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REVIEW

BOVINE CORONAVIRUS

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SUMMARY

This review aims to summarize current data describing the characteristics of bovine coronavirus (BCV) and the three clinical syndromes with which this virus is associated. The first half of this paper consists of a general description of the virus, commencing with a brief outline of the methods used for *in vitro* growth. The structure of the virus is then described in more detail, with particular reference to the structure and functions of the four major viral proteins. This is followed by an outline of the unique replication strategy adopted by coronaviruses. The second half of this review discusses the clinical significance of the virus, beginning with a detailed account of BCV-induced neonatal calf diarrhoea, the clinical syndrome with which this virus is most commonly associated. The clinical and epidemiological importance of BCV respiratory tract infection is then discussed, and finally the evidence supporting the aetiological role of BCV in outbreaks of winter dysentery in adult cattle is examined.

INTRODUCTION

Definition

Bovine coronavirus (BCV) was first reported by Mebus *et al.* (1972, 1973a), and is now widely recognized as an important cause of neonatal calf diarrhoea. The virus also infects the bovine respiratory tract and has been associated with winter dysentery in adult cattle. There has been a single report of accidental transmission of BCV from experimentally inoculated calves to a human investigator (Storz & Rott, 1981), but until further evidence becomes available the zoonotic potential of BCV must remain open to question.

Bovine coronavirus belongs to antigenic group 2 of the Coronaviridae family of viruses (coronaviruses), and for further information on BCV and other members

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of this family readers should refer to the recent reviews published by Babiuk *et al.* (1985), Siddell (1987), Spaan *et al.* (1988, 1990), Holmes (1990) and Lai (1990).

In vitro *growth*

Despite problems in virus isolation, BCV has now been grown in both tracheal and gut organ cultures (Stott *et al.*, 1976; Bridger *et al.*, 1978a, b; McNulty *et al.*, 1984) and also in a large number of cell lines including human rectal tumour-18 (HRT-18), Vero, Madin Darby bovine kidney (MDBK) and Madin Darby canine kidney 1 (MDCK1) (Laporte *et al.*, 1979; Dea *et al.*, 1980b; Schultze *et al.*, 1991b). Addition of exogenous trypsin enhances or promotes the growth of BCV in many cell lines including Vero, bovine foetal thyroid (BFTy), bovine foetal brain (BFB) and bovine embryonic lung (BEL) (Dea *et al.*, 1980b; Storz *et al.*, 1981; Toth, 1982). Virus growth in early passages in cell culture is typically without the production of a recognizable cytopathic effect (CPE). Later passages may result in a marked CPE characterized by syncytia formation and cell detachment, the precise CPE varying with both the virus strain and the host cell type. Plaques are observed when inoculated cultures are overlayed with agarose, and the addition of trypsin may enhance both CPE and plaque formation (Storz *et al.*, 1981; Vautherot, 1981; Hirano *et al.*, 1985; St. Cyr-Coats & Storz, 1988; Payne & Storz, 1990).

Animal models of infection

At present colostrum deprived gnotobiotic or conventional calves are the only animal models of BCV enteric infection. Dea *et al.* (1980a) failed to demonstrate BCV replication in other species, although a number of groups have demonstrated encephalitis in neonatal mice following inoculation with BCV via the intranasal or preferably the intracerebral route (Kaye *et al.*, 1975; Akashi *et al.*, 1981; Gerna *et al.*, 1981; Barthold *et al.*, 1990).

STRUCTURE

Morphology

Bovine coronavirus particles are pleomorphic to rounded in shape, varying in diameter from 80 to 160 nm and with a mean diameter of about 120 nm. Well preserved virions present a characteristic appearance when negatively stained preparations are examined under the electron microscope (EM). The virus envelope is seen as a distinct pair of electron dense shells from which the spike (S) glycoproteins (gps) radiate to form a fringe of surface projections (spikes or peplomers). These petal shaped spikes are about 20 nm long and lie external to a second fringe of shorter projections formed by the haemagglutinin-esterase (HE) gps. Both types of surface projection may be lost during sample storage and preparation, so virions which either partially or completely lack these surface projections are also frequently observed (Sharpee *et al.*, 1976; Bridger *et al.*, 1978b; Dea *et al.*, 1980a; Roseto *et al.*, 1982).

Virus proteins

Bovine coronavirus particles possess four major structural proteins: the nucleo-

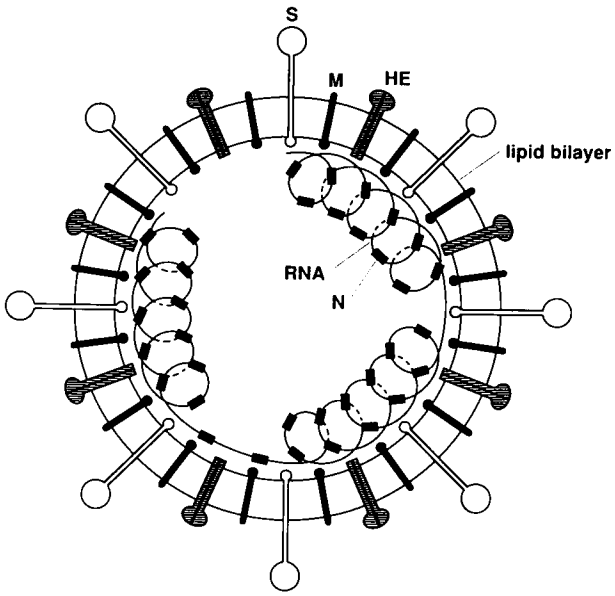

Fig. 1. A diagrammatic model of BCV structure. The viral envelope consists of a lipid bilayer derived from intracellular membranes of the host cell and three major virus proteins: M, S and HE. The M gp spans the viral envelope and interacts with the nucleocapsid, whilst the S and HE gps project from the viral envelope. The helical nucleocapsid lies internal to the envelope and consists of a single strand of non-segmented RNA and many molecules of the viral N protein.

capsid protein N, the integral membrane gp M (formerly matrix gp, E1), the spike gp S (formerly peplomer gp, E2) and the haemagglutinin-esterase gp HE (formerly haemagglutinin gp, E3) (King & Brian, 1982; Brian *et al.*, 1983; Deregt *et al.*, 1983, 1987; Vautherot & Laporte, 1983; St. Cyr-Coats *et al.*, 1988). The N protein lies internal to the virus envelope and is associated with the viral RNA, the M gp spans the viral envelope whilst the S and HE gps project from the envelope (Fig. 1). A number of minor structural and non-structural proteins have also been described (Babiuk *et al.*, 1985; Cox *et al.*, 1989; Abraham *et al.*, 1990b).

Nucleocapsid protein, N

The N protein is non-glycosylated, has a molecular weight (MW) of 50–52 kDa and can form disulphide-linked trimers (MW 160 kDa) under non-reducing conditions. The gene encoding the N protein has been sequenced in the Mebus (M) and F15 strains of BCV (Lapps *et al.*, 1987; Cruciere & Laporte, 1988): only minor sequence differences were detected between these two viruses. The encoded protein is rich in serine residues, and these may be phosphorylated. The N protein is also rich in basic amino acids, which are concentrated in regions representing the sites of genomic RNA binding. Many molecules of the N protein are associated with the genome to form a long, flexible, helical nucleocapsid.

Integral membrane glycoprotein, M

The M gp exists as a series of species which have different levels of glycosylation on an identical protein backbone. It is thought to originate as an unglycosylated precursor protein with a MW of about 22 kDa. Subsequent addition of one or two oligosaccharide side chains, each with a MW of about 2 kDa, results in species with MWs of about 24 and 26 kDa. The carbohydrates are joined by O-linked glycosidic bonds (King & Brian, 1982; Deregt *et al.*, 1987). The gene encoding the M gp of M strain BCV has been sequenced by Lapps *et al.* (1987), and analysis of sequence data for this and other coronaviruses has led to the proposal that the M gp has three distinct domains. A 5 kDa hydrophilic amino-terminal (N-terminal) domain lies external to the virus envelope, and contains all the carbohydrate present in the protein. The central domain forms three hydrophobic α-helices which span the lipid bilayer three times whilst the carboxy-terminal (C-terminal) domain lies on the inner surface of the viral envelope and interacts with the nucleocapsid.

Spike glycoprotein, S

The S gp exists either as an uncleaved 190 kDa precursor or as two cleavage products which migrate on polyacrylamide gel electrophoresis (PAGE) as a single band (100 kDa) or as two closely related bands (90 and 110 kDa). In the majority of cell lines the S gp is present as the cleaved form, but when the virus is grown in cells such as bovine foetal spleen (BFS) or BFB, exogenous trypsin is required for cleavage (Storz *et al.*, 1981; St. Cyr-Coats *et al.*, 1988). The genes encoding the S gp of six different strains of BCV have been sequenced, and have been found to be highly conserved (Abraham *et al.*, 1990a; Boireau *et al.*, 1990; Parker *et al.*, 1990b; Zhang *et al.*, 1991). The encoded protein contains 1363 amino acids and 19 potential N-linked glycosylation sites. A peptidase cleavage site has been identified at amino acids 764–768, allowing cleavage of the S precursor into S1 and S2 subunits which represent the N-terminal and C-terminal cleavage products respectively. The S2 subunit contains a C-terminal hydrophobic α-helix which anchors the Sgp in the virus membrane, whilst two amphipathic α-helices form the stalk which supports the outer bulbous S1 subunit.

The S gp has several important functions, the first of which is to bind to the host cell receptor during initiation of infection. Both isolated S gp and intact virions agglutinate mouse, rat and adult chicken red blood cells by binding to a receptor on the erythrocyte surface which contains a modified sialic acid residue, *N*-acetyl-9-O-acetylneuraminic acid (Neu 5, 9 Ac_2). It has been postulated that the same residue may act as a receptor on the target cell membrane during the infection process (Vlasak *et al.*, 1988b; Schultze *et al.*, 1990, 1991a). The S gp is also important in membrane fusion events: at the start of infection it induces fusion of the virus envelope with the cell membrane and later in infection it induces cell-to-cell fusion resulting in the spread of virus between cells and the formation of syncytia. Cleavage of the S gp is required for activation of its cell fusion activity, which is mediated by the S2 subunit (Storz *et al.*, 1981; Payne & Storz, 1988; St. Cyr-Coats *et al.*, 1988; Yoo *et al.*, 1991a). The S gp contains important neutralizing epitopes, and monoclonal antibodies (mAbs) directed against this protein neutralize the virus both *in vitro* and *in vivo* (Deregt *et al.*, 1989b). Strongly neutralizing mAbs have been mapped to two regions on the S1 subunit, and a further two regions

have been defined by non-neutralizing mAbs on each of the S1 and S2 subunits (Deregt & Babiuk, 1987; Deregt *et al.*, 1989a; Vautherot *et al.*, 1990; Yoo *et al.*, 1990, 1991b).

Haemagglutinin-esterase glycoprotein, HE

The HE gp is a disulphide linked dimer with a MW of 120–140 kDa. It is reduced by 2-mercaptoethanol to two identical monomers, each with a MW of 65 kDa. The gene encoding the HE gp has been sequenced in the Quebec and M strains of BCV, the reported sequences differing by only two bases which lead to a change in just one amino acid (Parker *et al.*, 1989; Kienzle *et al.*, 1990). The encoded protein contains 424 amino acids, nine potential sites for N-linked glycosylation and a C-terminal membrane anchoring domain. The primary translation product has a MW of 42.5 kDa. This protein is glycosylated to yield a 59 kDa monomer, which rapidly undergoes dimerization by the formation of disulphide bonds before further glycosylation yields the mature gp (Deregt *et al.*, 1987; Hogue *et al.*, 1989; Parker *et al.*, 1990a).

Bovine coronavirus induces both haemagglutination and haemadsorption, the latter term referring to the process by which erythrocytes are adsorbed to the membranes of infected cells (Sharpee *et al.*, 1976; Kienzle *et al.*, 1990; Payne & Storz, 1990). It was initially proposed that the HE gp was the sole haemagglutinating protein of BCV (King & Brian, 1982; King *et al.*, 1985) but it has now been demonstrated that both the S and the HE gps cause haemagglutination by binding to surface receptors containing Neu 5, $9Ac_2$ (Schultze *et al.*, 1991a, b). The HE gp is in fact a less potent haemagglutinin than the S gp, as it only agglutinates erythrocytes from mice and rats and not those from adult chickens which bear fewer surface receptors (Schultze *et al.*, 1991a). At present it is unclear why BCV contains two receptor-binding proteins, particularly since many coronaviruses lack the HE gp. In common with the S gp, the HE gp contains important neutralizing epitopes, and mAbs directed against the HE gp neutralize the virus both *in vitro* and *in vivo* (Deregt *et al.*, 1989b). Two independent epitopes (A and C) and a third overlapping epitope (B) have been defined on this protein; strongly neutralizing mAbs all mapped to epitope A (Deregt & Babiuk, 1987; Parker *et al.*, 1989, 1990a; Vautherot *et al.*, 1990).

The HE gp also contains a receptor destroying enzyme (RDE) which cleaves an ester linkage to release 9-O-acetyl residues from sialic acids and which therefore inactivates the erythrocyte receptor (Neu 5, $9Ac_2$) for BCV. Activation of this enzyme causes the virus to elute from erythrocytes when the temperature is raised to 37°C (Vlasak *et al.*, 1988a, b; Parker *et al.*, 1990a; Vautherot *et al.*, 1990; Schultze *et al.*, 1991b; Storz *et al.*, 1991). The RDE is probably involved in the early stages of virus replication, as enzyme inhibition greatly reduces viral infectivity (Vlasak *et al.*, 1988a).

The HE gp of BCV bears both a structural and functional resemblance to the HE protein of type C influenza viruses. An amino acid sequence homology of 29.7% has been demonstrated between the HE protein of BCV and the HA1 subunit of the influenza C virus HE protein (Kienzle *et al.*, 1990), and the HE proteins of both viruses induce haemagglutination, bind to receptors containing Neu 5, $9Ac_2$ and possess a receptor destroying acetylesterase enzyme (Vlasak *et al.*,

1988a, b; Schultze *et al.*, 1990, 1991a, b; Storz *et al.*, 1991). On the basis of these observations it has been proposed that coronaviruses may have acquired the HE gene from the influenza C viruses by a non-homologous recombination event (Luytjes *et al.*, 1988).

Genome

The BCV genome consists of a single strand of non-segmented RNA of positive sense (Guy & Brian, 1979). It is about 27–30 kb in size, and is organized into seven regions which each contain one or more open reading frames. The regions are separated by reinitiation sites (junction sequences), and these contain the signal for the transcription of the subgenomic messenger RNAs. The 5' end of the genome encodes for non-structural proteins including the viral RNA polymerase enzyme, whilst the gene order for the structural proteins is: 5'-HE-S-M-N-3' (Spaan *et al.*, 1988).

STRAIN VARIATION

The BCV isolates studied to date all belong to a single serotype as polyclonal sera have only detected very minor antigenic variations (Bridger *et al.*, 1978b; Dea *et al.*, 1982; El-Ghorr *et al.*, 1989; Heckert *et al.*, 1991c; Tsunemitsu *et al.*, 1991). Monoclonal antibodies have detected variations on the N, S and HE proteins and some isolates also vary in their physicochemical properties and in their ability to produce a CPE and form plaques in cultured cell monolayers (Dea *et al.*, 1980a; Vautherot & Laporte, 1983; Vautherot *et al.*, 1984; St. Cyr-Coats & Storz, 1988; Czerny & Eichhorn, 1989; Deregt *et al.*, 1989b; El-Ghorr *et al.*, 1989; Clark *et al.*, 1990; Hussain *et al.*, 1991).

REPLICATION

The unique replication strategy of coronaviruses has been described in several recent reviews (Siddell, 1987; Spaan *et al.*, 1988; Holmes, 1990; Lai, 1990). Studies on BCV indicate that this virus replicates in a manner similar to that of the other coronaviruses (Doughri *et al.*, 1976; Tektoff *et al.*, 1983; Keck *et al.*, 1988; Hofmann *et al.*, 1990), and the aim of this section is therefore merely to highlight the most important aspects of coronavirus replication.

It seems likely that BCV attaches via both the S and HE gps to receptors (possibly Neu 5, $9Ac_2$) on the target cell membrane (Vlasak *et al.*, 1988b; Schultze *et al.*, 1991a, b). The precise mechanism by which the virus then enters the cell is uncertain. Uptake may be achieved either by direct fusion of the virus envelope with the plasma membrane of the cell, or by endocytosis followed by fusion of the virus envelope with the membranes of endocytic vesicles (Payne & Storz, 1988; Payne *et al.*, 1990b; Yoo *et al.*, 1991a).

Once inside the cell virus replication occurs entirely within the cell cytoplasm. The genomic RNA first attaches to ribosomes and directs the synthesis of RNA dependent RNA polymerase. This enzyme directs transcription of a complementary full length (−) strand of RNA from the genomic (+) strand. The (−) strand

RNA serves as a template for synthesis of full length genomic RNA and also seven subgenomic mRNAs, which form a 3' nested set with common 3' ends. Each mRNA contains all the nucleotide sequence of the next smallest mRNA plus an extra gene at the 5' end which is referred to as the unique region. The 5' ends also have a common leader sequence of 60–70 bases, and it has been suggested that this leader sequence originates from the 3' end of the (–) strand RNA by a discontinuous leader primed transcription mechanism. In this model, leader RNA transcribed from the 3' end of the (–) strand RNA would separate from the rest of the template but still remain bound to the polymerase enzyme. The leader sequence–polymerase complex would then bind by base pairing at specific non-coding regions called reinitiation sites on the (–) strand RNA, where it would act as a primer for synthesis of the rest of the mRNA. It is likely that this proposed model of mRNA synthesis is still incomplete, particularly since it has recently been demonstrated that the mRNAs themselves undergo replication through a (–) strand copy of each mRNA (Hofmann *et al.*, 1990).

Only the unique regions of the mRNAs are translated, and most contain a single open reading frame which codes for only one protein. Translation of the mRNAs coding for the non-structural and N proteins occurs on free ribosomes in the cell cytoplasm, whilst the M, S and HE gps are synthesized on ribosomes at the rough endoplasmic reticulum (RER). The M gp is only glycosylated once it reaches the Golgi apparatus, whereas the S and HE gps are glycosylated at the RER during protein synthesis and the carbohydrate side chains subsequently modified during transport through the Golgi apparatus. Virus assembly occurs by budding at the membranes of the RER and Golgi apparatus, the site of assembly being determined by the restricted intracellular transport of the M gp. The N protein interacts with newly synthesized genomic RNA to form fragile nucleocapsids, which align on the cytoplasmic surface of the membranes of the RER and Golgi due to an interaction with the M gp. In these membranes the host cell proteins are replaced by viral gps, and whole virions are pinched off and released into the lumen.

Most BCV particles are released from intact cells using the normal cell secretory mechanisms, although a few particles are released by lysis of dying cells. Excess S and HE gps which have not been incorporated into virions are also transported to the plasma membrane of the cell, where they may act as targets for the host's immune response (Kienzle *et al.*, 1990; Payne *et al.*, 1990a).

BOVINE CORONAVIRUS AND NEONATAL CALF DIARRHOEA

Pathogenesis and pathology

The pathogenesis of BCV in the calf gastrointestinal tract and the associated pathological findings have been described in detail by several groups including Mebus *et al.* (1973b, 1975), Langpap *et al.* (1979), Bridger *et al.* (1978b) and Babiuk *et al.* (1985). The accompanying physiological changes have been reported by Lewis & Phillips (1978). These findings are briefly summarized below.

Calves may be infected with BCV by both the oral and respiratory routes. Virus infection of the enteric tract starts in the proximal small intestine and spreads

throughout the small and large intestines. Virus replication occurs in the surface epithelial cells, particularly in those on the distal half of the villi in the lower small intestine. Infected cells die, slough off and are replaced by immature cells. In the small intestine these changes result in stunting and fusion of adjacent villi, and in the large intestine they lead to atrophy of the colonic ridges. On histological examination it is seen that the tall columnar epithelial cells which normally line the small intestinal villi and colonic ridges are replaced by cuboidal and squamous epithelial cells, and in severe infections there may even be areas of complete desquamation. These changes are accompanied by a decrease in the number of goblet cells. Scanning electron microscopy (SEM) demonstrates that there are wide variations in the length and spacing of the microvilli on individual cells.

The absorptive capacity of the gut is severely diminished by the loss in surface area and by the presence of immature cells. These cells retain some of their secretory activity which leads to a further increase in the volume of fluid in the gut lumen. At the same time the immature cells are unable to secrete the normal digestive enzymes, so the digestive capacity of the gut is reduced. Undigested lactose accumulates in the gut lumen, leading to an increase in microbial activity and an osmotic imbalance which draws more water into the gut. The decrease in digestive and absorptive capacities leads to diarrhoea, with loss of water and electrolytes. In severe infections, diarrhoea may lead to dehydration, acidosis and hypoglycaemia, and death may occur due to acute shock and heart failure. More commonly the infection is self-limiting, as the virus rarely attacks crypt epithelial cells. The mitotic activity of these cells increases, producing immature cells which are more resistant to virus infection and which migrate up the villi to replace the damaged cells.

Clinical signs

The severity of BCV enteritis varies with both the age and immunological status of the calf and the infective dose and strain of virus, diarrhoea developing more quickly and being more severe in very young or colostrum deprived calves. The clinical signs associated with BCV enteritis are indistinguishable from those associated with rotavirus infection. A yellow diarrhoea develops about 48 h after experimental infection and continues for 3–6 days: the virus may be detected in the faeces throughout this period. Calves are often dull and anorexic during the acute stage of infection, and if diarrhoea is severe they may also become pyrexic and dehydrated. The majority of calves recover, but a few may die if diarrhoea is particularly severe (Mebus *et al.*, 1973b; Bridger *et al.*, 1978b; Reynolds *et al.*, 1985; Saif *et al.*, 1986).

Epidemiology

Bovine coronavirus has been reported in many countries and is probably distributed world wide (Mebus *et al.*, 1972; Acres *et al.*, 1975; Zygraich *et al.*, 1975; Woode *et al.*, 1978; de Visser *et al.*, 1987; Tsunemitsu *et al.*, 1991). The virus is widespread in cattle populations, and consequently serum antibodies to BCV can be detected in the majority of adult cattle (Hajer & Storz, 1978; Rodak *et al.*, 1982). The virus may be detected in both diarrhoeic and healthy calves, the reported incidence rates ranging from 8 to 69% and 0 to 24% for diarrhoeic and healthy calves

respectively (Marsolais *et al.*, 1978; Moon *et al.*, 1978; Woode *et al.*, 1978; Dea *et al.*, 1979; Langpap *et al.*, 1979; Reynolds *et al.*, 1986; Snodgrass *et al.*, 1986). The virus is frequently found in diarrhoeic faecal samples in conjunction with other enteropathogens, particularly rotavirus.

Bovine coronavirus causes enteritis in calves in both dairy and beef herds. Affected animals range in age from 1 day to about 3 months old, but diarrhoea typically occurs at between 1 and 2 weeks of age (Langpap *et al.*, 1979; Reynolds *et al.*, 1986; Mostl & Burki, 1988). Disease is more prevalent during the winter months, and this may reflect the enhanced capacity of the virus to survive in a cool, moist environment. Outbreaks of diarrhoea often occur in consecutive years on the same farm, and this may be because the virus remains viable in the environment from year to year. Bovine coronavirus is, however, a labile virus and outbreaks of diarrhoea still occur if cows are transferred to clean ground at calving. It is therefore more likely that clinically normal adult cows which are persistently infected with the virus act as a source of infection for susceptible calves. Bovine coronavirus has been detected in the faeces of a high proportion (over 70%) of clinically normal adult cows, despite the presence of specific antibodies in the serum and faeces (Crouch & Acres, 1984; Crouch *et al.*, 1985). The rate of virus excretion increases during the winter months and at parturition, and although the infectivity of BCV particles in faeces from clinically normal adult cattle has so far not been demonstrated, calves born to carrier animals have a significantly higher risk of developing diarrhoea (Collins *et al.*, 1987; Bulgin *et al.*, 1989). Subclinical persistent or recurrent infections are also common in both neonatal and older calves and virus excretion from these animals may maintain a reservoir of infection for susceptible calves (Heckert *et al.*, 1990, 1991c, d; Kapil *et al.*, 1990).

Diagnosis

Enteric BCV infections are generally diagnosed by examination of faecal samples for the virus. Initially negatively stained preparations were examined by electron microscopy, but accurate identification of the virus was often difficult (Stair *et al.*, 1972; Durham *et al.*, 1979). Virus recognition was facilitated by the use of specific antibodies and gold conjugated probes (Langpap *et al.*, 1979; Saif *et al.*, 1986; El-Ghorr *et al.*, 1988; Heckert *et al.*, 1989). Virus isolation is rarely used as a means of diagnosis as BCV is difficult to isolate, but some diagnostic tests are based on the haemagglutinating properties of the virus. The simplest of these methods is to use a haemagglutination (HA) test followed by a haemagglutination inhibition (HAI) test to check for BCV specificity (Sharpee *et al.*, 1976; Sato *et al.*, 1977). Faecal samples cause problems when tested in haemagglutination assays as they contain non-specific haemagglutinins, so modifications have been made to the basic HA test in an attempt to overcome this problem (Van Balken *et al.*, 1978; Sato *et al.*, 1984).

Enzyme linked immunosorbent assays (ELISAs) are probably the most widely used diagnostic test for BCV. Early assays were based on the use of polyclonal antisera (Reynolds *et al.*, 1984), but more recently mAbs have been incorporated to improve the specificity of these tests (Crouch *et al.*, 1984; Crouch & Acres, 1984; Czerny & Eichhorn, 1989). Recently dot blot hybridization assays have also been described which use a cDNA probe labelled with either phosphorus-32 (P32) or

biotin to detect BCV RNA (Shockley *et al.*, 1987; Verbeek & Tijssen, 1988, 1990; Verbeek *et al.*, 1990).

Treatment

Calves suffering from BCV enteritis should be treated by the standard methods used to treat other forms of calf diarrhoea. The aim of treatment is to replace the loss of fluids and electrolytes which can otherwise lead to dehydration and acidosis, and this is achieved by fluid replacement therapy which may also be accompanied by discontinuing the feeding of milk. The fluids used should be balanced electrolyte solutions and these may be given via the oral or intravenous routes. Oral administration of kaolin mixtures may be used to lessen the severity of diarrhoea. Affected animals should be segregated from healthy animals and kept in a warm area with fresh bedding.

Control

Protection against BCV diarrhoea is dependent on the presence of adequate levels of specific antibodies in the gut lumen. In neonatal calves this local immunity is passively acquired from the dam in the form of antibodies ingested in the colostrum and milk. The predominant isotype involved is IgG1, and this is largely derived from serum by a selective transport mechanism operating in the bovine mammary gland. The level of BCV specific antibodies in the lacteal secretions is very high at parturition but then declines rapidly (Rodak *et al.*, 1982; Crouch & Raybould, 1983). Duration of passively acquired immunity in the calf is prolonged by resecretion of absorbed antibodies back into the gut lumen and by adherence of immunoglobulins to the surface of the intestinal mucosa. Following natural exposure to infection, calves develop active immunity to the virus and it is probably IgA antibodies at the mucosal surface which protect against reinfection (Saif, 1987; Heckert *et al.*, 1991a, c). Diarrhoea is most likely to occur if the passively acquired immunity declines before an adequate level of active immunity has developed. The offspring of cows with low serum antibody titres and therefore low levels of antibody in their colostrum and milk are especially susceptible to infection, calves born to primiparous cows being particularly vulnerable (Woode & Bridger, 1975; Moon *et al.*, 1978; Crouch, 1985; Saif, 1985).

BCV diarrhoea can be prevented either by decreasing the challenge dose of virus to susceptible animals or by increasing their levels of specific immunity. The identification and segregation of carrier cows and calves should in theory decrease the challenge dose of virus, but in practice this approach may be impossible as BCV infections may be widespread even in a closed herd (Radostits & Acres, 1980; Crouch & Acres, 1984; Bulgin *et al.*, 1989; Heckert *et al.*, 1990). The immune status of susceptible calves may be raised either by parenteral vaccination of pregnant cows to increase the level of passively acquired immunity or by oral vaccination of neonatal calves to stimulate active immunity. At present there are no BCV vaccines available in the UK, although several vaccines are available in other countries.

Parenteral vaccination of pregnant cows has proved successful for controlling rotavirus diarrhoea in neonatal calves (Snodgrass *et al.*, 1980, 1982). Vaccination of pregnant mice has protected their offspring against BCV infection and the same approach should be possible in cattle (Bengelsdorff, 1988). Most adult cows

have measurable serum antibody titres to BCV as a result of natural infection (Rodak *et al.*, 1982), and the aim of vaccination is therefore to stimulate a secondary immune response which raises the level of protective antibody which is then ingested by the sucking calf in the colostrum and milk. The resulting level of passively acquired immunity in the calf should both prevent disease whilst at the same time permit subclinical infection to stimulate the development of active immunity. It has already been demonstrated that colostrum containing high levels of BCV specific antibodies will protect calves against clinical BCV infections, although it has also been found that high levels of passive immunity depress the active antibody response to whole BCV and the S and HE gps (Heckert *et al.*, 1991b, c). In contrast to these findings, several groups have found that vaccination of cows with either experimental or commercially available vaccines has little or no effect on the levels of BCV specific antibodies in the serum, colostrum and milk, and that adequate protection of calves is not achieved (Myers & Snodgrass, 1982; Rodak *et al.*, 1982; Waltner-Toews *et al.*, 1983; Collins *et al.*, 1987; Mostl & Burki, 1988). It has, however, been proposed that vaccination may prevent the increase in BCV shedding which occurs from carrier cows at parturition (Collins *et al.*, 1987).

Active immunization of calves by oral inoculation with live, attenuated BCV vaccines has protected colostrum deprived calves in experimental trials but has not proved effective in field trials (Thurber *et al.*, 1977). This is probably because the vaccine virus is neutralized in the gut by passively acquired maternal antibodies. Vaccine administration by the respiratory route has been proposed as an alternative method of stimulating active intestinal immunity without the need for primary infection of the gut, but there is still a danger that the virus is neutralized by maternal antibodies which have been absorbed systemically (Reynolds *et al.*, 1985; Saif *et al.*, 1986). There is also a danger that the neonate may fail to respond adequately to vaccines given by either the oral or respiratory routes due to immaturity of the immune system. *In utero* vaccination of bovine foetuses resulted in protective levels of immunity in 6-day-old calves, but the frequency of abortions and premature births precluded its practical application (Mullaney *et al.*, 1988).

BOVINE CORONAVIRUS AND RESPIRATORY TRACT INFECTIONS

In addition to being an enteric pathogen, BCV can also cause respiratory tract infections in a wide age range of calves. Infection is generally subclinical, but when clinical signs are present they are most commonly seen in calves between 2 and 16 weeks of age (Thomas *et al.*, 1982; McNulty *et al.*, 1984; Reynolds *et al.*, 1985; Singh *et al.*, 1985; Saif *et al.*, 1986; Saif, 1987; Mostl & Burki, 1989; Heckert *et al.*, 1990, 1991a, c, d; Tsunemitsu *et al.*, 1991). Within the respiratory tract the primary sites of infection are the epithelial cells of the nasal cavity and trachea, where infection may lead to mild upper respiratory signs such as rhinitis, sneezing and coughing. Immunofluorescent staining of nasal epithelial cells using BCV specific antibodies is a valuable method for diagnosing these infections (Reynolds *et al.*, 1985). The virus may also infect the lower respiratory tract and cause minor lung lesions, but clinical signs are generally absent. A more severe lower respiratory tract involvement has, however, also been reported (Kapil *et al.*, 1991). Bovine

coronavirus respiratory tract infections are generally not sufficiently severe as to require treatment, but they may predispose calves to more severe secondary lower respiratory tract infections.

Current data suggest that BCVs originating from the enteric and respiratory tracts are antigenically identical (Thomas *et al.*, 1982; Vautherot & Laporte, 1983; McNulty *et al.*, 1984; Reynolds *et al.*, 1985; Tsunemitsu *et al.*, 1991). Experimental investigations have demonstrated that the outcome of infection is largely unaffected by both the origin of the virus (enteric or respiratory) and the route of inoculation (nasal or oral) (Saif *et al.*, 1986). Infected calves frequently harbour BCV in both the enteric and respiratory tracts simultaneously (Reynolds *et al.*, 1985; Saif *et al.*, 1986; Heckert *et al.*, 1991d). The primary importance of BCV respiratory tract infections may be that they allow virus transmission by the aerosol–nasal route, in addition to the faecal–oral route generally used by enteric pathogens. Spread of virus by aerosol infection is assisted by prolonged and recurrent shedding of virus from the upper respiratory tract (Saif, 1987; Heckert *et al.*, 1989, 1991d). In a newly infected animal, initial virus replication in the upper respiratory tract may provide a large number of infective particles protected by mucus which are then transferred to the gut where a secondary infection occurs.

WINTER DYSENTERY

The precise cause of winter dysentery is still unclear, but in recent years BCV has been widely implicated as the aetiological agent. For a more detailed account, readers should refer to the review by Saif (1990). The disease occurs durng the winter months in housed adult dairy and beef cattle, and is characterized by an acute onset of dark, bloody diarrhoea accompanied by a dramatic fall in milk production. Affected animals may also develop a nasolacrimal discharge and cough, and become depressed and anorexic. The disease spreads rapidly within an affected herd leading to a very high morbidity (50–100%), but the mortality rate is very low (1–2%). Economic losses due to the drop in milk production can, however, be very high, as production may not return to normal for several months.

The clinical signs and pathological changes associated with winter dysentery are consistent with BCV being the causative agent (Van Kruiningen *et al.*, 1987). Seroconversion to BCV has been demonstrated in affected cattle, and the virus has frequently been both detected in and isolated from diarrhoeic faecal samples. It has yet to be proven whether this virus is a causative agent of winter dysentery, an opportunistic invader or merely part of the normal microflora of the adult bovine gut (Horner *et al.*, 1975; Durham *et al.*, 1979, 1989; Takahashi *et al.*, 1980, 1983; Espinasse *et al.*, 1982; Broes *et al.*, 1984; Saif *et al.*, 1988, 1991; Benfield & Saif, 1990). The epidemiology of winter dysentery is also consistent with BCV being a causative agent. Herds with a previous history of winter dysentery are more likely to suffer further outbreaks of disease, possibly due to the presence of carrier animals. Coronaviruses survive best at low temperatures and at low intensities of ultraviolet light, leading to higher levels of environmental contamination in winter (Pensaert & Callebaut, 1978). Since BCV may be transmitted by both the oral and

respiratory routes, spread of infection is facilitated by the close confinement of large numbers of cattle during the winter months.

The BCV associated with winter dysentery in adult cattle is closely related to the virus which causes diarrhoea in neonatal calves (Akashi *et al.*, 1980). Slightly different strains of the virus may, however, be involved in the two diseases, as Benfield & Saif (1990) demonstrated that two out of three winter dysentery isolates failed to agglutinate rat and mouse red blood cells, and that all three isolates failed to show strong cross-reactivity with M strain BCV in serum neutralization tests. Whilst present data strongly suggest an association between BCV and winter dysentery, it is not yet possible to induce diarrhoea consistently in adult cattle by oral inoculation with faeces collected from natural cases of the disease, although diarrhoea has been reproduced in gnotobiotic calves (Saif, 1990). Failure to reproduce the disease consistently in adult cattle may be because other risk factors are involved, and these may include both environmental stress factors such as changes in diet and low temperatures and also the presence of other microorganisms.

CONCLUDING REMARKS

Research on BCV is currently at an exciting stage, as not only has our knowledge of the virus advanced rapidly in recent years but a number of important questions are likely to be resolved in the near future. The precise functions of the S and HE gps in the initiation of infection remains to be elucidated, and the regions responsible for *in vivo* protection must be more fully mapped. The occurrence of infections in older animals, whether due to reinfection or the reactivation of subclinical persistent infections, should be further investigated, as should the possible role of BCV in winter dysentery. Finally, and most importantly, effective vaccines must be developed which protect calves against BCV diarrhoea and which may also protect adult animals against winter dysentery. These vaccines may be either conventional whole virus or subunit vaccines, but in either case the protective levels of antibody should be defined and the effect of passive immunity on the development of active immunity further examined.

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DEDICATION

This review is dedicated to my father, John Bryan Clark, who died suddenly during the completion of this manuscript: I hope there are hills and snow in heaven.

REFERENCES

ABRAHAM, S., KIENZLE, T. E., LAPPS, W. & BRIAN, D. A. (1990a). Deduced sequence of the bovine coronavirus spike protein and identification of the internal proteolytic cleavage site. *Virology* **176,** 296–301.

ABRAHAM, S., KIENZLE, T. E., LAPPS, W. E. & BRIAN, D. A. (1990b). Sequence and expression analysis of potential non-structural proteins of 4.9, 4.8, 12.7 and 9.5 kDa encoded between the spike and membrane protein genes of the bovine coronavirus. *Virology* **177,** 488–95.

ACRES, S. D., LAING, C. J., SAUNDERS, J. R. & RADOSTITS, O. M. (1975). Acute undifferentiated neonatal diarrhoea in beef calves. 1. Occurrence and distribution of infectious agents. *Can. J. comp. Med.* **39,** 116–32.

AKASHI, H., INABA, Y., MIURA, Y., TOKUHISA, S., SATO, K. & SATODA, K. (1980). Properties of a coronavirus isolated from a cow with epizootic diarrhoea. *Vet. Microbiol.* **5,** 265–76.

AKASHI, H., INABA, Y., MIURA, Y. *et al.* (1981). Propagation of the Kakegawa strain of bovine coronavirus in suckling mice, rats and hamsters. *Arch. Virol.* **67,** 367–70.

BABIUK, L. A., SABARA, M. & HUDSON, G. R. (1985). Rotavirus and coronavirus infections in animals. *Prog. vet. Microbiol. Immunol.* **1,** 80–120.

BARTHOLD, S. W., SOUZA, M. S. & SMITH, A. L. (1990). Susceptibility of laboratory mice to intranasal and contact infection with coronaviruses of other species. *Lab. anim. Sci.* **40,** 481–5.

BENFIELD, D. A. & SAIF, L. J. (1990). Cell culture propagation of a coronavirus isolated from cows with winter dysentery. *J. clin. Microbiol.* **28,** 1454–7.

BENGELSDORFF, H. J. (1988). Vaccination of cows against coronavirus-infection in their offspring: the mouse as a laboratory animal model. *Berl. Münch. tierärztl. Wschr.* **8,** 278–82.

BOIREAU, P., CRUCIERE, C. & LAPORTE, J. (1990). Nucleotide sequence of the glycoprotein S gene of bovine enteric coronavirus and comparison with the S proteins of two mouse hepatitis virus strains. *J. gen. Virol.* **71,** 487–92.

BRIAN, D. A., HOGUE, B., LAPPS, W., POTTS, B. & KAPKE, P. (1983). Comparative structure of coronaviruses. *Proc. 4th Int. Symp. neonatal Diarrhoea,* 100–16.

BRIDGER, J. C., CAUL, E. O. & EGGLESTONE, S. I. (1978a). Replication of an enteric bovine coronavirus in intestinal organ cultures. *Arch. Virol.* **57,** 43–51.

BRIDGER, J. C., WOODE, G. N. & MEYLING, A. (1978b). Isolation of coronaviruses from neonatal calf diarrhoea in Great Britain and Denmark. *Vet. Microbiol.* **3,** 101–13.

BROES, A., VAN OPDENBOSCH, E. & WILLEMANS, G. (1984). Isolement d'un coronavirus chez des bovins atteints d'enterite hémorragique hivernale (winter dysentery) en Belgique. *Ann. med. Vet.* **128,** 299–303.

BULGIN, M. S., WARD, A. C. S., BARRETT, D. P. & LANE, V. M. (1989). Detection of rotavirus and coronavirus shedding in two beef cow herds in Idaho. *Can. vet. J.* **30,** 235–9.

CLARK, M. A., CAMPBELL, I., EL-GHORR, A. A., SNODGRASS, D. R. & SCOTT, F. M. M. (1990). A comparison of bovine coronavirus strains using monoclonal antibodies. *Adv. exp. Med. Biol.* **276,** 461–6.

COLLINS, J. K., RIEGEL, C. A., OLSON, J. D. & FOUNTAIN, A. (1987). Shedding of enteric coronavirus in adult cattle. *Am. J. vet. Res.* **48,** 361–5.

COX, G. J., PARKER, M. D. & BABIUK, L. A. (1989). The sequence of cDNA of bovine coronavirus 32K nonstructural gene. *Nucl. Acids Res.* **17,** 5847.

CROUCH, C. F. (1985). Vaccination against enteric rota and coronaviruses in cattle and pigs: enhancement of lactogenic immunity. *Vaccine* **3,** 284–91.

CROUCH, C. F. & ACRES, S. D. (1984). Prevalence of rotavirus and coronavirus antigens in the feces of normal cows. *Can. J. comp. Med.* **48,** 340–2.

CROUCH, C. F. & RAYBOULD, T. J. G. (1983). Comparison of different antigen preparations as substrates for use in passive hemagglutination and enzyme-linked immunosorbent assays for detection of antibody against bovine enteric coronavirus. *J. clin. Microbiol.* **18,** 146–9.

CROUCH, C. F., RAYBOULD, T. J. G. & ACRES, S. D. (1984). Monoclonal antibody capture enzyme-linked immunosorbent assay for detection of bovine enteric coronavirus. *J. clin. Microbiol.* **19,** 388–93.

CROUCH, C. F., OHMANN, H. B., WATTS, T. C. & BABIUK, L. A. (1985). Chronic shedding of bovine enteric coronavirus antigen-antibody complexes by clinically normal cows. *J. gen. Virol.* **66,** 1489–500.

CRUCIERE, C. & LAPORTE, J. (1988). Sequence and analysis of bovine enteritic coronavirus (F15) genome. *Ann. Inst. Pasteur* **139,** 123–38.

CZERNY, C. P. & EICHHORN, W. (1989). Characterization of monoclonal and polyclonal antibodies to bovine enteric coronavirus: establishment of an efficient ELISA for antigen detection in feces. *Vet. Microbiol.* **20,** 111–22.

DEA, S., ROY, R. S. & BEGIN, M. E. (1979). Counterimmunoelectroosmophoresis for detection of neonatal calf diarrhea coronavirus: methodology and comparison with electron microscopy. *J. clin. Microbiol.* **10,** 240–4.

DEA, S., ROY, R. S. & BEGIN, M. E. (1980a). Physicochemical and biological properties of neonatal calf diarrhea coronaviruses isolated in Quebec and comparison with the Nebraska calf coronavirus. *Am. J. vet. Res.* **41,** 23–9.

DEA, S., ROY, R. S. & BEGIN, M. E. (1980b). Bovine coronavirus isolation and cultivation in continuous cell lines. *Am. J. vet. Res.* **41,** 30–8.

DEA, S., ROY, R. S. & ELAZHARY, M. A. S. Y. (1982). Antigenic variations among calf diarrhea coronaviruses by immunodiffusion and counterimmunoelectrophoresis. *Ann. rech. Vet.* **13,** 351–6.

DEREGT, D. & BABIUK, L. A. (1987). Monoclonal antibodies to bovine coronavirus: characteristics and topographical mapping of neutralizing epitopes on the E2 and E3 glycoproteins. *Virology* **161,** 410–20.

DEREGT, D., CROUCH, C. F., SABARA, M., GILCHRIST, J. E., BABIUK, L. A. & HUDSON, G. R. (1983). Preliminary studies of a bovine coronavirus (BCV) antigen responsible for neutralization. *Proc. 4th Int. Symp. neonatal Diarrhoea,* 117–33.

DEREGT, D., SABARA, M. & BABIUK, L. A. (1987). Structural proteins of bovine coronavirus and their intracellular processing. *J. gen. Virol.* **68,** 2863–77.

DEREGT, D., PARKER, M. D., COX, G. C. & BABIUK, L. A. (1989a). Mapping of neutralizing epitopes to fragments of the bovine coronavirus E2 protein by proteolysis of antigen–antibody complexes. *J. gen. Virol.* **70,** 647–58.

DEREGT, D., GIFFORD, G. A., IJAZ, M. K., *et al.* (1989b). Monoclonal antibodies to bovine coronavirus glycoproteins E2 and E3: demonstration of *in vivo* virus-neutralizing activity. *J. gen. Virol.* **70,** 993–8.

DOUGHRI, A. M., STORZ, J., HAJER, I. & FERNANDO, H. S. (1976). Morphology and morphogenesis of a coronavirus infecting intestinal epithelial cells of newborn calves. *Exp. mol. Pathol.* **25,** 355–70.

DURHAM, P. J. K., STEVENSON, B. J. & FARQUHARSON, B. C. (1979). Rotavirus and coronavirus associated diarrhoea in domestic animals. *N.Z. vet. J.* **27,** 30–2.

DURHAM, P. J. K., HASSARD, L. E., ARMSTRONG, K. R. & NAYLOR, J. M. (1989). Coronavirus-associated diarrhea (winter dysentery) in adult cattle. *Can. vet. J.* **30,** 825–7.

EL-GHORR, A. A., SNODGRASS, D. R. & SCOTT, F. M. M. (1988). Evaluation of an immunogold electron microscopy technique for detecting bovine coronavirus. *J. virol. Methods* **19,** 215–24.

EL-GHORR, A. A., SNODGRASS, D. R., SCOTT, F. M. M. & CAMPBELL, I. (1989). A serological comparison of bovine coronavirus strains. *Arch. Virol.* **104,** 241–8.

ESPINASSE, J., VISO, M., LAVAL, A., *et al.* (1982). Winter dysentery: a coronavirus-like agent in the faeces of beef and dairy cattle with diarrhoea. *Vet. Rec.* **110,** 385.

GERNA, G., CEREDA, P. M., REVELLO, M. G., CATTANEO, E., BATTAGLIA, M. & GERNA, M. T. (1981). Antigenic and biological relationships between human coronavirus OC43 and neonatal calf diarrhoea coronavirus. *J. gen. Virol.* **54,** 91–102.

GUY, J. S. & BRIAN, D. A. (1979). Bovine coronavirus genome. *J. Virol.* **29,** 293–300.

HAJER, I. & STORZ, J. (1978). Antigens of bovine coronavirus strain LY-138 and their diagnostic properties. *Am. J. vet. Res.* **39,** 441–4.

HECKERT, R. A., SAIF, L. J. & MYERS, G. W. (1989). Development of protein A-gold immunoelectron microscopy for detection of bovine coronavirus in calves: comparison with ELISA and direct immunofluorescence of nasal epithelial cells. *Vet. Microbiol.* **19,** 217–31.

HECKERT, R. A., SAIF, L. J., HOBLET, K. H. & AGNES, A. G. (1990). A longitudinal study of bovine coronavirus enteric and respiratory infections in dairy calves in two herds in Ohio. *Vet. Microbiol.* **22,** 187–201.

HECKERT, R. A., SAIF, L. J., MENGEL, J. P. & MYERS, G. W. (1991a). Isotype-specific antibody responses to bovine coronavirus structural proteins in serum, feces and mucosal secretion from experimentally challenge-exposed colostrum deprived calves. *Am. J. vet. Res.* **52,** 692–9.

HECKERT, R. A., SAIF, L. J., MENGEL, J. P. & MYERS, G. W. (1991b). Mucosal and systemic antibody responses to bovine coronavirus structural proteins in experimentally challenge-exposed calves fed low or high amounts of colostral antibodies. *Am. J. vet. Res.* **52,** 700–8.

HECKERT, R. A., SAIF, L. J., MYERS, G. W. & AGNES, A. G. (1991d). Epidemiologic factors and isotype-specific antibody responses in serum and mucosal secretions of dairy calves with bovine coronavirus respiratory tract and enteric tract infections. *Am. J. vet. Res.* **52,** 845–51.

HECKERT, R. A., SAIF, L. J. & MYERS, G. W. (1991c). Mucosal and systemic isotype-specific antibody responses to bovine coronavirus structural proteins in naturally infected dairy calves. *Am. J. vet. Res.* **52,** 852–7.

HIRANO, N., SADA, Y., TUCHIYA, K., ONO, K. & MURAKAMI, T. (1985). Plaque assay of bovine coronavirus in BEK-1 cells. *Jap. J. vet. Sci.* **47,** 679–81.

HOFMANN, M. A., SETHNA, P. B. & BRIAN, D. A. (1990). Bovine coronavirus mRNA replication continues throughout persistent infection in cell culture. *J. Virol.* **64,** 4108–14.

HOGUE, B. G., KIENZLE, T. E. & BRIAN, D. A. (1989). Synthesis and processing of the bovine enteric coronavirus haemagglutinin protein. *J. gen. Virol.* **70,** 345–52.

HOLMES, K. V. (1990). Coronaviridae and their replication. In *Virology,* eds B. N. Fields *et al.*, pp. 841–56. New York: Raven Press.

HORNER, G. W., HUNTER, R. & KIRKBRIDE, C. A. (1975). Coronavirus-like agent present in faeces of cows with diarrhoea. *N.Z. vet. J.* **23,** 98.

HUSSAIN, K. A., STORZ, J. & KOUSOULAS, K. G. (1991). Comparison of bovine coronavirus (BCV) antigens: monoclonal antibodies to the spike glycoprotein distinguish between vaccine and wild-type strains. *Virology* **183,** 442–5.

KAPIL, S., TRENT, A. M. & GOYAL, S. M. (1990). Excretion and persistence of bovine coronavirus in neonatal calves. *Arch. Virol.* **115,** 127–32.

KAPIL, S., POMEROY, K. A., GOYAL, S. M. & TRENT, A. M. (1991). Experimental infection with a virulent pneumoenteric isolate of bovine coronavirus. *J. vet. diagn. Invest.* **3,** 88–9.

KAYE, H. S., YARBROUGH, W. B. & REED, C. J. (1975). Calf diarrhoea coronavirus. *Lancet* **ii,** 509.

KECK, J. G., HOGUE, B. G., BRIAN, D. A. & LAI, M. M. C. (1988). Temporal regulation of bovine coronavirus RNA synthesis. *Virus Res.* **9,** 343–56.

KIENZLE, T. E., ABRAHAM, S., HOGUE, B. G. & BRIAN, D. A. (1990). Structure and orientation of expressed bovine coronavirus hemagglutinin-esterase protein. *J. Virol.* **64,** 1834–8.

KING, B. & BRIAN, D. A. (1982). Bovine coronavirus structural proteins. *J. Virol.* **42,** 700–7.

KING, B., POTTS, B. J. & BRIAN, D. A. (1985). Bovine coronavirus hemagglutinin protein. *Virus Res.* **2,** 53–9.

LAI, M. M. C. (1990). Coronavirus: organization, replication and expression of genome. *A. Rev. Microbiol.* **44,** 303–33.

LANGPAP, T. J., BERGELAND, M. E. & REED, D. E. (1979). Coronaviral enteritis of young calves: virologic and pathologic findings in naturally occurring infections. *Am. J. vet. Res.* **40,** 1476–8.

LAPORTE, J., L'HARIDON, R. & BOBULESCO, P. (1979). *In vitro* culture of bovine enteric coronavirus (BEC). *Colloq. de l'INSERM* **90,** 99–102.

LAPPS, W., HOGUE, B. G. & BRIAN, D. A. (1987). Sequence analysis of the bovine coronavirus nucleocapsid and matrix protein genes. *Virology* **157,** 47–57.

LEWIS, L. D. & PHILLIPS, R. W. (1978). Pathophysiologic changes due to coronavirus-induced diarrhea in the calf. *J. Am. vet. med. Ass.* **173,** 636–42.

LUYTJES, W., BREDENBEEK, P. J., NOTEN, A. F. H., HORZINEK, M. C. & SPAAN, W. J. M. (1988).

Sequence of mouse hepatitis virus A59 mRNA2: Indications for RNA recombination between coronaviruses and influenza C virus. *Virology* **166,** 415–22.

MARSOLAIS, G., ASSAF, R., MONTPETIT, C. & MAROIS, P. (1978). Diagnosis of viral agents associated with neonatal calf diarrhoea. *Can. J. comp. Med.* **42,** 168–71.

MCNULTY, M. S., BRYSON, D. G., ALLAN, G. M. & LOGAN, E. F. (1984). Coronavirus infection of the bovine respiratory tract. *Vet. Microbiol.* **9,** 425–34.

MEBUS, C. A., WHITE, R. G., STAIR, E. L., RHODES, M. B. & TWIEHAUS, M. J. (1972). Neonatal calf diarrhea: results of a field trial using a reo-like virus vaccine. *Vet. Med./Small Anim. Clin.* **67,** 173–8.

MEBUS, C. A., STAIR, E. L., RHODES, M. B. & TWIEHAUS, M. J. (1973a). Neonatal calf diarrhea: propagation, attenuation and characteristics of a coronavirus-like agent. *Am. J. vet. Res.* **34,** 145–50.

MEBUS, C. A., STAIR, E. L., RHODES, M. B. & TWIEHAUS, M. J. (1973b). Pathology of neonatal calf diarrhea induced by a coronavirus-like agent. *Vet. Pathol.* **10,** 45–64.

MEBUS, C. A., NEWMAN, L. E. & STAIR, E. L. (1975). Scanning electron, light and immunofluorescent microscopy of intestine of gnotobiotic calf infected with calf diarrheal coronavirus. *Am. J. vet. Res.* **36,** 1719–25.

MOON, H. W., MCCLURKIN, A. W., ISAACSON, R. E., *et al.* (1978). Pathogenic relationships of rotavirus, *Escherichia coli,* and other agents in mixed infections in calves. *J. Am. vet. med. Ass.* **173,** 577–83.

MOSTL, K. & BURKI, F. (1988). Incidence of diarrhoea and of rotavirus and coronavirus shedding in calves, whose dams had been vaccinated with an experimental oil-adjuvanted vaccine containing bovine rotavirus and bovine coronavirus. *J. vet. Med.* **35,** 186–96.

MOSTL, K. & BURKI, F. (1989). Bovine coronavirus is also a cause of respiratory disease in calves. *Proc. 11th Int. Symp. W.A.V.M.I.,* 191.

MULLANEY, T. P., NEWMAN, L. E. & WHITEHAIR, C. K. (1988). Humoral immune response of the bovine fetus to *in utero* vaccination with attenuated bovine coronavirus. *Am. J. vet. Res.* **49,** 156–9.

MYERS, L. L. & SNODGRASS, D. R. (1982). Colostral and milk antibody titers in cows vaccinated with a modified live-rotavirus-coronavirus vaccine. *J. Am. vet. med. Ass.* **181,** 486–8.

PARKER, M. D., COX, G. J., DEREGT, D., FITZPATRICK, D. R. & BABIUK, L. A. (1989). Cloning and *in vitro* expression of the gene for the E3 haemagglutinin glycoprotein of bovine coronavirus. *J. gen. Virol.* **70,** 155–64.

PARKER, M. D., YOO, D. & BABIUK, L. A. (1990a). Expression and secretion of the bovine coronavirus hemagglutinin-esterase glycoprotein by insect cells infected with recombinant baculoviruses. *J. Virol.* **64,** 1625–9.

PARKER, M. D., YOO, D., COX, G. J. & BABIUK, L. A. (1990b). Primary structure of the S peplomer gene of bovine coronavirus and surface expression in insect cells. *J. gen. Virol.* **71,** 263–70.

PAYNE, H. R. & STORZ, J. (1988). Analysis of cell fusion induced by bovine coronavirus infection. *Arch. Virol.* **103,** 27–33.

PAYNE, H. R. & STORZ, J. (1990). Scanning electron microscopic characterization of bovine coronavirus plaques in HRT cells. *J. vet. Med.* **37,** 501–8.

PAYNE, H. R., STORZ, J. & HENK, W. G. (1990a). Bovine coronavirus antigen in the host cell plasmalemma. *Exp. mol. Pathol.* **53,** 152–9.

PAYNE, H. R., STORZ, J. & HENK, W. G. (1990b). Initial events in bovine coronavirus infection: analysis through immunogold probes and lysosomotropic inhibitors. *Arch. Virol.* **114,** 175–89.

PENSAERT, M. & CALLEBAUT, P. (1978). The coronaviruses: clinical and structural aspects with some practical implications. *A. med. vet.* **122,** 301–22.

RADOSTITS, O. M. & ACRES, S. D. (1980). The prevention and control of epidemics of acute undifferentiated diarrhea of beef calves in western Canada. *Can. vet. J.* **21,** 243–9.

REYNOLDS, D. J., CHASEY, D., SCOTT, A. C. & BRIDGER, J. C. (1984). Evaluation of ELISA and electron microscopy for the detection of coronavirus and rotavirus in bovine faeces. *Vet. Rec.* **114,** 397–401.

REYNOLDS, D. J., DEBNEY, T. G., HALL, G. A., THOMAS, L. H. & PARSONS, K. R. (1985). Studies on the relationship between coronaviruses from the intestinal and respiratory tracts of calves. *Arch. Virol.* **85,** 71–83.

REYNOLDS, D. J., MORGAN, J. H., CHANTER, N., *et al.* (1986). Microbiology of calf diarrhoea in southern Britain. *Vet. Rec.* **119,** 34–9.

RODAK, L., BABIUK, L. A. & ACRES, S. D. (1982). Detection by radioimmunoassay and enzyme-linked immunosorbent assay of coronavirus antibodies in bovine serum and lacteal secretions. *J. clin. Microbiol.* **16,** 34–40.

ROSETO, A., BOBULESCO, P., LAPORTE, J., ESCAIG, J., GACHES, D. & PERIES, J. (1982). Bovine enteric coronavirus structure as studied by a freeze-drying technique. *J. gen. Virol.* **63,** 241–5.

SAIF, L. J. (1985). Passive immunity to coronavirus and rotavirus infections in swine and cattle: enhancement by maternal vaccination. In *Infectious Diarrhoea in the Young,* eds S. Tzipori *et al.*, pp. 456–67. Amsterdam: Elsevier Science Publishers, B.V. (Biomedical Division).

SAIF, L. J. (1987). Development of nasal, fecal and serum isotype-specific antibodies in calves challenged with bovine coronavirus or rotavirus. *Vet. Immunol. Immunopathol.* **17,** 425–39.

SAIF, L. J. (1990). A review of evidence implicating bovine coronavirus in the etiology of winter dysentery in cows: an enigma resolved? *Cornell Vet.* **80,** 303–11.

SAIF, L. J., REDMAN, D. R., MOORHEAD, P. D. & THEIL, K. W. (1986). Experimentally induced coronavirus infections in calves: viral replication in the respiratory and intestinal tracts. *Am. J. vet. Res.* **47,** 1426–32.

SAIF, L. J., REDMAN, D. R., BROCK, K. V., KOHLER, E. M. & HECKERT, R. A. (1988). Winter dysentery in adult dairy cattle: detection of coronavirus in the faeces. *Vet. Rec.* **123,** 300–301.

SAIF, L. J., BROCK, K. V., REDMAN, D. R. & KOHLER, E. M. (1991). Winter dysentery in dairy herds: electron microscopic and serological evidence for an association with coronavirus infection. *Vet. Rec.* **128,** 447–9.

SATO, K., INABA, Y., KUROGI, H., *et al.* (1977). Hemagglutination by calf diarrhea coronavirus. *Vet. Microbiol.* **2,** 83–7.

SATO, K., INABA, Y., TOKUHISA, S., *et al.* (1984). Detection of bovine coronavirus in feces by reversed passive hemagglutination. *Arch. Virol.* **80,** 23–31.

SCHULTZE, B., GROSS, H. J., BROSSMER, R., KLENK, H. D. & HERRLER, G. (1990). Hemagglutinating encephalomyelitis virus attaches to *N*-acetyl-9-*O*-acetylneuroaminic acid-containing receptors on erythrocytes: comparison with bovine coronavirus and influenza C virus. *Virus Res.* **16,** 185–94.

SCHULTZE, B., GROSS, H. J., BROSSMER, R. & HERRLER, G. (1991a). The S protein of bovine coronavirus is a hemagglutinin recognizing 9-*O*-acetylated sialic acid as a receptor determinant. *J. Virol.* **65,** 6232–7.

SCHULTZE, B., WAHN, K., KLENK, H. D. & HERRLER, G. (1991b). Isolated HE-protein from hemagglutinating encephalomyelitis virus and bovine coronavirus has receptor destroying and receptor-binding activity. *Virology* **180,** 221–8.

SHARPEE, R. L., MEBUS, C. A. & BASS, E. P. (1976). Characterization of a calf diarrheal coronavirus. *Am. J. vet. Res.* **37,** 1031–41.

SHOCKLEY, L. J., KAPKE, P. A., LAPPS, W., BRIAN, D. A., POTGIETER, L. N. D. & WOODS, R. (1987). Diagnosis of porcine and bovine enteric coronavirus infections using cloned cDNA probes. *J. clin. Microbiol.* **25,** 1591–6.

SIDDELL, S. (1987). The organization and expression of coronavirus genomes. In *The Molecular Biology of the Positive Strand RNA Viruses,* pp. 117–27. London: Academic Press.

SINGH, D. K., SINGH, N. P. & SINGH, G. K. (1985). Pneumoenteric syndrome in bovine neonates by bovine coronavirus. *Ind. J. vet. Med.* **5,** 55–7.

SNODGRASS, D. R., FAHEY, K. J., WELLS, P. W., CAMPBELL, I. & WHITELAW, A. (1980). Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. *Infect. Immun.* **28,** 344–9.

SNODGRASS, D. R., STEWART, J., TAYLOR, J., KRAUTIL, F. L. & SMITH, M. L. (1982). Diarrhoea in

dairy calves reduced by feeding colostrum from cows vaccinated with rotavirus. *Res. vet. Sci.* **32,** 70–3.

SNODGRASS, D. R., TERZOLO, H. R., SHERWOOD, D., CAMPBELL, I., MENZIES, J. D. & SYNGE, B. A. (1986). Aetiology of diarrhoea in young calves. *Vet. Rec.* **119,** 31–4.

SPAAN, W., CAVANAGH, D. & HORZINEK, M. C. (1988). Coronaviruses: structure and genome expression. *J. gen. Virol.* **69,** 2939–52.

SPAAN, W., CAVANAGH, D. & HORZINEK, M. C. (1990). Coronaviruses. In *Immunochemistry of Viruses, II. The Basis for Serodiagnosis and Vaccines,* eds M. H. V. Regenmortel & A. R. Neurath, pp. 359–79. Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division).

ST. CYR-COATS, K. & STORZ, J. (1988). Bovine coronavirus-induced cytopathic expression and plaque formation: host cell and virus strain determine trypsin dependence. *J. vet. Med.* **35,** 48–56.

ST. CYR-COATS, K., STORZ, J., HUSSAIN, K. A. & SCHNORR, K. L. (1988). Structural proteins of bovine coronavirus strain L9: effects of the host cell and trypsin treatment. *Arch. Virol.* **103,** 35–45.

STAIR, E. L., RHODES, M. B., WHITE, R. G. & MEBUS, C. A. (1972). Neonatal calf diarrhea: purification and electron microscopy of a coronavirus-like agent. *Am. J. vet. Res.* **33,** 1147–56.

STORZ, J. & ROTT, R. (1981). Reactivity of antibodies in human serum with antigens of an enteropathogenic bovine coronavirus. *Med. Microbiol. Immunol.* **169,** 169–78.

STORZ, J., ROTT, R. & KULUZA, G. (1981). Enhancement of plaque formation and cell fusion of an enteropathogenic coronavirus by trypsin treatment. *Infect. Immun.* **31,** 1214–22.

STORZ, J., HERRLER, G., SNODGRASS, D. R., *et al.* (1991). Monoclonal antibodies differentiate between the hemagglutinating and the receptor-destroying activities of bovine coronavirus. *J. gen. Virol.* **72,** 2817–20.

STOTT, E. J., THOMAS, L. H., BRIDGER, J. C. & JEBBETT, N. J. (1976). Replication of a bovine coronavirus in organ cultures of foetal trachea. *Vet. Microbiol.* **1,** 65–9.

TAKAHASHI, E., INABA, Y., SATO, K., *et al.* (1980). Epizootic diarrhoea of adult cattle associated with a coronavirus-like agent. *Vet. Microbiol.* **5,** 151–4.

TAKAHASHI, E., AKASHI, H. & INABA, Y. (1983). Bovine epizootic diarrhoea resembling winter dysentery caused by bovine coronavirus. *JARQ* **17,** 37–42.

TEKTOFF, J., DAUVERGNE, M., DURAFOUR, M. & SOULEBOT, J. P. (1983). Propagation of bovine enteritic coronavirus in three cell systems: electron microscopic studies. *Proc. 4th Int. Symp. neonatal Diarrhoea,* 134–53.

THOMAS, L. H., GOURLAY, R. N., STOTT, E. J., HOWARD, C. J. & BRIDGER, J. C. (1982). A search for new microorganisms in calf pneumonia by the inoculation of gnotobiotic calves. *Res. vet. Sci.* **33,** 170–82.

THURBER, E. T., BASS, E. P. & BECKENHAUER, W. H. (1977). Field trial evaluation of a reo-coronavirus calf diarrhea vaccine. *Can. J. comp. Med.* **41,** 131–6.

TOTH, T. E. (1982). Trypsin-enhanced replication of neonatal calf diarrhea coronavirus in bovine embryonic lung cells. *Am. J. vet. Res.* **43,** 967–72.

TSUNEMITSU, H., YONEMICHI, H., HIRAI, T., *et al.* (1991). Isolation of bovine coronavirus from feces and nasal swabs of calves with diarrhoea. *J. vet. med. Sci.* **53,** 433–7.

VAN BALKEN, J. A. M., DE LEEUW, P. W., ELLENS, D. J. & STRAVER, P. J. (1978). Detection of coronavirus in calf faeces with a haemadsorption-elution-haemagglutination assay (HEHA). *Vet. Microbiol.* **3,** 205–11.

VAN KRUININGEN, H. J., KHAIRALLAH, L. H., SASSEVILLE, V. G., WYAND, M. S. & POST, J. E. (1987). Calfhood coronavirus enterocolitis: a clue to the etiology of winter dysentery. *Vet. Pathol.* **24,** 564–7.

VAUTHEROT, J. F. (1981). Plaque assay for titration of bovine enteric coronavirus. *J. gen. Virol.* **56,** 451–5.

VAUTHEROT, J. F. & LAPORTE, J. (1983). Utilization of monoclonal antibodies for antigenic characterization of coronaviruses. *Ann. rech. Vet.* **14,** 437–44.

VAUTHEROT, J. F., LAPORTE, J., MADELAINE, M. F., BOBULESCO, P. & ROSETO, A. (1984). Antigenic and polypeptide structure of bovine enteric coronavirus as defined by monoclonal antibodies. In *Molecular Biology and Pathogenesis of Coronaviruses,* eds P. J. M. Rottier *et al.*, pp. 117–32. New York: Plenum Press.

VAUTHEROT, J. F., MADELAINE, M. F. & LAPORTE, J. (1990). Topological and functional analysis of epitopes on the S (E2) and HE (E3) glycoproteins of bovine enteric coronavirus. *Adv. exp. med. Biol.* **276,** 173–9.

VERBEEK, A. & TIJSSEN, P. (1988). Biotinylated and radioactive cDNA probes in the detection by hybridization of bovine enteric coronavirus. *Mol. cell. Probes* **2,** 209–23.

VERBEEK, A. & TIJSSEN, P. (1990). Polymerase chain reaction for probe synthesis and for direct amplification in detection of bovine coronavirus. *J. virol. Methods* **29,** 243–56.

VERBEEK, A., DEA, S. & TIJSSEN, P. (1990). Detection of bovine enteric coronavirus in clinical specimens by hybridization with cDNA probes. *Mol. cell. Probes* **4,** 107–20.

DE VISSER, N. A. P. C., BREUKINK, H. J., VAN ZIJDERVELD, F. G. & DE LEEUW, P. W. (1987). Enteric infections in veal calves: a longitudinal study on four veal calf units. *Vet. Q.* **9,** 289–96.

VLASAK, R., LUYTJES, W., LEIDER, J., SPAAN, W. & PALESE, P. (1988a). The E3 protein of bovine coronavirus is a receptor-destroying enzyme with acetylesterase activity. *J. Virol.* **62,** 4686–90.

VLASAK, R., LUYTJES, W., SPAAN, W. & PALESE, P. (1988b). Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. *Proc. Natl Acad. Sci. USA* **85,** 4526–9.

WALTNER-TOEWS, D., MARTIN, S. W., MEEK, A. H. & MCMILLAN, I. (1983). A field trial to test the efficacy of a combined rotavirus-coronavirus/*E. coli* vaccine in dairy cattle. In *Proc. 4th Int. Symp. neonatal Diarrhoea,* 456–83.

WOODE, G. N. & BRIDGER, J. C. (1975). Viral enteritis of calves. *Vet. Rec.* **96,** 85–8.

WOODE, G. N., BRIDGER, J. C. & MEYLING, A. (1978). Significance of bovine coronavirus infection. *Vet. Rec.* **102,** 15–16.

YOO, D., PARKER, M. D. & BABIUK, L. A. (1990). Analysis of the S spike (peplomer) glycoprotein of bovine coronavirus synthesised in insect cells. *Virology* **179,** 121–8.

YOO, D., PARKER, M. D. & BABIUK, L. A. (1991a). The S2 subunit of the spike glycoprotein of bovine coronavirus mediates membrane fusion in insect cells. *Virology* **180,** 395–9.

YOO, D., PARKER, M. D., SONG, J., COX, G. J., DEREGT, D. & BABIUK, L. A. (1991b). Structural analysis of the conformational domains involved in neutralization of bovine coronavirus using deletion mutants of the spike glycoprotein S1 subunit expressed by recombinant baculoviruses. *Virology* **183,** 91–8.

ZHANG, X., KOUSOULAS, K. G. & STORZ, J. (1991). Comparison of the nucleotide and deduced amino acid sequences of the S genes specific by virulent and avirulent strains of bovine coronaviruses. *Virology* **183,** 397–404.

ZYGRAICH, N., GEORGES, A. M. & VASCOBOINIC, E. (1975). Etiologie des diarrhées néonatales du veau. Résultats d'une enquête sérologique relative aux virus reo-like et corona dans la population bovine belge. *Ann. med. vet.* **119,** 105–13.

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