and had a bilateral seromucoid to mucopurulent nasal discharge. Single, dry, non-productive coughing was evident and their temperatures rose even higher (104-106.5°F). The nasal mucosae which were congested, were covered with numerous diphtheritic yellowish-brown lesions (Figure 76). Conjunctivitis accompanied by serous ocular discharge was a feature of every one of these animals. From day 4 to 7 p.i. they were seen to be tachypnoeic (RR = 40-70/min) and to be drooling saliva.

The 4 Group L steers which were the virus control group, developed clinical response, identical in severity to that of the Group K animals. The animals in Group M which had recovered from the field disease remained clinically normal throughout the period of the experiment.

The Group N steers which were monitoring the virus excretion by the vaccinated steers (Group K) developed typical clinical signs of IBR on day 6 after their introduction. The severity of the clinical signs was similar to those of both the vaccinated group (Group K) and the control group

Virus isolation

These results are presented in Table 28. The virus was recovered regularly from nasal and ocular discharges from day 4 to 12 and 4 to 10 p.i. respectively in Groups K and L. In Group N the virus was recovered from nasal and ocular discharges from day 7 to 14 and 7 to 10 p.i. respectively. In Group M, virus was recovered only from the nasal discharge of M2 from day 7 to 9 p.i..

Serological examination

The Group K animals were seronegative 3 weeks after the second vaccination. Following challenge with the virus both Groups K and L developed detectable serum neutralising antibodies between days 8 and 12 p.i.. Group M steers showed a slight rise in antibody titre on day 8 p.i. while Group N steers developed detectable antibodies on day 16 post contact.

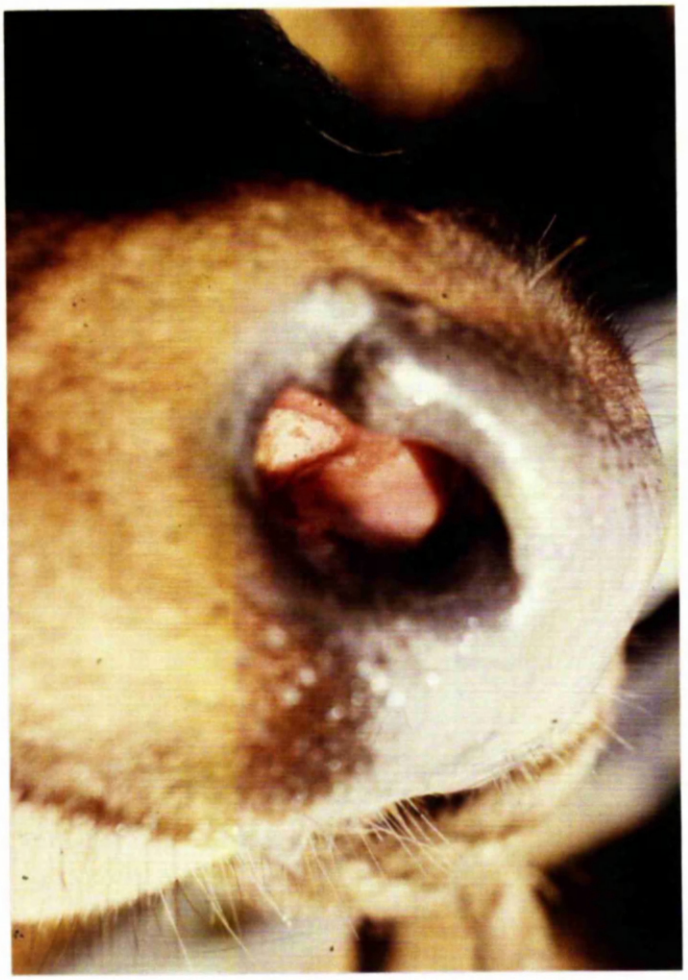


FIGURE 76. A close-up view of the nose of a stirk vaccinated with Pneumovac and challenged with the Strichen strain of infectious bovine rhinotracheitis virus 6 weeks later. Note the diphtheritic lesions on the mucosa of the nasal septum.

TABLE 28. The effects of challenge with the Strichen strain of infectious bovine rhinotracheitis virus on 7 control, 4 vaccinated and 4 recovered field cases of infectious bovine rhinotracheitis.

GROUPS	HISTORY	SEROLOGICAL STATUS AT TIME OF CHALLENGE	POST-CHALLENGE	
			(i) CLINICAL SIGNS	(ii) VIRUS EXCRETION
K	VACCINATED	4/4 SERONEGATIVE	4/4 (DAYS 3-12)	4/4 (DAYS 4-12)
L	NON-VACCINATED (CONTROL)	4/4 SERONEGATIVE	4/4 (DAYS 3-12)	4/4 (DAYS 4-12)
M	RECOVERED FIELD CASES	4/4 SEROPOSITIVE	0/4	1/4 (DAYS 7-9)
N	NON-VACCINATED (TRACERS)	3/3 SERONEGATIVE	3/3 (DAYS 6-12)	3/3 (DAYS 7-12)

DISCUSSION

Every susceptible animal challenged with the Strichen strain of IBR virus developed clinical signs similar to, but generally milder than, those observed in the field incidents (Chapter 2). There was a negative correlation between age and the severity of disease under these conditions of exposure, i.e. the clinical signs were most severe in the 2 week old calves and least severe in the 18 month old bullocks.

Slight to moderate dullness was usually the first sign of illness and this generally became apparent 2 to 3 days following infection. At the same time, pyrexia developed and achieved maximal values of 106 to 107°F on the fourth to fifth days after challenge. Pyrexia persisted for 2 days and 5 days in the 18 month and 2 week old animals respectively. Although the onset of fever was accompanied by a decrease in appetite, a reduction in the consumption of roughage alone, as observed in a few field outbreaks, was not a feature of the experimental disease.

The commonest presenting sign was a bilateral nasal discharge which initially was serous, but later became mucoid or mucopurulent. It would have been easy to have missed the discharge, which was mainly serous in the oldest (18 months) bullocks because they constantly licked their noses. In contrast in the younger animals the discharge inevitably became more copious and mucoid or mucopurulent in character. This sequential change in the character of nasal discharge was also noticed by Webster and Manktelow (1959) in New Zealand and by Markson and Darbyshire (1966) in England. In every group, the nasal mucosae were hyperaemic but pinpoint haemorrhages were observed only in the 6 and 18 month old animals. In addition, the mucosae appeared to be particularly friable since bleeding was easily induced on handling. However, in every age group, diphtheritic yellowish-brown plaques were subsequently visible on the nasal mucosae.

Single, sporadic, non-productive coughing was another common presenting clinical feature. Although the coughing generally began 3 days after challenge and persisted for about 7 days, 2 of the 5 week old calves continued to cough until they were slaughtered (33 days p.i.). Both these calves and 2 others in Group D (2 weeks old) developed

pneumonia. Although tachypnoea and hyperpnoea were common in every age group, dyspnoea was a feature of the disease only in the 6 month old animals.

Conjunctivitis developed in every group although not in every animal. In the oldest group it was relatively mild and persisted for up to 4 days. In contrast it was severe in a number of animals in all the other groups. Granular conjunctival lesions, which were observed in some of the field outbreaks, were also seen in particularly severely affected individuals. In the later cases, there was profuse lachrymation which resulted in the hair on the animals' cheeks becoming matted. Fine conjunctival haemorrhages as described by Dawson and others (1962) in the field disease and by Abinanti and Plumer (1961) in experiment infections were not observed in any of these experimentally infected animals. Similarly, keratitis did not develop; this agrees with the experimental findings of McKercher and others, (1959); Webster and Manktelow, (1959); Abinanti and Plumer, (1961) and Markson and Darbyshire, (1966). Only Hughes and others (1964) claims to have produced keratitis following the challenge of susceptible calves with IBR virus.

The drooling of clear ropy saliva which was seen in every one of these experimental animals was most profuse in those which were most severely affected. Hyperaemia of the oral mucosae was particularly noticeable in the young calves. However, oral lesions as described by Welleman and others (1974) were never seen. One of the 2 week old calves, which had developed diarrhoea a few days prior to challenge, continued to be diarrhoeic and was found dead 3 days after exposure to the IBR virus. Unfortunately, a post mortem examination was not possible, but it is very likely that this calf had died as a result of the enteric form of colibacillosis. None of the other experimental calves became diarrhoeic; this is in contrast to the observations of Markson and Darbyshire (1966) who reported that diarrhoea was common in their 2 month old calves following challenge with the Oxford strain of IBR. However the significance of this finding cannot be accurately assessed since there would appear to have been no control animals.

Although none of these animals died as a result of IBR, 3 of the 7 young calves would have died had they not been slaughtered for humane reasons on the days already stated.

On haematological examination there was a progressive decrease in the mean total white blood cell count in the Group A (18 month old) bullocks while that of Groups B and C increased until day 4 post exposure and thereafter decreased progressively until day 20. The leucopenia and neutropenia in the Group A bullocks is consistent to the haematological change resulting from a virus infection (Lewis and Shope, 1929). On the other hand there was a slight initial leucocytosis and neutrophilia in the Group B and C (6 month and 5 week old respectively) animals before the numbers of white blood cell count and neutrophils decreased. This possibly is an age related response since it has been shown that young cattle develop neutrophilia following a virus infection (Schalm, Jain and Carrol, 1975).

That the clinical disease was considerably more severe in the 2 and 5 week old calves than in either the 6 month old stirks or in the 18 month old bullocks is in keeping with the observations of Webster and Manktelow (1959) who found that the disease was more severe in week-old calves than in yearlings or adult cattle. This obvious age-related difference in susceptibility to the IBR virus is probably the result of an immature cell mediated defence mechanism in these young cattle.

The virus of IBR was isolated regularly from both nasal and ocular discharges for up to 13 days post challenge. Virus was recovered for a shorter period from the ocular discharges (average 6 days) than that from the nasal discharges (average 10 days). This could very well be the explanation for the difference in the recovery of virus from ocular and nasal swabs taken during the field outbreaks (Chapter 2). The re-isolated virus produced the typical BHV 1 cytopathic effect and was neutralised by specific hyperimmune serum. At necropsy, the virus was frequently recovered from the turbinates, the pharynx, the larynx, the upper and lower trachea as well as from the local lymph nodes. It is of considerable interest that the virus was not isolated from the lungs of any of these experimentally infected animals. On the other hand, virus was isolated on a few occasions from the liver and kidneys; this had possibly resulted from the migration of infected leucocytes from the respiratory tract to other organs (McKercher and others, 1963) since a true viraemia has not been established with either this strain (Msolla and Wiseman, 1979) or with other strains of IBR virus (McKercher and others, 1963).

In the current studies, all the experimental cattle had developed detectable serum neutralising antibodies within days of being exposed to the virus. The earliest time that antibodies were detected was 7 days after challenge (A4). In contrast in other experiments a significant proportion of animals, 25 per cent (Abinanti and Plumer, 1961) and 17 per cent (Hughes and others, 1964), did not produce a detectable titre of neutralising antibodies. The highest titres 3 weeks after challenge in individual animals were 1:128 (Group A), 1:32 (Group B), 1:16 (Group C) and 1:24 (Group D). The 18 month old bullocks (Group A) appear to have had a more efficient immunological response in that antibodies were detected much earlier and the 3 week titres were considerably higher than in the younger animals. This probably indicates that the older bullocks were immunologically more competent than the young calves.

The lesions found at necropsy were similar in distribution but were less severe than those seen in the field outbreaks (Chapter 2). There was rhinitis with diphtheritic lesions in the nasal mucosae, pharyngitis, laryngitis and tracheitis with petechial haemorrhages. Thrombosis of the pulmonary veins was not present in any of the experimentally infected animals and this was almost certainly the reason why renal infarction was never seen; these 2 lesions were commonly encountered in severe field cases. A clinically detectable pneumonia developed in the 2 week and 5 week old calves only and on macroscopic and microscopic examinations, these were confirmed as being acute exudative pneumonias.

The animals challenged with the Colorado and Oxford strain of IBR virus responded in a similar manner as those challenged with the Strichen strain. However, the clinical and pathological responses were much less severe with the Oxford strain which produced only very mild clinical signs and pathological lesions. Since the challenge dose of virus, the age of the experimental animals and their immunological status were the same prior to challenge, these findings confirm that the Strichen strain is much more virulent than the Oxford strain. On the other hand, the response with the Colorado strain was also less severe than that produced by the Strichen strain despite the fact that it had been isolated from original severe outbreaks in Colorado. However, the later virus had been passaged 28 times in bovine tissue culture systems

and perhaps this had adversely affected its pathogenicity.

Following recovery from primary infection with a herpes virus, it is common for the virus to persist in the host (Martin, 1976). This phenomenon which is usually referred to as latent infection, has been extensively studied by Paine (1964) who found that Herpes simplex in man was a persistent and recurrent infection which, under stress, could be reactivated giving rise to the characteristic acute clinical syndrome. Parallel studies in cattle infected with BHV 1 have confirmed that IBR virus is re-excreted by recovered animals; mild clinical disease has arisen following the administration of drugs with glucocorticoid activity (Sheffy and Davies 1972; Gibbs and others 1974), Caesarian section (Lomba and others 1976) and experimental Para-influenza type 3 virus infection (Mansik and others 1976). Following exposure of recovered cases of IBR to normal lungworm larvae, clinical signs typical of IBR developed from day 11 post challenge. Affected individuals became dull, their appetite decreased, pyrexia (103.2 - 105.2^{o}F) developed as well as a profuse bilateral, serous, nasal discharge. These animals also became tachypnoeic and they coughed frequently. The virus was recovered from nasal discharges and at necropsy from the turbinates and the upper trachea. Typical lesions of IBR were confirmed when animals were examined post mortem at 15 and 17 days after lungworm larval challenge. The onset of the clinical signs which were typical of IBR occurred earlier than would have been expected in a primary infection with a moderate number of D. viviparus larvae (Jarrett, Jennings, McIntyre, Mulligan and Urquhart, 1957). These experiments have demonstrated that challenge with D. viviparus larvae in numbers that can produce clinical signs of parasitic bronchitis can result in the re-activation and excretion of IBR virus. Although clinical signs did not develop in animals given only 1,000 larvae, nevertheless virus was isolated from the nasal discharge of one of them. In contrast, neither were confirmed in the animals given the lungworm vaccine "Dictol". Mild outbreaks of IBR have been diagnosed during the summer in grazing stirks (Imray, 1979b) and therefore it is possible that these incidents had resulted from the reactivation of latent infection by lungworm larvae.

When heifers with no detectable antibodies to IBR virus were challenged by the intravaginal route with the Strichen strain, they developed clinical signs similar to those observed in field outbreaks

of IPV (Collings and others 1972) and in the experimentally produced disease (McKercher and others, 1959). The intermittent recovery of the virus from the vaginal discharges for up to 21 days after challenge is in keeping with the findings of Huck, Miller and Woods (1973) who eventually recovered the virus from the vaginal discharges of experimentally infected heifers up to 358 days after infection.

A serological response to the IBR component of the inactivated vaccine, Pneumovac Plus, was not detected in any of the 4 calves 3 weeks after their second vaccination (i.e. 1 week prior to challenge). When challenged with the Strichen strain of virus, the development of clinical signs, the serological responses and the virus excretion patterns were virtually identical to those of the control groups. Susceptible calves housed with the vaccinates also developed typical signs of IBR. In marked contrast, the 4 animals, which had recovered from the field disease, did not react clinically following challenge and virus excretion was limited to only 1 stirk for a period of 3 days; this animal had the lowest serum antibody titre (1:4).

As a result of this study, it can be stated that Pneumovac Plus did not provoke a detectable serum neutralising antibody response and did not confer protection against challenge with the Strichen strain of IBR virus. Furthermore, replication of the challenge virus apparently occurred without hindrance since, within a few days of challenge, vaccinated calves were infective for in contact, seronegative individuals.

The most recent isolate of IBR virus in Britain (Strichen strain) produced characteristic clinical signs and pathological lesions in experimentally infected susceptible cattle. In common with other strains of this virus, the experimentally produced disease was less severe than the field incidents. The clinical disease was much more severe in young calves than in older cattle. The Strichen strain produced a more severe disease than did the Colorado strain and a considerably more severe disorder than the Oxford strain. Furthermore, the Strichen strain was confirmed as producing severe IPV when given intravaginally although this form of disease has not been confirmed in any of the field outbreaks of IBR investigated (Chapter 2).

It was of considerable interest to find that infection with even small numbers of D. viviparus larvae (1,000) could produce

reactivation and excretion of virus which could then be transmitted to susceptible in contact animals. The only vaccine against IBR currently available in Britain was unable to give a detectable measure of protection against challenge with the virulent Strichen strain of virus. These results confirm the need for a safe and effective vaccine against IBR in Britain.

CHAPTER 6

DIFFERENTIAL DIAGNOSIS OF INFECTIOUS BOVINE RHINOTRACHEITIS

INTRODUCTION

Infectious bovine rhinotracheitis is an acute, contagious condition characterised by severe inflammatory changes in the upper respiratory tract and trachea. There is usually pyrexia, dullness, reduced appetite and a bilateral nasal and ocular discharge with the drooling of saliva. Thus conditions which are likely to be confused with IBR are those which are characterised by ocular and upper respiratory tract lesions and salivation (Table 29). However diagnosis is not made on clinical examination alone since history and epidemiological features are equally important and in certain situations may be more valuable than the clinical examination in arriving at the diagnosis. Where appropriate these clinical findings may be supplemented with postmortem findings and together they should provide enough information where doubt exists on clinical and epidemiological findings. This discussion is based on clinical cases examined at the Veterinary School as well as experience gained during the numerous farm visits made in the course of this work.

DISCUSSION

Infectious bovine rhinotracheitis which has been encountered in all classes of cattle is most common in fattening animals (6 months to 2 years of age) during the winter housing period. In these types of animals, clinical signs of disease have appeared in purchased animals within 4 weeks of their arrival. Although the most severe incidents appear to have occurred in Scotland, this disorder has now been confirmed in virtually every part of the British mainland. The main presenting signs are reduced appetite, dullness, serous to mucopurulent ocular and nasal discharges, drooling saliva and frequent coughing. On clinical examination, pyrexia, conjunctivitis and diphtheritic lesions on the nasal mucosae are frequently present. For laboratory confirmation virus can be isolated from nasal and ocular swabs taken during the early stages of the disease. A rising antibody titre in a representative number of affected animals is also useful.

Foot and mouth disease, which is notifiable in Britain, must be considered first when several animals in the same group are seen to be drooling large amounts of saliva. In this disease, vesicles are almost always present in the tongue. In outbreak 12 foot and mouth disease was suspected until clinical examination of the oral cavities of affected

animals revealed the absence of lesions.

Transit fever is a commonly diagnosed condition in recently purchased animals, particularly in those areas where the incidence of IBR have been highest (Hepburn, 1925; Anderson, 1939; Pickering, 1939), i.e. the predominantly beef fattening areas of Britain. The incidence is highest in the late autumn when large numbers of suckled calves are sold at special sales. The clinical signs result from a severe exudative pneumonia which is thought to be the result of a primary virus infection (Parainfluenza virus type 3, IBR) followed by a secondary infection with Past. haemolytica or Past. multocida. For laboratory confirmation, Pasteurellae species can be isolated regularly from nasal swabs in early untreated cases. There is sudden onset extreme dullness, tachypnoea and anorexia with pyrexia (104-108°F). On auscultation fine crackles may be heard cranio-ventrally and thoracic pain may be detected on percussion. Latterly, expiratory grunting may develop and, if untreated, the animal will usually die within 72 hours. Although serous ocular and nasal discharges are present in many cases of transit fever, they are much less severe than animals suffering from IBR. In addition, pneumonia is not a feature in the early stages of IBR and therefore affected animals are much brighter than those with transit fever.

Infectious bovine keratoconjunctivitis (IBK) is a common disease of young cattle throughout the year. The major pathogen is considered to be Moraxella bovis, although other infectious agents may be necessary for this organism to exert its full pathogenic effect (Langford and Dorward, 1969; Wilcox, 1970). It has been proven experimentally that IBR virus enhances the pathogenicity of M. bovis (Pugh and others, 1976). Lesions are often confined to one eye and there is a copious serous lachrymation, conjunctivitis, keratitis and ulceration. In severe cases both eyes may be affected and there can be photophobia, blepharospasm and in some cases, slight fever. As the acute inflammation subsides, the ocular discharge becomes purulent, the central opacity gradually becomes smaller and recovery usually takes place within 3 to 5 weeks. For laboratory confirmation, M. bovis can be isolated from ocular swabs. Central keratitis has only been produced by one isolate of IBR virus (Hughes and others, 1964) following experimental infection but none of the calves given the Strichen strain of the virus have ever developed keratitis. Consequently, the presence of keratitis and ulceration should be considered as indicative of IBK

and not IBR. Of course, under field conditions, it is common for both the diseases to be present in one group of animals (Chapter 2).

When nasal discharge and drooling saliva occur together in the same animal, mucosal disease should be suspected. This condition which is prevalent in young cattle tends to be most common in winter. The clinical signs of mucosal disease, which is also known as the sporadic form of bovine virus diarrhoea, develop in a proportion of cattle following infection with a Togavirus. For laboratory confirmation, the virus of mucosal disease can be isolated from blood and faeces. Unlike IBR and transit fever, nasal swabs are of little use in confirming this disease. In the early stages, there is slight dullness, anorexia and pyrexia (103-107°F), usually with a bilateral serous nasal discharge which in severe cases becomes mucopurulent (Figure 77). Lachrymation often occurs but it is not a major feature. The oral mucosae initially appears salmon pink in colour but later superficial erosions appear with the drooling of copious amounts of viscous saliva (Figure 78). Superficial erosions may also appear on the muzzle which can become "crusty". One of the main characteristic features of this disorder is profuse diarrhoea which is the result of ulceration along virtually the entire length of the gastrointestinal tract. Another major feature is lameness resulting from interdigital ulceration. Unlike mucosal disease, the presence of oral lesions, diarrhoea and lameness are not features of IBR.

Malignant catarrhal fever is an acute, infectious non contagious disease which is most common in 1 to 3 year old cattle. Cases usually arise in the early summer in animals which have had some association with lambing ewes (Selman, Wiseman, Max Murray and Wright, 1974). Since the aetiological agent of malignant catarrhal fever in Europe has yet to be identified, diagnosis must be confirmed at necropsy. Affected individuals are moderately to severely dull and anorexic with pyrexia (105-108°F). There is usually a bilateral serous nasal discharge which later becomes mucopurulent (Figure 79). The eyelids are swollen and there is severe conjunctivitis. Photophobia with blepharospasm are usually evident. Keratitis, which is the most obvious clinical evidence of panophthalmitis, begins at the corneal-scleral junction and spreads centripetally finally leading to total blindness. Corneal ulceration is not a feature of malignant catarrhal fever. There is a bilateral mucopurulent nasal discharge and cracking of the muzzle

frequently occurs. The drooling of saliva can frequently be seen and on opening the animal's mouth, hyperaemia of the oral mucosae is present in the early stages of the disease. Later shallow, irregular erosions with diphtheresis may become evident. Necrosis and haemorrhage of the tips of the oral papillae is common. Other characteristic features include marked superficial lymph node enlargement, dermatitis with erythema and eczema, incoordination and head pressing. Although the ocular and nasal signs are more severe in malignant catarrhal fever than in IBR, the large number of the other characteristics of the former disease, including keratitis, ensures that there is usually little difficulty in differentiating between them.

Photosensitisation is the name given to the dermatitis which develops when non-pigmented sensitised areas of skin are exposed to strong light. A particularly severe form of photosensitisation has been encountered in grazing Hereford cross heifers. Lesions are commonly seen around the eyes and muzzle. The non-pigmented parts of the eyelids become inflamed, conjunctivitis also develops as well as a profuse serous lachrymation which results in the medial canthi becoming "scalded" (Figure 80). The non-pigmented areas of the muzzle crack and peal off leaving unprotected underlying tissues which may bleed easily (Figure 81). There is usually a bilateral seromucoid nasal discharge. Several days after these signs have developed, the skin over the rest of the face and the other non-pigmented areas of the body becomes hard and it too sloughs off eventually. Swelling of the vulva and hardening of the lateral aspects of the teats also commonly occurs. The nature of the lesions and their obvious confinement to the non-pigmented areas of the skin is pathognomonic of this condition.

Although IBR has been confirmed in grazing cattle, the clinical signs are much less severe than in housed animals. Parasitic bronchitis should be the first disease to be considered when immature grazing cattle develop respiratory disease. With parasitic bronchitis there is neither ocular nor nasal discharge and tachypnoea and hyperpnoea are characteristic features in addition to frequent coughing. On auscultation, squeaks and crackles can often be heard of the caudal lung lobes. Laboratory confirmation depends on the identification of D. viviparus larvae in the faeces.

Infectious bovine rhinotracheitis was confirmed in young calves on a single farm and the clinical signs were identical to those which developed in fattening cattle. There was neither an increase in the incidence of neonatal diarrhoea as has been reported from Belgium by Wellemans and others (1974) nor an increase in neonatal mortality.

In adult cattle, frequent coughing often associated with the feeding of mouldy hay and the subsequent development of farmer's lung disease in a number of the older animals (Wiseman, 1978). Farmer's lung is an allergic pneumonia with neither upper respiratory tract nor ocular involvement suggestive of IBR virus infection.

The severe form of IBR is unlikely to be confused with any of the diseases described above. However, as infection with the present wild strain of the virus becomes endemic, it is likely that the clinical disease will become less severe and more variable, such as the development of severe conjunctivitis with minimal upper respiratory tract signs.

TABLE 29. The differentiation of infectious bovine rhinotracheitis from other similar conditions.

Clinical sign	Infectious bovine rhinotracheitis	Transit Fever	Mucosal disease	Malignant Catarrhal Fever	Photo-sensitisation	Infectious bovine keratoconjunctivitis
Dullness	++	+++	++	+++	+	+
Reduced appetite	++	+++	++	+++	+	−
Pyrexia	+++	+++	+++	+++	+ −	−
Ocular Discharge	++	+	++	+++	++	++
Conjunctivitis	++	±	+	+++	++	+++
Keratitis	−	−	−	+++	−	+++
Nasal discharge	+++	+++	+++	+++	++	−
Nasal lesions	+++	−	+	++	++	−
Coughing	+++	±	+	±	−	−
Tachypnoea	++	++	+	++	−	−
Hyperpnoea	++	++	+	++	+ −	−
Dyspnoea	+	+++	−	++	−	−
Pneumonia	±	+++	±	±	−	−

TABLE 29 Cont'd.

Clinical sign	Infectious bovine rhinotracheitis	Transit Fever	Mucosal disease	Malignant Catarrhal Fever	Photo-sensitisation	Infectious bovine keratoconjunctivitis
Drooling saliva	++	–	+++	+++	–	–
Oral lesions	–	–	+++	+++	±	–
Diarrhoea	–	±	+++	±	–	–
CNS - signs	–	–	–	+	–	–
Morbidity %	> 80	< 10	5-10	< 1	Variable	Variable
Mortality %	2-3	< 5	> 90	99	0	0
Other features	–	Thoracic pain	Interdigital ulcerations	Superficial lymph node enlargement	Skin necrosis in non-pigmented areas	Corneal ulceration

– ± + ++ +++ Indicates frequency of occurrence of clinical sign.

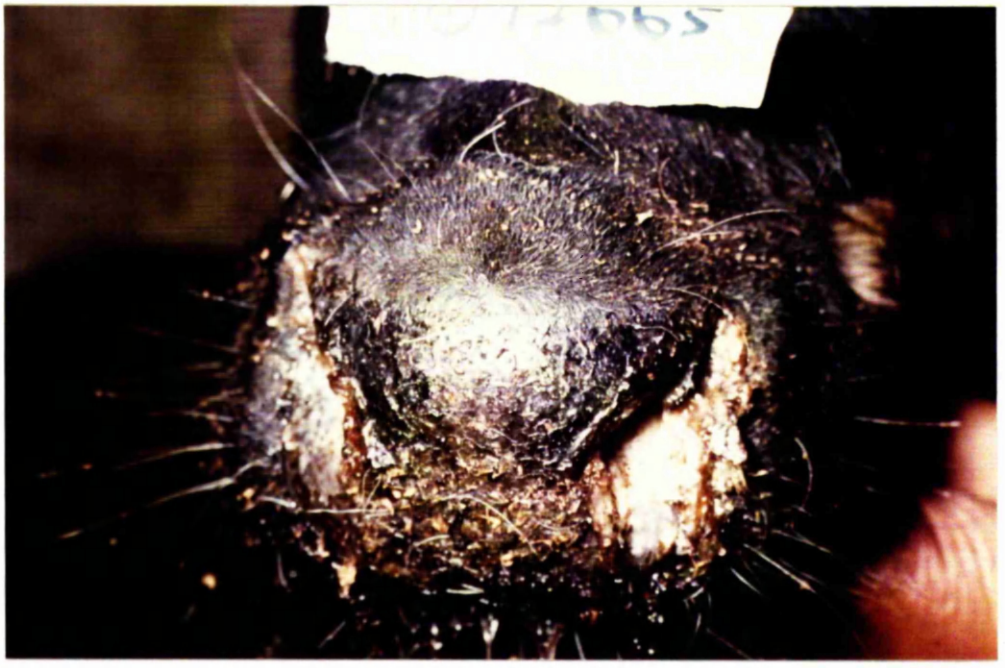


FIGURE 77. A close-up view of the muzzle of a stirk suffering from mucosal disease. Crusting of the muzzle and a bilateral mucopurulent nasal discharge can be seen.

FIGURE 78. A close-up view of the muzzle of an animal suffering from mucosal disease. A moderate amount of frothy saliva can be seen drooling from its mouth. Encrustation of the muzzle can be observed particularly at the skin-muzzle junction.

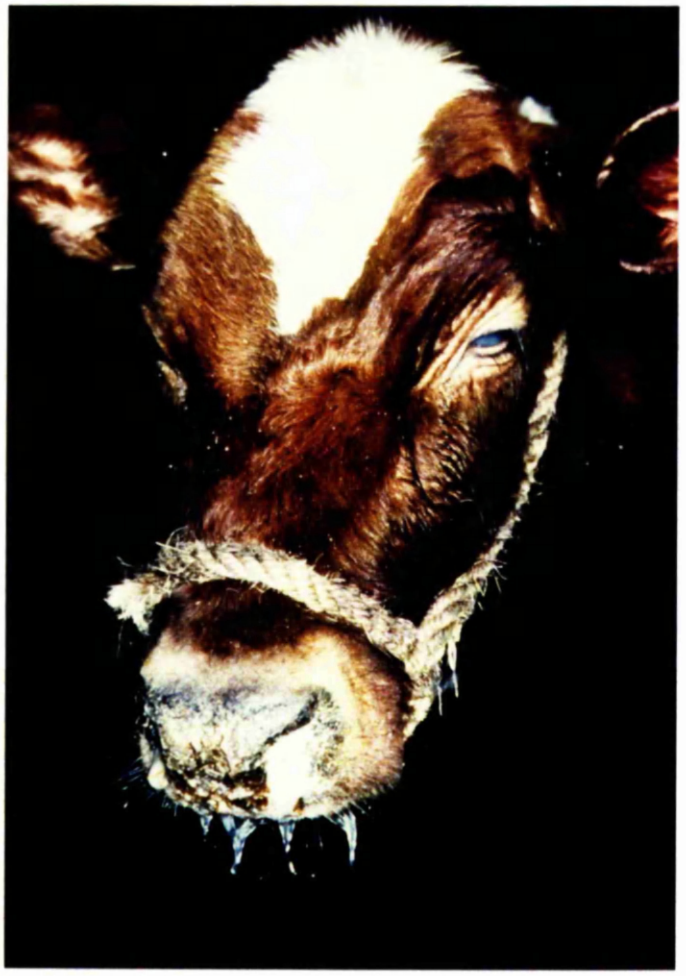


FIGURE 79. A close-up view of the head of an animal suffering from malignant catarrhal fever. Swelling of the eyelids and keratitis can be seen. There is a profuse bilateral mucopurulent nasal discharge with cracking of the muzzle. Drooling of saliva is also evident.

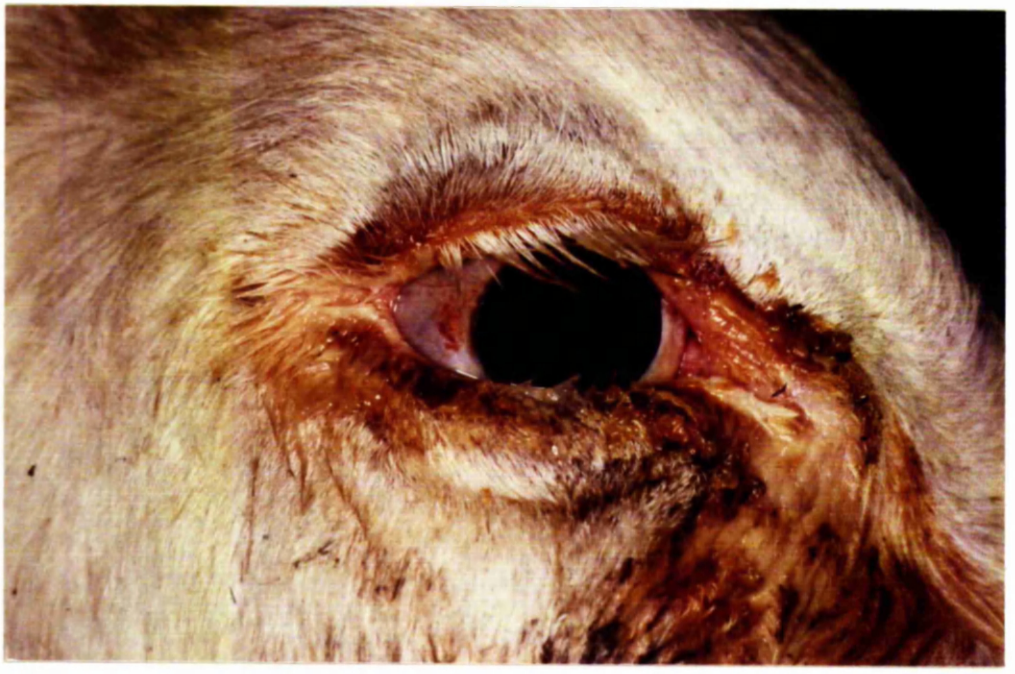


FIGURE 80. A close-up view of the eye of a Hereford cross heifer suffering from photosensitisation. There is conjunctivitis, erosion of the epithelium around the eye particularly at the medial canthus and profuse lachrymation. Note that there is no keratitis.

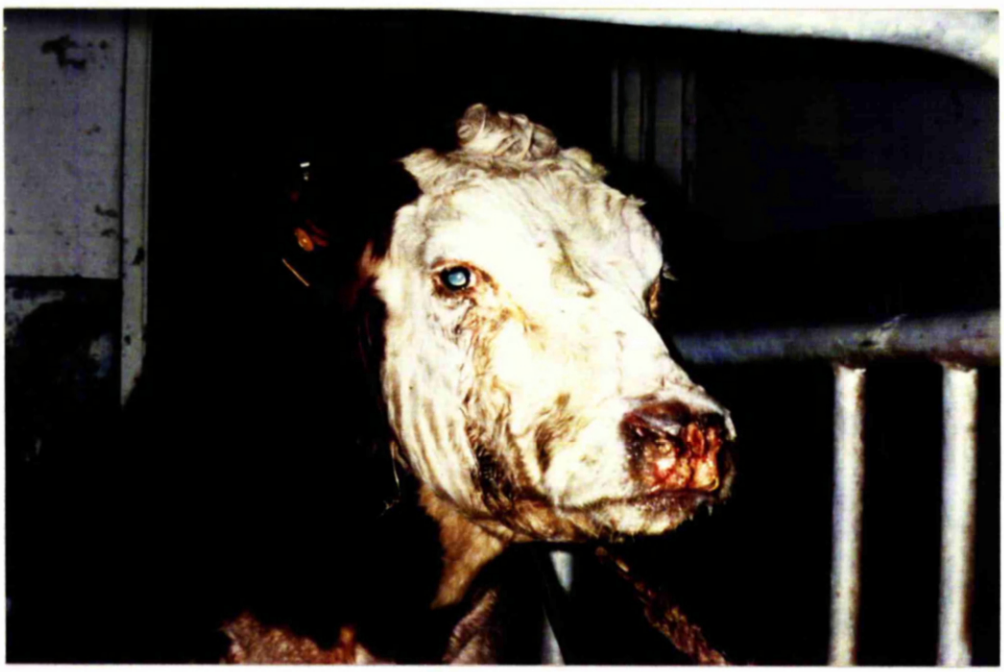


FIGURE 81. A close-up view of the head of a Hereford cross heifer suffering from photosensitisation. There is profuse lachrymation resulting in matting the hair on the right cheek. The surface epithelium of the muzzle has cracked and has pealed off revealing subepithelial tissues. A slight serous nasal discharge is also evident.

GENERAL DISCUSSION

GENERAL DISCUSSION

It was during the early months of the 1977-78 winter housing period that a number of outbreaks of a hitherto unrecognised respiratory disorder were seen in the north east of Scotland. Since this "new" condition had almost invariably developed in recently purchased cattle, it was often referred to as "funny" or "peculiar" transit fever. These were considered to be "atypical" cases of transit fever because affected animals had clinical signs of a severe upper respiratory tract disease with conjunctivitis, but no evidence of pneumonia. In contrast, the signs of "typical" transit fever are those of a severe exudative pneumonia with minimal upper respiratory tract and ocular involvement (Hepburn, 1925). The "new" respiratory disease was subsequently confirmed as being a particularly severe form of IBR (Wiseman and others, 1978).

The farmer's first indication of illness was usually one or more animals which were slightly dull and reluctant to eat. Other common signs of disease noticed by the farmers included ocular and/or nasal discharges, a sudden increase in the frequency of coughing, an increased rate and depth of respiration, a reduced milk yield and the drooling of saliva. When they were examined clinically, all affected individuals were found to be pyrexic. There was no relationship between the severity of the dullness and the degree of pyrexia since apparently healthy animals often had temperatures well in excess of 103°F.

The most common clinical sign was profuse serous nasal discharge which later became mucoid or mucopurulent in severe cases. It was not always immediately obvious that a number of cattle did have a serous nasal discharge because affected individuals were constantly licking their noses clean. However, copious amounts of clear discharge were seen when animals lowered their heads to eat or to drink. The nasal mucosae in such cases was obviously congested and frequently small diphtheritic plaques were seen; these plaques later coalesced to produce large areas of diphtheresis. The severity of the nasal lesions together with the presence of marked halitosis, respiratory distress and the fluid tracheal noises which could be heard all over the thorax, confirmed that the "new" form of IBR was considerably more severe than that previously reported (Dawson and others, 1962; Darbyshire and Shank, 1963; Collings and others, 1972). The extent

of the upper respiratory tract and tracheal involvement meant that the degree of pulmonary involvement was difficult to assess critically except in terminal cases.

A moderate to severe conjunctivitis, which resulted in profuse, serous lachrymation developed in a large proportion of the animals in all but 2 of the 15 incidents investigated. The eyelids of severe cases were slightly swollen and congested and this gave white-faced animals the appearance of having a pink "halo" round each eye. Although marked conjunctival oedema was sometimes present in severely affected animals, haemorrhages were never observed either in the field incidents or following experimental infection. It is interesting that this has also been the experience of other workers who have studied the present epidemic in detail (Cuthbertson and Wood, 1979; Imray, 1979a). A central keratitis, with or without ulceration, was not uncommon in these 15 outbreaks. However, this was always considered to have been IBK since the virus of IBR has consistently failed to produce this type of lesion under controlled conditions (McKercher and others, 1955; Webster and Marktelow, 1959; Abinanti and Plumer, 1961; Markson and Darbyshire, 1966). On the other hand, Pugh and others (1964) established that IBR virus infection increases the severity of Morexella bovis infection.

A particularly interesting feature of these recent outbreaks has been the large number of animals which have drooled saliva. While congestion of the oral mucosae was commonly observed, ulceration was never seen. Miller (1955) and McKercher (1959) also reported that the drooling of saliva (excessive salivation) in the absence of oral lesions was a particular feature of some incidents. It is probable that this had resulted from an unwillingness to swallow because of the pain associated with severe pharyngitis and laryngitis. It is also likely to have been the reason for cattle on a few farms tending to reject roughage while continuing to eat concentrates, albeit more slowly.

A marked clinical improvement occurred usually within a week in mildly affected individuals, but it took considerably longer for more severely affected cases which had not been treated. The condition of a small but variable proportion of severely affected animals deteriorated markedly; they became very dull, anorexic and dyspnoeic even at rest.

The type of dyspnoea was unusual in that initially these cases inhaled through their mouths and exhaled through their noses. It was subsequently confirmed at necropsy that the nasal passages and larynx of these animals were almost completely obstructed with necrotic debris. This unusual form of dyspnoea has not been commented upon previously. The vast majority of severely affected individuals died despite almost continual antibiotic therapy. Several animals were found dead following a relatively short illness (less than 48 hours) in the 2 incidents in which the mortality rate was highest. In both outbreaks, the cattle were being fattened under very intensive conditions in slatted floor buildings. Under feedlot conditions in North America apparent sudden deaths have also been reported (Miller, 1955).

The recent syndrome described by Wiseman and others (1978), Cuthbertson and Wood (1979) and Imray (1979a) was considerably more severe than the disease originally described in Britain by Dawson and others (1962) and by Darbyshire and Shanks (1963). The major clinical and epidemiological features of this epidemic were similar to those in the original descriptions of IBR in the United States of America (Shroeder and Moys, 1954; Miller, 1955; McKercher and others, 1957) and the recent outbreaks in continental Europe (Zygraich and others, 1974; Dannacher and Fedida, 1977; Straub, 1978b). Therefore, it can be inferred that the "new" form of IBR in this country has resulted from infection with a particularly virulent strain of virus.

Infection with IBR virus (bovine herpesvirus 1) can produce clinical syndromes other than respiratory disease, e.g. encephalitis, an increased neonatal calf mortality and genital infections. None of these was confirmed in any of the present outbreaks. However, in one incident not described in detail in this thesis, several weaned suckled calves died having developed clinical signs suggestive of encephalitis. It was speculated that IBR virus was involved because many of the other stirks in the same shed had developed characteristic respiratory and ocular signs. However, Haemophilus somnus was isolated on the only occasion that the brain of an affected animal was examined and, on histopathological examination, lesions typical of infectious thromboembolic meningoencephalitis (TEM) were found. Although it was the first occasion that this manifestation of Haemophilus somnus infection had been confirmed in Scotland, IBR and TEM have been reported as occurring in the same group of cattle (Griner, Jensen and Brown,

1956). Indeed, it has even been suggested in North America that IBR and other respiratory infections predispose feedlot cattle to TEM (Kennedy, Biberstein, Howarth, Frazier and Dungworth, 1960).

Abortion has occurred sporadically in pregnant females following the development of classical IBR and also, on a few occasions, in cows which had not developed respiratory signs. On the other hand, abortion storms similar to those which appear to be common in North America (Kahrs, 1977) have not as yet been reported.

The greatest number of new cases usually developed during the second or third week of the incident but, even in a stable population, new cases continued to arise for up to 5 weeks after the first one had been noticed. Although the morbidity rate varied considerably, it was in excess of 90 per cent in most outbreaks. The mortality rate also varied markedly; in several outbreaks, no fatalities occurred whilst in others up to 8 per cent of the animals at risk died or were culled. These morbidity and mortality rates were similar to those reported by McKercher (1959). Until 1977, deaths from IBR virus infection had not been confirmed in Britain, although Collings and others (1972) reported that 3 cows had died following an outbreak of IBR. However, they failed to carry out a detailed postmortem examination in any of these cases.

In the most severe incident discussed here, the financial loss from IBR was estimated by the farmer to have been around £20,000 (Wiseman and others, 1979). However, the value of the animals which had died or had been culled in all 15 incidents, represented only 23 per cent of the total losses. In the 10 beef fattening herds, the most important cost was loss of production during the recovery period. Cattle which appeared to have made a complete clinical recovery did not gain weight for up to 8 weeks; this represented almost two thirds of the financial loss on these units. When it is considered that the average loss sustained on the 10 beef farms was £5,600 compared with an average of only £800 on the 4 dairy farms, it can be appreciated why one farmer was on the verge of a nervous breakdown while a few others considered that a second year of this disease would seriously jeopardise their future in agriculture.

Although IBR has been confirmed in young calves, store cattle, beef suckler cows and in dairy cows, the incidence of disease has been greatest on fattening beef units. The mortality rates have been highest and the economic losses greatest on intensive units into which cattle have been bought regularly through markets. From this it can be deduced that managemental practices play a most significant role in the spread of IBR virus infection and in the expression of its full pathogenic effect.

The crowding together of large numbers of susceptible cattle in a single building facilitates the spread of an infection such as IBR which requires close contact. It has also been suggested that the virulence of this pathogen actually increases when it is introduced into a large, susceptible population (McKercher and Theilen, 1963). Dusty food appeared to have been important too since a hammer milled, barley-based ration had been fed on both farms where the mortality rate was greatest. Food particles were seen adhering to the nasal mucous membranes several centimetres from the nares of animals on these farms. The fact that dust exacerbated the clinical severity of IBR was referred to by Miller (1955) and McKercher (1959).

The greatest single environmental factor was undoubtedly the buying-in of stock from livestock markets; the close association between the purchase of cattle and their developing typical signs of IBR a few weeks later have been commented upon in every publication relating to the recent epidemic (Wiseman and others, 1978; Cuthbertson and Wood, 1979). This association also explains the epidemiological features of severe IBR during the last 2 years; the incidence has been greatest in areas into which the largest number of cattle are brought in to fatten, i.e. the northern and eastern parts of Scotland and England. Also, there is a sudden large increase in the number of cattle sold for fattening during the months of September to December when weaned suckled calves are sold. These animals presumably become infected during their sojourn through market so introducing the infection onto the purchaser's premises. Such animals are subjected to a number of stress factors including weaning, castration, fatigue, fear and transportation and the additive effect of these must be to reduce the weaned suckled calf's resistance to infection; this results in an especially severe form of the disease. As has already been

referred to, the crowding together of animals under one roof enables the IBR virus to spread easily. This explains the seasonal distribution of these severe incidents, for, although the disease occurs in grazing cattle, it is generally acknowledged as being relatively mild. In one of the incidents in dairy cattle described in this thesis, the herd was owned by a cattle dealer who was known to have moved several cows to more than one market. Indeed, one of the animals which died had been sold twice and returned twice before succumbing on the dealers premises to IBR.

The reason for the importance of buying and selling cattle in the spread of IBR virus is not the inefficiency of the marketing organisations but the peculiar characteristics of the causal agent itself. Bovine herpesvirus 1, in common with many other members of the herpesvirus group, has the ability to become "latent" in a host animal recovering from a primary infection.

The exact sites of viral persistance have not been identified nor has the state in which it persists i.e. as complete or incomplete particles. The available evidence indicates that it is likely to be the sensory ganglia of the nerves which innervate the upper respiratory tract and pharynx. Following infection via the intra-nasal route, IBR virus was frequently detected in the mandibular and maxillary branches of the trigeminal nerve (Bagust and Clark, 1972). Narita, Inui, Namba and Shimizu (1978) have established that a possible site is the trigeminal ganglion since ganglionitis developed and the virus was isolated from the ganglion following the treatment of recovered IBR cases with drugs having glucocorticoid activity. The virus subsequently appeared in the Schwann cells, the neuroglial cells and finally in the nasal secretions. The pyrexia which developed following viral reactivation was found to be associated with the appearance of virus on the nasal mucosa and before it was detected in the nasal secretions (Pastoret, Burtonboy, Aquilar-Setien and Schoenaers, 1978; Pastoret, Aquilar-Setien, Burtonboy and Schoenaers, 1978).

The treatment of recovered cases of IBR with glucocorticoid drugs under experimental conditions induces activation and excretion of virus (Kubin, 1969; Bottcher and Mahler, 1970; Sheffy and Rodman, 1973; Bistch, 1973). The virus has also been isolated from recovered cases

following abrupt dietary changes (Chow, Palotay, Deem, 1956; Crane and others, 1964). Therefore, the inevitable stresses, which accompany weaning and marketing, must contribute to a significant degree to the close association between the onset of disease and the introduction of newly purchased animals on to a farm. Individual animals excreting the virus could introduce the infection to the susceptible animals, which are also under a considerable degree of stress.

Reactivation and excretion of IBR virus from recovered cases of disease was achieved in the current studies using both injections of corticosteroids and natural stress factors. In the latter category, a procedure such as dehorning, even under local anaesthetic, resulted in the excretion of virus by 3 of 4 calves (Msolla and Wiseman, 1979). Challenge with variable doses of the cattle lungworm also caused reactivation although the vaccine did not do so. This fact might explain the incidents of IBR which have been said to occur in grazing cattle (Imray, 1979b). It was interesting that the animal from which IBR virus was recovered following ingestion of 1,000 D. viviparus larvae had the lowest serum neutralising antibody titre (1/4). Virus excretion may have occurred in the others too, but the quantity of virus may have been so small as to have been quickly neutralised by the greater amount of circulating antibody. IgG is considered to play a role in recovery from secondary or tertiary herpesvirus infections but not from primary infections (Rouse and Babiuk, 1978). Recovery from a primary infection is said to be mainly due to cell mediated immunity (Rouse and Babiuk, 1978). The high mortality rate in cases given corticosteroids can therefore be explained since this type of drug is known to inhibit to a significant degree the development of cell mediated immunity.

Since the IBR virus is a herpesvirus and latent carrier animals develop following primary infection, eradication of the disease is not possible even if the necessary huge financial resources were available. Experience from North America suggests that economically significant losses would continue to occur annually on units on which large numbers of purchased cattle were fattened. Consequently, effective control measures are required. A system based on daily physical examination of individual animals and administering antibiotic therapy to every one found to be pyrexic, has been shown to minimise the clinical effects of IBR (Imray, 1979a). Clearly, this type of approach

is not feasible on farms where there is a large turnover of animals. Unfortunately the only vaccine available, until October, 1979 (Pneumovac Plus) did confer a detectable degree of protection to susceptible cattle challenged with the most recent isolate of the IBR virus (Strichen strain).

It has been shown in the United States that inactivated vaccines are of limited use in controlling IBR in feedlots and consequently, control of this disease on this type of beef unit is based upon the use of modified live virus vaccines (Kahrs, 1977). However, the abortifacient property of this type of vaccine precluded their use in breeding females. A new type of live vaccine was developed in Belgium in response to the wave of severe IBR which swept across north west Europe (Zygraich, Huygelen and Vascoboinic, 1974). This vaccine has been tested extensively under laboratory and field conditions both in Belgium and in the United States of America. It has been proven to be safe in that it does not produce abortion and to be effective in providing protection from challenge with field strains of the IBR virus. A further advantage is that protection is afforded within 48-72 hours of vaccination because this vaccine is given intranasally. In the latter half of 1979, in response to increasing pressure from the agricultural lobby, the Minister of Agriculture agreed to license this new vaccine (Tracherine: Smith Kline Animal Health Ltd., England) for use in Britain. It is the first live bovine virus vaccine licensed for use in this country.

The vaccine strain of IBR virus can be differentiated from other strains because of its temperature specific marker. However, field isolates cannot be differentiated one from another using routine laboratory techniques. When susceptible cattle were exposed intranasally to the Strichen strain, they developed typical clinical signs 2-3 days later. The experimental clinical syndrome was most severe 4-5 days post infection but by 14 days after exposure the animals appeared to have recovered completely. The Strichen strain produced a slightly more severe clinical and pathological syndrome than did the Colorado strain. However, both these strains produced a considerably more severe disease than did the Oxford strain. Although the experimental disease produced by all 3 strains was less severe than the equivalent naturally occurring disease, the results reflected the

respective pathogenicity of these strains in the field. The fact that the clinical severity was negatively correlated with age agrees with the field evidence, although the effect of stress factors on the pathogenicity must be borne in mind because they may very well be the dominant factors. In addition, the Strichen strain also produced IPV so agreeing with Straub's observation that the "newer" respiratory strains of BHV 1 will produce both respiratory and genital disease (Straub, 1978b).

When the physical characteristics of the Strichen strain were compared with those of the well established Colorado and Oxford strains, it was found that their one-step growth curves and plaque morphology were identical. However, the mean plaque diameter of the Strichen and Colorado strains was significantly greater than that of the Oxford strain. This would suggest a possible relationship between plaque size and virulence for IBR virus since this association has been confirmed with other animal viruses. Furthermore it has been found that the Strichen strain is less susceptible to neutralisation with both homologous and heterologous antisera than either of the other 2 strains examined. Therefore, the ability of this latest isolate to persist for a relatively longer period, even in the presence of specific neutralising antibodies, is likely to be a significant contributing factor to its ability to produce severe disease.

The Strichen strain of IBR virus would appear to have been in Britain for only a very short time. It is highly unlikely that it was carried over here from the continent by migrating birds as has been suggested for other diseases, e.g. foot and mouth disease. It is possible that it developed following the mutation of a domestic strain although this does not appear to be a common characteristic of the herpesviridae (Gibbs and Rweyemamu, 1977). The recent severe outbreaks of IBR in Europe appeared in certain areas only and in herds clustered round those into which Holstein cows from North America had been introduced. Hence, the name for this disease in Belgium "la grippe Canadienne" (Canadian 'flu). Serological studies undertaken by French workers have confirmed that the prevalence of antibodies to IBR virus is much higher in Holstein cattle imported from North America than in cattle imported from elsewhere or in domestic French cattle (Dannacher and Fedida, 1978). They claim that the virus responsible

for the recent wave of severe IBR in Europe was imported with Holstein cattle from North America.

Holstein cattle imported in Britain during the last decade have been blamed for introducing enzootic bovine leukosis (Watson, 1979) and Mycoplasma bovis infection (Wilson, 1979). Several dairy herds in the Grampian region contained Holstein cattle and the percentage of seropositive animals in these herds was considerably greater than that in the other dairy herds in that region. Since the proportions of positive animals was almost identical to those found by the French workers (Dannacher and Fedida, 1977), it is likely that the Strichen strain of IBR virus had also been imported into Britain with female Holstein cattle.

These serological investigations also revealed that there had been a marked increase in recent IBR virus infections in the Grampian region since the incidence of antibodies in dairy heifers was significantly greater than that in the dairy cows. Furthermore, it was also significantly greater than the incidence in dairy cows and heifers in the Strathclyde region. This could reflect the higher incidence of field disease which had occurred in the Grampian region even though this was mainly a feature of fattening cattle operations. Furthermore, the overall figure of 12 per cent of animals with antibodies is considerably in excess of the initial findings of Dawson and others (1962) and also those of Kirby and others (1977) bearing in mind that the latter workers claimed that their test system (IHT test) was 10 times as sensitive as the serum neutralisation test which was used here.

It has been confirmed, as a result of this multidisciplinary investigations, that a severe form of IBR is present in Britain. The disease has been most severe on intensive beef units in which large numbers of purchased cattle are regularly fattened. The strain of virus responsible, the Strichen strain, has properties which are significantly different from those of the prototype British strain (the Oxford strain) and these specific characteristics could be responsible for its tremendous virulence. The source of the Strichen strain of IBR virus would appear to have been imported Holstein cattle although the evidence for this deduction is wholly circumstantial. The appearance of a severe form of IBR in the British Isles soon after other diseases had

been confirmed in animals imported into this country, highlights the need for constant vigilance and awareness of the dangers to the British livestock industry as a whole, of importing live animals which have not been shown to be free from specific, exotic infections.

APPENDIX 1

OUTBREAK I

This outbreak has been described in detail in Chapter II.

OUTBREAK 2

DATE November 1978.

FARMER'S COMPLAINT

A gradual drop in food consumption and "pneumonia" in most of the recently housed fattening stirks. They had runny eyes and runny noses.

ANIMALS AT RISK

280, 12-18 months old fattening stirks.

109 homebred stirks.

37 bought-in from a neighbour.

134 bought-in stirks.

HUSBANDRY SYSTEM

The animals were housed in a large slatted floor shed which was subdivided into eight pens, four on either side with a central feeding passage. The ration consisted of a mixture of home milled maize, biscuit meal, barley as well as molasses, oat seconds, protein balance and romensin (Elanco Ltd. - Hants, England). This mixture was generally dusty. There was communal watering facility between pens.

HISTORY OF INCIDENT

4 - 8 weeks after the farmer had purchased several groups of animals from various markets, he noticed that they were dull and food consumption was dropping gradually. There was sporadic coughing and affected individuals developed bilateral serous ocular and nasal discharges. There was increased respiratory rates.

CLINICAL FEATURES

Dullness. Reduced appetite. Temperature (104.5-106^{o}F). Purulent ocular discharge. Conjunctivitis. IBK present in several animals but no change of incidence from previous years. Seromucoid to mucopurulent nasal discharge. Congested nasal mucosa without obvious lesions. Coughing. Tachypnoea. Dyspnoea and pneumonia in severely affected animals. Drooling saliva. Neither diarrhoea nor nervous signs were seen.

Marked weight loss in protracted cases (4-5 weeks).

Morbidity - 80%. Mortality - 7%. Several animals found dead after being ill for a short time. Several salvaged.
Duration of clinical signs - 4 weeks.
Treatment with various antibiotics gave a poor response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATIONS

Nasal swabs - 7/8 IBR +ve
Ocular swabs - 2/2 IBR +ve

SEROLOGY

Acute samples - Not available.
Convalescent samples - 21/42 seropositive.
Reciprocal titres - 4,4,6,6,6,6,6,6,8,8,8,8, 8,12,12,12,12,16,24,48, 96.
Mean titre - 14.8

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION (Summary of six cases)

Dullness. Reduced appetite. Temperature (105°F).
Purulent ocular discharge. Severe conjunctivitis.
No IBK. Bilateral seromucoid nasal discharge. Diphtheritic lesions in nasal mucosa. Coughing. Tachypnoea. Dyspnoea. Drooling saliva. No diarrhoea. No nervous signs. Marked weight loss.
Duration of illness - 2 weeks.
Treated with antibiotics - poor response.

POSTMORTEM FINDINGS

Turbinates	-	congestion, rhinitis
Nasopharynx	-	severe pharyngitis
Larynx	-	severe laryngitis
Trachea	-	congestion and haemorrhages. Tracheitis and covered with a yellowish diphtheritic membrane.
Lungs	-	consolidation of the cranial lobes. Severe exudative pneumonia. Interstitial emphysema.
Abomasum	-	a few haemorrhagic spots in the pylonic region.
Kidneys	-	infarcts present
CNS	-	normal

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	-ve	Pasteurella haemolytica Proteus sp.	ND
Ocular swabs	ND	ND	ND
Turbinates	IBR +ve	ND	ND
Nasopharynx	IBR +ve	ND	ND
Trachea	IBR +ve	Pasteurella haemolytica	M.dispar M.bovirhinis
Lung	-ve	Neisseria spp.	M.dispar M.bovis

OUTBREAK 3

DATE November 1978.

FARMER'S COMPLAINT

Coughing, profuse salivation, tachypnoea and bilateral serous nasal discharge in a group of recently purchased stirks.

ANIMALS AT RISK

150 weaned suckled calves and bullocks.

HUSBANDRY SYSTEM

The animals were housed in three separate courts in one building. They were fed bruised barley, silage and hay. Store animals are purchased and fattened during winter. Each court had its own water trough.

HISTORY OF INCIDENT

Clinical signs developed about four weeks after the first group of purchased animals had been introduced.

CLINICAL FEATURES

Dullness. Anorexia. Temperature (103.5-107°F). No ocular discharge. IBK present in several animals, but more cases this year than previous years. Serous to mucopurulent nasal discharge with diphtheritic lesions in the nasal mucosa. Coughing. Tachypnoea. Dyspnoea. Stertorous respirations. Drooling saliva. A few severe cases. No nervous signs. Marked loss of body weight.
Morbidity - 90%. Mortality - 6.7%.
Duration of clinical signs - 4 weeks.
Treatment with antibiotics and corticosteroids gave variable response. Animals were treated for a few days and then put outside to recover.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATIONS

Nasal swabs - 5/5 IBR +ve
Ocular swabs - ND

SEROLOGY

Acute samples - Not available.
Convalescent samples - 17/17 seropositive
Reciprocal titres - 8,8,12,12,12,12,12,12,12,16,16,24,24,24,24,24,96.
Mean titre - 20.5

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION

Dullness. Reduced appetite. Temperature (106°F). Slight serous ocular discharge. Mucopurulent nasal discharge. Diphtheritic lesions on nasal mucosa. Coughing. Tachypnoea. Dyspnoea. Drooling saliva.
Duration of illness - 3 weeks.
Treated with antibiotics - poor response.

POSTMORTEM FINDINGS

Turbinates	-	severe congestion and rhinitis
Nasopharynx	-	pharyngitis with widespread haemorrhages
Larynx	-	laryngitis. Yellowish diphtheritic material on the larynx, causing obstruction
Trachea	-	tracheitis, widespread haemorrhages and yellowish necrotic debri covering the whole length of the trachea
Lungs	-	consolidation of the middle and cranioventral aspect of the diaphragmatic lobe, and covered by fibrinous pleurisy. There were thrombi in the large vessels.
Abomasum	-	congested and oedematous
Kidneys	-	several infarcts
CNS	-	normal

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	IBR +ve	ND	ND
Ocular swabs	ND	ND	ND
Turbinates	-ve	B.alvei	ND
Nasopharynx	IBR +ve	ND	ND
Trachea	IBR +ve	Acinetobacter lwoffi Neiserria flavescens	ND
Lung	-ve	Pasteurella multocida	M.dispar

OUTBREAK 4

DATE January 1978.

FARMER'S COMPLAINT

In early January recently purchased bullocks developed frequent coughing and "bad eyes".

ANIMALS AT RISK

400 feeding cattle - 100 home-bred, 300 brought-in. Also 70 winter calving beef suckler cows and calves and another group of 40 spring calving beef suckler cows.

HUSBANDRY SYSTEM

The 400 feeding cattle were housed in a large slatted floor shed with a central feeding passage. Weaned suckled calves and stirks are bought in the autumn and sold fat during the winter or as forward stores in the spring.
The group of 40 cows were under the same roof, but separated from the 400 feeding cattle by a wall and a gap of 5 metres. The winter calving cows and calves were in a cubicle house on the other side of the farm. The animals were fed a mixture of barley and silage and also hay. There were communal water troughs between pens.

HISTORY OF INCIDENT

About 6 weeks after a group of Friesian bullocks had been purchased, they developed ocular/nasal discharges and were heard to cough frequently. Animals in adjacent pens then became affected as did the animals on the other side of the feeding passage. One group of 55 stirks were moved into a vacant pen in the slatted floor house, 10-14 days before the first farm visit and they too became infected. Clinical signs of IBR were not seen in either of the two groups of cows.

CLINICAL FEATURES

Dullness. Drop in food consumption by about 50% for 3-4 days. Temperature (104-106°F). Seromucoid ocular discharge. Conjunctivitis. IBK - present but no obvious increase in new cases. Bilateral serous to mucoid nasal discharge. Frequent coughing. Tachypnoea. Pneumonia in severely affected animals. Only a few were drooling saliva.

Diarrhoea present in one animal which died from mucosal disease.

There was no obvious weight gain for 4-6 weeks. No nervous signs.

Morbidity - 50%. Mortality - 1%.

Duration of clinical signs - 6 weeks.

Treatment with various antibiotics gave a poor response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

Six cows 8 months in-calf aborted within one week, 6 weeks after the IBR outbreak began.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATIONS

Nasal swabs	-	5/5 IBR +ve
Ocular swabs	-	2/2 IBR +ve

SEROLOGY

Acute samples	-	not available
Convalescent samples	-	20/25 seropositive
Reciprocal titres	-	6,6,6,8,8,8,8,8,8, 12,12,12,12,12,12, 16,16,24,24,24.
Mean titre	-	12.1

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION

Bright. Reduced appetite. Temperature (103.5°F).
Ocular discharge. Severe conjunctivitis.
Bilateral serous nasal discharge. Diphtheritic lesions in nasal mucosae. Dyspnoea. Respiratory stertor. Drooling saliva.
Duration of illness - 4 weeks.
Treated with antibiotics - poor response.

POSTMORTEM FINDINGS

Turbinates	-	normal
Nasopharynx	-	normal
Larynx	-	necrotising laryngitis
Trachea	-	congestion and tracheitis
Lungs	-	oedematous and consolidation of the anterior lobes. Many small haemorrhages in non-pneumonic areas. Extensive interstitial emphysema.
Abomasum	-	normal
Kidneys	-	normal
CNS	-	normal.

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	-ve	ND	ND
Ocular swab	-ve	ND	ND
Turbinates	-ve	ND	ND
Nasopharynx	-ve	Proteus sp.	A. laidlawii
Trachea	-ve	Proteus sp.	A. laidlawii
Lung	-ve	Proteus sp.	A. laidlawii

OUTBREAK 5

DATE January 1979.

FARMER'S COMPLAINT

Anorexia, tachypnoea, ocular and nasal discharges.

ANIMALS AT RISK

120 bulling and in-calf heifers.
180 milking cows and heifers.
55 calves. 4 bulls.

HUSBANDRY SYSTEM

The heifers had been served outside and then housed in a slatted floor building in October 1978. This bull was then sold because a number of heifers in the group in which this trouble started were not in-calf. The milking cows were in 2 groups in cubicles under one roof with a central feeding passage. Both silage and hay were available. The heifer and cow accommodation was separated by about 30 yards.

HISTORY OF INCIDENT

8 heifers became severely ill and were treated over 2 days during the first week in January. Others subsequently were heard to cough and developed ocular and nasal discharge. The oldest animals were those worst affected. During the first week in February, a cow was also seen to be ill and over the next 3 weeks another 10 cows from both sides of the shed were treated. Only 1 cow was treated twice.

CLINICAL FEATURES

Slight dullness. Reduced appetite. Temperature (104.5 - 106.0°F). Purulent ocular discharge. Mild to moderate conjunctivitis. IBK present but no increase in incidence. Profuse serous nasal discharge without obvious nasal lesions. Coughing. Wheezing respirations in cows. Tachypnoea. Hyperpnoea. A few animals drooling saliva. Decreased milk yield by 3-4 gallons but came back to normal production after recovery. One heifer aborted. Low conception rates (50%) to first service with animals returning to the bull after 6 weeks. Diarrhoea was observed in one cow.

No nervous signs. Two calves (10 days old) affected and showed conjunctivitis and bilateral purulent nasal discharge. Morbidity in heifers - 80%, cows - 50%. One calf died 4 weeks after becoming blind.
Duration of clinical signs - 6 weeks.
Treatment with Penicillin and/or Terramycin for 3 consecutive days gave good response.
4-5 years ago, IPV was diagnosed in the herd.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATIONS

Nasal swabs - 6/9 IBR +ve
Ocular swabs - 1/1 IBR +ve

SEROLOGY

Acute samples - not available.
Convalescent samples - 19/36 seropositive.
Reciprocal titres - 4,6,6,6,6,6,8,8,12,12, 12,12,12,12,12,12,12, 16,16.
Mean titre - 10.0

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

No individual animal was available for further detailed studies.

OUTBREAK 6

DATE February 1979.

FARMER'S COMPLAINT

Ocular and nasal discharge in recently bought-in animals, which were not eating roughage.

ANIMALS AT RISK

100 store cattle 6-8 months old.

HUSBANDRY SYSTEM

Store cattle are purchased during the winter, housed in cubicles, fed hay and silage and fattened at grass the following summer.

HISTORY OF INCIDENT

Three days after being purchased 3 of 14 stirks were seen to be ill. The disease spread slowly to involve the other four groups during the following four weeks.

CLINICAL FEATURES

Slight dullness. Reduced appetite - particularly for roughage. Temperature (106-107^{o}F). Mucopurulent ocular discharge. Conjunctivitis. No IBK. Bilateral mucopurulent nasal discharge. No obvious nasal lesions in the nasal mucosae. Frequent coughing. Tachypnoea. Respiratory stertor. No salivation. No diarrhoea. No nervous signs.

Morbidity - 90%. Mortality - 0%.

Duration of clinical signs - 4-5 weeks.

Treatment with oxytetracycline gave a fair clinical response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATIONS

Nasal swabs - 3/5 IBR +ve

Ocular swabs - Not done.

SEROLOGY

Acute samples - 4/16 seropositives.

Reciprocal titres - 4,6,8,16.

Convalescent samples - 14/16 seropositives.

Reciprocal titres - 4,6,6,8,12,12,12,12, 12,16,24,24,48,96.

Mean titre - 20.9

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

No individual sick animal was purchased for further detailed studies.

OUTBREAK 7

DATE January 1979.

FARMER'S COMPLAINT

Dullness, anorexia, runny eyes and runny nose in 2 recently purchased weaned calves.

ANIMALS AT RISK

20 large bullocks purchased during October/November
7 stirks purchased on 15th December
6 weaned suckled calves purchased 28th December.

HUSBANDRY SYSTEM

Store cattle are bought and fattened. The 2 groups of 13 and 20 animals were kept on straw in 2 courts under the same roof but separated by a silage pit.

HISTORY OF INCIDENT

The 6 weaned calves had come from the Western Isles and when purchased, they were being sold for the second time. Nine days after their arrival, 2 of the 6 calves became ill. The other 11 animals all subsequently developed mild clinical signs.

At the same time the bullocks in the second court were heard to be coughing frequently, although other clinical signs were not seen.

The dealer from whom the 6 weaned calves were bought had a similar syndrome affect his own cattle at the same time.

CLINICAL FEATURES

Slight dullness. Selective anorexia; animals eating barley and silage mix, but not hay. Temperature (103-105^{o}F). Seromucoid ocular discharge. Conjunctivitis. One or two animals had conjunctival oedema. White faced animals had obvious 'red-eyes'. IBK - 1 to 2 cases, incidence less than previous years. Seromucoid nasal discharge. Coughing. Drooling saliva. One heifer aborted - not Brucellosis. As a group, there was no weight-gain for 4 weeks.

Morbidity - 100%. Mortality - 8%.

Duration of clinical signs 3-4 weeks.

Treatment - with oxytetracycline and etamiphylline camsylate for 3 days: One animal - good clinical response while the other had no detectable clinical response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATION

Not done.

SEROLOGY

Acute - not available.

Convalescent - 5/12 seropositive.

Reciprocal titres - 8,12,16,24,24.

Mean titre - 16.8

CLINICAL FINDINGS ON ADMISSION

The animal was dead on arrival.
Duration of illness - 3 weeks.

POSTMORTEM FINDINGS

Turbinates	-	Rhinitis.
Nasopharynx	-	Pharyngitis.
Larynx	-	Very severe obstructive laryngitis with yellowish-brown necrotic material.
Trachea	-	Severe tracheitis with haemorrhagic necrosis.
Lungs	-	Consolidation of the cranial lobes. No emphysema present.
Abomasum	-	Normal
Kidneys	-	Normal
CNS	-	Normal

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	IBR +ve	ND	ND
Ocular swabs	ND	ND	ND
Turbinates	IBR +ve	ND	ND
Nasopharynx	IBR +ve	ND	ND
Trachea	IBR +ve	ND	ND
Lung	-ve	ND	ND

OUTBREAK 8

DATE January 1978.

FARMER'S COMPLAINT

A sudden drop of 2-3 gallons in milk yield in the high yielding cows and 1-2 gallons in the low yielders, together with frequent coughing and profuse serous nasal discharge.

ANIMALS AT RISK

105 Friesian heifers and cows, 41 in-calf and bulling heifers, 30 calves and 4 bulls.

HUSBANDRY SYSTEM

The milking cows and the young heifers were loose-housed in kennels adjacent to each other and separated at several points by ordinary gates. Silage was also fed to the cows in troughs because the face of the self-fed pit was relatively small. The heifers were not fed near the cows.

HISTORY OF INCIDENT

On 13th January the first signs of disease were seen in a recently calved heifer and during the following 3 weeks virtually all the lactating animals became affected.

In-calf heifers had been grazed away during the summer (1977) but 2 bullocks from an adjacent field had grazed with them for 2 weeks. They came home in mid-November but they were not housed until mid-December. Only 3-4 of the animals developed mild clinical signs.

Bulls - one of the immature bulls was taken to a local market on 11th January and brought home again.
Another which had been to another farm on loan returned in August.
A third young one had been purchased 9 months prior to the incident.
The bulls which were being used did show mild respiratory signs towards the end of the incident. None of the calves were considered to have been affected.

CLINICAL FEATURES

Slight dullness. Selective anorexia for 3 weeks; animals eating concentrates, but not silage. Temperature (104-106°F). Serous ocular discharge. Oedematous conjunctivitis. No IBK. Copious bilateral serous nasal discharges seen hanging 12-14 inches down from the animal's nose. No obvious nasal lesions. Frequent non-productive coughing. Tachypnoea. Hyperpnoea. A few animals drooling saliva. Two animals diarrhoeic.

In 1977-78 three animals, all served by one bull, aborted - not Brucellosis.

In 1978-79 two heifers and two cows aborted - not Brucellosis. Leptospiral infection diagnosed. A total of 8 heifers had difficulty in settling and two aborted. Drop in milk yield. Nervous signs and calf mortality - not detected. Morbidity - 100% in lactating animals. Mortality - about 2%. Duration of clinical signs - 4 weeks.

Treatment with antibiotics gave poor response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATION - HERD

VIRUS ISOLATION

Not done.

SEROLOGY

Acute samples - not available.

Convalescent samples - 6/83 seropositive.

Reciprocal titres - 6,6,8,12,12,16.

Mean titre - 10.0

FURTHER INVESTIGATION - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION

Slightly dull. Anorexia. Temperature (104.5°F).
Bilateral serous ocular discharge. Conjunctivitis. No IBK.
Bilateral serous nasal discharge. Diphtheritic lesions in nasal mucosae. Coughing. Tachypnoea (RR = 60/min).
Hyperpnoea. Drooling saliva. One small pustule in vagina.
Pustular dermatitis of udder and hindquarters. No diarrhoea.
No nervous signs.
Duration of illness - 3 weeks.

POSTMORTEM FINDINGS

Turbinates	-	rhinitis with numerous haemorrhages 0.5 - 1.0cm throughout the nasal passages.
Nasopharynx) Larynx) Trachea)	-	congestion and numerous haemorrhages.
Lungs	-	congestion and numerous haemorrhages.
Abomasum	-	congestion around the pylonic region and many petechial haemorrhages.
Kidneys	-	grossly enlarged, congested, many pale-yellowish areas and numerous petechial haemorrhages.
CNS	-	normal.

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	ND	ND	ND
Ocular swabs	ND	ND	ND
Turbinates	ND	ND	ND
Nasopharynx	IBR +ve	-ve	ND
Trachea	IBR +ve	-ve	ND
Lung	-ve	-ve	ND

OUTBREAK 9

DATE June 1978.

FARMER'S COMPLAINT

A cow was seen to be dull, to have a bloody discharge on her tail and a scabby nose.

ANIMALS AT RISK

33 single suckler beef cows, 35 calves and 18 stirks.

HUSBANDRY SYSTEM

The cows calved from January to March inside, in a cubicle house. Hay and silage are fed and the cows have access to one side of a large trough. On the other side, which was a slatted floor area, were housed his own 1977 weaned calves together with 50 bought-in weaned calves which had been purchased in November.

HISTORY OF INCIDENT

On 5th June one cow had been seen to be standing by herself, and to have a bloody discharge on her tail. She was thought to have calved 6-8 weeks previously. During the 1977 winter housing period, neither ocular or nasal discharges had been seen in any of the different age groups. However, just prior to the animals being put outside in May, there was a big increase in the amount of coughing in the weaned calves only.

CLINICAL FEATURES

Slight dullness. Bilateral serous ocular discharge. Conjunctivitis. IBK - present and incidence higher than in previous years. Bilateral seromucoid nasal discharge. Diphtheritic lesions in nasal mucosae - affected cow had a few haemorrhagic lesions in addition to diphtheresis in the nasal mucosae. Coughing was a major feature in all the affected animals, while as a group, frequent coughing was the only clinical feature. Anorexia, diarrhoea, nervous signs, salivation and abortion - not detected.
Morbidity - 10%. Mortality - 0%.
Duration of clinical signs 3-4 weeks.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATION

Not done.

SEROLOGY

Acute samples - not available.

Convalescent samples - 9/86 seropositive.

Reciprocal titre - 4,4,6,6,6,6,6,8 and 192.

Mean titre - 26.4

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION

Dullness. Anorexia. Temperature (104.5°F). Serous ocular discharge. Conjunctivitis. No IBK. Mucopurulent nasal discharge. Diphtheritic lesions in the nasal mucosae. Haemorrhagic lesions in the nares. Frequent coughing. No drooling of saliva. Diarrhoea, nervous signs - not seen.
Duration of illness - 3 weeks.
Treatment was not attempted.

POSTMORTEM FINDINGS

Not done.

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	IBR +ve	ND	ND
Ocular swabs	IBR +ve	ND	ND
Turbinates	ND	ND	ND
Nasopharynx	ND	ND	ND
Trachea	ND	ND	ND
Lung	ND	ND	ND

OUTBREAK 10

DATE January 1978.

FARMER'S COMPLAINT

Recently purchased animals went off their food, began to drool saliva, their rate and depth of respiration increased and some were heard to snore.

ANIMALS AT RISK

About 300 bullocks and heifers of various breeds aged approximately 10-15 months.

HUSBANDRY SYSTEM

Forward stores are bought in regularly to be fattened in about 8 weeks on a barley/protein mix. They were loose housed in one shed with a central feeding passage and bedded in straw. During January and February about 300 cattle had been sold and an equivalent number bought-in. Water troughs were shared between pens.

HISTORY OF INCIDENT

After Christmas 1977, clinical signs appeared in a group of recently purchased animals from 10 days to 3 weeks after their arrival. Later animals in almost every group became affected.

CLINICAL FEATURES

Dullness. Anorexia. Temperature (103.5-106°F). Serous to mucoid ocular discharge. Conjunctivitis. IBK - a few cases but no obvious increase in incidence. Bilateral mucoid to mucopurulent nasal discharge. Diphtheritic lesions in nasal mucosae. Coughing. Tachypnoea (RR = 50-60/min). Hyperpnoea. A few cases had rhonchi on the cranio-ventral aspect on the right side of the thoracic cavity. In a few cases, fluid sounds were heard in the trachea. Slight subcutaneous emphysema over the thoracic area. Drooling saliva very common. Diarrhoea only in one animal. Marked weight loss in severely affected animals. No abortions. No nervous signs.

Morbidity - 90%. Mortality - 4%.

Duration of clinical signs - 8 weeks.

Treatment of 30 cases with oxytetracycline - fair response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATION - HERD

VIRUS ISOLATION

Nasal swabs - 1/5 IBR +ve

Ocular swabs - not done

SEROLOGY

Acute samples - 4/4 (100%) seropositive.

Reciprocal titres - 12,12,16,48.

Convalescent samples - 3/3 (100%) seropositive.

Reciprocal titres - 12,12,12.

Mean titre - 12.0

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION

Dullness. Anorexia. Temperature (104.2°F). Bilateral mucopurulent nasal discharge. Diphtheritic lesions in nasal mucosae. Coughing. Tachypnoea (RR = 50/min). Fluid sounds in the trachea. Slight subcutaneous emphysema over the thoracic area. Animal lame on the left rear fetlock joint. Marked weight loss.
Duration of illness - 3 weeks.

POST MORTEM FINDINGS

Turbinates - rhinitis with a lot of necrotic debri in nasal cavity.

Nasopharynx), Larynx), Trachea) - diffuse pinkish congestion and covered with a frothy mucopus.

Lungs - gross severe pneumonia with widespread adhesions to the thoracic wall and diaphragm.
L. lung: multiple areas of suppuration.
R. lung: enlarged, with an area of coagulative necrosis. There was thrombosis of blood vessels as well as interstitial emphysema in non-pneumonic diaphragmatic lobes.

Abomasum - severe ostertagiasis.

Kidneys - renal infarcts.

CNS - normal

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	IBR +ve	ND	ND
Ocular swabs	ND	ND	ND
Turbinates	-	-	-
Nasopharynx	IBR +ve	-	-
Trachea	-	P.haemolytica	-
Lung	-	-	-

OUTBREAK 11

DATE April 1977.

FARMER'S COMPLAINT

Hyperpnoea and coughing in a recently purchased heifer.

ANIMALS AT RISK

53 Ayrshire cows, 3 heifers and 1 bull.

HUSBANDRY SYSTEM

The cows were tied in stalls, in a single byre extending along 3 sides of a square. The 3 heifers and bull were in a separate byre away from the milking cows.

HISTORY OF INCIDENT

Two newly calved heifers were bought-in to replace 2 cull cows. A few days later they became anorexic, pyrexic and were seen to be hyperpnoeic and coughing. Two weeks later the cows in the adjacent stalls developed clinical signs. During the next 3 weeks the disease spread slowly but relentlessly along the byre.

CLINICAL FEATURES

Slight dullness. Reduced appetite. Temperature (104-107.5°F). No increase in IBK cases. Bilateral serous nasal discharges but no obvious nasal lesions. Frequent coughing - often productive, resulting in mucus or heavily blood stained mucus being seen on the walls in front of the cows. A few animals drooling saliva. Marked drop in milk yield. Transient diarrhoea in a few cases. Ocular discharge, conjunctivitis, nervous signs, abortion - not seen.

Morbidity >90%. Mortality - 0%.

Treatment of 2 original animals with oxytetracycline - good response.

None of the three heifers and bull were affected.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATION

Not done.

SEROLOGY

Acute samples - 6/51 seropositive.

Reciprocal titres - all >2

Convalescent samples - 42/51 seropositive.

Reciprocal titres - 2-128

Mean titre - Not possible to work-out because only a few samples were assayed.

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

No individual sick animal was purchased for further detailed studies.

OUTBREAK 12

DATE February 1979.

FARMER'S COMPLAINT

Animal slow to come to feeding trough.
Dullness, anorexia, drooling saliva, ocular discharge.

ANIMALS AT RISK

64 bullocks about 8 months old.

HUSBANDRY SYSTEM

Store cattle are bought-in regularly throughout the year to be fattened. The winter accommodation consists of 2 inside sheds, each holding 40 animals, and 2 self-feed silage pits.
Animals were fed on silage and barley.
Water troughs - shared between pens.

HISTORY OF INCIDENT

Three weeks after the introduction of a group of purchased bullocks, one animal was seen to be ill. It was not one of those most recently purchased. During the next 4 days, a further 14 bullocks developed severe clinical signs. All those ill on the first 2 days were on the same side of the unit.

CLINICAL FEATURES

Dullness. Reduced appetite. Temperature (104-107°F). Serous ocular discharge. Conjunctivitis. Occasional coughing. Tachypnoea. Hyperpnoea. Harsh respirations. Respiratory stertor and tracheal fluid sounds. Bilateral mucopurulent nasal discharges. No obvious nasal lesions. Profuse frothy salivation. No diarrhoea. No nervous signs as such although severely affected individuals shook their heads frequently.
Morbidity - about 90%. Mortality - 1 animal culled (1.4%).
Duration of clinical signs - 1 week.
Treatment with either chlortetracycline or oxytetracycline - good response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATION

Nasal swabs - 5/6 IBR +ve
Ocular swabs - 2/2 -ve

SEROLOGY

Acute samples - 8/46 seropositive.
Reciprocal titres - 4,6,6,8,12,16,16,24.

Convalescent samples - 40/46 seropositive.
Reciprocal titres - 4,6,6,6,6,8,8,8,8,8,8,8,
8,12,12,12,12,12,12,12,12,
12,12,12,12,12,12,12,12,12,
12,16,16,24,24,24,24,24,32
and 96.
Mean titre - 14.7

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION

Dullness. Anorexia. Temperature (106.5°F). Serous ocular discharge. No conjunctivitis. No IBK. Bilateral serous to mucoid nasal discharge. No lesions in nasal mucosae. No coughing. Drooling saliva. Diarrhoea.
Nervous signs - not detected.
Animal had been ill for ten days.
Treatment with oxytetracycline poor response.

POSTMORTEM FINDINGS

Turbinates - rhinitis.
Nasopharynx - severe pharyngitis.
Larynx - severe laryngitis.
Trachea - severe tracheitis.
Lungs - extensive pneumonia with much interstitial emphysema. Some of the pneumonic lesions were necrotic. There were several large pulmonary haemorrhages.
Abomasum - normal.
Kidneys - pale and infarcts present.
CNS - normal.

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swab	ND	ND	ND
Ocular swab	ND	ND	ND
Turbinates	ND	ND	ND
Nasopharynx	ND	Streptococcus faecalis	M. bovis
Trachea	ND	Aerococcus viridans	M. bovis
Lung	ND	Micrococcus sp. Actinobacillus lignieresii	M. bovis

OUTBREAK 13

DATE March 1979.

FARMER'S COMPLAINT

Coughing, tachypnoea, runny eyes and runny nose in recently purchased animals.

ANIMALS AT RISK

130 feeding cattle 8-12 months old.

HUSBANDRY SYSTEM

Store animals are purchased and fattened in a slatted floor house with a central feeding passage. Hay is fed together with a home-made meal based on barley. This was fine but not very dusty.

HISTORY OF INCIDENT

About 10 days after a group of 52 bullocks was housed, several animals were seen to be ill. Other purchased groups have been introduced and the disease has slowly spread to involve them as well as older animals already in the shed.

CLINICAL FEATURES

Slight dullness. Selective appetite, eating hay and leaving concentrates. Temperature (105-106°F). Mucopurulent ocular discharges. Conjunctivitis. IBK became apparent after IBR was confirmed and 12 animals had severe lesions. Bilateral mucopurulent nasal discharges. A few individuals had diphtheritic lesions in nasal mucosae. Coughing. Tachypnoea. Hyperpnoea. Several animals had respiratory stertor. Drooling of saliva not common. Marked weight loss. No diarrhoea. No nervous signs.
Morbidity - 90%. Mortality 5%.
Duration of clinical signs - 5 weeks.
Treatment with oxytetracycline - variable response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATION

Nasal swabs - 4/5 IBR +ve

Ocular swabs - Not done.

SEROLOGY

Not done.

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION

Dullness. Anorexia. Temperature (101.0°F). Purulent ocular discharge. Congested conjunctival mucosae. No IBK. Severe halitosis. Bilateral mucopurulent nasal discharge. Diphtheritic lesions in nasal mucosae. Blood streaks in the nares. Coughing frequently. Tachypnoea. Harsh respirations. Rhonchi and crackles on the anterior ventral aspect of both sides of the thoracic cavity. Not drooling saliva. No diarrhoea. No nervous signs.
Duration of illness 4 weeks.

POSTMORTEM FINDINGS

Turbinates	-	rhinitis with a lot of necrotic debri.
Pharynx	-	pharyngitis, necrosis and haemorrhage.
Larynx	-	severe laryngitis and haemorrhages.
Trachea	-	severe tracheitis and haemorrhages.
Lungs	-	marked interstitial pneumonia with pleurisy and emphysema.
Abomasum	-	normal.
Kidneys	-	kidney infarcts present.
CNS	-	normal.

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	ND	ND	ND
Ocular swabs	ND	ND	ND
Turbinates	IBR +ve	Pseudomonas aeruginosa	M. bovis
Nasopharynx	ND		ND
Trachea	IBR +ve	Acinetobacter lwoffi Aeromonas vividans	M. bovis
Lung	-ve	Flavobacterium spp.	M. bovis

OUTBREAK 14

DATE March 1979.

FARMER'S COMPLAINT

Frequent coughing, serous ocular and nasal discharge and reduced appetite in recently purchased bullocks.

ANIMALS AT RISK

42, eight to ten months old fattening stirks and 50 six months old stores.

HUSBANDRY SYSTEM

The cattle were housed in one slatted floor shed with central feeding passage. Stirks are bought-in to fatten or to be sold as forward stores in the spring.

HISTORY OF INCIDENT

Three weeks after 28 Friesian stirks, aged 8 months, had been purchased, coughing and oculo-nasal discharge were observed.

CLINICAL FEATURES

Dullness. Reduced appetite. Temperature (103-106^{o}F).
Bilateral serous ocular discharge. Moderate conjunctivitis.
IBK 1-2 cases per year. Incidence not higher during outbreak. Bilateral mucoid to mucopurulent nasal discharge.
Diphtheritic plaques in nasal mucosa. Coughing.
Tachypnoea. Drooling saliva. Marked loss of weight in severely affected animals. As a group there was no obvious weight gain for 2-3 weeks. No diarrhoea. No nervous signs.
Morbidity - 90%. Mortality - 1%.
Duration of clinical signs 3-4 weeks.
Treatment with oxytetracycline - variable response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATION

Nasal swabs	-	5/5	IBR +ve
Ocular swabs	-	2/2	-ve

SEROLOGY

Not done.

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

No individual sick animal was purchased for further detailed studies.

OUTBREAK 15

DATE February 1979.

FARMER'S COMPLAINT

Agalactia, anorexia, pyrexia, dullness, ocular and nasal discharge.

ANIMALS AT RISK

Byre - 42 cows and heifers 1 Bull.
Court - variable number of cattle in-transit.

HUSBANDRY SYSTEM

There was two separate populations of cattle: milking cows in a traditional double byre and a "flying" herd of cows which were bought-in and then sold immediately after calving; average time on the farm for the latter animals is 3-4 days. The turnover in this latter unit is about 70 animals per week. However, only 4 cows from the milking byre have been sold and been replaced. During the winter housing period, the dairy cows had been allowed outside every day.

HISTORY OF INCIDENT

From 3-9th February, 4 lactating cows suddenly went off their milk, they became anorexic and developed severe respiratory signs. The second cow seen to be ill had been sold through a market twice and had been returned twice, on both occasions within 7 days of being sold. Her illness had initially been diagnosed as transit fever.

CLINICAL FEATURES

Dullness. Anorexia. Temperature (105-107^{o}F). Bilateral serous ocular discharge. Conjunctivitis. IBK - one case, no change in incidence. Bilateral mucoid nasal discharge. Coughing. Tachypnoea. Hyperpnoea. Drooling saliva. Sudden onset agalactia. No diarrhoea. No nervous signs. No abortions. No fertility problems.
Morbidity - about 30%. Mortality 5%.
Duration of clinical signs - 2 weeks.
Treatment with penicillin - poor response.

CLINICAL DIAGNOSIS

Infectious bovine rhinotracheitis.

FURTHER INVESTIGATIONS - HERD

VIRUS ISOLATION

Nasal swabs - 1/3 IBR +ve.
Ocular swabs - ND.

SEROLOGY

Acute samples - no samples available.
Convalescent samples - 16/22 seropositive.
Reciprocal titres - 6,6,6,6,8,8,8,8,12,12,12, 16,16,16,24,24.
Mean titre - 10.25

FURTHER INVESTIGATIONS - INDIVIDUAL ANIMAL

CLINICAL FINDINGS ON ADMISSION

Dullness. Anorexia. Temperature (99°F). Serous ocular discharge. Conjunctivitis. No IBK. Bilateral mucopurulent nasal discharge. No evidence of diphtheritic plaques. No coughing. Halitosis. Drooling saliva. Diarrhoea and nervous signs not seen. Animal recumbent on admission. Duration of illness - 2 weeks.

POSTMORTEM FINDINGS

Not done.

MICROBIOLOGY

SAMPLE	VIROLOGY	BACTERIOLOGY	MYCOPLASMOLOGY
Nasal swabs	IBR +ve	ND	ND
Ocular swabs	IBR +ve	ND	ND
Turbinates	ND	ND	ND
Nasopharynx	ND	ND	ND
Trachea	ND	ND	ND
Lung	ND	ND	ND

APPENDIX 2

THE RESULTS OF THE PREVALENCE OF NEUTRALISING ANTIBODIES TO INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS IN SCOTTISH HERDS

ABBREVIATIONS

The following abbreviations have been used in this appendix.

G	=	Grampian region
S	=	Strathclyde region
C	=	Central region
H	=	Highland region
DG	=	Dumfries and Galloway region

Thus:

GB	=	Banff and Buchan
GG	=	Gordon
GM	=	Moray
SC	=	Cunninghame
SCD	=	Cumnock and Doon Valley
SE	=	Eastwood
SEK	=	East Kilbride
SKL	=	Kilmarnock and Loudon
SL	=	Lanark
SR	=	Renfrew
SS	=	Kelvin
C	=	Central
DG	=	Dumfries and Galloway
H	=	Highland

TABLE 1. The results of the survey on the prevalence of neutralising antibodies to infectious bovine rhinotracheitis virus in the Grampian region.

Farm	Herd Size	Animals sampled		No. Positive	Reciprocal Titre	Type of herd	Other Information
		No.	Age				
GB1	90	10	10c	-	-	S/C	
GB2	79	10	10c	1c	8	Bought-in	Signs of IBR 1978
GB3	180	10	9c,1h	-	-	S/C	-
GB4	75	10	10c	-	-	S/C	-
GB5	200	10	10c	-	-	S/C	Holstein bull from Canada
GB6	150	10	10c	1c	4	S/C	-
GB7	65	10	10c	1c	4	Bought-in	-
GB8	65	11	11c	-	-	Bought-in	-
GB9	70	10	10c	1c	16	S/C	-
GB10	135	10	7c,3h	2c	6,6	S/C	-
GB11	75	10	10c	1c	16	Bought-in	-
GB12	300	10	10c	3c	4,4,6	S/C	Holsteins
GB13	75	10	10c	-	-	Bought-in	-
GB14	254	10	10c	6c	4,6,6,8, 12,12	S/C	Holsteins
GB15	200	10	10c	-	-	S/C	-
GB16	95	10	6c,4h	1c	4	S/C	-
GB17	70	10	10c	2c	12,12	S/C	-
GB18	45	10	9c,1h	-	-	Bought-in	-
GB19	90	10	7c,3h	-	-	S/C	-
GB20	170	10	10c	8c	4,6,8,8, 12,16,16, 24	S/C	Holsteins

Table 1. (Cont'd)

Farm	Herd Size	Animals sampled		No. Positive	Reciprocal Titre	Type of herd	Other Information
		No.	Age				
GG1	200	10	9c,1h	3c	6,12,16	S/C	-
GG2	100	10	7c,3h	-	-	S/C	-
GG3	170	10	10c	-	-	S/C	-
GG4	170	10	10c	1c	8	S/C	-
GG5	160	10	10c	1c	12	S/C	-
GG6	180	10	10h	4h	12,12,24, 32	S/C	-
GG7	350	10	10c	3c	6,8,12	S/C	-
GG8	60	10	10c	-	-	S/C	-
GG9	35	10	10c	1c	4	S/C	-
GG10	30	10	10c	-	-	S/C	-
GG11	110	10	10c	-	-	S/C	-
GG12	150	10	10c	-	-	S/C	Signs of IBR 1977-78
GG13	90	10	10c	-	-	S/C	-
GM1	80	10	5c,5h	-	-	S/C	-
GM2	120	10	10c	-	-	S/C	-
GM3	130	10	8c,2h	-	-	S/C	-
GM4	150	10	9c,1h	-	-	S/C	-
GM5	200	10	10c	1c	8	S/C	-
GM6	250	18	18c	6c	4,6,6,8, 12,12	S/C	Holsteins
GM7	85	10	8c,2h	-	-	S/C	-

TABLE 2. The results of the survey on the prevalence of neutralising antibodies to infectious bovine rhinotracheitis virus in the Strathclyde region.

Farm	Herd Size	Animals sampled		No. Positive	Reciprocal titre	Type of Herd	Other Information
		No.	Age				
SC1	130	10	10c	2c	4,12	S/C	-
SC2	100	10	5c,5h	2c	12,24	S/C	-
SC3	140	10	10c	1c	6	S/C	Signs of IBR 1977-78
SC4	165	10	10c	-	-	S/C	-
SC5	110	10	10c	-	-	Bought-in	
SC6	100	10	5c,5h	3c,2h	4,8,12,16 16	Bought-in	
SC7	60	10	10c	-	-	Bought-in	-
SC8	84	10	10c	-	-	S/C	-
SCD1	90	10	10c	-	-	S/C	-
SCD2	45	10	10c	2c	6,12	Bought-in	-
SCD3	85	10	9c,1h	-	-	S/C	-
SCD4	80	10	6c,4h	-	-	S/C	-
SCD5	65	10	7c,3h	-	-	S/C	-
SCD6	95	10	9c,1h	3c	6,8,12	S/C	Signs of IBR 1969-70
SCD7	115	10	4c,6h	2c	24,24	S/C	-
SCD8	70	10	10c	-	-	Bought-in	-
SCD9	85	10	9c,1h	4c	4,6,12, 12	Bought-in	-
SCD10	35	10	6c,4h	1c	8	S/C	-
SE1	39	10	10c	2c	6,12	S/C	-

Table 2. (Cont'd)

Farm	Herd Size	Animals sampled		No. Positive	Reciprocal titre	Type of herd	Other Information
		No.	Age				
SEK1	85	10	10c	1c	4	S/C	-
SEK2	55	10	10c	-	-	S/C	IBR signs seen in 1978
SEK3	85	10	6c,4h	-	-	S/C	-
SEK4	100	10	8c,2h	-	-	S/C	-
SEK5	80	10	10c	8c	4,4,4,12, 12,12,24, 32	S/C	-
SEK6	100	10	7c,3h	-	-	S/C	-
SEK7	120	10	10c	6c	12,12,12, 12,12,12	S/C	-
SEK8	50	10	10c	1c	16	S/C	-
SEK9	70	10	9c,1h	-	-	S/C	-
SEK10	70	10	4c,6h	1c	12	S/C	-
SEK11	85	10	8c,2h	4c	12,12,12, 24	S/C	-
SKL1	140	10	10c	2c	6,12	S/C	-
SKL2	120	10	10c	1c	6	S/C	-
SKL3	92	10	10c	-	-	S/C	-
SKL4	52	10	8c,2h	1c	8	S/C	-
SKL5	100	10	10c	3c	6,12,12	S/C	-
SKL6	140	10	6c,4h	-	-	S/C	-
SKL7	130	10	6c,4h	-	-	S/C	-
SKL8	90	10	7c,3h	2c	12,12	S/C	-

Table 2. (Cont'd)

Farm	Herd Size	Animals sampled		No. Positive	Reciprocal titre	Type of Herd	Other Information
		No.	Age				
SL1	13	13	13h	-		Beef	-
SL2	53	10	10c	2c	8,12	Beef	-
SL3	121	10	10c	-	-	Beef	-
SL4	112	10	6c,4h	2c,1h	6,8,12	Beef	-
SL5	60	6	5c,1h	1c	12	Beef	-
SL6	11	11	11c	5c	6,8,12, 12,96	Bought-in Beef	
SL7	157	10	10h	-		S/C	-
SL8	11	11	5c,6h	3c	6,6,16	Beef	-
SR1	62	10	10c	-	-	S/C	-
SR2	80	10	10c	-	-	S/C	-
SR3	95	10	4c,6h	-	-	S/C	-
SR4	50	10	7c,3h	-	-	S/C	-
SR5	190	10	8c,2h	-	-	S/C	-
SR6	75	10	10c	4c	16,24,24, 24	S/C	-
SR7	55	10	10c	-	-	S/C	-
SR8	56	10	9c,1h	-	-	S/C	-
SR9	130	10	9c,1h	-	-	S/C	-
SR10	38	10	8c,2h	1c	4	S/C	IBR signs 1977-78
SR11	80	10	9c,1h	-	-	S/C	-

Table 2. (Cont'd)

Farm	Herd Size	Animals sampled		No. Positive	Reciprocal titre	Type of Herd	Other Information
		No.	Age				
SS1	45	10	9c,1h	-	-	S/C	-
SS2	60	10	9c,1h	-	-	S/C	-
SS3	130	10	8c,2h	1h	12	S/C	-
SS4	45	10	7c,3h	1c	12	S/C	-
SS5	55	10	8c,2h	1c	16	S/C	-
SS6	120	10	10c	-	-	S/C	-

TABLE 3. The results of the survey on the prevalence of neutralising antibodies to infectious bovine rhinotracheitis virus in the Central, Highland, Dumfries and Galloway regions.

Farm	Herd Size	Animals sampled		No. Positive	Reciprocal titre	Type of Herd	Other Information
		No.	Age				
C1	70	10	10c	3c	6,8,12	S/C	-
C2	85	10	10c	2c	8,12	S/C	-
C3	70	10	10c	2c	12,24	S/C	-
C4	65	10	10c	1c	4	S/C	-
DG1	100	10	10h	-	-	S/C	-
DG2	51	10	10h	-	-	S/C	-
DG3	99	10	10h	-	-	S/C	-
H1	20	10	10c	3c	6,6,8	Beef S/C	-
H2	50	8	5c,3h	3c,1h	6,8,8,8	Beef Bought-in	IBR previous years
H3	60	10	10c	1c	24	Beef S/C	-
H4	30	14	14c	1c	4	Beef S/C	-

APPENDIX 3

TABLE 1. Results of neutralisation of Strichen rabbit antiserum with the Strichen strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	78,82,95,76	82.75	1.0	0.00
5 "	72,76,76,74	74.5	0.9	-0.05
10 "	76,74,64,64	69.5	0.84	-0.08
15 "	46,44,44,48	45.5	0.55	-0.26
20 "	30,25,24,31	27.5	0.33	-0.48
25 "	18,18,25,22	20.75	0.25	-0.60
30 "	17,15,10,16	14.5	0.18	-0.75

TABLE 2. Results of neutralisation of Strichen rabbit antiserum with the Colorado strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	68,71,81,76	74.0	1.00	0.00
5 "	70,50,62,81	65.75	0.89	-0.05
10 "	35,34,34,31	33.5	0.45	-0.35
15 "	18,16,20,18	18	0.24	-0.62
20 "	10, 8,13,12	10.75	0.15	-0.82
25 "	4, 6, 9, 9	7	0.09	-1.05
30 "	5, 6, 3,10	6	0.08	-1.10

TABLE 3. Results of neutralisation of Strichen rabbit antiserum with Oxford strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	85,84,94,103	91.5	1.00	0.00
5 "	81,85,76,74	79.0	0.86	-0.07
10 "	61,42,69,69	60.25	0.66	-0.18
15 "	27,28,29,32	29.0	0.32	-0.50
20 "	15,13,18,18	16.0	0.17	-0.77
25 "	5, 6, 9, 4	6.0	0.07	-1.16
30 "	3, 4, 6, 6	4.75	0.05	-1.30

TABLE 4. Results of neutralisation of Strichen bovine antiserum with Strichen strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	72,74,60,80	71.5	1.00	0.00
5 "	73,72,67,66	69.5	0.97	-0.01
10 "	52,65,66,68	62.75	0.88	-0.06
15 "	60,67,52,46	56.25	0.79	-0.10
20 "	54,52,44,48	49.5	0.69	-0.16
25 "	50,38,33,37	39.5	0.55	-0.26
30 "	30,25,29,30	28.5	0.40	-0.40

TABLE 5. Results of neutralisation of Strichen bovine antiserum with Colorado strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	46,50,52,54	50.5	1.00	0.00
5 "	50,49,45,48	48.0	0.95	-0.02
10 "	54,42,43,37	44.0	0.87	-0.06
15 "	36,38,30,28	33.0	0.65	-0.19
20 "	20,23,20,27	22.5	0.46	-0.34
25 "	14,17,12,19	15.5	0.31	-0.51
30 "	6,11, 6,11	8.5	0.17	-0.77

TABLE 6. Results of neutralisation of Strichen bovine antiserum with Oxford strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	90,86,92,96	91.0	1.00	0.00
5 "	93,88,87,86	88.5	0.97	-0.01
10 "	76,70,84,64	73.5	0.81	-0.09
15 "	45,68,52,61	56.5	0.62	-0.29
20 "	40,55,60,30	46.25	0.51	-0.29
25 "	33,39,33,41	36.5	0.40	-0.40
30 "	19,19,25,25	22.0	0.24	-0.62

TABLE 7. Results of neutralisation of Colorado rabbit antiserum with Colorado strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	50,63,60,62	58.75	1.00	0.00
5 "	62,64,44,55	56.25	0.96	-0.02
10 "	30,32,37,32	32.75	0.56	-0.25
15 "	26,20,22,18	21.5	0.37	-0.43
20 "	12,15,10,11	12.0	0.20	-0.70
25 "	8, 8, 6, 9	7.75	0.13	-0.89
30 "	1, 3, 3, 5	3.0	0.05	-1.30

TABLE 8. Results of neutralisation of Colorado rabbit antiserum with Strichen strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	47,34,42,45	42.0	1.00	0.00
5 "	38,35,44,27	36.0	0.86	-0.06
10 "	33,30,32,35	32.5	0.77	-0.11
15 "	30,26,28,28	28.0	0.67	-0.17
20 "	17,17,33,29	24.0	0.57	-0.24
25 "	12,12,15,11	11.5	0.27	-0.57
30 "	7,10, 5, 5	6.75	0.16	-0.79

TABLE 9. Results of neutralisation of Colorado rabbit antiserum with Oxford strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNT	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	35,34,31,38	34.5	1.00	0.00
5 "	30,29,27,26	28.0	0.81	-0.09
10 "	25,23,25,24	24.25	0.70	-0.15
15 "	20,22,18,20	20.0	0.56	-0.25
20 "	10,13,15,14	13.0	0.38	-0.42
25 "	9, 9, 8, 8	8.5	0.25	-0.60
30 "	4, 4, 3, 5	4.0	0.12	-0.92

TABLE 10. Results of neutralisation of Oxford rabbit antiserum with Oxford strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNTS	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	116,118,122,116	118.0	1.00	0.00
5 "	102,104, 99,113	104.5	0.89	-0.05
10 "	96, 84, 94, 92	91.5	0.78	-0.11
15 "	84, 72, 78, 70	76.0	0.64	-0.19
20 "	62, 63, 60, 74	64.75	0.55	-0.26
25 "	52, 50, 48, 46	49.0	0.42	-0.38
30 "	46, 36, 33, 36	37.75	0.32	-0.49

TABLE 11. Results of neutralisation of Oxford rabbit antiserum with Strichen strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNTS	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	64,50,54,52	55.0	1.00	0.00
5 "	49,50,37,47	45.75	0.83	-0.08
10 "	45,44,41,32	40.5	0.74	-0.13
15 "	36,37,40,42	38.75	0.71	-0.15
20 "	33,31,27,25	29.0	0.53	-0.27
25 "	25,19,22,20	21.5	0.39	-0.41
30 "	18,19,11,12	15	L.27	-0.57

TABLE 12. Results of neutralisation of Oxford rabbit antiserum with Colorado strain of infectious bovine rhinotracheitis virus.

TIME	PLAQUE COUNTS	MEAN PLAQUE COUNTS	$^{vt}/_{vo}$	Log $^{vt}/_{vo}$
0 minutes	71,64,81,72	72.0	1.00	0.00
5 "	53,57,71,59	60.0	0.83	-0.08
10 "	46,45,57,59	51.75	0.72	-0.14
15 "	45,48,42,48	45.75	0.64	-0.19
20 "	33,28,39,35	33,75	0.47	-0.33
25 "	21,22,25,30	24.5	0.34	-0.47
30 "	21,21,21,17	20	0.28	-0.55

APPENDIX 4

Abbreviations used in this Appendix

C = Congestion of nasal and vaginal mucosa

SND = Serous nasal discharge

SMD = Seromucoid nasal discharge

MND = Mucopurulent nasal discharge

SOD = Serous ocular discharge

POD = Purulent ocular discharge

ds = drooling saliva

WL = Weight loss

VD = Vaginal discharge

$\pm$)
)
++) = Frequency/Degree of severity
)
+++)

TABLE 1. The details of the clinical response following intranasal challenge of the 18 month (Group A) old bullocks with the Strichen strain of infectious bovine rhinotracheitis virus.

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	A1	A2	A3	A4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.6	101.0	102.0	101.5
PULSE (PR/min)	80	80	84	84
RESPIRATORY SYSTEM (RR/min)	30	30	30	30
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 1 cont'd. DAY 1 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	A1	A2	A3	A4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.8	101.2	102.0	101.6
PULSE (PR/min)	80	80	84	84
RESPIRATORY SYSTEM (RR/min)	30	30. C	30	30
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 1 cont'd

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	A1	A2	A3	A4
DEMEANOUR	Sl. dull	Sl. dull	Sl. dull	Sl. dull
TEMPERATURE °F	102.0	101.5	102.4	101.6
PULSE (PR/min)	80	80	84	84
RESPIRATORY SYSTEM (RR/min)	30. C.	30. C. SND	36. C.	30
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 1 cont'd. DAY 3 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	A1	A2	A3	A4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	103.6	105.0	104.0	102.2
PULSE (PR/min)	84	96	90	84
RESPIRATORY SYSTEM (RR/min)	30. C. SND	48. C. SMD	30. SMD. Haemorrhages Coughing +	30. SND
EYES	-	-	-	-
OTHER SIGNS	Red. appetite ds	Red.appetite ds	Red.appetite ds	Red.appetite ds

TABLE 1 cont'd.

DAY 4 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	A1	A2	A3	A4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	103.6	104.2	104.2	102.0
PULSE (PR/min)	90	108	84	84
RESPIRATORY SYSTEM (RR/min)	30. SMD. Diph.lesions.	60. SMD. Diph.lesions. Hyperpnoea.	30. SMD. Diph.lesions.	30. C. SND
EYES	-	--	-	-
OTHER SIGNS	Anorexia	Anorexia ds	Red.appetite	Red.appetite

TABLE 1 cont'd. DAY 5 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	A1	A2	A3	A4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	103.0	103.3	102.5	101.0
PULSE (PR/min)	84	96	84	80
RESPIRATORY SYSTEM (RR/min)	30. MND. Diph.lesions. Coughing +	36. MND. Diph.lesions. Coughing ++	30. SMD. Diph.lesions	30. SND. Diph.lesions.
EYES	Conjunctivitis SOD	-	Conjunctivitis SOD	-
OTHER SIGNS	Red.appetite ds	Anorexia ds	Red.appetite ds	-

TABLE 1 cont'd.

DAY 6 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	A1	A2	A3	A4
DEMEANOUR	Sl. dull	Bright	Bright	Bright
TEMPERATURE °F	102.5	102.0	101.8	101.0
PULSE (PR/min)	96	108	90	80
RESPIRATORY SYSTEM (RR/min)	30. SMD. Diph.lesions. Coughing ++	36. SMD. Diph.lesions Coughing +	30. SND. Diph.lesions	30. SND. Diph.lesions.
EYES	Conjunctivitis SOD	-	Conjunctivitis SOD	-
OTHER SIGNS	ds	ds	ds	-

TABLE 1 cont'd. DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
DEMEANOUR				
TEMPERATURE °F				
PULSE (PR/min)				
RESPIRATORY SYSTEM (RR/min)				
EYES				
OTHER SIGNS				

TABLE 2. The details of the clinical response following intranasal challenge of the 6 month (Group B) old stirks with the Strichen strain of infectious bovine rhinotracheitis virus.

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	B1	B2	B3	B4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	102.0	102.5	102.2	101.6
PULSE (PR/min)	72	72	90	80
RESPIRATORY SYSTEM (RR/min)	30. SND.	36. SND.	30	30. SND.
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 2 cont'd. DAY 1 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	B1	B2	B3	B4
DEMEANOUR	Bright	Bright	Quiet	Bright
TEMPERATURE °F	102.2	102.5	102.5	102.2
PULSE (PR/min)	84	84	96	96
RESPIRATORY SYSTEM (RR/min)	48. SND.	50. SND.	36. SND.	50. SND.
EYES	-	-	SOD	-
OTHER SIGNS	-	-	-	-

TABLE 2. cont'd.

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	B1	B2	B3	B4
DEMEANOUR	Sl. dull	Quiet	Sl. dull	Sl. dull
TEMPERATURE °F	103.2	103.6	103.0	102.4
PULSE (PR/min)	96	96	100	96
RESPIRATORY SYSTEM (RR/min)	40. SMD. Coughing	50. SMD.	48. SMD. Hyperpnoea Harsh resp.	48. SMD.
EYES	-	-	-	Conjunctivitis SOD
OTHER SIGNS	-	-	-	-

TABLE 2 cont'd DAY 3 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	B1	B2	B3	B4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	104.6	105.1	104.7	103.0
PULSE (PR/min)	86	96	120	96
RESPIRATORY SYSTEM (RR/min)	40. SMD. C. Coughing ++	60. SMD. Coughing + Diph.lesions	48. SMD. Diph.lesions Harsh resp.	36. SMD. Coughing + Harsh resp.
EYES	Conjunctivitis SOD	-	Conjunctivitis SOD	Conjunctivitis SOD
OTHER SIGNS	-	Red.appetite	Red.appetite	-

TABLE 2 cont'd

DAY 4 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	B1	B2	B3	B4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	106.1	105.3	105.3	105.0
PULSE (PR/min)	144	144	96	108
RESPIRATORY SYSTEM (RR/min)	60. MND. Coughing +++ Diph.lesions	60. MND. Dyspnoea. Diph.lesions Coughing +	60. SMD. Dyspnoea. Diph.lesions Coughing +	50. SMD. Diph.lesions Coughing +
EYES	Conjunctivitis ++ SOD	-	Conjunctivitis SOD	Conjunctivitis SOD
OTHER SIGNS	Anorexia ds WL	Anorexia ds WL	Anorexia WL	Anorexia ds WL

TABLE 2 cont'd DAY 5 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	B1	B2	B3	B4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	105.4	104.3	105.0	103.9
PULSE (PR/min)	96	96	96	96
RESPIRATORY SYSTEM (RR/min)	36.Harsh resp MND Diph.lesions Coughing ++	60.harsh resp MND Diph.lesions Coughing +	50.Harsh res SMD Hyperpnoea. Diph.lesions Coughing +	48.Harsh resp. MND Diph.lesions Coughing +++
EYES	Conjunctivitis SOD	Conjunctivitis SOD	Conjunctivitis SOD	Conjunctivitis SOD
OTHER SIGNS	Anorexia ds	Anorexia ds	Anorexia ds	Anorexia ds

TABLE 2 cont'd

DAY 6 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	B1	B2	B3	B4
DEMEANOUR	Sl. dull	Sl. dull	Sl. dull	Sl. dull
TEMPERATURE °F	104.6	104.4	103.9	103.8
PULSE (PR/min)	96	96	84	84
RESPIRATORY SYSTEM (RR/min)	48.Harsh resp MND Diph.lesions	60.Harsh resp MND Hyperpnoea	48.Harsh resp SMD Hyperpnoea Diph.lesions	36.Harsh resp. SMD Diph.lesions Coughing ++
EYES	Conjunctivitis SOD	SOD	Conjunctivitis SOD	SOD
OTHER SIGNS	Red.appetite ds	Red.appetite	Red.appetite	Red.appetite ds
	KILLED TODAY			KILLED TODAY

TABLE cont'd DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
DEMEANOUR				
TEMPERATURE °F				
PULSE (PR/min)				
RESPIRATORY SYSTEM (RR/min)				
EYES				
OTHER SIGNS				

TABLE 3. The details of the clinical response following intranasal challenge of the 5 week (Group C) old calves with the Strichen strain of infectious bovine rhinotracheitis virus.

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.5	101.5	102.0	102.2
PULSE (PR/min)	90	80	84	80
RESPIRATORY SYSTEM (RR/min)	30. SND.	30	30. SND.	30
EYES	-	-	-	-
OTHER SIGNS	-	-	-	Sl. diarrhoea

TABLE 3 cont'd. DAY 1 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Sl. dull	Sl. dull	Sl. dull	Sl. dull
TEMPERATURE °F	102.0	101.5	102.0	102.0
PULSE (PR/min)	84	96	84	72
RESPIRATORY SYSTEM (RR/min)	30	30	30	30
EYES	-	Conjunctivitis SOD	-	Conjunctivitis
OTHER SIGNS	-	-	-	-

TABLE 3 cont'd.

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Sl.dull	Sl.dull	Sl.dull	Very dull
TEMPERATURE °F	102.5	102.4	102	102
PULSE (PR/min)	84	96	84	84
RESPIRATORY SYSTEM (RR/min)	30. C. SND.	30. SMD. Coughing +	36. C. SMD.	30. C. MND.
EYES	Conjunctivitis SOD	-	-	Conjunctivitis POD
OTHER SIGNS	-	-	-	-

TABLE 3 cont'd. DAY 3 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	104.6	103.0	102.8	101.6
PULSE (PR/min)	72	84	84	60
RESPIRATORY SYSTEM (RR/min)	30. SMD. Diph.lesions Coughing ++	30. SMD. Diph.lesions Coughing ++	36. C. SMD. Coughing ++	30. SMD. Diph.lesions
EYES	Conjunctivitis SOD	Conjunctivitis SOD	-	Conjunctivitis SOD
OTHER SIGNS	Red.appetite ds	Red.appetite ds	Red.appetite	Red.appetite ds

TABLE 3 cont'd.

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Sl.dull	Sl.dull	Sl.dull	Very dull
TEMPERATURE °F	102.5	102.4	102	102
PULSE (PR/min)	84	96	84	84
RESPIRATORY SYSTEM (RR/min)	30. C. SND.	30. SMD. Coughing +	36. C. SMD.	30. C. MND.
EYES	Conjunctivitis SOD	-	-	Conjunctivitis POD
OTHER SIGNS	-	-	-	-

TABLE 3 cont'd. DAY 3 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	104.6	103.0	102.8	101.6
PULSE (PR/min)	72	84	84	60
RESPIRATORY SYSTEM (RR/min)	30. SMD. Diph.lesions Coughing ++	30. SMD. Diph.lesions Coughing ++	36. C. SMD. Coughing ++	30. SMD. Diph.lesions
EYES	Conjunctivitis SOD	Conjunctivitis SOD	-	Conjunctivitis SOD
OTHER SIGNS	Red.appetite ds	Red.appetite ds	Red.appetite	Red.appetite ds

TABLE 3 cont'd.

DAY 4 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Dull	Dull	Very dull	Dull
TEMPERATURE °F	104.8	103.5	103.8	103.6
PULSE (PR/min)	84	80	120	80
RESPIRATORY SYSTEM (RR/min)	30. SMD. Diph.lesions	30. SMD. Diph.lesions	48. SMD. Diph.lesions Coughing ++	30. MND. Diph.lesions
EYES	Conjunctivitis SOD	-	Conjunctivitis SOD	Conjunctivitis
OTHER SIGNS	Anorexia ds	Anorexia ds KILLED TODAY	Anorexia	Anorexia ds

TABLE 3 cont'd. DAY 5 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Dull	-	Very dull	Very dull
TEMPERATURE °F	103.2		102.5	102.6
PULSE (PR/min)	84		90	90
RESPIRATORY SYSTEM (RR/min)	32. SMD.- Diph.lesions Harsh resp.		36. MND. Diph.lesions Coughing ++ Harsh resp. Dyspnoea.	40. SMD. Diph.lesions Harsh resp.
EYES	Conjunctivitis SOD.		Conjunctivitis SOD	Granular conjunctivitis POD
OTHER SIGNS	Anorexia ds		Anorexia	Anorexia ds

TABLE 3 cont'd.

DAY 6 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	C1	C2	C3	C4
DEMEANOUR	Dull	-	Dull	Dull
TEMPERATURE °F	102.0		102.0	101.8
PULSE (PR/min)	96		90	90
RESPIRATORY SYSTEM (RR/min)	30. SMD. Diph.lesions Harsh resp.		36. MND. Diph.lesions Coughing ++ Harsh resp. Dyspnoea	36. MND. Diph.lesions
EYES	Conjunctivitis SOD		-	Granular Conjunctivitis POD.
OTHER SIGNS	Red.appetite ds		Anorexia ds KILLED TODAY	-

TABLE cont'd. DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
DEMEANOUR				
TEMPERATURE °F				
PULSE (PR/min)				
RESPIRATORY SYSTEM (RR/min)				
EYES				
OTHER SIGNS				

TABLE 4. The details of the clinical response following intranasal challenge of the 2 week old calves (Group D) with the Strichen strain of infectious bovine rhinotracheitis virus.

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	D1	D2	D3	D4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.8	102.5	101.5	101.5
PULSE (PR/min)	96	96	96	96
RESPIRATORY SYSTEM (RR/min)	30	30	32	30
EYES	-	-	-	-
OTHER SIGNS	Slight pasty yellowish brown diarrhoea.	-	-	-

TABLE 4 cont'd. DAY 1 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	D1	D2	D3	D4
DEMEANOUR	Sl.dull	Dull	Sl.dull	Sl.dull
TEMPERATURE °F	102.0	103.5	101.8	102.2
PULSE (PR/min)	84	96	90	84
RESPIRATORY SYSTEM (RR/min)	30. SMD. C.	32. MND.	32 Harsh resp.	30
EYES	SOD	-	-	Conjunctivitis SOD
OTHER SIGNS	Yellowish brown pasty diarrhoea	-	-	-

TABLE 4 cont'd

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	D1	D2	D3	D4
DEMEANOUR	Dull	Very dull	Sl. dull	Dull
TEMPERATURE °F	103.2	103.5	103.0	102.5
PULSE (PR/min)	96	96	96	84
RESPIRATORY SYSTEM (RR/min)	30. SND Diph.lesions	32. MND	32. SND	30. SND
EYES	Conjunctivitis POD	-	Conjunctivit s SOD	Conjunctivitis SOD
OTHER SIGNS	Diarrhoea	-	-	-

TABLE cont'd DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	D1	D2	D3	D4
DEMEANOUR	Very dull	Dull	Dull	Dull
TEMPERATURE °F	102.0	104.5	106.0	104.3
PULSE (PR/min)	96	96	96	90
RESPIRATORY SYSTEM (RR/min)	42.Harsh resp MND Diph.lesions	34.Harsh resp SND Diph.lesions Coughing ++	36.Harsh res MND Diph.lesions	32.Harsh resp. SND Diph.lesions Coughing +
EYES	Conjunctivitis SOD	Con junctivitis SOD	Conjunctivit s SOD	Conjunctivitis SOD
OTHER SIGNS	Diarrhoea Red.appetite ds DEAD BY NOON	Red.appetite ds	Red.appetite ds	Red.appetite ds

TABLE 4 cont'd

DAY 4 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	D1	D2	D3	D4
DEMEANOUR		Dull	Dull	Dull
TEMPERATURE °F		105.1	106.0	104.5
PULSE (PR/min)		96	96	96
RESPIRATORY SYSTEM (RR/min)		36. SMD. Diph.lesions	40. SMD. Diph.lesions	36.Harsh resp. SMD Diph.lesions
EYES		Conjunctivitis SOD	Conjunctivitis SOD	-
OTHER SIGNS		Anorexia ds	Anorexia ds	Anorexia ds

TABLE 4 cont'd DAY 5 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	D1	D2	D3	D4
DEMEANOUR		Dull	Dull	Dull
TEMPERATURE °F		104.5	106.6	104.5
PULSE (PR/min)		96	96	90
RESPIRATORY SYSTEM (RR/min)		40. SMD. Diph.lesions	36. SMD. Diph.lesions	36. Harsh resp. MND Diph.lesions
EYES		Conjunctivitis SOD	Conjunctivitis SOD	Conjunctivitis SOD
OTHER SIGNS		Red.appetite ds	Red.appetite KILLED TODAY	Red.appetite ds

TABLE 4 cont'd

DAY 6 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	D1	D2	D3	D4
DEMEANOUR	DEAD	Dull		Dull
TEMPERATURE °F		106.8		103.5
PULSE (PR/min)		96		96
RESPIRATORY SYSTEM (RR/min)		48.Harsh resp Dyspnoea SMD Diph.lesions		42.Harsh resp. SMD Diph.lesions
EYES		Conjunctivitis SOD		Conjunctivitis SOD
OTHER SIGNS		Diarrhoea Red.appetite ds		Diarrhoea Red.appetite

TABLE cont'd DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
DEMEANOUR				
TEMPERATURE °F				
PULSE (PR/min)				
RESPIRATORY SYSTEM (RR/min)				
EYES				
OTHER SIGNS				

TABLE 5. The details of the clinical response following intranasal challenge of the 6 month old (Group E) stirks with the Colorado strain of infectious bovine rhinotracheitis virus.

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	E1	E2	E3	E4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.5	101.8	101.2	101.5
PULSE (PR/min)	80	84	84	84
RESPIRATORY SYSTEM (RR/min)	30	30	30	30
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 5. cont'd

DAY 1 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	E1	E2	E3	E4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.6	102.2	101.8	102.4
PULSE (PR/min)	84	90	84	84
RESPIRATORY SYSTEM (RR/min)	30	60. Harsh resp Coughing ++	30	30
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 5. cont'd

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	E1	E2	E3	E4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	105.2	102.5	104.2	103.8
PULSE (PR/min)	90	96	96	96
RESPIRATORY SYSTEM (RR/min)	40.Harsh resp SND Coughing ++	60.Harsh resp SND Coughing ++	48 SND Coughing ++	40.Harsh resp. SND Coughing ++
EYES	-	-	SOD	-
OTHER SIGNS	-	-	-	-

TABLE 5 cont'd DAY 3 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	E1	E2	E3	E4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	106.2	104.7	105.1	105.8
PULSE (PR/min)	90	96	96	90
RESPIRATORY SYSTEM (RR/min)	40.Harsh resp SND Diph.lesions Coughing ++	60.Harsh resp SND Diph.lesions Coughing	48.Harsh res SMD Diph.lesions Coughing	40.Harsh resp. SND Coughing
EYES	Conjunctivitis SOD	-	SOD	-
OTHER SIGNS	ds	ds	-	ds

TABLE 5 cont'd

DAY 4 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	E1	E2	E3	E4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	105.6	10.8	105.4	105.2
PULSE (PR/min)	90	96	90	90
RESPIRATORY SYSTEM (RR/min)	48.Harsh resp. SND Diph.lesions Coughing ++	66 Harsh resp SND Diph.lesions Coughing ++	48.Harsh resp SND Diph.lesions Coughing ++	48.Harsh resp. SND Diph.lesions Coughing ++
EYES	Conjunctivitis SOD	-	-	-
OTHER SIGNS	ds	-	-	-
	KILLED TODAY			

TABLE 5 cont'd. DAY 5 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	E1	E2	E3	E4
DEMEANOUR		Sl.dull	Dull	Sl.dull
TEMPERATURE °F		102.8	104.2	103.0
PULSE (PR/min)		96	96	84
RESPIRATORY SYSTEM (RR/min)		60.Harsh resp SMD Diph.lesions	40.Harsh resp SND Diph.lesions	36 SND Diph.lesions Coughing ++
EYES		-	-	-
OTHER SIGNS		-	-	-

TABLE 5 cont'd

DAY 6 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	E1	E2	E3	E4
DEMEANOUR		Dull	Sl.dull	Sl.dull
TEMPERATURE °F		104.0	102.7	102.2
PULSE (PR/min)		96	90	90
RESPIRATORY SYSTEM (RR/min)		48.Harsh resp Resp.stertor MND Diph.lesions	40.Harsh resp SMD Diph.lesions	40 SMD Diph.lesions Coughing ++
EYES		-	-	-
OTHER SIGNS		KILLED TODAY	-	-

TABLE cont'd. DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
DEMEANOUR				
TEMPERATURE °F				
PULSE (PR/min)				
RESPIRATORY SYSTEM (RR/min)				
EYES				
OTHER SIGNS				

TABLE 6. The details of the clinical response following intranasal challenge of the 6 month (Group F) stirks with the Oxford strain of infectious bovine rhinotracheitis virus.

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	F1	F2	F3	F4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.5	102.0	101.5	102.0
PULSE (PR/min)	90	90	90	90
RESPIRATORY SYSTEM (RR/min)	30	30	30	30
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 2 cont'd. DAY 1 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	F1	F2	F3	F4
DEMEANOUR	Bright.	Bright	Bright	Bright
TEMPERATURE °F	101.8	101.5	102.5	102.5
PULSE (PR/min)	90	96	96	96
RESPIRATORY SYSTEM (RR/min)	30	42.Harsh resp Coughing +	30 Coughing +	60 Harsh resp.
EYES	-	SOD	-	-
OTHER SIGNS	-	-	-	-

TABLE 6 cont'd

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	F1	F2	F3	F4
DEMEANOUR	Sl.dull	Sl.dull	Sl.dull	Sl.dull
TEMPERATURE °F	102.2	103.0	103.0	102.5
PULSE (PR/min)	96	96	96	96
RESPIRATORY SYSTEM (RR/min)	30 SND	36.Harsh resp	32.Harsh resp Coughing +	36.Harsh resp.
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 6 cont'd. DAY 3 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	F1	F2	F3	F4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	102.0	103.5	102.5	102.0
PULSE (PR/min)	90	96	96	96
RESPIRATORY SYSTEM (RR/min)	30. SND. C.	36.Harsh resp SND	36.Harsh resp	42.Harsh resp
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 6 cont'd

DAY 4 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	F1	F2	F3	F4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	102.0	102.5	103.5	102.8
PULSE (PR/min)	90	96	96	96
RESPIRATORY SYSTEM (RR/min)	32. SND. C.	36. C.	36.Harsh resp Diph.lesions	48.Harsh resp.
EYES	-	-	-	-
OTHER SIGNS	-	-	KILLED TODAY	-

TABLE 6 cont'd. DAY 5 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	F1	F2	F3	F4
DEMEANOUR	Sl.dull	Sl.dull	-	Sl.dull
TEMPERATURE °F	102.1	103.1		101.8
PULSE (PR/min)	90	90		96
RESPIRATORY SYSTEM (RR/min)	30. SND. C.	32.Harsh resp. C.		40.Harsh resp. C.
EYES	-	-		-
OTHER SIGNS	-	-		-

TABLE 6 cont'd

DAY 6 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	F1	F2	F3	F4
DEMEANOUR	Sl.dull	Sl.dull	-	Sl.dull
TEMPERATURE °F	102.0	102.4		101.5
PULSE (PR/min)	90	90		96
RESPIRATORY SYSTEM (RR/min)	30. SND C.	32.Harsh resp. C. Coughing ++		42. Harsh resp. C.
EYES	-	-		-
OTHER SIGNS	KILLED TODAY	-		-

TABLE cont'd. DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
DEMEANOUR				
TEMPERATURE °F				
PULSE (PR/min)				
RESPIRATORY SYSTEM (RR/min)				
EYES				
OTHER SIGNS				

TABLE 7. The details of the clinical response following intravaginal challenge of the 12 month (Group G) heifers with the Strichen strain of infectious bovine rhinotracheitis virus.

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	G1	G2	G3	G4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.4	101.2	101.6	101.6
PULSE (PR/min)	-	-	-	-
RESPIRATORY SYSTEM (RR/min)	-	-	-	-
EYES	-	-	-	-
REPRODUCTIVE SIGNS	-	-	-	-

TABLE 7 cont'd DAY 1 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	G1	G2	G3	G4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.6	101.2	101.4	101.2
PULSE (PR/min)	-	-	-	-
RESPIRATORY SYSTEM (RR/min)	-	-	-	-
EYES	-	-	-	-
REPRODUCTIVE SIGNS	C. Small vesicles	C. Small vesicles	C. Small vesicles	C. Pustules

TABLE 7 cont'd

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	G1	G2	G3	G3
DEMEANOUR	Sl. dull	Sl. dull	Sl. dull	Sl. dull
TEMPERATURE °F	101.2	101.0	102.0	101.2
PULSE (PR/min)	-	-	-	-
RESPIRATORY SYSTEM (RR/min)	-	-	-	-
EYES	-	-	-	-
REPRODUCTIVE SIGNS	C. Pustules +++ VD Tail swishing	C. Pustules +++ VD Tail swishing Ulceration	C. Pustules ++ VD Tail swishing	C. Pustules +++ VD Tail swishing

TABLE 7 cont'd DAY 3 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	G1	G2	G3	G4
DEMEANOUR	Sl.dull	Sl.dull	Sl.dull	Sl.dull
TEMPERATURE °F	101.0	101.5	101.2	101.2
PULSE (PR/min)	-	-	-	-
RESPIRATORY SYSTEM (RR/min)	-	-	-	-
EYES	-	-	-	-
REPRODUCTIVE SIGNS	C. Pustules + VD Constant micturition Tail swishing	C. Pustules + Tail swishing	C. Pustules + VD Constant micturition Tail swishing	C. Pustules Ulcerations VD Tail swishing

TABLE 7 cont'd

DAY 4 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	G1	G2	G3	G4
DEMEANOUR	Sl.dull	Bright	Bright	Bright
TEMPERATURE °F	101.2	102.0	101.5	101.7
PULSE (PR/min)	-	-	-	-
RESPIRATORY SYSTEM (RR/min)	-	-	-	-
EYES	-	-	-	-
REPRODUCTIVE SIGNS	C. Pustules + Tail swishing KILLED TODAY	C. Pustules + Tail swishing Ulceration	C. Pustules +++ VD Ulceration Tail swishing	C. Pustules +++ VD Tail swishing KILLED TODAY

TABLE 7 cont'd

DAY 5 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	G1	G2	G3	G4
DEMEANOUR	-	Bright	Bright	-
TEMPERATURE °F		102.0	101.8	
PULSE (PR/min)		-	-	
RESPIRATORY SYSTEM (RR/min)		-	-	
EYES		-	-	
REPRODUCTIVE SIGNS		C. Ulcers + Tail swishing	Pustules + Yellowish-white purulent dis. Tail swishing Constant micturition.	

TABLE 7 cont'd

DAY 6 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	G1	G2	G3	G4
DEMEANOUR	-	Bright	Bright	-
TEMPERATURE °F		101.8	102.2	
PULSE (PR/min)		-	-	
RESPIRATORY SYSTEM (RR/min)		-	-	
EYES		-	-	
REPRODUCTIVE SIGNS		C. Pustules ++ Ulcers Tail swishing	C. Pustules Purulent yellowish white vaginal discharge Tail swishing	

TABLE cont'd DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
DEMEANOUR				
TEMPERATURE °F				
PULSE (PR/min)				
RESPIRATORY SYSTEM (RR/min)				
EYES				
REPRODUCTIVE SIGNS				

TABLE 8. The details of the clinical response following Lungworm (D.viviparus) larvae infection of stirks (Group H) which had recovered from an experimental infection with the Strichen strain of infectious bovine rhinotracheitis virus.

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	H1	H2	H3	H4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	101.5	102.0	101.8	102.5
PULSE (PR/min)	80	72	80	84
RESPIRATORY SYSTEM (RR/min)	36	30	30.Harsh resp	36.Harsh resp.
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 8. cont'd DAY 11 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	H1	H2	H3	H4
DEMEANOUR	Sl.dull	Bright	Sl.dull	Sl.dull
TEMPERATURE °F	101.2	102.0	102.0	102.5
PULSE (PR/min)	80	72	80	84
RESPIRATORY SYSTEM (RR/min)	36 Coughing +	30	30.Harsh resp Coughing +	36.Harsh resp. Coughing ++
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 8. cont'd.

DAY 12 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	H1	H2	H3	H4
DEMEANOUR	Sl. dull	Sl. dull	Sl. dull	Sl. dull
TEMPERATURE °F	101.2	102.1	102.2	102.5
PULSE (PR/min)	80	72	80	84
RESPIRATORY SYSTEM (RR/min)	36. Coughing ++	30	32.Harsh resp Coughing ++	36.Harsh resp. Coughing ++
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 8 cont'd. DAY 13 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	H1	H2	H3	H4
DEMEANOUR	Sl.dull	Sl.dull	Sl.dull	Sl.dull
TEMPERATURE °F	102.2	102.5	102.2	102.6
PULSE (PR/min)	84	80	84	90
RESPIRATORY SYSTEM (RR/min)	36.Harsh resp SND Coughing	32.Harsh resp Coughing	36.Harsh resp SND Coughing	40.Harsh resp. SMD Coughing +++
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 8 cont'd.

DAY 14 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	H1	H2	H3	H4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	104.1	104.1	104.0	105.1
PULSE (PR/min)	84	92	96	100
RESPIRATORY SYSTEM (RR/min)	78.Harsh resp SND Coughing ++	96.Harsh resp Coughing +	102.Harsh resp SMD Dyspnoea Coughing ++	72.Harsh resp. SMD Coughing ++ Rhonchi
EYES	–	–	–	–
OTHER SIGNS	–	–	–	–

TABLE 8 cont'd. DAY 15 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	H1	H2	H3	H4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	104.2	104.0	103.7	105.6
PULSE (PR/min)	96	84	90	100
RESPIRATORY SYSTEM (RR/min)	84.Harsh resp SMD Coughing	72.Harsh resp SMD Muzzle encrusted and peeling	84.Harsh resp SMD Coughing	84.Harsh resp. SMD Coughing ++ Diph.lesions
EYES	–	Conjunctivitis in left eye	–	–
OTHER SIGNS	Red.appetite KILLED TODAY	Red.appetite	Red.appetite	Anorexia

TABLE 8.

DAY 16 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	H1	H2	H3	H4
DEMEANOUR		Dull	Dull	Dull
TEMPERATURE °F		103.0	103.4	104.5
PULSE (PR/min)		90	90	96
RESPIRATORY SYSTEM (RR/min)		66.Harsh resp Coughing ++	80.Harsh resp Coughing ++	72.Harsh resp. SND Diph.lesions
EYES		-	-	--
OTHER SIGNS		Red.appetite	-	Red.appetite

TABLE 8. cont'd DAY 17 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	H1	H2	H3	H4
DEMEANOUR		Dull	Dull	Dull
TEMPERATURE °F		103.0	103.4	104.5
PULSE (PR/min)		90	90	96
RESPIRATORY SYSTEM (RR/min)		66.Harsh resp Coughing ++	80.Harsh resp Coughing ++	72.Harsh resp. SND Diph.lesions
EYES		-	-	-
OTHER SIGNS		Red.appetite	-	Red.appetite KILLED TODAY

TABLE 9. The details of the clinical response following intranasal challenge with the Strichen strain of infectious bovine rhinotracheitis virus on "Pneumovac Plus" vaccinated animals (Group K).

DAY 0 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	K1	K2	K3	K4
DEMEANOUR	Bright	Bright	Bright	Bright
TEMPERATURE °F	102.0	102.0	102.0	102.5
PULSE (PR/min)	80	80	80	72
RESPIRATORY SYSTEM (RR/min)	30	30	30	28
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 9 cont'd. DAY 1 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	K1	K2	K3	K4
DEMEANOUR	Bright	Bright	Sl.dull	Bright
TEMPERATURE °F	102.1	102.0	102.2	102.5
PULSE (PR/min)	80	80	84	72
RESPIRATORY SYSTEM (RR/min)	30	30	30	30
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 9 cont'd

DAY 2 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	K1	K2	K3	K4
DEMEANOUR	Bright	Sl.dull	Sl.dull	Bright
TEMPERATURE °F	102.1	103.5	104.2	102.0
PULSE (PR/min)	84	80	80	84
RESPIRATORY SYSTEM (RR/min)	30. SND	30. SND	32. SND	30. SND
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE 9 cont'd DAY 3 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	K1	K2	K3	K4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	106.0	106.0	105.6	104.2
PULSE (PR/min)	96	96	90	80
RESPIRATORY SYSTEM (RR/min)	60.Harsh resp SMD Diph.lesions Respstertor	90.Harsh resp SND Diph.lesions Coughing	80.Harsh res MND Diph.lesions Coughing	70.Harsh resp. SND Diph.lesions
EYES	SOD	POD	POD	-
OTHER SIGNS	Red.appetite ds	Anorexia ds	Anorexia	Red.appetite

TABLE 9 cont'd

DAY 4 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	K1	K2	K3	K4
DEMEANOUR	Dull	Dull	Dull	Dull
TEMPERATURE °F	104.6	104.2	104.3	104.0
PULSE (PR/min)	96	96	90	80
RESPIRATORY SYSTEM (RR/min)	70.Harsh resp. MND Resp.stertor Coughing Diph.lesions	50.Harsh resp MND Diph.lesions	48.Harsh resp SND Coughing Diph.lesions	48.Harsh resp. SND Diph.lesions Coughing
EYES	SOD	Conjunctivitis POD	Conjunctivitis POD	-
OTHER SIGNS	Anorexia ds	Anorexia ds	Anorexia ds	Anorexia

TABLE 9 cont'd. DAY 5 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	K1	K2	K3	K4
DEMEANOUR	Sl.dull	Sl.dull	Sl.dull	Sl.dull
TEMPERATURE °F	103.5	102.3	102.7	102.6
PULSE (PR/min)	80	86	80	80
RESPIRATORY SYSTEM (RR/min)	36.Harsh resp. MND Diph.lesions	50.Harsh resp MND Diph.lesions Coughing	40 SMD Diph.lesions	40 SND Diph.lesions Coughing
EYES	-	SOD	-	SOD
OTHER SIGNS	Red.appetite	Red.appetite ds	-	-

TABLE 9 cont'd.

DAY 6 POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
	K1	K2	K3	K4
DEMEANOUR	Sl.dull	Bright	Bright	Bright
TEMPERATURE °F	103.2	102.0	102.2	102.0
PULSE (PR/min)	80	80	72	80
RESPIRATORY SYSTEM (RR/min)	40.Harsh resp SMD Diph.lesions	40.Harsh res SMD Diph.lesions Coughing	30 SND Diph.lesions	36.Harsh resp. SMD Diph.lesions
EYES	-	-	-	-
OTHER SIGNS	-	-	-	-

TABLE cont'd. DAY POST-INFECTION

CLINICAL PARAMETER	EXPERIMENTAL ANIMAL NO.			
DEMEANOUR				
TEMPERATURE °F				
PULSE (PR/min)				
RESPIRATORY SYSTEM (RR/min)				
EYES				
OTHER SIGNS				

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