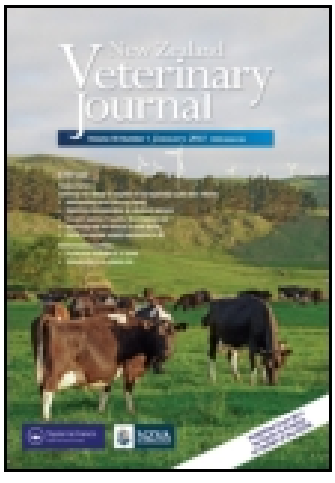

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Rotavirus and coronavirus associated diarrhoea in domestic animals

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Rotavirus and coronavirus associated diarrhoea in domestic animals

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ABSTRACT

Ultracentrifuged faeces from a variety of species of domestic animals with diarrhoea were examined by electron microscopy. Rotaviruses were detected in faeces of cattle, pigs and horses from neonates to 6 months of age. Infections were most common in the early post-natal period. Rotavirus infection was usually associated with a history of recurrent outbreaks of severe diarrhoea that was unresponsive to conventional antibacterial and symptomatic treatment. Coronaviruses were found in faeces of cattle, sheep, deer and horse, and were associated with sudden out-breaks of profuse, watery diarrhoea. A wide range of ages were represented in the infected group.

Direct electron microscopy and immune-electron microscopy of faeces clarified by centrifugation in a microhaematocrit centrifuge, proved to be useful ancillary techniques of examination.

INTRODUCTION

Diarrhoea frequently occurs in young animals, and has been ascribed to a variety of causes such as nutritional imbalance, faulty management, bacteria, coccidia, chlamydiae and viruses. In recent years, an understanding of the problem has been complicated by the incrimination of rotaviruses and coronaviruses as causative agents, especially in the early post-natal animal.

Rotaviruses (reo-like, orbi-like viruses, duoviruses) have been associated with diarrhoea in a number of mammalian species, including calves[18] [26] [30], children[6] [14], mice[1] [20], piglets[31], foals[13], lambs[24], rabbits[8] and deer[27]. Coronaviruses have been implicated in causing diarrhoea in calves[19] [23] [25], piglets[21], dogs[5], foals[4] and man[10]. Experimental transmission and detailed pathological studies with both classes of viruses have confirmed their pathogenic potential[16] [17].

In New Zealand, both viruses have been detected. Rotaviruses were recently reported as causing neonatal diarrhoea in calves[9] and children[3], and coronaviruses were detected in the faeces of scouring adult cattle[15].

Since late 1975, we have been using electron microscopy to examine faeces submitted as diagnostic specimens from scouring young and adult domestic animals. We report here our findings of rotaviruses and coronaviruses in these species.

MATERIALS AND METHODS

Samples

Faeces' samples were sumbitted from a variety of species and locations, all from animals with histories of diarrhoea. Whilst most were from animals of only a few weeks of age, a number of samples were from older animals. Histories in most cases were not very detailed. Following receipt, faeces samples were held frozen at -18°C pending examination, which was usually carried out within 7 days.

Electron Microscopy

The faeces were made up to a 10% suspension in tissue-culture medium, and clarified by centrifugation at 6000 G for 20 minutes in a refrigerated centrifuge. The clear supernatants were then centrifuged at 100 000 G for 1-5 hours. The resultant pellets were re-suspended in a few drops of medium, placed on formvar, carbon-coated, electron-microscope grids and stained with 2% sodium phosphotungstate at pH 6.8. A minimum of five grid squares were then examined electron-microscopically.

A number of very liquid faeces were also processed by centrifugation in a capillary tube in a microhaematocrit centrifuge for 2 to 8 minutes. A portion of the clarified supernatant was then stained and examined with the electron microscope as above. Immune electron microscopy was also used on a number of faeces, using a modification of previously described methods[7] [12] [22]. A drop of clarified centrifuged supernatant was mixed with a drop of rotavirus antiserum for 5 to 90 minutes at room temperature. The combined drop was then re-mixed and immediately placed on a coated grid for 30 seconds, and then stained and examined as before. A further drop of supernatant was processed similarly using negative serum, as a control.

RESULTS (Tables I, II and III.)

In many cases, very large numbers of rotaviruses were seen in the stained pellet suspension; generally, little searching was required to find this agent. Coronaviruses, on the other hand, required considerably more searching, and numbers were never very numerous. Routine identification of both viruses was based on their size, shape and structure, as described in published reports[25] [28].

The use of the microhaematocrit procedure proved adequate where virus particles were reasonably numerous. Immune electron microscopy was also found to be a very satisfactory method with rotaviruses, as large clumps of aggregated rotaviruses settled rapidly onto the coated grid, making detection both easy and specific. The elimination of unnecessary centrifugation procedures saved considerable time, and an incubation period of 20 minutes was found to be quite sufficient.

No mixed infections of rotavirus and coronavirus were found in single faeces samples, though both viruses were occasionally demonstrated on the same property at different times and, in one case, mixed infection was demonstrated in a sample of pooled faeces from several calves.

Clinical Histories

The clinical disease seen in all the species yielding rotaviruses or coronaviruses conformed to 2 patterns. In general, rotavirus-positive faeces came from properties with histories of recurrent outbreaks of diarrhoea among a number of young

TABLE 1: RESULTS OF ELECTRON-MICROSCOPIC EXAMINATION OF FAECES OF SCOURING ANIMALS

Species	Number of faeces examined	Number of faeces positive		
		Rotavirus	Coronavirus	Mixed Infection
Cattle	124	33	28	1*
Pig	8	3	–	–
Sheep	10	–	3	–
Deer	20	–	2	–
Horse	15	1	1	–
Possum+	1	–	–	–
Dog	7	–	–	–
Goat	1	–	–	–

* Pooled sample
\+ Trichosurus vulpecula.

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TABLE 11: AGES OF ANIMALS WITH ROTAVIRUS INFECTION

Species	<2 wk	2-4 wk	4-8 wk	8-12 wk	12-26 wk	6-12 mth	>1 yr
Cattle	9	7	8	4	5	1	–
Pig	1	1	1	–	–	–	–
Horse	–	1	–	–	–	–	–

animals. In some cases, the problem had apparently been present for 4 years or more. Routine bacteriological monitoring of faeces samples revealed the presence of *Escherichia coli* in a number of cases. However, the use of appropriate antibiotics, even after sensitivity testing, generally brought little, or only transient, response to therapy. Likewise, improved management and hygiene did little to clear the problem.

Affected animals were sometimes reported to be febrile, and most became dehydrated. Morbidity and mortality rates were usually about 30% and 15% respectively but, on some properties, rates reached twice this level. A proportion of the recovered animals was reported to be stunted in development subsequently. The contagiousness of the disease was well illustrated on one cattle property, where an outbreak of rotavirus diarrhoea followed 4 days after the introduction of a calf from another property with a recent history of rotavirus diarrhoea. The faeces of affected animals were generally voluminous, pasty to watery in consistency, sometimes mucoid or blood-flecked and, generally, with little smell. The colour of the faeces ranged from white to yellow though, in some animals, a grey or green colour was seen.

TABLE 111: AGES OF ANIMALS WITH CORONAVIRUS INFECTION

Species	<2 wk	2-4 wk	4-8 wk	8-12 wk	12-26 wk	6-12 mth	>1 yr
Cattle	2	–	2	2	1	8	14
Sheep	–	–	–	–	3	–	–
Deer	–	1	–	–	2	–	–
Horse	–	–	–	1	–	–	–

Coronavirus-positive faeces came from properties with a history of a sudden outbreak of a profuse, watery diarrhoea. The colour of the faeces usually remained unchanged from that normal for the age and diet of the animal. Occasional blood-flecks were seen and there was little smell. Affected animals were frequently reported to be febrile and dehydrated. Morbidity and mortality rates varied considerably, but were usually about 40% and 15% respectively though, in some cases, reached double these levels. Antibiotic therapy only occasionally appeared to have a beneficial effect.

One beef-cattle property was unusual in the late onset of rotavirus infection. This property had had outbreaks of severe diarrhoea in its calves for 4 years in succession, confirmed in the last 2 years as due to rotavirus infection. The disease usually became apparent at about 3 months old. In one year, a mixed infection of both rotavirus and coronavirus was confirmed in pooled faeces from 6-month-old calves.

DISCUSSION

Electron microscopy of negatively stained, ultracentrifuged, faecal sediment had been used by a number of authors for detection of rotavirus and coronavirus infections. Many of these authors[18] [23] [25] [26] [28] have used a sucrose-gradient, purification step before attempting to demonstrate the viruses. This makes the procedure difficult to apply in a routine diagnostic situation involving a large number of faeces. The simplified procedure[11] [12] used here was found to be both successful and easily applied to routine diagnosis. England el at.[11] found the simplified procedure more reliable and sensitive for the routine diagnosis of intestinal rotavirus and coronavirus infections than either viral isolation or fluorescent antibody test.

The direct, electron-microscopic examination of clarified fluid from faeces after microhaematocrit centrifugation provided a rapid and simple means of rotavirus detection, as sufficient virus was frequently present for detection without concentration or purification procedures. Even coronaviruses were occasionally detected, despite lower numbers. This method has been previously described in a Nordern Laboratories' publication[2]. It is considered, however, that the method would not be sufficiently sensitive to detect low levels of virus.

The use of immune electron microscopy to diagnose rotavirus infection has been described by several authors[7] [12] [22] who found it to be a sensitive and specific technique. The method described in this paper is a simplified procedure considered more suitable for routine diagnosis.

Rotavirus and coronavirus particles were discovered during this survey in a number of previously unreported species in New Zealand from a variety of locations. Rotavirus infection, though mainly found in the early post-natal period similar to overseas reports, was also found in a number of older calves. These mainly came from beef cattle properties, so the later onset of infection may be due to management practices.

The majority of cases of coronavirus infection were found in animals 3 months old or more, in contradistinction to most overseas reports which, apparently, did not examine many older animals. Outbreaks of coronavirus-associated diarrhoea have been reported in 16-20 year-old humans[10] and in adult cows[15].

Further investigation is required to evaluate the economic significance of intestinal rotavirus and coronavirus infections in New Zealand and to gain a greater understanding of their inter-relationships with microbial causes of diarrhoea. The pathogenic effect of these two viruses may well be exacerbated by some bacteria[29]. There is little doubt at this stage, however, that rotavirus infection has a severe effect on calf-rearing programmes on some properties in this country, with a consequent need for some means of controlling, or eliminating, the problem.

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