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Epidemiological studies of parvovirus infections in calves on endemically infected properties

P. J. K. DURHAM*, R. H. JOHNSON, H. ISLES, R. J. PARKER, R. G. HOLROYD, I. GOODCHILD,
Department of Tropical Veterinary Science, James Cook University, Townsville, Queensland 4811, Australia

Bovine parvovirus serology and virus excretion were monitored in calves located on three endemically infected North Queensland properties. Maternally derived serum antibody to bovine parvovirus was found to have a half-life of 19 days. On all three properties, calves developed intestinal bovine parvovirus infection with seroconversion soon after weaning. This occurred more promptly where the environment was subject to heavier bovine parvovirus contamination due to management practices. The concurrent presence of moderate levels of residual serum antibody had only minor influence on the onset of the infection. On one beef cattle property, onset of intestinal bovine parvovirus infection was associated with an outbreak of post-weaning diarrhoea. Anthelmintic treatment trials indicated that this syndrome was unrelated to helminth burdens, though coccidiosis appeared responsible for occasional subsequent cases of dysentery. It was considered that bovine parvovirus may have significantly contributed to the development of the diarrhoea syndrome, in conjunction with substantial weaning stresses.

BOVINE parvovirus appears to be widespread in the world cattle population, judged by the results of serological surveys and virus isolation reports originating from many countries. Most isolations have originated from calves with diarrhoea in animals a few weeks to six months old (Siegl 1976).

Despite the considerable evidence indicating the frequently endemic nature of bovine parvovirus in cattle, relatively little investigation has been made of the role of the virus in causing enteric disease. Experimental studies have, however, shown the virus to be capable of inducing mild to moderate diarrhoea in seronegative calves (Spahn et al 1966, Storz and Bates 1973, Storz et al 1978, Weiblen et al 1983, Durham et al 1985).

No study has apparently yet been made of the behaviour of the virus in calves reared on endemically infected properties. The present investigation was, therefore, undertaken to gain a better understanding of the virus-host interaction under such conditions.

* Present address: Animal Health Reference Laboratory, Wallaceville Animal Research Centre, Private Bag, Upper Hutt, New Zealand

Materials and methods

Three endemically infected properties were selected. They were all government research farms located in tropical North Queensland and represented a range of environmental and managemental conditions.

Property A

This was an irrigated dairy farm located in a dry tropical zone with a predominantly summer (December to March) rainfall of 1092 mm. The Australian Friesian Sahiwal cross calves were born throughout the year, with seasonal peaks in February and May. Following removal from their dams a few days after birth, the calves were moved around small grassed paddocks while fed whole milk via a calfeteria system, plus lucerne hay and maize ad libitum. The calves were weaned at 60 kg liveweight, during which time they were confined in covered pens for two weeks while being fed maize and lucerne chaff ad libitum. They were subsequently put out on irrigated pasture and fed a maize meal supplement. The animals were vaccinated with *Leptospira pomona* and polyvalent clostridial vaccines at weaning and were also treated with levamisole at weaning and at monthly intervals thereafter.

On this property diarrhoea was not regarded as a serious problem and only occurred sporadically, mainly in younger calves.

Property B

This dairy property was located on the North Queensland tablelands, where it was subject to occasional severe winter frosts. Mean rainfall of 1260 mm occurred predominantly in summer, although some

rain was recorded in all months, being supplemented by irrigation in the drier periods.

Calving on this property occurred mainly between late November and March. The calves, mainly Australian Friesian Sahiwal crosses, were held in a small 'permanent' grassed calf paddock until weaning, lactating cows were brought in twice daily to allow multiple suckling at a rate of three calves per cow. While sucking, the calves had access to good quality hay, grain and water. At weaning at six to eight weeks, the calves were treated with levamisole and vaccinated with *L pomona* and polyvalent clostridial vaccines and then turned out onto another grass paddock while being fed a grain supplement.

Although diarrhoea had been a problem for some years on this property, it was minimal after the introduction of multiple suckling.

Property C

This beef cattle property was located in a dry tropical zone. The mean rainfall of 850 mm occurred almost entirely between December and March.

Several hundred Brahman cross Shorthorn and Sahiwal cross Shorthorn calves were born between late October and January and were run with the cows on unimproved native pasture until weaning at about six months old. At this time calves were separated from their dams, treated with levamisole, yarded at a density of one beast per $4 \cdot 6$ m^2 and fed Townsville stylo-speargrass hay from racks with water ad libitum. After 10 days the calves were branded, dehorned and vaccinated with a polyvalent clostridial vaccine and then turned out into a paddock of Townsville stylo, Verano and native pasture mixture at a density of 13 beasts ha^{-1}. Ten days later the calves were given access to similar pasture, and the stock density was reduced to $2 \cdot 5$ beasts ha^{-1}.

This property had in recent years suffered a fairly severe problem of post-weaning diarrhoea, with some deaths. Coccidial involvement had been indicated on the basis of post mortem findings and oocyst excretion rates (R. J. Parker, personal communication).

Experimental procedures

On all three properties, faeces and blood samples were collected from calves at about two weekly intervals. Faeces consistency was assessed visually and scored on a 0 to 4 score, representing a range from normal to watery consistency.

On property A, 25 autumn born calves were sampled from birth over one season. On property B summer born calves were sampled from birth over two seasons, 25 animals being involved the first year and 14 the next.

Property C was also examined over a two year period. In 1981, 80 Brahman cross Shorthorn calves were selected at weaning from a group of 307 calves and allocated to two groups, each consisting of equal numbers of half and threequarter cross Brahmans of both sexes. The calves in one group were treated with injectable levamisole on days 2, 29 and 57 after weaning. All 307 calves were run together during the observation period. Sampling of experimental groups began at weaning. Virological monitoring was confined to the untreated group.

In 1982, 20 randomly selected calves on property C were dosed at weaning with an anticoccidial drug (Monensin; Elanco) administered in a slow release intraruminal capsule (Pasture Research Laboratory, CSIRO, Armidale, NSW). These calves, together with an equivalent number of control animals, were sampled periodically while running with the remainder of the calves. Additionally, 10 randomly selected calves were bled 12 weeks and five weeks before weaning to give an indication of maternally derived antibody levels.

Laboratory procedures

Ten per cent suspensions of faeces in phosphate buffered saline (PBS) were frozen and thawed three times, then clarified by two 20 minute cycles of centrifugation at 2000 *g* and 9000 *g*. The supernatant fluids were then ultracentrifuged at 250,000 *g* for 90 minutes and the pellets resuspended in Dulbecco's modified Eagle's medium (DMEM) to 1/20 of the original volume, being subsequently inoculated into actively growing bovine embryonic lung cells in DMEM with 10 per cent fetal calf serum and antibiotics. The inoculated cultures were passaged three times by cell subculture at five day intervals. Where enteroviruses were initially isolated, the isolation procedures were repeated following heat inactivation (56°C for 60 minutes) of the sample. Cultures showing cytopathic effect and third passage cultures were tested for haemagglutinating (HA) activity with $0 \cdot 5$ per cent human erythrocytes. Isolates were variously identified on the basis of cytopathic effect, HA activity, chloroform and bromodeoxyuridine sensitivity, heat stability and cationic stabilisation at 56°C and neutralisation by specific antisera.

Faeces from property C were also examined for parasite eggs, using standard parasitological procedures.

The sera were heat inactivated at 56°C for 30 minutes and, following pre-treatment with acid washed kaolin and packed erythrocytes, were subjected to a standard haemagglutination inhibition (HI) test (Joo et al 1976), using 4 HA units of bovine parvovirus 267 (Wosu et al 1979) as antigen, and $0 \cdot 5$ per cent human erythrocytes.

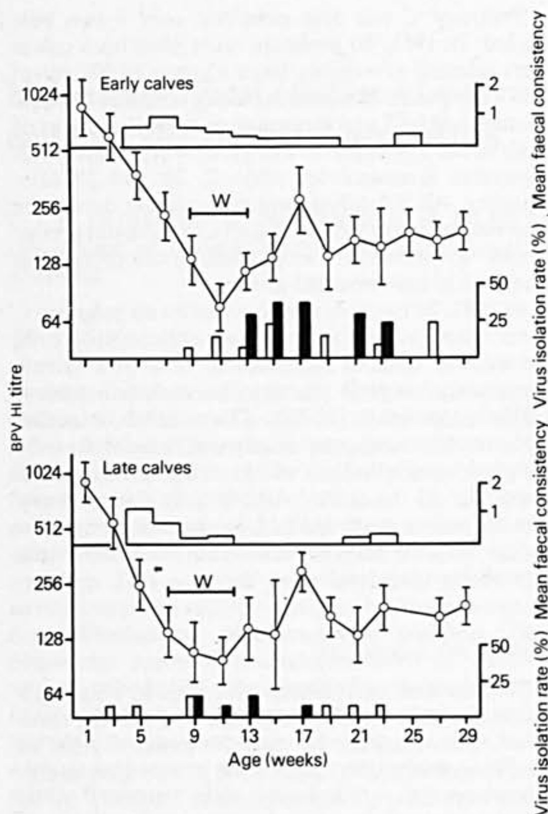

FIG 1: Mean faecal consistency and bovine parvovirus antibody level in calves on property A, from birth until after weaning (W). Black (bovine parvovirus) and clear (enterovirus) histograms represent isolation rate from faeces. Bars represent standard error

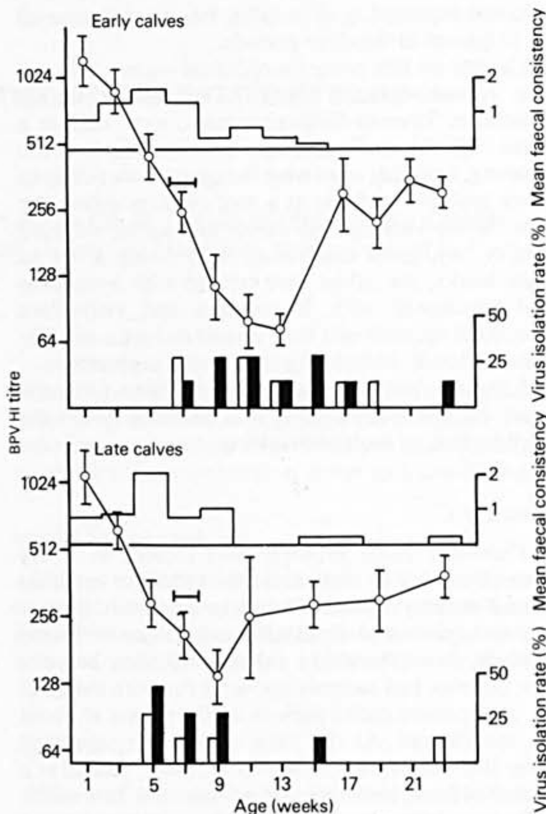

FIG 2: Mean faecal consistency and bovine parvovirus antibody level in calves on property B, from birth until after weaning (W) in the first year. Black (bovine parvovirus) and clear (enterovirus) histograms represent isolation rates from faeces. Bars represent standard error

Results

Property A

The results were analysed into two groups of 12 calves, representing the earlier and later born calves. The mean serological results from birth for each group are shown in Fig 1, which also indicates the pattern of virus isolations, weaning period and changes in mean faecal consistency. Diarrhoea was not a major problem, occurring mainly in younger calves.

Active production of bovine parvovirus antibody was found to begin in calves before total loss of maternal antibody. While many calves reached peak antibody production about four to six weeks after weaning, others maintained static titres of antibody between 16 and 64. Enterovirus isolations were made over a broad time span, some from as early as two weeks after birth. In contrast, bovine parvovirus isolations occurred mainly over a limited period, with initial isolations occurring one to four weeks after weaning, younger weaned calves tending to show earlier bovine parvovirus isolation. Bovine parvovirus HI titres at the time of initial isolation ranged between 32 and 256. As many as three bovine parvovirus or enterovirus isolations were made from some individual calves over a two month period.

Property B

The results obtained on this property are shown in Figs 2 and 3, after analysis of two groups representing earlier and later born calves.

Once again, the main feature in both years of observation was the failure of passive antibody to decline below detectable levels, before onset of active antibody production. Seroconversion occurred four to six weeks after weaning in earlier born calves, in contrast to later born calves which showed seroconversion soon after weaning and earlier bovine parvovirus isolation, in some cases even before weaning. Most of the bovine parvovirus and entero-

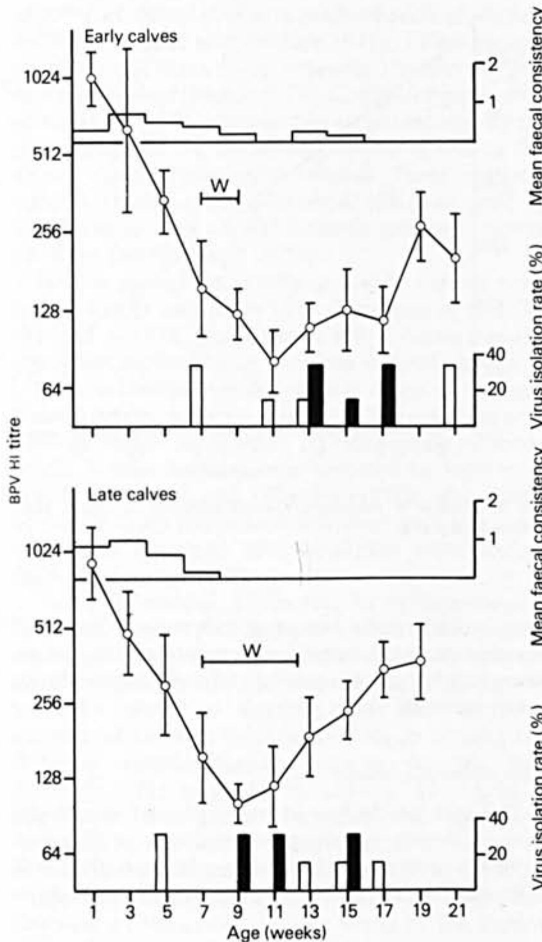

FIG 3: Mean faecal consistency and bovine parvovirus antibody level in calves on property B, from birth until after weaning (W) in the second year. Black (bovine parvovirus) and clear (enterovirus) histograms represent isolation rates from faeces. Bars represent standard error

virus isolates were from normal calves, though both viruses were occasionally recovered from calves with moderate diarrhoea. In one case, bovine viral diarrhoea virus was recovered from the faeces of a four-week-old calf with diarrhoea.

Overall, calves weaned younger tended to show earlier bovine parvovirus excretion, although this was modified to some degree by earlier onset of virus excretion in calves possessing lower levels of maternally derived antibody. One earlier born calf, which possessed no maternally derived antibody to bovine parvovirus, nevertheless did not show excretion and seroconversion until four weeks after weaning.

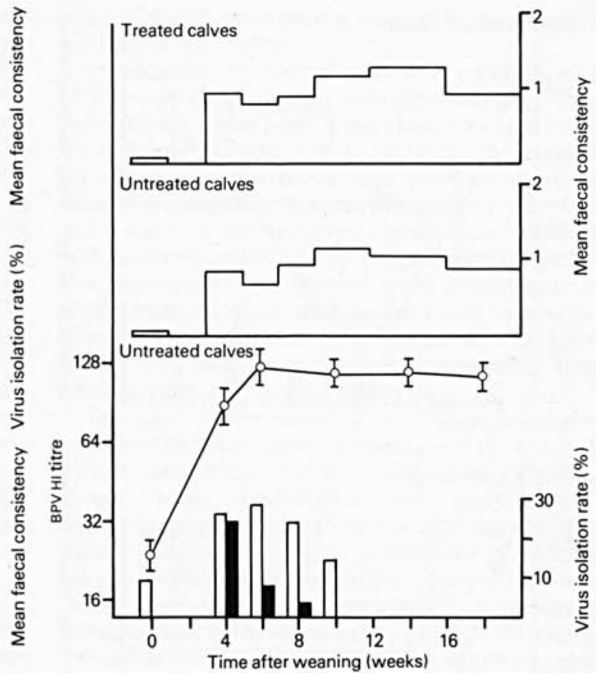

FIG 4: Mean faecal consistency in anthelmintic treated and untreated calves on property C in the first year. The lower graph shows mean bovine parvovirus antibody titres and faecal virus isolation rate of untreated calves. Black (bovine parvovirus) and clear (enterovirus) histograms represent isolation rate from faeces. Bars represent standard error

Property C

In both years of observation, there was a marked rise in serum bovine parvovirus antibody about three to six weeks after weaning. This was coincident with the onset of moderate diarrhoea and of bovine parvovirus excretion in faeces (Figs 4 and 5). Enterovirus isolations were broadly distributed, reaching a peak at four to six weeks. Both bovine parvovirus and enterovirus were isolated from one calf with mild diarrhoea four weeks after weaning. The calves varied considerably in the degree of diarrhoea at times of sampling, some being severely affected though no deaths occurred. No consistent differences were noted between half and three-quarter cross Brahman calves, nor between sexes.

Anthelmintic treatment of calves in the first season appeared to have no effect on the incidence of post-weaning diarrhoea (Fig 4) although helminth egg counts in treated animals were all less than 100 eggs per gram (epg). Untreated calves possessed mean helminth egg counts of 620 epg at weaning, which declined to 345 epg six weeks after weaning and thereafter remained relatively constant. Individual counts ranged from 100 epg to 1300 epg and showed no

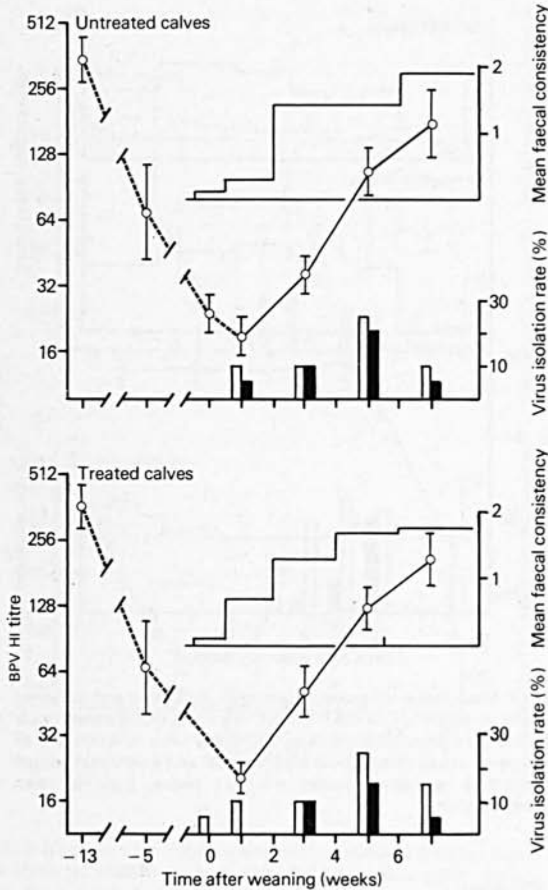

FIG 5: Mean faecal consistency and bovine parvovirus antibody level in monensin treated and untreated calves on property C in the second year. Black (bovine parvovirus) and clear (enterovirus) histograms represent isolation rates from faeces. Bars represent standard error

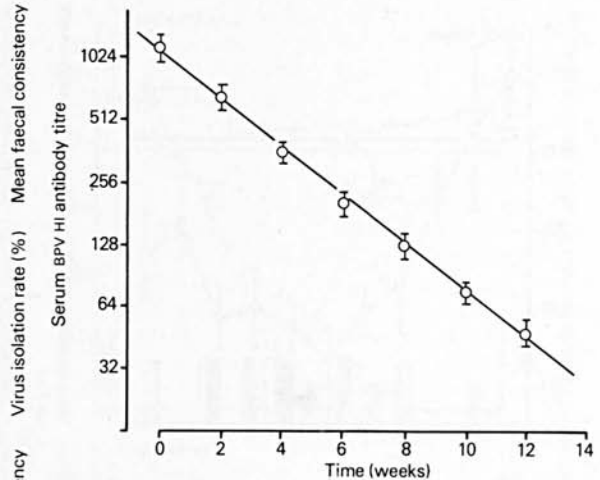

FIG 6: Decline in maternally derived antibody in calves from properties A and B

correlation with faecal consistency. Small numbers of coccidial oocysts were present in most faeces, peak excretion being detected four weeks after weaning when nearly 40 per cent of the calves had oocyst counts greater than 2000 opg and 10 per cent greater than 5000 opg. The majority of oocysts in the high counts were *Eimeria zurnii.*

In the second season monensin treatment had no effect on the overall incidence of diarrhoea, although oocyst excretion was suppressed in treated animals for several weeks, no cases of dysentery being seen. In contrast, several high *E zurnii* oocyst counts were recorded in untreated calves, one being associated with dysentery. An association between *E zurnii* coccidiosis, dysentery, weight loss and death was later recorded in several calves following cessation of virological examinations (R. J. Parker, personal communication). Both groups of calves were found to possess similar helminth egg counts in the faeces, averaging 530 epg at weaning and declining to 300 epg seven and nine weeks later.

Miscellaneous results

The rate of decline in mean titre of maternally derived bovine parvovirus HI antibody in 20 calves from properties A and B is shown in Fig 6, the result indicating the antibody to have a half-life of 19 days.

Discussion

The rates of decline in colostrum-derived antibody to bovine parvovirus (Figs 1 to 3) were complicated by factors which precluded accurate estimation of bovine parvovirus antibody half-life. These included failure to obtain some early serum samples, sampling before attainment of peak antibody levels from colostrum and irregular sampling times with regard to birth date. Consequently, half-life data were derived from sequential sera collected from 20 calves which were uncomplicated by such factors. The half-life of 19 days obtained for colostrum-derived bovine parvovirus antibody was found to be in close agreement with results obtained by other workers (MacDougall and Mulligan 1969, Logan et al 1973, Cunningham 1978).

It is clear from present and previous work (Bates et al 1972, Storz and Bates 1973) that bovine parvovirus can replicate in the intestine of calves which possess substantial levels of serum antibody to it. Bovine parvovirus thus showed similar behaviour to a

number of other enteric organisms such as *Escherichia coli* (Logan and Penhale 1971), *Vibrio cholera* (Welliver and Ogra 1978), rotavirus (Hess et al 1981) and coronavirus (Mebus 1978). Continued replication of the virus in the intestine may be potentiated by the peculiarities of the intestinal immune system as this shows considerable independence from systemic control (Bazin 1979, Husband 1980) as well as appearing to lack normal immune memory systems (Watson and Husband 1977).

Bovine parvovirus is able to invade various body organs (Storz and Bates 1973, Sugimura et al 1974, Storz et al 1978, Bodine et al 1981). Consequently, continued replication of the virus in intestinal tissues is likely to lead to some degree of invasion of the body tissues, despite systemic immunity. The resultant antigenic challenge could boost previous serum antibody levels. Similar findings were reported by Scott et al (1970) and Scott and Gillespie (1971), who found active antibody production in kittens with low levels of passive immunity after challenge with virulent feline panleucopenia virus.

Some substantial differences in epidemiological behaviour were noted between properties A and B. Although onset of seroconversion and bovine parvovirus replication in the intestine appears to be generally related to weaning, there seem to be a number of possible factors involved in causing the differing epidemiological patterns on the two properties. On property A, calving was virtually continuous throughout the year and, therefore, would permit a continuous build up of bovine parvovirus in the weaning area, so that all calves would be weaned in a fairly contaminated environment. Confinement of the calves for two weeks in the limited area of the weaning pens would also exacerbate contamination of the environment, especially for the earlier born calves, as they were born during a peak calving period and consequently more calves were confined in the weaning pens. This probably explains the higher rate of virus excretion in these calves.

On property B, calving was not continuous and the calves born first were weaned in an area which was relatively uncontaminated with bovine parvovirus. The larger grassed area in which the calves were confined during weaning would also lead to reduced contamination of the environment. These two factors probably account for the delayed onset of virus excretion and seroconversion in the earlier born calves, especially in the calf which failed to obtain maternal bovine parvovirus antibody. In contrast, the later born calves were introduced into an environment contaminated with virus from the previous calves, with consequent earlier and heavier challenge and hence earlier virus excretion and seroconversion. The tendency for earlier infection in the calves with lower levels of maternal antibody or those which were weaned slightly younger is a reasonable consequence of this heavier challenge.

On property C, bovine parvovirus excretion and seroconversion also began soon after weaning, antibody titres reaching peak values about six weeks later. This process coincided in both years with the onset of an outbreak of diarrhoea. The problem appeared unrelated to helminth burdens as egg counts were low and it was of similar incidence in anthelmintic treated and untreated animals. In the second year of observation intraruminal administration of monensin in a slow release capsule at weaning was found to have no effect on the incidence of mild to moderate diarrhoea in the first weeks after weaning, suggesting that coccidia were not involved at this time.

The main feature observed on all three properties was the correlation between weaning and the onset of bovine parvovirus excretion and seroconversion, though some modification was noted under conditions of probable higher virus challenge or lower passive immunity. A number of possible explanations may account for this association. Thus, it has been claimed that serum IgG_1 may transfer to the small intestinal mucosa in young calves and that the degree of transfer is related to serum IgG_1 levels (Newby and Bourne 1976). Similarly, in sheep, Cripps et al (1974) found most of the intestinal IgG_1 to be derived from serum. Consequently, the onset of bovine parvovirus activity in the intestine could be explained by loss of mucosal IgG_1 following reduction in maternally acquired serum antibody with time. However, this does not appear to be a very likely explanation in the present study as viral activity was predominantly related to the process of weaning, regardless of the age at which weaning was carried out and only showed a slight tendency to occur earlier in calves with low serum antibody levels. Furthermore, the calf on property B which failed to acquire maternal antibody to bovine parvovirus did not seroconvert until after weaning, despite having been apparently susceptible for over eight weeks.

The presence of viral inhibitors in ingested milk may offer an alternative explanation as they disappear from the intestinal tract after weaning. Milk from immune dams would be expected to contain specific immunoglobulins against bovine parvovirus, mainly IgG_1 with smaller amounts of IgA and IgM (Butler 1969). Although levels would be relatively low compared with colostrum, they would still have some antiviral activity in the intestine (Watson and Husband 1977, Woolcock 1979). In addition, milk contains small amounts of non-specific antimicrobial substances such as glycoproteins (Matthews et al 1976, Snodgrass and Wells 1976, Thouless et al 1977), lactoferrin, mucin and lysozyme (Watson and Husband 1977, Spik et al 1978, Bazin 1979, Bullen and Armstrong 1979). The cumulative

action of the above inhibitors may thus be sufficient to restrict intestinal colonisation by bovine parvovirus under conditions of low to moderate challenge from a contaminated environment. Sudden removal of milk from the diet would, however, leave the animal at risk.

The animal at weaning would also be expected to be subject to considerable changes in the gastrointestinal microflora, which may well lead to a degree of digestive upset, with consequent increase in the rate of epithelial cell turnover in the intestine. This process could be further aggravated by accelerated epithelial cell demand caused by concurrent infection by other cytocidal organisms. As bovine parvovirus replication is dependent on an actively dividing host cell population, such increases in cell turnover would be conducive to active bovine parvovirus colonisation of the intestine.

Enterovirus isolations were distributed over a broader timespan than bovine parvovirus and although some increase in isolation frequency was seen after weaning, the correlation was much less clear. However, it is possible that enteroviruses may have contributed to the post-weaning diarrhoea syndrome.

Whatever the explanation, it is clear that bovine parvovirus infection was frequently activated in calves at weaning and that this was associated on property C with a substantial problem of post-weaning diarrhoea, the initiation of which appeared to be unrelated to helminth burdens. The reason for this association may be related to the substantially greater stress imposed on calves at weaning on property C, in comparison with the other two properties. Calves on property C were subjected to a harsher environment with poorer nutrition. On weaning, the calves were densely stocked in open dusty yards, where they frequently refused to eat for the first 24 hours after weaning (R. G. Holroyd, personal communication) and were subjected to the stresses of branding and dehorning. They were also weaned older when the level of passive antibody protection was considerably lower.

On the basis of the above findings, it is considered that bovine parvovirus infection may have been a contributory factor in the aetiology of post-weaning diarrhoea on property C. It is clear that the virus deserves further investigation as to its possible role in the aetiology of the post-weaning diarrhoea syndrome.

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