Rotavirus Infection in Dairy Calves in Southern Vietnam

Helena Kyle

Supervisor: Camilla Björkman
Department of Clinical Sciences

Assistant supervisor: Stefan Alenius
Department of Clinical Sciences

ABSTRACT

Rotavirus A is a double stranded RNA commonly considered to cause diarrhoea in young calves. There is no specific treatment for the disease and it can become economically devastating to the farmer. In this investigation 120 faecal samples were collected from dairy calves in South Vietnam in order to estimate the occurrence and importance of rotavirus infection in the country. The samples were also used for a co-operating study of the protozoan parasite *Cryptosporidium parvum.* The calves were younger than two months old and both diarrhoeic and non diarrhoeic individuals were sampled. Half the samples were collected at state farms with herd sizes of several hundred cows and the other half were from household farms with 1 and 100 cows. The samples were analysed for presence of rotavirus A by ELISA.

18 samples were positive for rotavirus, 2 of these samples were co-infections with *C. parvum*. 17 of the positive samples were found in state farms. Only 2 samples in total were found positive in household farms.

45 samples out of 120 came from calves suffering from diarrhoea. Twelve samples from calves with rotavirus infection were identified as diarrhoeic, 2 of these were co-infected samples. The number of diarrhoeic samples free from infection of rotavirus indicates that also other enteropathogens are involved in causing diarrhoea in South Vietnam.

SAMMANFATTNING

Rotavirus A är ett dubbelsträngat RNA-virus som är en vanlig orsak till kalvdiarré. Det finns ingen effektiv behandling för rotavirusinfektion och ett sjukdomsutbrott kan bli ekonomiskt förödande för bonden. I denna studie samlades 120 prover in från mjölkraskalvar i södra Vietnam för att undersöka förekomsten av infektion med rotavirus. Proverna testades i en parallell studie även för *Cryptosporidium parvum.* Kalvarna i studien var yngre än två månader, och både kalvar med och utan diarré provtogs. Hälften av proverna togs på statligt ägda gårdar med flera hundra kor medan den andra hälften var från familjejordbruk med 1-100 kor. Proverna anlyserades med avseende på förekomst av rotavirus med antigen-ELISA.

18 prover fanns vara positiva för rotavirus och i 3 av dessa prover påträffades även *C. parvum*. Sjutton av de rotavirus- positiva proverna kom från statligt ägda gårdar. Sammanlagt var endast 2 prover tagna på familjejordbruk positiva.

45 av de 120 proverna kom från kalvar med diarré. 12 av kalvarna med diarré var positiva för rotavirus, och 2 av dessa var saminfektioner med *C. parvum*. Antalet negativa prover från kalvar med diarré visar att andra patogener troligen är vanliga inslag i infektionspanoramat vid kalvdiarré i södra Vietnam.

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1. INTRODUCTION

1.1 Calf Diarrhoea

Diarrhoea is one of the most common causes of sickness and mortality among newborn calves. Rotavirus, *Cryptosporidium parvum*, coronavirus och *Escherichia coli* F5 (previously named *E. coli* K99) are internationally considered the most common pathogens causing neonatal calf diarrhoea. The clinical signs are similar for all the pathogens, but the symptoms tend to be more severe if the pathogens occur in a coinfection (Tzipori, 1981).

In a French survey (Bendali et al., 1999b) it was reported that lack of mineral and vitamin supplementation, dirty cows or no periodical cleaning of stalls after calving season, no *E. coli* vaccination of cows, calf birth assistance due to dystocia, stress due to dyspné, inadequate colostrum feeding, many calves on a small surface and introduction of new cows to the herd were factors that contributed to diarrhoea in the calves. There was no relationship between treating the navel and the presence of diarrhoea. The number of births from a cow, herd size and type of housing were not relevant (Bendali et al.,1999b).

Diarrhoeic and unhealthy calves are an important source of economic loss for the farmer. The average cost associated with prevention and mortality of gastrointestinal disease has been estimated to 33.46 US Dollars per calf per year. It is not only a loss of the present value of the calf but also loss of genetic potential for herd improvement (Kaneene & Hurd, 1990).

1.2 Rotavirus

A calf with rotavirus infection can show a wide spectrum of clinical signs. The infection may be subclinical, or associated with mild diarrhoea. In more severe cases the calf can express depression, anorexia, diarrhoea and dehydration. Calves seem to be most susceptible to rotavirus infection at the age 1 day - 3 weeks, but infections do also occur in older individuals. The diarrhoea may last for 1-2 days in mild cases, and 3-5 days in cases with a secondary bacterial infection (Torres-Medina et al. 1985).

In studies performed in Sweden between 1993 and 2006, the prevalence of rotavirus among calves with diarrhoea was 24-47% (de Verdier 2006). In UK the prevalence has been reported to be 42% (Reynolds et. al., 1986) and in Costa Rica 7% (Perez, et. al., 1998). In Argentina the prevalence of rotavirus during a 10-year period was 62% among calves with diarrhoea (Garaicoechea et al. 2006). Rotavirus was found in 37% of diarrhoeic calves in France (de Rycke et al. 1986). The overall prevalence of rotavirus was 16% in calves in Ohio (Lucchelli et al., 1992) and 47% in a survey with calves from south-west France (Bendali et al. 1999a)

1.2.1 Structure

Rotaviruses are a primary cause of diarrhoea in calves, lambs, piglets, foals, kids and children. The genus rotavirus belongs to the family of Reoviridae, a double stranded RNA virus. The genome consists of 11 double stranded RNA segments, which is surrounded by a three layered protein capsule (Fig. 1). The genus is grouped into 7 species; Rotavirus A – G. Group A rotaviruses are the most frequent serotype causing diarrhoea in farm animals and humans. Rotavirus serogroup B and C are also seen in cattle. The rotavirus serogroups are further divided into serotypes based on the outer capsid proteins, VP7 (G types) and VP4 (P types). At least 14 G and 12 P serotypes are recognized in group A rotaviruses (Radostits et al., 2000). The rotaviruses are species specific, and therefore not zoonotic.

1.2.2 Pathogenesis

The virus infects the established enterocytes by endocytosis at the small intestine villi. The viral genome is transcribed in the cell and causes degenerative changes that make the cell exfoliate. A massive loss of enterocytes leads to fusion of the villi. Squamous or cuboidal epithelium replaces the columnar epithelium. The diarrhoea is caused by the lack of cells that are able to process lactose and the reduced mucus area cause an increase of glucose and galactose in the lumen. In the large intestine the amount of lactose in the lumen leads to a reduced absorption of water because of osmotic pressure. Mature absorbing cells in the small intestine are replaced by immature cells with secretory function. Functional balance may therefore change from absorption to secretion (Scott et. al., 2004).
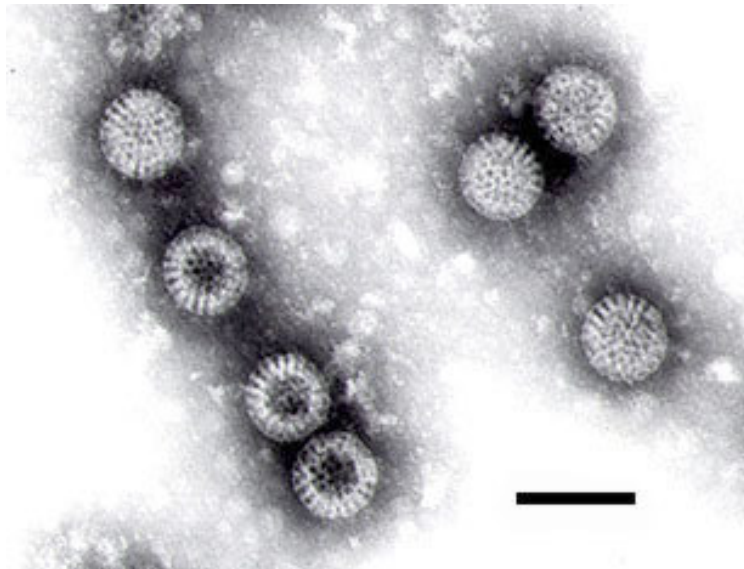
www.epa.gov/microbes/rota.htm. Photo credit: F. P. Williams, U.S. EPA

Fig 1. Electronmicroscope picture of rotavirus particles

1.2.3 Transmission

Rotavirus is endemic and present in all cattle herds (Scott et. al, 2004). The pathogen is being shed by both calves and adult cows. The calves become infected by the faecal-oral route by their dam or by other calves. The colostrum contains specific anti-rotaviral antibodies, that protect the calf from infection during the first few days after birth. The incubation period is 18-24 h. The distinct drop of colostral antibodies at the age of 3 days match the peak of diarrhoea at the age of 5-7 days (Radostits et al., 2000).

1.2.4 Diagnosis

Diagnosis of rotavirus infection is based on finding the pathogen in the faeces from a calf. The two diagnostic methods most often used are electron microscopy and ELISA. Using electron microscopy one can clearly see the virions, but the method is fairly insensitive. If several samples are to be analysed at the same time, ELISA is a rapid and effective diagnostic method (Klingenberg, 1995, Radostits et al., 2000).

Presence of rotavirus in the faeces does not necessarily mean that the diarrhoea is caused by rotavirus infection. Many calves without diarrhoea excrete rotavirus. To evaluate an outbreak of calf diarrhoea on a farm, it is recommended to take 4 samples from diarrhoeic and 4 samples from healthy calves for comparison (Scott et. al, 2004).

1.2.5 Risk Factors

Risk factors that influence rotavirus infection and severity of the disease:

- Age of the animal. Calves are most susceptible to rotavirus infection at the age of 1-3 weeks (Radostits et al., 2000).
- Colostrum intake. Colostrum contains specific antirotaviral antibodies that prevent infection and diarrhoea. The levels decline rapidly, and about three days post partum the concentration is thought to be non-protective. Colostrum intake decreases the mortality (Radostits et al. 2000), but delays rather than prevents rotaviral infection (Scott et. al, 2004).
- Immune status of the dam. The level of specific antibodies in the colostrum is lower in heifers than in dams (Radostits et al., 2000).
- Ambient temperature (Radostits et al., 2000).
- Presence of other enteropathogens. The results of several investigations indicate that mixed infections are more common than infections with single pathogens (Radostits et al., 2000).
- Population density (Tzipori, 1981, Murphy et. al, 1999)

Rotavirus is mostly found in calves kept in large groups. The morbidity rate varies from herd to herd and from one year to another. The survival of rotavirus in air and on surfaces is influenced by the air humidity (Radostits et al., 2000).

1.2.5 Treatment and prophylaxis

There is today no efficient treatment for the pathogens causing neonatal diarrhoea. The way to manage calf diarrhoea is therefore prophylactic. Prevention of calf diarrhoea is difficult because of the large number of pathogens that may be involved, but there are also many different environmental factors associated with calf diarrhoea, as mentioned above. The adequate prophylaxis is concentrated to colostrum intake and a reduction of environmental risk factors. A rich and balanced diet and a well cleaned environment seem to decrease the rate of diarrhoea (Bendali et al., 1999b).

Even more important than a clean environment is the local immunity of the gut lumen of the calf. The rotaviral antibodies from the colostrum, circulating in the blood, are only important the first days. After that the antibodies in the gut are the most important, and feeding of small amounts of colostrum for a longer period can prevent rotaviral infection. (Murphy et al., 1999)

In a survey from Japan a correlation between neonatal diarrhoea and virus neutralizing maternal antibodies against rotavirus was examined. The titers of antibodies were significantly higher in the calves with no diarrhoea than in diarrhoeic calves. (Kohara & Tsunemitsu, 2000) This shows that colostrum intake is an important part in preventing calf diarrhoea.

1.3 Cattle in Vietnam

The following information was extracted from Tuyen & Giao. (2002).

Eighty percent of the Vietnamese population of 80 million people lives in the countryside and are dependent on farming products for their income. The local Vietnamese cattle are small and produce low levels of milk and meat (Fig. 2). Some factors that have a negative effect on dairy production in the country are that there is not enough land for cattle breeding, the farmers are still inexperienced in cattle feeding and management and the animal health service is not effective.

There is a growing demand for dairy products in Vietnam and measures have been taken by the government to enhance the dairy production. In October 2001, the government issued a 10 year plan for dairy development in the whole country. One measure was to select first-class local cows to be crossbred with Holstein-Friesians. Frozen semen from Holstein-Friesian bulls, and also heifers and cows are imported from e.g. Australia. The crossbreeds are well suited to the Vietnamese management system and the tropical climate. The milk production has successfully increased since the project started.

Fig 2. Local breed Vietnamese cows.

The dairy cows in Vietnam are mostly found in the southern parts of the country. Most farms are household farms with less than 10 animals, but some have 10-100 cows. There are also 9 state farms with 500 to 1000 animals. The cattle on the state farms altogether constitute almost 50% of the total cattle population in the country.

1.4 Aim of the study

The purpose of this study was to investigate the occurrence of rotavirus infection in dairy calves in South Vietnam and to evaluate the importance of group A rotavirus as a cause of neonatal enteritis in the country.

2. MATERIALS AND METHODS

2.1 Selection of farms and cattle

Cattle from 6 districts, Cu Chi, Ho Chi Minh City, Long Thanh, Binh Thanh, Hoc Mon and Chau Thanh, in south of Vietnam were included in the study. The farms were participating in an ongoing Dairy Cow Project and not selected especially for the purpose of this study. Two exceptions were the districts of Hoc Mon and Chau Thanh, where local veterinarians guided us, and farms were chosen by convenience.

Samples were collected from 39 dairy farms from September to November 2006. Half of the samples were collected in four of the nine state farms in Vietnam. The state farms housed several hundred cows each. The remaining samples were from household farms which were divided into three categories: 1-5 cows (15 farms), 5-20 cows (14 farms) and 20-100 cows (6 farms).

Calves younger than 60 days were included in the study. Both diarrhoeic and non diarrhoeic calves were sampled from the same farm. At farms with less than 20 cows all calves were sampled. In farms with herd size over 20 cows, the number of calves was chosen by convenience. No more than 20 samples were collected at any farm.

2.2 Sample collection

Faeces were sampled from the rectum using a disposable latex glove (Fig. 3). In rare cases when the rectum was empty, the top layer of a fresh dropping on the floor was collected, but only if it could be proven to belong to the right calf. The samples were transported to Nong Lam University and stored at a temperature of - 20°C until analysis.

The farmers were asked about age and breed of the calves, and the gender and the diarrhoeic status were noted. Diarrhoea was defined as faeces so loose that it would not hold its shape.

Fig 3. Collecting faecal samples at a state farm.

2.3 Analysing samples

The IDEIA™ Rotavirus antigen ELISA (Enzymelinked Immunosorbent Assay) specific to group A rotavirus (DakoCytomation Ltd, Cambridgeshire) was used in this study and the test was performed according to the instructions from the manufacturer. Sample diluent was added to faecal material to obtain a 10% suspension. The faecal suspension and positive and negative controls were added to the wells coated with rotavirus specific polyclonal antibodies, together with a rotavirus specific polyclonal antibody conjugated to hydrogen peroxidase (the conjugate). If rotavirus was present in the sample it was captured between the coated antibodies and the conjugate. After 60 minutes incubation at room temperature the plate was washed to remove redundant sample material and unbound conjugate. A chromogen was added and the plate incubated for 10 minutes at room temperature. Presence of bound conjugate in the wells caused a change in colour, which is stopped by adding sulphuric acid. Colour intensity significantly higher than the colour in the negative control well indicated that the sample was positive for group A rotavirus. The results were first read visually. If any of the wells on a plate looked positive, the optical density at 450 nm was measured by a spectrophotometer.. The producer reports the sensitivity and specificity of the test to be 93-100% and 94-100%, respectively.

Fig 4. Laboratory at Nong Lam University.

2.4 Modification of the study

There were some adjustments made to the original plan for the study. Each calf participating was supposed to be checked for level of total protein in the blood. There was also a questionnaire prepared to be used for gathering information about the history of diarrhoea of the calves.
Information about the level of total protein is interesting because in young calves it indicates the colostral intake. A low level of antibodies in the blood is indicated

by a low total protein value (Radostits et al. 2000). A connection between low antibody levels and diarrhoea could have been searched for. Also, knowing if the calves had been suffering from diarrhoea at an earlier stage of life would have been valuable information. It would have given a better picture of the number of calves shedding rotavirus without suffering from diarrhoea. When the calf is sampled only at one occasion, symptoms from rotavirus infection could have ceased at the time of sampling.

The young calves are valuable to the farmers of Vietnam. If the calves weaken, the farmer may suffer an economical loss. Veterinarians are seldom trusted to inject the calves because of fear that this will lead to infection. A foreigner is even less trusted. Mostly because of linguistic difficulties it was also difficult to get information from the farmers about the diarrhoeic state of the calves. As a consequence of this, few blood samples were collected (Fig. 5) and the study had to be based on the diarrhoeic status at the day of sampling.

Fig 5. Collecting blood samples from the jugular vein using vacutainer.

3. RESULTS

In total, 120 samples were collected from 39 dairy herds and analysed for presence of group A rotavirus. The samples were also used in a co-operating study to estimate the prevalence of the protozoan parasite *Cryptosporidium parvum*. The *C. parvum* results are presented here but discussed in a separate report (Kjellén, 2007).

Sixteen faecal samples were collected from the category "1-5 cows", 27 samples from "5-20 cows" and 20 samples from the category "20-100 cows". Fifty-seven of the faecal samples were collected from state farms.

In total 18 (15%) samples were positive for rotavirus and 10 (8%) samples were positive for *C. parvum*. 45 of the 120 samples were from calves with diarrhoea. 4 (9%) calves suffering from diarrhoea were positive for *C. parvum* and 10 (22%) were positive for group A rotavirus. Another two samples were co-infected with the two pathogens.

Seventy-five calves had formed faeces at the time of sampling. Five (7%) of them were positive for rotavirus, 3 (4%) were positive for *C. parvum* and one was positive for both pathogens (Fig.6).
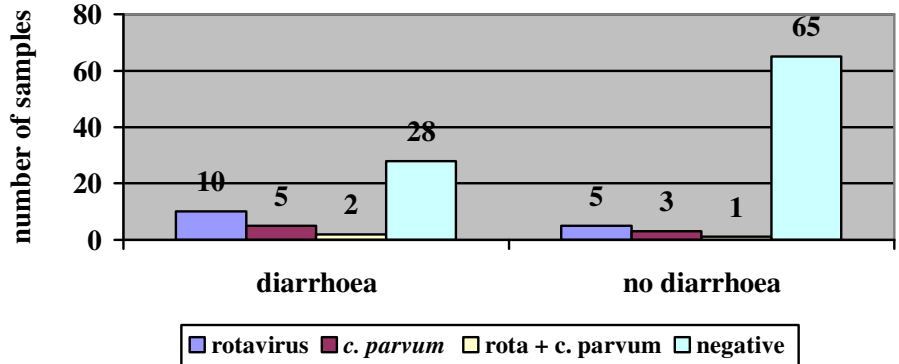

Fig. 6. *Cryptosporidium parvum and rotavirus infection in calves with and without diarrhoea.*

3.1 State farms

The majority of the samples with rotavirus and *C. parvum* were from calves held in state farms (Fig. 7). Of the samples from state farms, 14 were positive for rotavirus and 5 were positive for *C. parvum.* Three calves were co-infected with both pathogens.
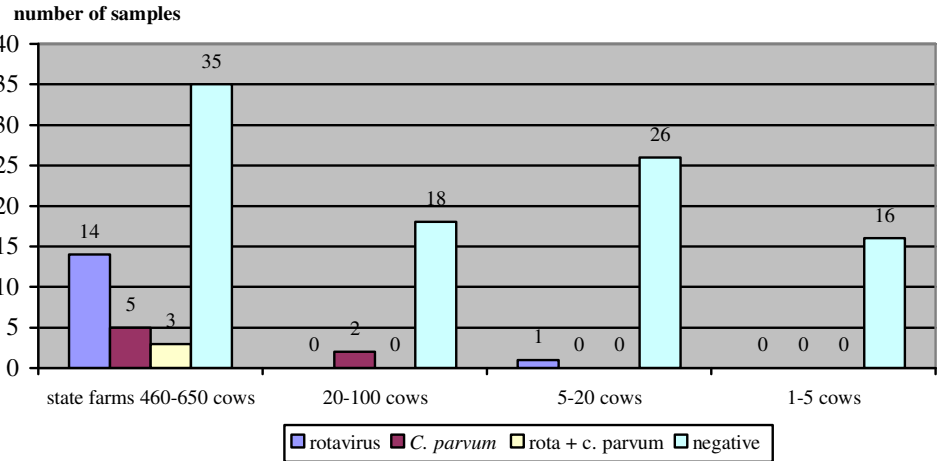

Fig. 7. *Presence of rotavirus and* Cryptosporidium parvum *in calves from farms of different size.*

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The overall occurrence of rotavirus and *C. parvum* among the samples collected from state farms was 30% and 14% respectively. The average presence of diarrhoea was 54%. There were individual differences between the farms (Table 1).

In the samples from state farm number 3 the occurrence of diarrhoea was 75%. There was also a high number of samples positive for rotavirus and *C. parvum,* 10 (50%) and 2 (10%) respectively. Co-infection with both pathogens was found in 3 samples (15%). State farm number 1 had a low occurrence of diarrhoea and no pathogens were found in any sample from this farm.

Ten of the samples (50%) at state farm number 2 were from diarrhoeic calves. Two of them (10%) were shedding rotavirus and 2 (10%) were shedding *C. parvum*.

Because of lack of analysing reagents only 5 samples were collected at state farm number 4. Rotavirus and *C. parvum* was found in 1 (20%) sample each.

Table 1. Diarrhoea, rotavirus and Cryptosporidium parvum *infection in dairy calves from 4 state farms in South Vietnam*

Herd number	Herd size (number of cows)	Number of samples	Diarrhoea (%)	No diarrhoea (%)	Rotavirus (%)	C. parvum (%)	Rota + c.parvum (%)
1	460	12	4 (33)	8 (67)	0 (0)	0 (0)	0 (0)
2	650	20	10 (50)	10 (50)	2 (10)	2 (10)	0 (0)
3	450	20	15 (75)	5 (25)	10 (50)	2 (10)	3 (15)
4	450	5	2 (40)	3 (60)	1 (20)	1 (20)	0 (0)
Total		57	31 (54)	26 (46)	13 (23)	5 (9)	3 (5)

3.2 Household farms

Few positive samples were found in the household farms. The farms were divided into 3 groups referring to herd size. Only 1 sample in the group of 5-20 cows was positive for rotavirus, and 2 samples in the group of 20-100 cows were had *C. parvum* (Table 2). The occurrence of diarrhoea was higher than the occurrence of pathogens in the household farms. The rate of diarrhoea seemed to increase with herd size.

Table 2. Number of diarrhoea, rotavirus and Cryptosporidium parvum *infection in calves from household farms*

Herd size (number of cows)	Number of farms	Number of samples	Diarrhoea (%)	No diarrhoea (%)	Rota-virus (%)	C. parvum (%)	Rota + C. parvum (%)
1-5	15	16	2 (13)	14 (87)	0 (0)	0(0)	0 (0)
5-20	14	27	7 (26)	20 (74)	1 (4)	0 (0)	0 (0)
20-100	6	20	7 (35)	13 (65)	0 (0)	2(10)	0 (0)
Total	35	63	16 (25)	47 (75)	1 (2)	2 (3)	0 (0)

4. DISCUSSION

In this first study on rotavirus infection in calves in Vietnam the prevalence was 15% which can be considered low to moderate in comparison with findings from other parts of the world where prevalences have been reported to vary from 7 to 62% (de Rycke et. al. 1986, Reynolds et. al. 1986, Perez et. al. 1997, Garaicoechea 2005, de Verdier 2006). The majority of these investigations exclusively monitor calves with diarrhoea. However, a study from Ohio including samples from both diarrhoeic and healthy calves showed a rotavirus prevalence of 16% (Lucchelli et. al. 1992). Furthermore, a survey in south-west France investigating both healthy and diarrhoeic calves reported a rotavirus prevalence of 47% (Bendali et. al. 1999a).

The most interesting result was that the levels of both rotavirus and *C. parvum* infection were so much higher in samples collected in state farms than in household farms. Out of 57 samples collected from calves held in state farms there were 17 samples with rotavirus. This is a prevalence of 30%, a level twice as high as the overall prevalence in this study. Three of these samples were coinfected with *C. parvum*. Coinfections were not seen at all in the household farms. The high rate of infection can be explained by a combination of different factors.

The state farms are large, holding several hundreds of cows, and the calves are kept together in big groups. Small calves are held in separate boxes, but they are still able to be in contact with each other and with elder calves that tend to run free close to the younger ones. Age sequencing is sometimes used, but the all-in-all-out system is uncommon. The continuous breeding system is often used, and this causes difficulties to clean and dry the pens properly before new calves are put in. This provides a good environment for the rotavirus to spread within a herd and to stay there for a long time once it has been manifested there. There are also more movements of animals and people to and from the state farms which make it easier to spread the rotavirus. In a large herd it is hard for the farmers to pay enough attention to every calf, and the infections are allowed to spread in the herd. These are all factors that in other studies have been reported to influence the rate of calf diarrhoea, and may contribute to the high level of rotaviral infections

in calves in the state farms. (Tzipori, 1981, Bendali et. al., 1999b, Radostits, 2000).

The level of diarrhoea in state farms was also higher than in household farms. Fifty four percent of the calves sampled at the state farms were suffered from diarrhoea. This rate can be considered as moderate to high. In household farms the rate of diarrhoeic samples was 25%.

Sixty three samples were collected from 35 household farms. Rotavirus was found in only 1 sample, *C. parvum* was found in 2 samples. This indicates that the prevalences of these two pathogens are very low in household farms. The factors contributing to this low rate of rotavirus are several. There is not much traffic to and from household farms, keeping the environment more closed. The small farms that have very few cows often have only one calf at a time, creating a kind of all-in-all-out system. The farmers can be very specific and attentive to their calves, giving them a better protection against infections.

Many samples from all herd sizes were from calves suffering from diarrhoea, but containing no rotavirus or *C. parvum*. This indicates that there are other pathogens or factors causing calf diarrhoea in the farms in South Vietnam.

The results of this study indicate that there still are a lot of questions that need to be answered about the pattern of calf diarrhoea in South Vietnam. Rotavirus plays a role in the infection panorama of calf diarrhoea in South Vietnam, but there are most probably other pathogens that are more important and there is still more work to be done.

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