CRYPTOSPORIDIUM PARVUM INFECTION IN DAIRY CALVES IN SOUTH VIETNAM

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ABSTRACT

Infectious diarrhoea of neonatal calves is common worldwide. Several pathogens, e.g. rotavirus and coronavirus, enterotoxigenic *Eschericia coli* bacteria and the protozoan parasite *Cryptosporidium parvum* have the potential to cause diarrhoea in calves. In this study, which was part of a larger project on calf diarrhoea in South Vietnam, the occurrence of *C. parvum* in dairy calves was investigated.

One hundred and twenty faecal samples were collected from dairy calves in 4 state farms, each housing several hundred cows, and 35 household farms with 1-100 cows in 6 districts between September and November 2006. Both diarrhoeic and non-diarrhoeic calves, aged 1-60 days, were sampled. Fifty seven samples were collected from state farms and 63 samples were collected from household farms. Analyses were conducted by *C. parvum* antigen ELISA. The samples were also used in a cooperating study to estimate the prevalence of rotavirus.

In total, 10 samples (8%) were positive for *C. parvum,* 3 of these were co-infected with rotavirus. Forty five samples were from calves with diarrhoea. Four of them were infected by *C. parvum*, and another 2 were co-infected with *C. parvum* and rotavirus. Of the 75 samples collected from clinically healthy calves, 3 had *C. parvum* only and 1 was co-infected with *C. parvum* and rotavirus.

Eight (80%) of the *C. parvum* positive samples were collected in state farms and the remaining two were from household farms with 5-20 cows. The 3 samples that were coinfected with *C. parvum* and rotavirus were from state farm calves. The average occurrence of diarrhoea among the calves sampled in state farms was 54%, whereas 25% of calves sampled in household farms were diarrhoeic.

The results show that *C. parvum* and rotavirus are present among calves in South Vietnam and might have clinical significance. They indicate that calves in large sized state farms are infected by *C. parvum* to a higher extent than calves in household farms, and also that calf diarrhoea is more common in the state farms.

SAMMANFATTNING

Spädkalvsdiarré är vanligt över hela världen. Rotavirus, coronavirus, enterotoxisk *Eschericia coli* och den encelliga parasiten *Cryptosporidium parvum* är smittämnen som ofta orsakar diarré hos kalvar. I den här studien, som är en del av ett större projekt rörande kalvdiarré i södra Vietnam, undersöktes förekomsten av *C. parvum* hos kalvar i mjölkkobesättningar.

Under tidsperioden september-november 2006 insamlades i 6 distrikt 120 träckprover från mjölkraskalvar i 4 statsägda besättningar med vardera flera hundra kor, och 35 småskaliga jordbruk med 1-100 kor. Kalvar som var 1-60 dagar gamla ingick i undersökningen och både kalvar med och utan diarré provtogs. Femtiosju prover insamlades från statsägda besättningar och 63 från småskaliga jordbruk. Proverna analyserades med avseende på förekomst av *C. parvum* med antigen ELISA. Proverna användes även i en parallell studie för att uppskatta prevalensen av rotavirus.

Totalt 10 prover (8 %) var positiva för *C. parvum* och 3 av dessa var saminfekterade med rotavirus. Fyrtiofem prover kom från kalvar med diarré varav 4 var infekterade med *C. parvum* och ytterligare 2 var saminfekterade med *C. parvum* och rotavirus. Av de 75 kliniskt friska kalvar som provtogs hade 3 stycken *C. parvum*-infektion och endast en kalv var saminfekterad med *C. parvum* och rotavirus.

Åtta (80 %) av proverna som var positiva för *C. parvum* hade tagits på statsägda besättningar och de resterande 2 kom från småskaliga jordbruk med 5-20 kor. De 3 prover som var saminfekterade med *C. parvum* och rotavirus var alla från statsägda besättningar. Förekomsten av diarré hos provtagna kalvar var 54 % i statsägda besättningar medan 25 % av kalvar i småskaliga jordbruk hade diarré vid provtagningstillfället.

Resultaten visar att både *C. parvum* och rotavirus förekommer hos kalvar södra Vietnam och kan vara av klinisk betydelse. Resultaten indikerar även att kalvar från stora besättningar i större utsträckning är infekterade med *C. parvum* än kalvar från småskaliga jordbruk samt att diarré är vanligare i statsägda besättningar.

INDEX

1. INTRODUCTION

1.1 Milk production and cattle in Vietnam

Eighty percent of the Vietnamese population of 80 million people lives in the countryside and are dependent on farm products for their income. The local Vietnamese cattle are small and produce low levels of milk and meat. Some of the problems affecting 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 (Tuyen et al. 2002).

Due to a growing demand for dairy products, 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 factor was to select first-class local cows to be crossbred with Holstein-Friesians. Holstein-Friesian heifers and cows and frozen semen from bulls are imported from countries like Australia. The crossbreeds are well suited to the Vietnamese management system and tropical climate. The milk production has successfully increased since the project started (Tuyen et al. 2002).

The dairy cows in Vietnam are mostly concentrated to the southern parts of the country. Most farms are household farms with less than 10 animals. There are some farms with a herd size of 10-100 cows. There are also 9 state farms with 500 to 1000 animals. The populations of these state farms altogether constitute almost 50% of the total cattle population in the country (Tuyen et al. 2002).

1.2 Calf diarrhoea

Infectious diarrhoea of neonatal animals is a common disease (Holland 1990). The disease not only causes a loss of the present value of the calf but also of the genetic potential for herd improvement (Bruning-Fann et al. 1992).

Coronavirus, *Cryptosporidium parvum,* Enterotoxigenic Escherichia coli (ETEC) and rotavirus are well known causes of enteric infections in neonatal farm animals (Holland 1990). These pathogens are collectively responsible for 75-95% of infections in neonatal calves around the world (Radostits et al. 2000). Another common cause of diarrhoea is *Salmonella spp.* (Reynolds et al. 1986). The clinical signs tend to be non-specific but do generally include watery to semi formed faeces, weakness, anorexia and dehydration (Holland 1990).

Mixed infections of different enteropathogens are common and will often exaggerate the clinical outcome of the disease (*Holland* 1990). Rotavirus and *Cryptosporidium parvum* are often mentioned as the most important infectious agents causing neonatal calf diarrhoea (Snodgrass et al. 1986).

According to Blowey (2003) diarrhoea in calves only a few days old is often caused by bacterial infections such as *Escherichia coli* or *Clostridium welchii*. Cryptosporidiosis and infections of rotavirus and coronavirus commonly occur at

an age of 10-14 days whereas *Salmonella* infection can occur at any age (Blowey et al. 2003).

Prevention of calf diarrhoea is difficult because of the large number of pathogens that may be involved. Different management and environmental factors have also been associated with the disease. Dirty cows or no periodical cleaning of stalls after calving seasons have been reported to contribute to diarrhoea in the calves. Other factors mentioned are calf birth assistance due to dystocia, stress due to dyspné, many calves on a small surface and introduction of new cows to the herd (Bendali et al. 1999b).

1.3 *Cryptosporidium parvum*

1.3.1 *The parasite*

Cryptosporidium is a genus in the family Cryptosporidiidae, suborder Eimeriina, order Eucoccidiida, subclass Coccidia and class Sporozoa (Tzipori 1983). *Cryptosporidium* are intracellular parasites found in the digestive and respiratory tract in a variety of vertebrate hosts worldwide (Fig. 1) (Tzipori 1983). Based on molecular phylogenetical analyses, the *Cryptosporidium* species are currently under evaluation (Kosek et al. 2001). Thus, some parasites previously recognised as genotypes within the species *C. parvum* have recently been recognised as separate species. E.g. *C. hominis* which infects mainly humans was previously known as *C. parvum* genotype I. The remaining *C. parvum* (previously genotype 2) infects a variety of animal species including cattle and man, and is zoonotic (Huetink et al. 2001, Bowman et al. 2003).

Today 3 species are known to regularly infect cattle: *C. parvum*, *C. andersoni* and the recently described *C. bovis* (Fayer et al. 2005, Langkjaer et al. 2007, Thompson et al. 2007). *C. andersoni* is found mainly in adult cattle but has also been found in young stock and *C. parvum* usually infects calves younger than one month (Olson et al. 2004). *C. bovis* tends to infect older calves and young stock (Langkjaer et al. 2007).

C. parvum, *C. hominis* and *C. bovis* can not be separated based only by traditional diagnostic methods such as microscopy or ELISA test, but DNA analysis has to be applied in order to distinguish between the species.

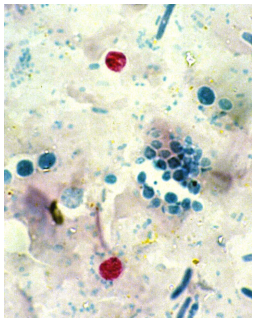

Fig. 1. Cryptosporidium parvum in acid staining (http://www.k-state.edu/parasitology/625tutorials/index.html [2007-01-15]).

1.3.2 Life cycle

The life cycle of *C. parvum* is direct (Fig. 2) (Radostits et al. 2000). First, the oocyst is ingested by the host animal. In the intestinal lumen it opens along a preexisting suture line and four infective sporozoites are released through excystation (Bowman et al. 2003, Radostits et al. 2000). The sporozoites invade the microvillous brush border of the enterocytes and differentiate into trophozoites. Via merogony asexual multiplication of the nuclei of the mature trophozoites follows. The outcome is a development of either meronts I or II, which are released to invade previously uninfected host cells (Holland 1990). Meronts I initiate another phase of merogony, while meronts II initiate a sexual phase called gametogony (Radostits et al. 2000). Merogony results in microgametocytes (males) or macrogametocytes (females) and by fertilization, zygotes are produced (Holland 1990). The zygotes develop into oocysts. Oocyst walls are formed and new sporozoites are developed inside through sporongy. The oocysts sporulate within the host cell and are infective when passed with faeces (Holland 1990, Radostits et al. 2000).

The parasite is intracellular but extracytoplasmatic (Holland 1990). Infection is mostly concentrated to the lower parts of the small intestine (Bowman et al. 2003). The sporozoites can also invade the microvillous border of the gastric glands, bile duct or respiratory tract (Ballweber 2001). The intracellular stages are formed within a parasitophoros vacuole, confined to the microvillous region of the host cell. The sporozoites become incorporated with the microvillous membrane and are internalized in a membrane sac of the host cell (Radostits et al. 2000).

The new oocysts are produced within 72 hours, and so the prepatent period could be as short as three days. Some oocysts are made thin-walled to break and re-infect the host. The rest are thick-walled to be able to pass in faeces (Ballweber 2001).

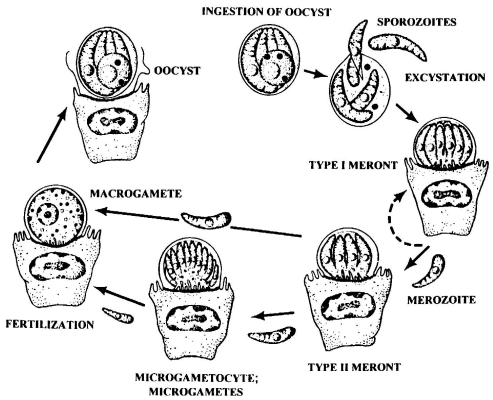

Fig. 2. Life cycle of Cryptosporidium parvum (http://www.k-state.edu/parasitology/625tutorials/index.html [2007-01-15]).

1.3.3 The oocysts

A calf can shed millions of oocysts during the first two weeks of infection (Fayer et al 1998). The oocysts are 4-5 µm in diameter and are excreted in the faeces in a fully infective state (Zajac et al. 2006). They sporulate within the host cell and the

infection persists until the immune response of the animal eliminates the parasite (Radostits et al. 2004).

The oocysts are robust and resistant to desiccation and most disinfectants (Tzipori 1983). They can however be destroyed by some concentrated disinfectants such as 5% ammonia and 10% formol saline or by freeze-drying (Bowman et al. 2003). The infectivity is also destroyed if they are exposed to temperatures below -20°C for 3 days and above 64, 2°C for 5 minutes (Fayer 1997). The oocysts remain infective for months outside the host, especially in moist environments (Radostits et al. 2000).

1.3.4 Transmission

Transmission of *C. parvum* is conducted by faecal contamination (Radostits et al. 2000). The parasite can be transmitted from animals to humans and transmission from human to human has also been shown. The latter transmission route seems to be more common than the first (Bowman et al. 2003).

Groundwater contaminated by domestic or wild animal faeces is an identified route of transmission besides the transmission by faecal contamination directly between animals and humans (Tzipori 1983). For example, studies have shown *C. parvum* to be rather prevalent in Norwegian water sources (Robertsson et al. 2001). Infection is also said to be transmitted mechanically through vectors (Radostits et al. 2004).

1.3.5. *Cryptosporidiosis in humans*

Cryptosporidium infection in humans causes acute, self-limiting diarrhoea in immunocompetent persons. Immunocompromised persons may suffer from a severe, life threatening, chronic diarrhoea and malabsorption (Casemore et al. 1985). Other possible symptoms are abdominal pain, fever and vomiting (Tzipori 1983). Risk groups are elderly people, children in day care centres and travellers (Holland 1990). Human cryptosporidiosis is in general more common in children than in adults. Infection rates are predicted to be highest in developing countries and the children of these countries are said to constitute an important risk group. Further on, the infection causes a significant public health problem in developing countries since AIDS is endemic (Casemore et al. 1985). In AIDS patients, *Cryptosporidium* spp. can generate life-threatening infections (Bowman et al. 2003).

1.3.6 Cryptosporidiosis in cattle

In most cases, outbreak of disease occurs at young age, in calves less than three weeks old, or in immunocompromised individuals (Ballweber 2001). Cryptosporidiosis is characterized by high morbidity and the most common clinical sign is diarrhoea (Holland 1990). Other symptoms that might show at the same time are depression, various degrees of apathy, inappetence and poor condition. Only at some occasions does severe dehydration and collapse occur (Radostits et al. 2000).

The symptoms may appear 2-7 days after inoculation and the calf will recover within 6 -10 days after the appearance of diarrhoea. Oocysts are shed in faeces for 3-12 days (Radostits et al. 2000). The illness is usually self-limiting in healthy animals but it enhances in severity in the presence of other pathogens such as rotavirus in calves (Holland 1990).

The protozoa cause tissue reactions in the intestines, which may lead to diarrhoea. Villous atrophy, a shortening of microvilli, villous fusion and cryptitis can be seen. The intestinal digestion and absorption of dietary nutrients is decreased due to the loss of microvilli and decreased activity in mucosal enzymes and villous function. It is mostly the lower part of the small intestine that is infected but cecum and colon can occasionally be affected (Radostits et al. 2000). Sometimes the parasite causes bacterial overgrowth by decreasing disaccharidase activity. Less disaccharidase reduces the breakdown of sugars and causes a good environment for bacterial growth (Ballweber 2001).

Once the calves have been exposed to *C. parvum,* it has been indicated that they will become resistant to further infections of the pathogen (Harp et al. 1990). Calves that have been isolated from the parasite and then experimentally challenged to an infection have acquired diarrhoea, oocyst shedding and a raised antibody titre. At a second challenge, no symptoms of infection, no oocyst shedding and no increase in antibody titres were observed (Harp et al. 1990). Age-related resistance, unrelated to prior exposure, has been observed in lambs but not in calves (Radostits et al. 2000). Separating the effects of non-immunological age-related resistance from immunity through natural exposure on older individuals is difficult since *C. parvum* is common in the environment and the animals could be assumed to continuously be exposed for an infection (Harp et al. 1990).

Cryptosporidium parvum is often the only pathogen found in diarrhoeic calves (Singh et al. 2006). It has been reported from Norway that the prevalence of the parasite seems to increase as the number of calves in a herd increases, and that small farms tend to have fewer problems with cryptosporidiosis (Hamnes et al. 2006). However, the importance of *C. parvum* infection as a cause of calf diarrhoea is under debate since *C. parvum* is commonly found in healthy animals. In some studies, no association between infection with *C. parvum* and diarrhoea or other clinical signs were found (de Rycke et al. *1986*, Huetink et al. 2001). However, results from Canada showed a three time higher risk for calves shedding oocysts to be diarrhoeic than non-infected calves (Trotz-Williams et al. 2005a). Another example is from India, where results show a 1.59 times greater risk for a calf to suffer from diarrhoea if infected (Singh et al. 2006). According to a study by Reynolds et al. (1986), there are more enteropathogenes, such as *C. parvum,* found in diarrhoeic calves than in clinically healthy calves.

1.3.7 Diagnosis

C. parvum is diagnosed by identifying oocysts in faeces. Faecal smears are often dried, stained and analysed by microscopy (Urquhart et al. 1996, Radostits et al. 2000). One staining method is the Ziehl-Nielsen technique, which stains granules of the sporozoites with a bright red colour, but there are also other staining

techniques, including immunofluorescence (Urquhart et al. 1996). Other methods of diagnosis are to use immunoassays e.g. enzyme linked immuno assay (ELISA) and to histologically demonstrate the endogenous parasite stages attached to the brush border of epithelial cells (Radostits et al. 2000).

1.3.8 Treatment and prophylaxis

There is no specific effective treatment for *C. parvum* infection (Bowman et al. 2003). Many antimicrobial and antiprotozoal drugs have been tested, but so far only two substances have been found to have some beneficial effects. Halofuginone has been reported to reduce oocyst output and prevent diarrhoea in lambs and calves. It is suggested to prevent re-infection of sporozoites and recycling merozoites in the gut. The drug will allow some intestinal infection, which is good for the development of immunity. Paromomycin sulphate has been tested on goat kids and has been found effective (Radostits et al. 2000).

Calves suffering from diarrhoea need fluids and electrolytes orally and parenterally. The calves should be fed milk continuously to prevent loss of body weight and digestion function. Parenteral nutrition could be considered in cases of valuable calves. Further transmission should be avoided through isolation of unhealthy calves and feeding calves adequate amounts of colostrums. It is useful to keep the farm clean and to use an all-in, all-out system so that all areas could be properly cleaned. Rodents and pets should not have access to the calves to avoid transmission through vectors (Radostits et al. 2000). Fresh bedding in combination with strict sanitation is probably the best prophylaxis against calf diarrhoea (Krogh et al. 1985).

Fig. 3. Household farm in South Vietnam.

1.4 Aim of the study

The purpose of this study was to investigate the occurrence of *C. parvum* infection in healthy and diarrhoeic dairy calves younger than 60 days in household farms and state farms in Vietnam. Comparisons were made between *C. parvum* infected calves with or without symptoms and associations to environment and housing were looked for. The samples collected for this occasion were also analysed for rotavirus in a co-operating survey (Kyle, 2007).

2. METHODS AND MATERIALS

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 Vietnam were included in the study. All of them were reachable within a few hours drive from Nong Lam University, Ho Chi Minh City. The farms were not selected especially for the purpose of this study. In order to collect samples we followed the schedule of the Dairy Cow Project. 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 on 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. 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) (Fig. 3).

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. If the farm had more than 20 cows, the number of calves was chosen by convenience. No more than 20 samples were collected at any farm.

Fig. 5. Collection of faecal samples in state farm.

2.2 Sample collection

Faeces were sampled from rectum of the calves using a disposable latex glove (Fig. 5). 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 -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 wouldn't hold its shape.

2.3 Sample analyses

In this project an ELISA test (ProSpecT® *Cryptosporidium* Microplate Assay; Remel, Santa Fe) was used for analyses. Analyses were conducted at Long Nam University (See Fig. 6) following the manufacturer's instructions.

The ELISA-kit consisted of a micro titre plate with wells inoculated with anti-*Cryptoporidium* specific antigen (anti-CSA) antibodies. Swabs of the faecal samples were mixed in one ml dilution and then placed into the wells. After incubation for 60 minutes, the wells were washed with buffer to remove unbound material.

The next step was to add monoclonal anti-CSA antibodies labelled with horse radish peroxidase (conjugate) to each well. The plates were incubated for another 30 minutes and washed to remove unbound conjugate A substrate was then added. If a sample is positive, the substrate will change colour. After 10 minutes incubation a stop solution was added. The results shall be analysed within 10 minutes, either visually or using a spectrophotometer. In this study, the results were analysed visually since there wasn't a spectrophotometer available at all occasions of analysing.

In all analyses, one positive and one negative control sample were included to discover mistakes in testing procedure or malfunctioning analysing kits.

Fig. 6. Laboratory at Long Nam University.

2.4 Adjustments of the study

There were minor adjustments made to the original plan for the survey. A refractometer and material for collecting blood samples were brought for analysing the total protein of the calves. Each calf participating in the study was supposed to be checked for level of total protein in the blood. There were also questionnaires used to gather information about the history of diarrhoea of the calves.

Information about the level of total protein is interesting because it indicates the colostral intake of the calf. A low level of antibodies in the blood is indicated by a low total protein (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 oocysts without suffering from diarrhoea. When the calf is sampled at one occasion, symptoms from cryptosporidiosis 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, no blood samples were collected and the study had to be based on the diarrhoeic status at the day of sampling.

3. RESULTS

One hundred and twenty samples were collected from dairy calves younger than two months old and analysed regarding the presence of *C. parvum*. The same samples were also used in a co-operating study to estimate the prevalence of group A rotavirus. The rotavirus results are presented here but are discussed in a separate report (Kyle, 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". *57* of the faecal samples were collected from state farms.

In total, 10 of the samples (8%) were positive for *C. parvum* and 18 samples (15%) were positive for rotavirus. Forty five (38%) out of the 120 sample were collected from calves suffering from diarrhoea. Four (9%) of the diarrhoeic calves were positive for *C. parvum,* 10 (22%) were positive for rotavirus and 2 (4%) were co-infected with *C. parvum* and rotavirus (Fig. 7).

Seventy five calves were clinically healthy when sampled. Three (4%) were positive for *C. parvum* and 5 (7%) were positive for rotavirus. One clinically healthy calf was co-infected with *C. parvum* and rotavirus (Fig. 7).

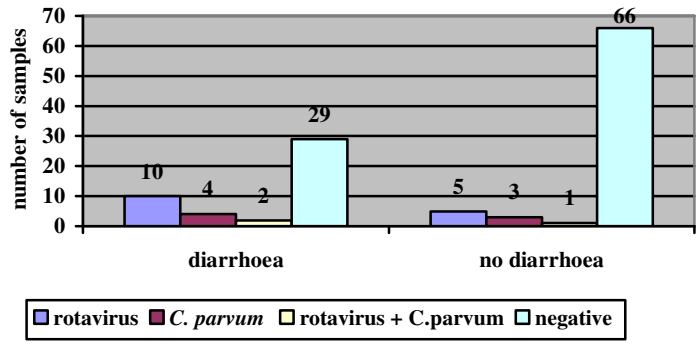

Fig. 7. Cryptosporidium parvum and rotavirus infection in calves with and without diarrhoea.

3.1 State farms

Most of the *C. parvum* positive samples were from the 57 calves held in the state farms. The occurrence of *C. parvum* and rotavirus in the state farm samples was 5 (9%) and 14 (25%), respectively. An additional three samples were infected with both *C. parvum* and rotavirus. The average occurrence of diarrhoea among the sampled calves was 54% (Table 1 and Fig. 8).

Three out of four state farms had sampled calves with *C. parvum* and rotavirus infection. In state farm number 3, two samples were positive for *C. parvum* and there was a high prevalence of rotavirus. There were 3 calves co-infected with *C. parvum* and rotavirus, differing from the other farms where no co-infections were found. There was also a higher percentage (75%) of diarrhoea in the sampled calves on this farm than on the others. None of the pathogens were found on state farm number 1, which also had a lower occurrence of diarrhoea.

Table 1. Diarrhoea and infection of *Cryptosporidium parvum* and rotavirus in calves of state farms in South Vietnam

Herd number	Herd size (number of cows)	Number of samples	Diarrhoea (%)	No diarrhoea (%)	Rotavirus (%)	C. parvum (%)	Rota-virus + *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)	2 (40)	1 (20)	0 (0)
Total		57	31 (54)	26 (46)	14 (25)	5 (9)	3 (5)

3.2 Household farms

There were few *C. parvum* and rotavirus positive samples from household farms. Two samples were positive for *C. parvum* in farms with a herd size of 20-100 cows and one sample was positive for rotavirus in herd sizes of 5-20 cows. There were no cases of co-infections with *C. parvum* and rotavirus found in any of the farms (Table 2 and Fig. 8).

The occurrence of diarrhoea was higher than the occurrence of pathogens in the samples collected in the household farms. Sixteen calves out of 63 sampled at household farms suffered from diarrhoea, i.e. 25%, in comparison to 54% in the state farms (Table 2).

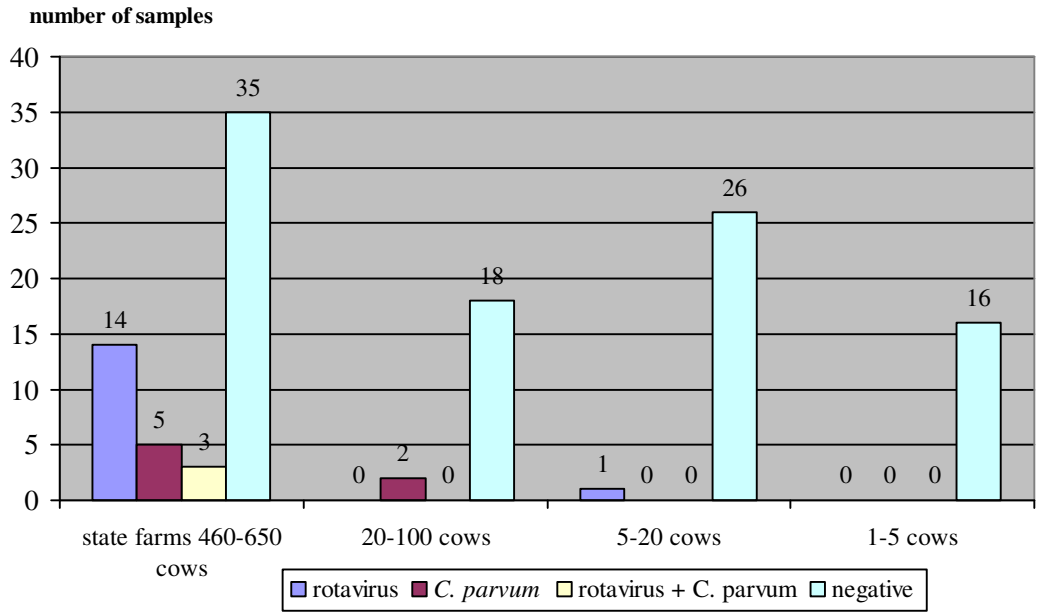

Fig.8. Cryptosporidium parvum and rotavirus in farms of different size.

Table 2. The occurrence of diarrhoea, *Cryptosporidium parvum* and rotavirus infection at household farms of different size

Herd size (number of cows)	Number of farms	Number of samples	Diarrhoea (%)	No diarrhoea (%)	Rota-virus (%)	C. parvum (%)	Rotavirus + *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

The results of this study show that 10 (8%) of 120 samples were infected by *C. parvum.* The prevalence of rotavirus was 15%. There is no research previously published in English regarding the prevalence of *C. parvum* in South Vietnam

There is a variety of results from studies concerning the prevalence of *C. parvum* in calves worldwide. Some African, Central American and European studies have shown a prevalence of *C. parvum* from 0 to 23% (Reynolds et al. 1986, Abraham et al. 1992, Perez et al. 1998, Bendali et al. 1999a, Björkman et al. 2003). Other studies from North America, Central Asia and Europe report prevalences from 12 to 95% (Naciri et al. 1999, Trotz-Williams et al. 2005, Singh et al. 2006, Hamnes et al. 2006), but they used a sucrose flotation method which concentrates the oocysts and makes it possible to detect lower levels of infection (Bowman et al.

2003). The prevalence of *C. parvum* in the present study could be considered low to moderate in comparison to the set of results first mentioned which applied methods with similar sensititivities as the ELISA method used here.
An important finding in this study was that infections with *C. parvum* and rotavirus were foremost occurring in samples from state farms, and that the prevalence in household farms was markedly low. Also worth noting was that none of the samples infected with both *C. parvum* and rotavirus were collected at the household farms. The indication that the herd size influences the rate of infection is supported by a study from Norway (Hamnes et al. 2006) showing a trend for increasing prevalence of *Cryptosporidium* with increasing number of calves in the herd.

The high rate of infection in state farms might be explained by a combination of different factors. In some of the farms the young calves are separated in pens during the first weeks of age and then kept in groups. Other farms keep calves of all ages together in larger areas. An important risk of contamination is to keep calves in groups, referring to Radostits et al. (2000) who indicate that *C. parvum* is transmitted via faecal contamination or via vectors. In a study from France (Naciri et al. 1999), nose-to-nose contact was considered an important rote of transmission which is enabled in a group. Even in the farms with separated housing system the calves were occasionally still able to have contact with each other and with older calves kept next to the pens. The all-in all-out system is uncommon and a continuous breeding system is often used. This causes difficulties to clean and dry the pens properly before new calves are put in, an environment where infections are easily spread and maintained in the hot, humid climate. The size of the state farms can be considered a risk since many animals and large areas make cleaning and elimination of manifested pathogens difficult. The study from Norway, earlier mentioned, shows a significantly higher prevalence of *Cryptosporidium* in calves housed in shared pens that were poorly washed (Hamnes et al. 2006). In a large herd it is also hard for the farmers to pay enough attention to every calf, and the infections are allowed to spread. Another factor that could be of importance is that state farms hold several hundred cows and could be assumed to be more trafficked and to have more cattle- and staff exchange than household farms. The risk of introducing pathogens to the herd increases.

A risk of spreading disease that was foremost observed in household farms was the lack of separation of farm animals. The contact between birds, calves, pigs and dogs enables contamination of *C. parvum* via vectors. The low occurrence of infection in the household farms could be due to the low number of cows. Housing of a few calves at a time creates an all-in all-out system and simplifies caretaking and cleaning. A household farm could also be assumed to have less contact with people, new animals and vehicles than the state farms.

The level of diarrhoea in state farms was higher than in household farms. Thirty one (54%) samples out of 57 collected at the state farms came from calves suffering from diarrhoea. In household farms the rate of diarrhoeic samples was 25%.

The infection of *C. parvum* and rotavirus seems to be a contributory factor to the rate of diarrhoea in calves of Vietnam. Four of the sampled calves infected by *C. parvum* were suffering from diarrhoea. Ten diarrhoeic calves were infected by rotavirus and an additional two calves were co-infected with both pathogens. Forty five calves out of 120 suffered from diarrhoea at the time of sampling but 29 diarrhoeic samples were negative for both *C. parvum* and rotavirus. Out of the 63 samples from household farms 16 samples were diarrhoeic. Two samples were positive for *C. parvum* and one sample was positive for rotavirus. Thirteen samples (81%) of the diarrhoeic samples from household farms were diarrhoeic but negative. This indicates that the dairy calves in Vietnam may also be affected by other enteropathogens causing diarrhoea.

Out of the 75 clinically healthy calves, 4% were infected by *C. parvum* and 8% by rotavirus. This is in agreement with the results of previous studies showing that calves infected by enteropathogens can be subclinically infected (Björkman et al. 2003, Trotz-Williams et al. 2005, Singh et al. 2006). Another possibility is that the calves already recovered from diarrhoea caused by an infection.

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

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