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Chapter 20

Acute Respiratory/Enteric Disease in Calves and Sheep

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INTRODUCTION

Reoviruses in general, including those associated with infections of cattle and sheep, are classified as a genus of the family Reoviridae, and are frequently referred to as the classical reoviruses. The reoviruses of mammalian origin have been divided into three serotypes, although cross-reactions occur. Serological surveys conducted in various parts of the world have indicated that reovirus infections are widely distributed in cattle and sheep populations. Nevertheless, there is still little evidence for the implication of reoviruses as pathogens of the respiratory or alimentary tracts of either species, particularly when compared with the effects produced with other known disease agents.

It has been postulated (Darbyshire and Roberts, 1968; Lamont et al., 1968; Phillip and Darbyshire, 1971) that reoviruses of cattle are of less importance in the etiology of respiratory disease than are a number of other viruses that have been implicated as primary pathogens. Reoviruses are likely to initiate tissue damage in the respiratory tract, thereby enabling secondary agents to invade and replicate to advantage. In the case of the alimentary tract, their role as pathogens is even less well defined.

In sheep, evidence for pathogenic effects of reoviruses is also limited. Reovirus type 1 has been recovered from sheep showing signs of concurrent respiratory and alimentary tract disease, and the virus isolated was then utilized to reproduce the condition experimentally. It seems that the behaviour of reoviruses as pathogens of sheep, as in cattle, depends upon the activities of secondary agents in precipitating disease.

VIRUS PROPERTIES

Physical and chemical characteristics

Virus infectivity is stable between pH 2.2 and 8.0, and resists the effects of ether and chloroform. The virus also resists the effects of 1% phenol for at least 1 h, but is inactivated by 70% ethanol or 3% formalin at 56°C. When heated to 55°C in the presence of magnesium ions, virus infectivity is actually increased; the reverse is true at temperatures below 0°C. Infectivity can also be enhanced by treating the virus with proteolytic enzymes. Virions become cytotoxic following exposure to ultraviolet irradiation.

The efficiencies of various chemical agents for the disinfection of premises do not appear to have been assessed in much detail. The virus is known to survive 1% phenol and 20% lysol for 1 h at room temperature; it is unknown

Chapter 20 references, p. 224

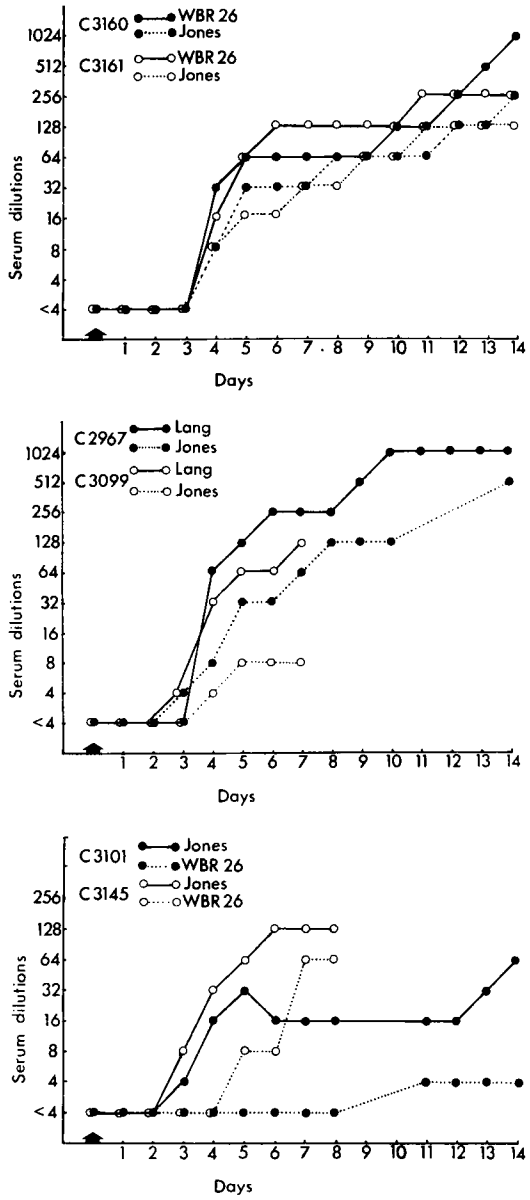

Fig. 89. HI reovirus antibody responses in calves inoculated with (a) bovine strain WBR 26 type 1; (b) human strain Lang type 1; and (c) human strain Jones type 2; note the heterotypic responses to the human strain Jones type 2 (a and b) and bovine strain WBR 26 type 1 (c) (data with permission from Lamont et al., 1968).

whether longer periods in the presence of such chemicals may reduce infectivity. The persistence of reoviruses in animal products has not been reported to date.

Antigenic properties

In respect of their antigenic structure, mammalian reoviruses are comprised of three serotypes. This has been determined both by cross-neutralization and HI tests. Heterotypic reactions can, however, be demonstrated by both techniques (Flammini et al., 1980a, b) as exemplified by Lamont et al. (1968), who used calves infected with reovirus types 1 or 2 and found heterotypic responses in HI tests (Fig. 89).

Isolations of the three reovirus serotypes have been made from animals and

man. All three types occur in cattle and sheep; a report (Kurogi et al., 1974) of the isolation of two further distinct types from cattle was subsequently modified: the viruses were found related to type 2 (Kurogi et al., 1980). The human isolates of all three serotypes are evidently indistinguishable from those recovered from animals, but the five described serotypes of avian origin form a separate group. Strains of reoviruses within the three types recovered from various animal species have usually proved to be indistinguishable, and will apparently cross the species barrier. Human strains of types 1 and 2 have been shown capable of infecting calves (Lamont et al., 1968).

All three reovirus serotypes possess two group-specific antigens in common; each type possesses a type-specific antigen as determined by immunodiffusion. However, immunoelectrophoretic analysis indicates that the reoviral antigens can be further resolved into four group-specific and two type-specific antigens.

EPIZOOTIOLOGY

The host range of the three mammalian reovirus serotypes is very wide indeed: they are found in most animals, including primates, domesticated livestock and wild animals. Reoviruses are also found in birds, including chickens, but these form a separate group. Reservoir hosts may exist but are unknown at the present time; reoviruses have been recovered from culicine mosquitoes, which may act as true vectors or merely as mechanical carriers (Parker et al., 1965). Reoviruses of cattle have been reported in various countries, including North America, Europe, Africa and the Far East. They have been found in sheep in Europe and Australasia.

Reoviruses evidently spread easily among cattle or sheep populations. There have been reports of inapparent infections among cattle in the course of vaccination trials (Blackmer, 1976; Thurber et al., 1977; Morzaria et al., 1979). The economic consequences of reovirus infection are difficult to assess in view of the mild character of the effects of infection. In serological surveys carried out on bovine respiratory disease in the United Kingdom, Phillip et al. (1968) reported that about 26% of cattle associated with outbreaks had a significant increase in reovirus antibody. In Belgium incidence was much lower (Wellemans, 1969) In a later survey in the United Kingdom, Darbyshire and Roberts (1968) recorded an incidence of 22% for reovirus type 2 during a 3-year period of serum monitoring.

Similar seroepidemiological surveys have shown that sheep populations experience infection widely as well (Stanley et al., 1964; Pringle and Cartwright, 1969; McFerran et al., 1973; Munz et al., 1974). Epizootics may occur in sheep (Belák and Palfi, 1974; Belák et al., 1974), and it would appear from the literature that reoviruses initiate a more severe condition of respiratory disease in sheep than in cattle.

PATHOGENESIS

In both cattle and sheep, reoviruses initiate infection in the respiratory and alimentary tracts. The virus enters the cell by phagocytosis and becomes associated with lysosomes. Viral RNA synthesis proceeds in the cytoplasm, and infected cells later display intracytoplasmic inclusions in the Golgi apparatus which accumulate as perinuclear masses within which virus particles may be observed.

After experimental infection of the respiratory and alimentary tracts in cattle there is a latent period of less than 24 h. This is followed by a period of

Chapter 20 references, p. 224

viremia which may last for up to one week, depending on the virus strain. Virus can also be recovered from nasal discharge and feces, as well as from the conjunctival sac. Following experimental infection, virus may be recovered for up to 2 weeks.

Once reovirus has initiated infection it spreads to various tissues, probably by means of a viremia. Most virus strains have a tropism for respiratory tract tissue, including the nasal turbinate mucosa, tonsils, trachea, lung and mediastinal lymph nodes. In addition, virus may be demonstrated in the spleen and kidney; it can be found throughout the alimentary tract and in the mesenteric lymph nodes for at least a week after infection.

It has been shown by Phillip et al. (1968) that *Chlamydia* will act synergistically with reoviruses to induce a more severe pneumonopathy than either agent alone. Nonetheless, the clinical response is still mild, although lesions observed at necropsy of such dually infected animals are more severe than those induced by reovirus alone. The conclusion drawn from experimental evidence is that reoviruses are only mild pathogens of the bovine respiratory and alimentary tracts.

The clinical response of sheep to infection, however, would seem to be more pronounced. A strain of reovirus type 1 which had been isolated originally from sheep (Belák and Palfi, 1974a; Belák et al., 1974) in an epidemic of respiratory and enteric disease in a flock was used to inoculate lambs (Belák and Palfi, 1974b, c). After an incubation period of 4–6 days, the lambs showed pyrexia and ocular and nasal discharges; there was clinical evidence of pneumonia accompanied by diarrhea. These clinical signs abated within 3 weeks of infection. Neutralizing antibody titers of 64–128 were demonstrable.

Virus was recovered from the conjunctiva and internal organs of succumbed animals. Such experimental evidence suggests that reoviruses, at least those of serotype 1, are capable of inducing respiratory and intestinal disease in young sheep. There is no evidence for persistence or latency of reoviruses in sheep or cattle.

DISEASE SIGNS

In calves experimentally infected with reovirus types 1 or 2, only rectal temperatures were elevated (Lamount et al., 1968). This accords with a previous report (Abinanti, 1963) that bovine reovirus strains do not produce clinical illness in calves. Later, Phillip et al. (1968) were able to enhance the response by adding *Chlamydia* to reovirus and administering the mixture intratracheally. Diarrhea occurred within 3 days p.i., accompanied by a mucopurulent nasal discharge during the first week.

In lambs, laboratory evidence suggests that reovirus type 1 is capable of producing disease, whereas types 2 and 3 are not (McFerran and Baskerville, 1972; Snodgrass et al., 1976). However, this does not rule out that strains of both types may act as pathogens. Reovirus type 1 administered experimentally produces pyrexia, together with ocular and nasal discharge, sneezing and some dyspnea, all within a period of 4–6 days p.i. In the following week, diarrhea may be evinced, varying in severity and even leading to death before the end of the second week p.i. (Belák and Palfi, 1974c).

In natural outbreaks in sheep attributed to reovirus (Belák et al., 1974), mild respiratory and enteric signs were noted. Lambs with less vitality than the remainder of the flock succumbed, accounting for some 13% of the total number of animals affected. *Pasteurella* organisms were isolated from the lungs, which suggests that the pneumonia resulted from an invasion of secondary organisms following the virus infection.

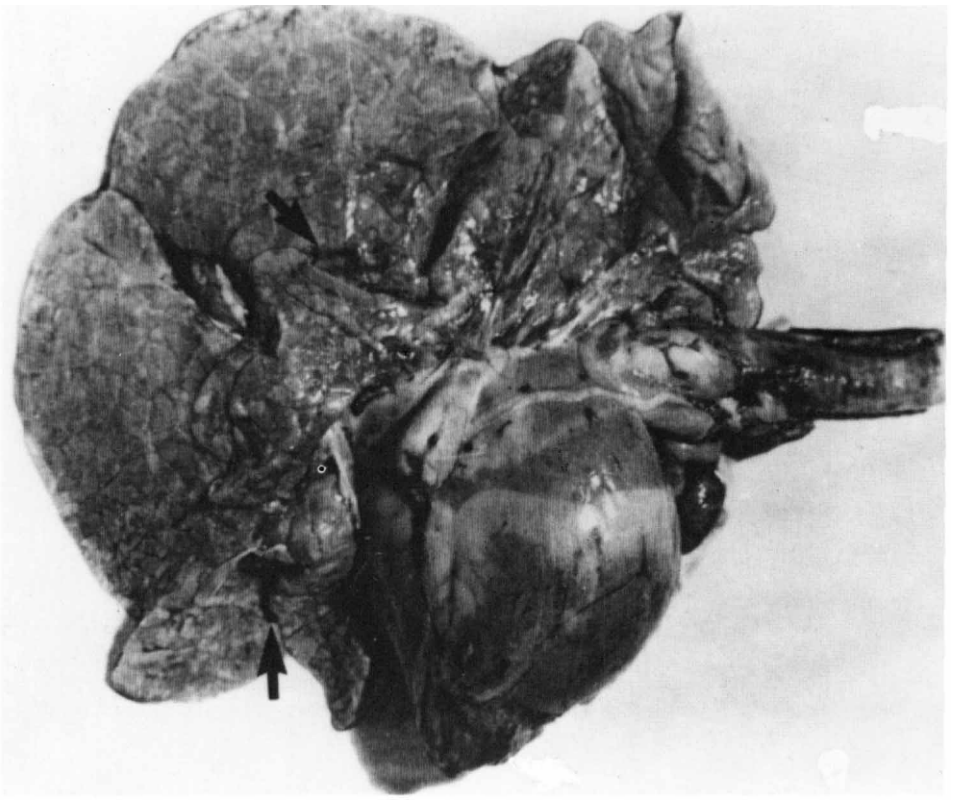

Fig. 90. Ventral surface of the lungs of a calf inoculated with reovirus type 1 (strain WBR 26); arrows indicate the areas of consolidation.

PATHOLOGY

Cattle

The pathology and histopathology of reovirus infections has been deduced from experimental infections with BRD in calves (Lamont et al., 1968). Colostrum-deprived calves inoculated both intranasally and intratracheally with human strains of types 1 and 2, or with a bovine strain of type 1, all produced similar responses.

There were no overt clinical signs apart from pyrexia, but on necropsy lesions of pneumonia were observed (Fig. 90). Histologically, the findings were similar to those noted in other virus infections of the respiratory tract. They include an extensive epithelialization of the lung alveoli, with pseudo-epithelialization of septal cells (Figs. 91, 92). Hepatocytes are often shed into the bloodstream with hemosiderosis.

Alveolar septal cell necrosis has also been observed. No intracytoplasmic inclusions have been described in histological lesions, but the alveolar walls have a marked reticulin content. Small islets of red cells, forming "blood lakes", may be observed.

IMMUNE REACTION

CMI and the development of specific immunoglobulin in bovine and ovine reovirus infections have not been described. In calves, HI antibody can be demonstrated subsequent to infection. For such tests a hemagglutinating

Chapter 20 references, p. 224

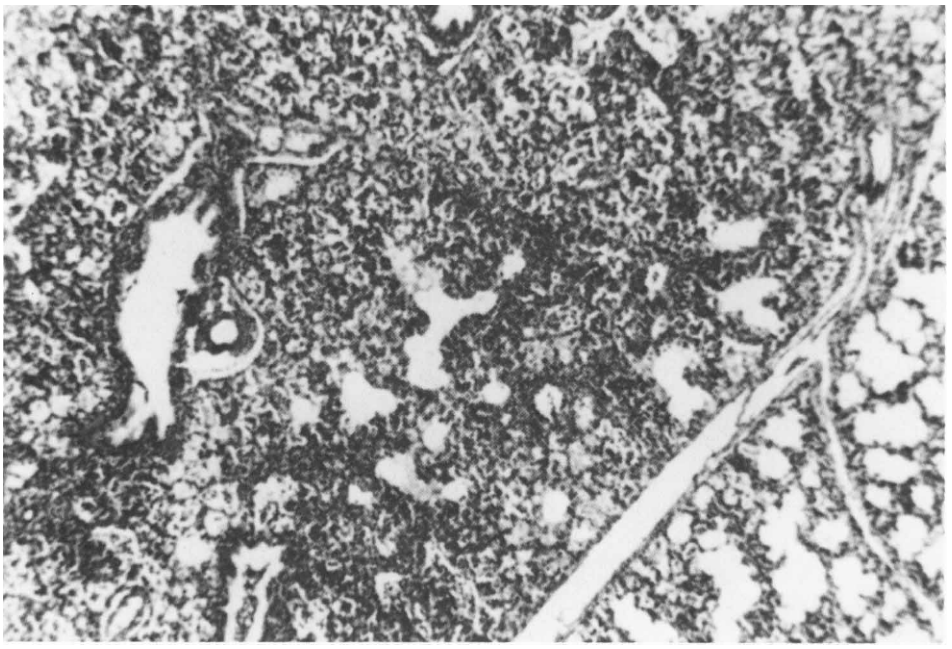

Fig. 91. Section of lung from a calf, 4 days after inoculation with reovirus, showing an early exudative reaction with a peribronchiolar, lymphoreticular hyperplasia and emphysema (data with permission from Lamont et al., 1968)

strain of reovirus is used as antigen. Some bovine strains, originally isolated in pig kidney cell cultures, may require at least one passage in monkey kidney cells to enhance hemagglutinating activity. Antibody becomes demonstrable within 3–4 days p.i. The homotypic response is followed by a heterotypic HI response. A similar heterotypic response had been demonstrated previously in man (Rosen et al., 1963a) as well as in naturally occurring infections in cattle (Rosen et al., 1963b). Such antibody may attain maximum titer within 14 days,

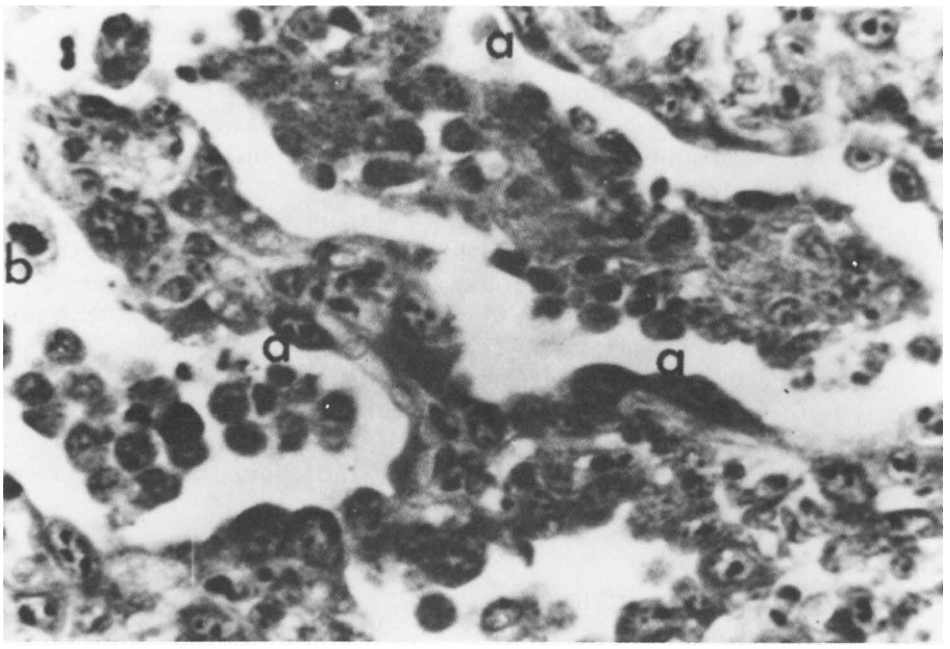

Fig. 92. Section of lung from a calf, 7 days after inoculation with reovirus, showing early epithelization; cell marked "a" shows cytoplasmic attenuation and basophilia; cell marked "b" is in mitosis (data with permission from Lamont et al., 1968).

although occasionally this may take a further 14 days; the levels are maintained for up to a year. Lamont et al. (1968) did not consider HI titers as a true indicator of protection against challenge infection.

Neutralization tests using calf kidney or lamb kidney cultures have also been employed to titrate antibodies in calves or sheep. In the latter, the results from HI and neutralization tests show a good correlation (McFerran et al., 1973).

LABORATORY DIAGNOSIS

Association of reoviruses with a clinical picture must be interpreted with considerable reserve in the first instance. This association may be easier to prove in sheep than in cattle. Nasal swabs should be taken from any discharges and used to infect culture monolayers of pig kidney or lamb kidney cells.

Antigen for HI tests is produced by growing virus in cynomolgus monkey kidney cell monolayers. The fluids are harvested and clarified by centrifugation, the supernatant being used as the antigen without further concentration. The antigen is tested against human erythrocytes group O, to determine the concentration of the hemagglutinin. As mentioned, some bovine strains may require additional passage in monkey kidney cells to produce a hemagglutinating activity. The timing of the collection of samples is probably most important. For the recovery of virus, swabs need to be made within the first few days of onset of illness. The first of paired serum samples should also be collected at this time. The swabs should be placed in a transport medium, such as sterile broth or tissue culture medium containing 0.5% bovine albumin, incorporating antibiotics such as streptomycin, penicillin or polymixin B. The swab fluids are kept at 4°C as long as possible until used to inoculate cultures.

With the collection of second samples from the same animals the sera may be examined for HI and neutralizing antibodies. The neutralization tests are performed in the conventional manner, using a standard virus dose of approximately 100 $TCID_{50}$ against dilutions of serum; 4-fold increases are significant. Neutralizing antibody titers of 1/64 to 1/128 in single serum samples can occur in lambs following either experimental or natural infections. In cattle, similar titers would be significant, whereas HI titers of comparable levels would also be indicative of a recent infection. The latter tests should be conducted against hemagglutinins of all three reovirus types in order to ascertain the highest, and thereby the homotypic titer.

PROPHYLAXIS AND CONTROL

There have been but a limited number of attempts to evaluate vaccines against reoviruses. Phillip et al. (1973) examined the efficacy of 2 commercial vaccines against BRD, both inactivated multifactorial vaccines. One preparation contained PI3 virus, BAV-3 and *Chlamydia*; the other vaccine was directed against PI3 virus, BAV-3, reovirus type 1 and BVD virus. These vaccines were evaluated solely on the basis of antibody responses, and comparisons were made of liveweight gains. Whereas calves were evidently protected on one farm, antibody titers fell sharply on another. The work of Philip et al. (1973) was extended by Morzaria et al. (1979) with a vaccine containing reovirus 1. It was found that whereas humoral responses were obtained in calves, the presence of maternal antibodies interfered with the response to vaccination.

Twiehaus et al. (1975) surveyed the opinions of veterinarians who used oral reovirus vaccines in the field. Performance ratings indicated that such vac-

Chapter 20 references, p. 224

cines were possibly efficacious in reducing calf diarrhea in vaccinated beef and dairy herds. A combined reovirus and coronavirus vaccine used in calves, however, did not produce any significant difference between vaccinated and unvaccinated animals (Blackmer, 1976). Thurber et al. (1977) also conducted field trials with reovirus vaccines in calves, but considered them of use only when reovirus infections were not very prevalent. In sheep, there are apparently no reports of any vaccine trials or use.

REFERENCES

Abinanti, F.R., 1963. Respiratory disease viruses of cattle and observations on reovirus infections of cattle. Am. Rev. Resp. Dis. Suppl., 88: 290–305.

Belák, S. and Palfi, V., 1974a. Isolation of reovirus type 1 from lambs showing respiratory and intestinal symptoms. Arch. Ges. Virusforsch., 44: 177–183.

Belák, S. and Palfi, V., 1974b. Experimental infection of lambs with a reovirus of sheep origin. Magy. Allatorv. Lap., 30: 329–331 (in Hungarian).

Belák, S. and Palfi, V., 1974c. Experimental infection of lambs with reovirus type 1 isolated from sheep. Acta Vet. Arch. Sci. Hung., 24: 241–247.

Belák, S., Palfi, V. and Huszar, E., 1974. A mass respiratory and intestinal disease of lambs caused by a reovirus. Magy. Allatorv. Lap., 29: 14–16.

Blackmer, P.E., 1976. A practitioner's experience with experimental reo-coronavirus calf diarrhoea vaccine. Vet. Med. Small Anim. Clin., 71: 351–354.

Darbyshire, J.H. and Roberts, D.H., 1968. Some respiratory virus and mycoplasma infections of animals. J. Clin. Pathol., Suppl. 2: 61–87.

Flammini, C.F., Bottarelli, E., Allegri, G., Giulioni, A. and Scatozza, F., 1980a. Serological reactions to reovirus types 1 and 3 in experimentally infected guinea pigs and in intensively reared calves. Microbiologica, 3: 485–489.

Flammini, C.F., Bottarelli, E., Allegri, G. and Giulioni, A., 1980b. Neutralizing antibody to reovirus types 1 and 3 in calves. Microbiologica, 3: 491–496.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K., Goto, Y., Omori, T. and Matumoto, M., 1974. New serotypes of reoviruses isolated from cattle. Arch. Ges. Virusforsch., 45: 157–160.

Kurogi, H., Inaba, Y., Takahashi, E., Sato, K. and Omori, T., 1980. Serological relationship between Japanese strains of reovirus of bovine origin and type 2 reovirus strains. Natl. Inst. Avian Health Q. (Tokyo), 20: 32–33.

Lamont, P.H., Darbyshire, J.H., Dawson, P.S., Omar, A.R. and Jennings, A.R., 1968. Pathogenesis and pathology of infection in calves of strains of reovirus types 1 and 2. J. Comp. Pathol., 78: 23–33.

McFerran, J.B. and Baskerville, A., 1972. Unpublished observations. Cited in: McFerran et al., 1973. Arch. Ges. Virusforsch., 40: 80.

McFerran, J.B., Nelson, R. and Clarke, J.K., 1973. Isolation and characterization of reoviruses isolated from sheep. Arch. Ges. Virusforsch., 40: 72–81.

Morzaria, S.P., Richards, M.S., Harkness, J.W. and Mallard, B.A., 1979. A field trial with a multicomponent inactivated respiratory viral vaccine. Vet. Rec., 105: 410–414.

Munz, E., Reimann, M. and Ackermann, E., 1974. Seroepidemiological studies on reovirus infections of man, domestic and wild animals in Tanzania. Acta Trop., 36: 277–288.

Parker, L., Baker, E. and Stanley, N.F., 1965. The isolation of reovirus type 3 from mosquitoes and a sentinal mouse. Aust. J. Exp. Biol. Med. Sci. 43: 167.

Phillip, J.I.H., Clegg, F.G., Halliday, G.J., Cross, M.H., Hardy, R. and Maund, B.A., 1973. An examination of two bovine respiratory disease vaccines. Vet. Rec., 92: 420–424.

Phillip, J.I.H. and Darbyshire, J.H., 1971. Respiratory viruses of cattle. Adv. Vet. Sci. Comp. Med., 15: 159–199.

Phillip, J.I.H., Omar, A.R., Popovic, V., Lamont, P.H. and Darbyshire, J.H., 1968. Pathogenesis and pathology in calves of infection by *Bedsonia* alone and by *Bedsonia* and reovirus together. J. Comp. Pathol., 78: 89–99.

Pringle, C.R. and Cartwright, L.N., 1969. An inhibitor of reovirus in sheep serum. J. Med. Microbiol., 2: 117–123.

Rosen, C., Evans, H.E. and Spickard, A., 1963a. Reovirus infections in human volunteers. Am. J. Hyg., 77: 29–37.

Rosen, C., Abinanti, F.R. and Hovis, J.F., 1963b. Further observations on the natural infection of cattle with reoviruses. Am. J. Hyg., 71: 250–257.

Snodgrass, D.R., Burrells, C. and Wells, P.W., 1976. Isolation of reovirus type 2 from respiratory tract of sheep. Arch. Virol., 52: 143–149.

Stanley, N.F., Leak, P.M., Grieve, G.M. and Peret, D., 1964. The ecology and epidemiology of reovirus. Aust. J. Exp. Biol. Med. Sci., 42: 373–384.

Thurber, E.T., Bass, E.P. and Beckenhauer, W.H., 1977. Field trial evaluation of a reo-coronavirus calf diarrhoea vaccine. Can. J. Comp. Med., 41: 131–136.

Twiehaus, M.J., Mebus, C.A. and Bass, E.P., 1975. Survey of the field efficacy of reoviral calf diarrhoea vaccine. Vet. Med. Small Anim. Clin., 70: 23–25.

Wellemans, G., 1969. Serological examination of bovine herds affected by respiratory troubles. Ann. Med. Vet., 113: 47–60.