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## Cryptosporidiosis in Dairy Calves: Risk Factors, Diagnosis, and Zoonotic Potential

By Lise A. Trotz-Williams, BVetMed, MSc, PhD; Andrew S. Peregrine, BVMS, PhD, DVM;  
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**Infection with the parasite, *Cryptosporidium parvum*, is a common cause of diarrhea in calves on dairy farms. This issue of *Large Animal Veterinary Rounds* reviews diagnostic tests and treatment for *C. parvum*, risk factors for infection, and the zoonotic potential of the parasite in neonatal dairy calves, with emphasis on the results of research conducted in southern Ontario during 2003–2005.**

Parasites of the genus *Cryptosporidium* are capable of establishing infections in many different host species, including mammals, birds, and reptiles. In neonatal dairy calves, infection with *Cryptosporidium* often results in diarrhea, weakness, and dehydration. Severe cases of cryptosporidiosis may result in mortality, and the expenses associated with morbidity and mortality in affected animals can represent substantial financial losses to dairy producers. The most common species of *Cryptosporidium* infecting dairy calves <1-month-old is *Cryptosporidium parvum*, so named because of the small size of the oocysts (3 to 5 microns in diameter) that are often excreted in large numbers in the feces of infected calves (Figure 1).<sup>1</sup> These features of *C. parvum*, as well as the resistance of oocysts to most commonly-used disinfectants and to moderate changes in environmental conditions, facilitate the rapid spread of infection among calves on a farm. Furthermore, since at least one genetic subtype of *C. parvum* in calves is known to infect humans, there is the potential for zoonotic spread of infection to persons in close contact with infected animals. The small size and resistant nature of the oocysts give them an ability to evade routinely used drinking water purification methods. As a result, contamination of drinking water sources by viable oocysts has been implicated as the cause of several water-borne outbreaks of disease in humans in North America, Europe, and elsewhere.<sup>2,3</sup> There are no licensed medications effective against *C. parvum* available for routine use in Canada. Treatment of cryptosporidiosis is usually symptomatic and the control of the parasite relies heavily on prevention. This issue reviews the importance and epidemiology of *C. parvum* in dairy herds, and the methods for controlling this parasite on farms.

### Diagnosis of *C. parvum* infection

Several techniques have been developed for the detection of *Cryptosporidium* infection in animals. Histological examination of stained sections of ileum, retrieved at post mortem or by biopsy, reveals acid-fast organisms in parasitophorous vacuoles at the luminal surface of intestinal epithelial cells. However, diagnosis of infection is most often achieved by examination of fecal specimens for evidence of *Cryptosporidium* oocysts. Diagnostic methods usually involve concentrating the oocysts present in the specimen, followed by staining. The most effective concentration is achieved by the addition of solutions (eg, sucrose or zinc sulphate) with subsequent flotation and, in some cases, centrifugation of the suspension.<sup>4</sup> Commonly-used stains include Ziehl-Nielsen and auramine-rhodamine.<sup>4</sup> In addition, diagnostic tests to detect *Cryptosporidium* antigen in fecal specimens, which were originally developed for use in humans, are often employed on samples from



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**Figure 1: An oocyst of *Cryptosporidium parvum* in an unstained fecal wet smear from a calf (mag.  $\times 400$ ).** The small size of these organisms and their resistance to most commonly-used disinfectants can make control of the parasite difficult to achieve.



calves.<sup>5</sup> Enzyme-linked immunoassays (ELISAs) and polymerase chain reaction (PCR) have been used for research purposes. PCR is of particular value, not only for the detection of *C. parvum* in fecal samples, but also, in conjunction with restriction fragment length polymorphism (RFLP) and sequencing of PCR products, for the molecular characterization of *Cryptosporidium* isolates from various sources.<sup>6</sup>

Commonly-used diagnostic methods are time-consuming and, in some cases, costly; as a result, diagnostic testing for *Cryptosporidium* is rarely done in practice. To investigate potential options of simple and inexpensive testing for *C. parvum* in calves, a sucrose wet mount test and a lateral immunochromatography (LI) test were evaluated at the Ontario Veterinary College. Parameters assessed were epidemiological sensitivity and specificity, cost per test, and time required per sample tested.<sup>7</sup> Polymerase chain reaction-restriction length fragment polymorphism (PCR-RFLP) of the *Cryptosporidium* oocyst wall protein (COWP) gene locus with gel electrophoresis was used as a gold standard.

The sucrose wet mount test is a 3-step procedure in which a mixture of the fecal specimen and a modified Sheather's solution is examined for *C. parvum* oocysts on a slide at magnification  $\times 400$  (Figure 2). The LI test is a rapid assay developed by BioX Diagnostics, Belgium for the detection of *C. parvum* antigen in calf feces, using test strips that are immersed for a few minutes in a mixture of the test feces and the reagent provided. Results of the evaluation, using 199 fecal specimens from calves 7- to 21-days-old with and without diarrhea, revealed good agreement between the two diagnostic techniques. Agreement between each of the two tests and the gold standard was also good. The sensitivity and specificity of the sucrose wet

**Figure 2: Sucrose wet mount procedure**

- Prepare a modified Sheather's sucrose solution (specific gravity 1.32) by dissolving 2 kg of granulated white sugar in 1000 ml of tap water, using gentle heat.
- Mix 5 g fresh feces with 3 ml sucrose solution.
- Place 1 drop of the suspension on a slide
- Position a coverslip over the drop, using gentle pressure to eliminate air bubbles.
- Examine using  $400\times$  magnification.
- Oocysts appear as refractile, pink-tinged spherical structures approx  $4\times 5\ \mu\text{m}$  in diameter.



mount test were 88.6% and 93.8%, respectively, and 78.3% and 93.3% for the LI test. Both tests were inexpensive: the consumables required per sample tested, cost US\$ 0.36 (CAD\$ 0.41) for the wet mount test and approximately US\$ 3.00 (CAD\$ 3.46) for the LI test. Both tests were also easy to use; however, LI was faster and simpler to perform than the sucrose wet mount test and, generally, less complicated. The conclusion was that both tests provided inexpensive, simple, and reliable methods for the detection of *C. parvum* shedding in young calves and could readily be used in practice.

### Control of *C. parvum* using halofuginone lactate

Most licensed chemotherapeutic agents effective against coccidial parasites show little or no activity against *Cryptosporidium* at recommended doses. In addition, trials of most chemoprophylactic agents (eg, metronidazole, monensin, decoquinat, lasalocid, and amprolium) have also demonstrated limited efficacy, toxicity at effective doses, or prohibitively high costs due to the requirement for very large doses.<sup>8</sup> The resistance of the parasite to these medications is thought to be due, in part, to its development in the sheltered environment of a parasitophorous vacuole, just beneath the surface of the intestinal epithelial cell.

Trials have shown that halofuginone lactate and paromomycin, an aminoglycoside, can be successfully used as prophylactic medications to control the intensity and severity of infection in calves.<sup>9-11</sup> Both medications appear to delay the onset of oocyst shedding and diarrhea. In one study, naturally infected neonatal dairy calves treated with halofuginone lactate at 100  $\mu\text{g}/\text{kg}$  daily for the first 7 days after birth demonstrated the same overall risk of diarrhea as untreated controls; however, halofuginone-treated calves had a decreased risk of shedding *C. parvum* oocysts and, in

this group, oocyst shedding was also significantly delayed.<sup>11</sup> In another trial, naturally infected suckling calves received halofuginone at 120 µg/kg for 7 consecutive days and 44% fewer of the treated calves developed diarrhea than untreated controls.<sup>10</sup> A reduced risk of diarrhea was also recorded in a trial using doses of 120 µg/kg in experimentally infected dairy calves.<sup>9</sup> Moreover, this study reported reduced severity of clinical signs in treated animals. Lallemand and coworkers reported no effects of halofuginone hydrobromide administered at 100 mg/kg on the risk of diarrhea or oocyst shedding in calves;<sup>12</sup> however, there is limited information on the bioavailability of this form of halofuginone in calves. Halofuginone lactate (Halocur™-Intervet, Whitby, Ontario) is available in Canada as a solution of halofuginone lactate 0.5 mg/mL. This medication is not licensed in Canada for routine use, but may be obtained by Canadian farmers through application with the issue of an Emergency Drug Release certificate.

Generally, cryptosporidiosis is a self-limiting disease in otherwise healthy calves. Treatment of calves is usually supportive and, in most cases, clinical signs and shedding of oocysts resolve within 2 weeks. Because the parasite is resistant to most medications, control of *C. parvum* relies primarily on prevention through maintaining good management practices. However, in outbreak situations, halofuginone lactate appears to be a useful tool for controlling *Cryptosporidium* infections.

### Factors associated with *C. parvum* shedding

Various studies have been carried out to determine whether particular management factors and farm characteristics may influence the risk of infection with *C. parvum* in young dairy calves. Research conducted in the United States with 7,369 calves up to weaning age on 1,103 farms in 28 states found that infected calves were more likely to be found on farms using multi-cow maternity facilities than on those using single-cow facilities.<sup>13</sup> Calves were also more likely to be infected on farms with > 100 milking cows. In another study, which sampled calves up to 3-months-old on 11 northeastern US dairy farms, changing bedding in the calf housing area > 12 times per year and increased contact between calves were associated with an increased risk of shedding *Cryptosporidium* oocysts.<sup>14</sup> Research on 2,943 dairy cattle of various ages on 109 farms in New York State demonstrated that feeding milk replacer and the presence of fan or exhaust ventilation in calf-rearing areas were associated with a decreased risk of shedding *C. parvum* oocysts.<sup>15</sup>

### Factors associated with calf-level risk of *C. parvum* shedding

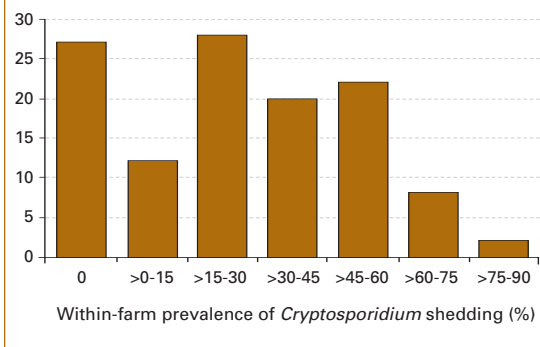
In a longitudinal observational study conducted at the Ontario Veterinary College, 11 dairy farms in southwestern Ontario were visited weekly during the summer of 2003 and the winter of 2004. All farms had a history of calf diarrhea. Weekly fecal samples were collected for the first 4

weeks of life from each of 1,045 calves and producers were asked to complete a questionnaire on hypothesized calf-level management practices for each calf enrolled in the study. Fecal samples were assigned a score to reflect the presence or absence of diarrhea. Samples were also examined for *C. parvum* oocysts and were used in testing a subset of calves for *Salmonella*, enterotoxigenic *Escherichia coli* (ETEC), bovine rotavirus, and bovine coronavirus. In addition, one blood sample taken from each calf in the first week of life was used to assess passive transfer of maternal antibodies and to test for bovine viral diarrhea virus (BVDV) by PCR. Statistical regression models were employed to examine these data for associations between various factors and the calf-level risk of shedding *C. parvum* oocysts, and between those factors and the calf-level risk of diarrhea. Results revealed that 78% of the calves shed *C. parvum* oocysts and 73% were diarrheic in the first month of life. Of all of the enteropathogens for which the calves were tested, only *C. parvum* was associated with diarrhea when controlling for all other significant factors; calves shedding *C. parvum* oocysts had 5.3 (95% CI, 4.4–6.4) times the odds of diarrhea than non-shedding calves. Furthermore, calves shedding higher numbers of oocysts were more likely to develop diarrhea than those shedding lower numbers. Leaving calves with their dams for more than an hour after birth appeared to significantly increase the risk of calf diarrhea and calves born in the summer had a significantly higher risk of diarrhea than winter-born calves. Significant predictors of an increased calf-level risk of *C. parvum* shedding included birth of calves in the summer rather than winter and birth of calves in individual as opposed to multi-cow calving pens. However, to a large extent, many of the factors investigated represent management practices to which calves within individual farms are uniformly exposed and the risk of shedding *C. parvum* is also highly correlated within farms. In light of these facts and because the small number of farms was not ideal for investigating factors that are essentially uniform within herds, a herd-level study was conducted as a follow-up to the calf-level investigation.

### Factors associated with herd-level risk of *C. parvum* shedding

Investigation of risk factors for the herd-level prevalence of *C. parvum* shedding in calves was carried out from May to October 2004. In this study, a single fecal sample was collected from each of 1,089 calves, 7- to 28-days-old, from 119 dairy herds in southern Ontario, with a maximum of 15 calves sampled per herd. Fecal specimens were examined for *C. parvum* oocysts and each producer was asked to complete a questionnaire on hypothesized farm-level risk factors for *C. parvum* shedding. Results indicated that 30% of the calves were shedding *C. parvum* and 77% of the 119 herds were found to contain at least one shedding calf. Within-herd prevalence of *C. parvum* ranged from 0 to 80% (Figure 3). Regression analysis revealed that the use of calf-

**Figure 3: Within-herd prevalence of *Cryptosporidium parvum* shedding in calves 7 to 28 days old on 119 dairy herds in Southern Ontario.** Study farms included some that were experiencing a high incidence of neonatal diarrhea at the time the work was carried out, as well as others with no reported diarrhea problems. Numbers of calves sampled per farm ranged from 1 to 13 (median 10).



scour prophylaxis in cows and in calves, as well as the feeding of milk replacer to calves in the first week of life, were significantly associated with an increased within-herd prevalence of the parasite. On the other hand, the presence of concrete flooring in calf-housing areas and the use of soap or detergent when washing calf-feeding utensils were significantly associated with decreased within-herd prevalence of shedding.

While some of the management factors or farm characteristics found to be significantly associated with the risk of *C. parvum* shedding in various studies may be true protective or causative factors, it is possible that others may in fact be merely predictors of the risk of shedding without having any causative association with *C. parvum* infection. Therefore, results of risk factor studies should be interpreted with caution. Nevertheless, such investigations often provide useful information on probable relationships between *C. parvum* in dairy herds and prevalent management practices, some of which may be modified to assist in controlling this parasite.

### Zoonotic potential of *C. parvum* in calves

The molecular characterization of isolates of *Cryptosporidium* at various gene loci has greatly enhanced epidemiological knowledge and understanding of this parasite. In fact, it has led to a re-naming of some species of *Cryptosporidium* and the discovery of genetically distinct sub-genotypes (subtypes) within previously recognized species. Most notably, the morphologically indistinguishable *C. parvum* genotypes 1 and 2 are now known to be two separate species of the parasite: *C. hominis* and *C. parvum*, respectively.<sup>16</sup> *C.*

*hominis* is principally a parasite of humans and has only rarely been identified in natural infections of other hosts. Some subtypes of *C. parvum* have been reported only in cattle, while others have been identified in humans as well as other mammalian hosts and, therefore, appear to be zoonotic.<sup>17</sup> Using this information, it has become possible to identify possible sources of infection in sporadic as well as outbreak cases of human cryptosporidiosis. Genetic characterization of *Cryptosporidium* isolates from humans indicates that the majority of isolates originating in the United Kingdom and mainland Europe are various subtypes of *C. parvum*, some of which are zoonotic,<sup>17,18</sup> whereas most isolates from North America and elsewhere are identified as various subtypes of *C. hominis*.<sup>6</sup> This would suggest that most human infections in North America originate from human sources.

In Ontario, however, genotyping of 44 *Cryptosporidium* isolates from dairy calves < 1-month-old, as well as 11 isolates from sporadic human cases of cryptosporidiosis, suggested that cattle and other ruminants may be a source of sporadic human infections.<sup>19</sup> In this study, all calf isolates were identified as zoonotic subtypes of *C. parvum*. In the 11 human isolates, 4 were *C. hominis*, 6 were *C. parvum*, and 1 was *Cryptosporidium* cervine genotype, a genotype possibly of wildlife or livestock animal origin that is rarely reported in humans. For the 6 human *C. parvum* isolates, 3 were identified as the same subtype as the calf isolates, implying a possible zoonotic origin. Therefore, in treating cases of calf cryptosporidiosis, the potential for infection transmission to people, either by direct contact with contaminated calf feces or by indirect transmission of oocysts via food or drinking water, is an important issue. Appropriate hygienic measures should be taken to minimize this risk.

### Summary

In general, attention to good calf-management practices, in conjunction with high standards of hygiene when treating diarrheic calves and the strategic use of halofuginone lactate in herds with a high incidence of cryptosporidiosis, can be used to minimize the risk of calf-to-calf and calf-to-human transmission of infection.

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## Abstracts of Interest

### Effect of halofuginone lactate on the occurrence of *Cryptosporidium parvum* and growth of neonatal dairy calves.

JARVIE BD, TROTZ-WILLIAMS LA, MCKNIGHT DR, LESLIE KE, WALLACE MM, TODD CG, SHARPE PH, PEREGRINE AS. GUELPH ONTARIO

Thirty-one Holstein bull calves were purchased at birth from 3 dairy farms in Eastern Ontario. Each calf was assigned at random to oral treatment with either 5 mg of halofuginone lactate in 10.0 mL of aqueous carrier solution (Halocur, base comprised 10 mg of benzoic acid, 100 mg of lactic acid, and 0.3 mg of tartrazine) or 10 mL of placebo (Halocur base minus the active ingredient, halofuginone lactate) administered 15 to 30 min after morning milk feeding for the first 7 d of life. Intakes of milk, calf starter, and water, and fecal consistency score were recorded daily for 56 d. Calf weights were recorded weekly for 56 d. Fecal samples were taken from all calves at approximately 2, 7, 14, 21, and 28 d of age for isolation of *Cryptosporidium parvum* oocysts. Logistic and linear regression analyses were used to assess the effect of treatment on the incidence of diarrhea and *C. parvum* infection status. The odds of *C. parvum* shedding among calves in the halofuginone lactate-treated group was 70% lower than the odds of shedding among calves in the placebo group. In calves treated with halofuginone lactate, no oocyst shedding occurred until 2 wk of age, whereas 12.5% of calves in the placebo group began shedding oocysts during wk 1. From all ages of placebo-treated calves, 31 of 73 samples (42.5%) were positive for *C. parvum*, whereas only 15 of 67 samples (22.4%) from all ages of halofuginone lactate-treated calves tested positive. The largest number of *C. parvum*-positive samples occurred in the third week of life. There was a significant delay of 3.1 d in the incidence of diarrhea among calves treated with halofuginone lactate. Intake of milk and starter, body weight gains, and age at weaning were not significantly different between treatment groups.

*J Dairy Sci* 2005;88(5):1801-1806.

### Natural transmission of *Cryptosporidium parvum* between dams and calves on a dairy farm

FAUBERT GM, LITVINSKY Y. STE. ANNE-DE-BELLEVUE, QUEBEC

The transmission of *Cryptosporidium parvum* between dams and their respective calves was studied. For this purpose, fecal specimens taken from the rectum of preparturient, parturient, and postparturient dams were analyzed for *C. parvum* oocysts. Fecal specimens were taken from the newborn calf 4 hr after birth. Because the environment can be a source of contamination to the animals, specimens taken from inside and outside the barn were analyzed. The sucrose concentration method together with the Zielh-Nielsen acid-fast staining method were employed to increase the chances of oocyst detection. We are reporting that at parturition, the dams shed a higher number of oocysts by comparison to the preparturient and postparturient periods. Neonates acquire the infection at birth mainly because of the high number of oocysts shed by the dams at parturition. The management practice of moving calves 4 hr after birth away from the dams and the barn reduces the number of clinical cases because they are no

longer in contact with an environment that is highly contaminated. We hypothesize that the increase in the number of oocysts sheds by dams at parturition might be due to a depression of the T helper 1-type of immune response during that period.

*J Parasitol* 2000;86(3):495-500.

### **Survival of *Cryptosporidium parvum* oocysts in calf housing facilities in the New York City watersheds**

COLLICK AS, FOGARTY EA, ZIEGLER PE, WALTER MT, BOWMAN DD, STEENHUIS TS.

Pathogen contamination of the public drinking water supply in the New York City watersheds is a serious concern. New York City's Watershed Agriculture Program is working with dairy farms in the watersheds to implement management practices that will reduce the risk of pathogens contaminating the water supply. Solar calf housing (SCH) was suggested as a best management practice (BMP) to control *Cryptosporidium parvum*, a common protozoan parasite that causes disease in humans. This BMP targets young calves because they are the primary source of *C. parvum* in dairy herds. The objective of this project was to assess and compare the survivability of *C. parvum* in SCH and in conventional calf housing (CCH), usually located in the main barn. *C. parvum* oocysts were secured in sentinel chambers and placed in SCH and CCH bedding on four farms. The chambers were in thermal, chemical, and moisture equilibrium with their microenvironments. An oocyst-filled control chamber, sealed from its surroundings, was placed near each chamber. Chambers and controls were sampled after 4, 6, and 8 wk. Oocyst viability in the chambers decreased to less than 10% in warm months and between 15 and 30% in the winter months. The viability of the control oocysts was similar to the chambers during warm months and generally higher during winter months. There was no significant ( $P > 0.05$ ) difference in the viability decrease between SCH and CCH. Although oocyst viability was similar in both types of calf housing, SCH allow contaminated calf manure to be isolated from the main barn manure and potentially managed differently and in a way to decrease the number of viable oocysts entering the environment during field spreading.

*J Environ Qual.* 2006 Mar 1;35(2):680-7.

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*Drs. Trotz-Williams, Peregrine and Leslie have stated that they have no disclosures to announce in relation to the contents of this issue.*

## **Upcoming Meetings**

13-18 May 2007

### **II International *Giardia* and *Cryptosporidium* Conference**

Centro Cultural Universitario,  
Universidad Michoacana de san Nicolás de Hidalgo,  
Morelia-Michoacán, México

CONTACT: Conference secretariat

Website: <http://cinvestav.mx/giardiacrypto/>

E-mail: [giardiacrypto07@cinvestav.mx](mailto:giardiacrypto07@cinvestav.mx)

20-22 June 2007

### **International Calf Management Conference**

Steinkjer, Norway

CONTACT: Hanne Solheim Hansen

(e-mail [Hanne.Hansen@hint.no](mailto:Hanne.Hansen@hint.no))

Or Aud Sakshaug

(e-mail [Aud.sakshaug@hint.no](mailto:Aud.sakshaug@hint.no))

Website: <http://www.hint.no/calfmanagement/>

7-11 August 2007

### **Society for Theriogenology/ American College of Theriogenologists (SFT/ACT)**

#### **Annual Conference and Symposium**

Monterey, California

CONTACT: [www.therio.org](http://www.therio.org)

20-22 September 2007

### **American Association of Bovine Practitioners 40<sup>th</sup> Annual Convention**

Vancouver, British Columbia

CONTACT: AABP

Website: <http://www.aabp.org/>

26-30 September 2007

### **International Veterinary Emergency and Critical Care Symposium**

New Orleans, Louisiana

CONTACT: [www.veccs.org](http://www.veccs.org)

18-21 October 2007

### **American College of Veterinary Surgeons Symposium**

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