

THE OCCURRENCE AND EPIDEMIOLOGY OF ENTEROPATHOGENS AND DIARRHOEA IN NEONATAL LAMBS

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SUMMARY

Two hundred and fifty one faecal samples were collected from 25 neonatal lambs (from birth to one month of age) and examined for enteropathogens, namely rotavirus, *Cryptosporidium*, *Salmonella* and *Clostridium*. Rotavirus and *Cryptosporidium* were isolated from 6 (24%) and 9 (36%) lambs, respectively. Diarrhoea was observed in 13 lambs (52%) and in six lambs it was associated with either *Cryptosporidium* (3 lambs) or *Cryptosporidium* and rotavirus (3 lambs). Mixed infections (both agents) were seen in four lambs. Rotavirus was excreted between days 3 and 15 of age for 1 to 4 days while *Cryptosporidium* was excreted between days 11 and 19 of age for 1 to 4 days also.

Keywords: Neonate, rotavirus, *Cryptosporidium*, diarrhoea, lambs.

Several organisms have been associated with diarrhoea in lambs. These include rotavirus, *Cryptosporidium*, *Salmonella* spp enterotoxigenic *Escherichia coli* (ETEC), *Clostridium perfringens* and coronavirus (Barker and Carbonell, 1974; McNulty *et al.*, 1976; Snodgrass *et al.*, 1976b; Berg *et al.*, 1978; Auon *et al.*, 1985; Nagy *et al.*, 1983; Ramiisse *et al.*, 1984; Linklater and Mitchell, 1985). This report describes the first study conducted in Malaysia to determine the occurrence, aetiology and epidemiology of diarrhoea in neonatal lambs.

The study was conducted between June and July 1992 in a sheep farm in the southern part of West Malaysia. Weaned and adult animals on the farm grazed on pasture during the day and returned to the sheds in the evening. Newborn lambs (Commercial Merino Border Leicester crosses) were kept in slatted, raised floor wooden sheds and allowed to suckle the ewes until they were weaned at the age of 2-3 months. Faecal samples were collected per rectum from each lamb on alternate days from birth to about one month of age. Faecal smears were made and air dried while the remaining samples were kept at 4°C until sent in ice to the Faculty of Veterinary Medicine and Animal Science (twice a week) where the samples were processed within 24 h and examined immediately or stored at -70°C until examined. The faecal consistency was also graded as diarrhoeic or non-diarrhoeic, the former being either soft, not pelleted or watery.

Faecal samples were examined for rotavirus by polyacrylamide gel electrophoresis (Herring *et al.*, 1982) and for *Cryptosporidium* by the modified Ziehl Neelsen technique (Henriksen and Pohlenz, 1981). Smears were examined microscopically for *Cryptosporidium* oocysts under 400X magnification and confirmed under 1000X magnification. Faeces were also cultured for aerobic and anaerobic bacteria, particularly *Salmonella* spp. and *Clostridium perfringens*.

Table 1 gives the occurrence of enteropathogens and diarrhoea. Two hundred and fifty one faecal samples were collected from 25 lambs. At other times the lambs did not have faeces in the rectum. Twenty one faecal samples from 11 lambs (44%) contained enteropathogens; rotavirus was isolated from nine faecal samples of 6 (24%) of the lambs and *Cryptosporidium* from 13 faecal samples of 9 (36%) of the lambs. Of the 11 lambs with enteropathogens, one had a concurrent infection (one sample) while three had subsequent infections (13 samples) of rotavirus and *Cryptosporidium*, two had rotavirus only (two samples) and five (five samples) had *Cryptosporidium* only. In subsequent infections, the interval between the excretion of these two organisms varied, ranging from one to three days and rotavirus was excreted first. The rotavirus had the electrophoretype of group A. Enteropathogens were isolated more often from diarrhoeic lambs ($p > 0.05$, $\chi^2 = 0.05$). This correlation

Table 1. Occurrence of enteropathogens and diarrhoea in lambs

Number	Non-diarrhoeic	Diarrhoeic	Total
Lambs	12	13	25
Total rotavirus +	4	2	6
Total <i>Cryptosporidium</i> +	3	6	9
Rotavirus + only	2	0	2
<i>Cryptosporidium</i> + only	2	3	5
Rotavirus + <i>Cryptosporidium</i> + concurrently	0	1	1
Rotavirus + <i>Cryptosporidium</i> + subsequently	1	2	3
Faecal samples	207	44	251
Total rotavirus +	7	2	9
Total <i>Cryptosporidium</i> +	6	7	13
Rotavirus + only	7	1	8
Total <i>Cryptosporidium</i> + only	6	6	12
Rotavirus + <i>Cryptosporidium</i> + concurrently	0	1	1

was found in lambs excreting *Cryptosporidium* ($p > 0.05$, $\chi^2 = 1.21$) but not with rotavirus which was found more often in non-diarrhoeic lambs ($p > 0.05$, $\chi^2 = 1.10$). Rotavirus and *Cryptosporidium* were excreted in lambs up to three weeks of age. Rotavirus was excreted between days 3 and 15 of age for 1 to 4 days and there was no trend between the presence of diarrhoea and duration of excretion. *Cryptosporidium* was excreted between days 11 and 19 of age for 1 to 4 days also and single isolation was common in non-diarrhoeic lambs. Faeces was watery, the appetite remained normal and minimal supportive treatment was required. Furthermore neither contents nor colour of the faeces were abnormal.

Escherichia coli was isolated from faeces of both diarrhoeic and non-diarrhoeic lambs. However, no attempt was made to identify their serogroups or whether they were ETEC or non-ETEC because these antisera were not available in the country. Salmonella was not isolated from any of the lambs. *Clostridium perfringens* was also not isolated from diarrhoeic faeces.

Investigations of diarrhoea in lambs revealed that several organisms were involved and that more than one organism may be detected in one outbreak and the prevalence rates differ between locations. Fassi-Fehri *et al.* (1988) found rotavirus in 30% and ETEC in 10% of diarrhoeic neonatal lambs of Morocco, while Ramisse *et al.* (1984) found rotavirus in 64% and *Cryptosporidium* in 36% of 25 diarrhoeic lambs from 16 flocks in France. In the latter work, lambs infected with rotavirus were concurrently infected with *Cryptosporidium* (16%), *Eimeria* (4%), *Clostridium perfringens* type A (4%) and ETEC (4%). In addition, Nagy *et al.* (1983) found *Cryptosporidium* in 23% of 53 diarrhoeic lambs from five of 14 flocks in Hungary and concurrent infections with coronavirus, ETEC or coccidia were detected in three flocks. Unlike these

studies, the present one selected 25 newborn lambs and followed them up to one month of age for the development of diarrhoea and the excretion of organisms usually associated with diarrhoea, particularly *Cryptosporidium* and rotavirus. Diarrhoea developed in 13 lambs and *Cryptosporidium* and rotavirus were the two organisms detected, the prevalence rates being 46% and 15%, respectively. *Cryptosporidium* and rotavirus were also detected in the 12 non-diarrhoeic lambs at the prevalence rates of 25% and 33%, respectively. The overall prevalence rates of *Cryptosporidium* and rotavirus in the 25 lambs were 35% and 24%, respectively. Gorman *et al.* (1990) had detected *Cryptosporidium* in diarrhoeic and non-diarrhoeic lambs, the prevalence rates being higher in the latter (3.5% versus 7.9%). Rotavirus too had been identified in diarrhoeic and non-diarrhoeic lambs (Auon *et al.*, 1985; Ellis and Daniels, 1988) and the prevalence rate was higher in diarrhoeic lambs (20% versus 11%, Ellis and Daniels, 1988).

The age at which *Cryptosporidium* and rotavirus started to be excreted (11 to 19 days and 3 to 15 days, respectively) and the duration (1-4 days) was within the ranges reported previously and the clinical signs of watery diarrhoea but mild effects were similar to some reports but contrary to others in which lambs had severe watery diarrhoea leading to dehydration and often death. In natural infections, rotavirus has been isolated in lambs as young as three days and as old as eight months, while experimentally infected neonatal lambs were found to excrete the virus 24 h post-infection and continued up to seven days, the duration being shorter in older lambs (McNulty *et al.*, 1976; Snodgrass *et al.*, 1976a, 1976b). Clinical signs developed 11 to 18 h post-infection and diarrhoea continued for three to four days. Clinical signs in day-old lambs included watery faeces, abdominal distension and discomfort while in 12-day old lambs diarrhoea

was mild with no anorexia or other clinical signs and virus excretion may continue for several days after clinical recovery. *Cryptosporidium* has been isolated from lambs as young as five days and as old as 3.5 months (Tzipori *et al.*, 1981; Ramisse *et al.*, 1984). These lambs either died after two to three days of illness or recovered after approximately seven days of illness and the prepatent period was four to six days. Anderson (1982) found that infected neonatal lambs had diarrhoea which was profuse, watery and yellow lasting for four days but appetite remained good and only minimal supportive treatment was required. The protozoa has also been isolated in healthy and diarrhoeic lambs less than 3.5 months old (Ramisse *et al.*, 1984; Gorman *et al.*, 1990).

In conclusion, the results of the present study indicated that there was no association of either rotavirus or *Cryptosporidium* with diarrhoea.

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RINGKASAN

KEJADIAN DAN EPIDEMIOLOGI ENTEROPATOGEN DAN CIRIT-BIRIT DALAM ANAK BEBIRI NEONAT

Dua ratus lima puluh satu sampel tinja dikumpul daripada 25 ekor anak bebiri neonat (umur daripada baru lahir hingga satu bulan) dan diperiksa untuk enteropatogen, terutama sekali *Cryptosporidium*, *Salmonella* dan *Clostridium*. Rotavirus dan *Cryptosporidium* telah dipencil masing-masing daripada 6 (24%) dan 9 (36%) ekor anak bebiri. Cirit-birit telah dicerap dalam 13 ekor (52%) anak bebiri dalam dalam 6 ekor daripadanya cirit-birit tersebut berkaitan samada *Cryptosporidium* (3 ekor) atau *Cryptosporidium* dan rotavirus (3 ekor). Jangkitan campuran (kedua-dua agen) didapati dalam 4 ekor anak bebiri. Rotavirus telah dikumuhkan di antara umur 3 dan 15 hari selama 1 hingga 4 hari, sambil *Cryptosporidium* pula dikumuhkan di antara umur 11 dan 19 hari juga selama 1 hingga 4 hari.