Number of *Cryptosporidium parvum* oocysts or *Giardia* spp cysts shed by dairy calves after natural infection

Daryl V. Nydam, DVM; Susan E. Wade, PhD; Stephanie L. Schaal, BA; Hussni O. Mohammed, BVSc, MPVM, PhD

**Objective**—To determine the total number of *Cryptosporidium parvum* oocysts and *Giardia* spp cysts shed by dairy calves during the period when they are most at risk after natural infection.

**Animals**—478 calves naturally infected with *C parvum* and 1,016 calves naturally infected with *Giardia* spp.

**Procedure**—Oocysts or cysts were enumerated from fecal specimens. Distribution of number of oocysts or cysts versus age was used to determine the best fitting mathematical function. Number of oocysts or cysts per gram of feces for a given duration of shedding was computed by determining the area under the curve. Total number of oocysts or cysts was calculated by taking the product of the resultant and the expected mass of feces.

**Results**—Intensity of *C parvum* oocyst shedding was best described by a second-order polynomial function. Shedding increased from 4 days of age, peaked at day 12, and then decreased. An infected 6-day-old calf would produce $3.89 \times 10^5$ oocysts until 12 days old. Pattern of shedding of *Giardia* spp cysts was best described by exponential functions. Intensity of shedding increased from 4 days of age, peaked at day 14, and then decreased. An infected calf would produce $3.8 \times 10^4$ cysts from day 50 until day 56.

**Conclusions and Clinical Relevance**—The large number of oocysts and cysts shed indicates that shedding by dairy cattle poses a risk for susceptible calves and people. Estimates reported here may be useful to aid in designing cost-effective strategies to manage this risk. (Am J Vet Res 2001;62:1612–1615)

*Cryptosporidium parvum* and *Giardia duodenalis* (also known as *G intestinalis*) are zoonotic protozoan parasites that have recently come to the attention of livestock producers and public health officials. *Cryptosporidium parvum* has a wide host range with infections being reported in humans and at least 79 species of domestic animals and wildlife, including cattle. *Giardia* spp also has a wide host range. Humans, dogs, cats, and some wildlife are the principal reservoirs, although *Giardia* infections also have been reported in cattle, pigs, sheep, and other animals. They can cause self-limiting gastroenteritis in immunocompetent hosts and life-threatening disease in immature or immunocompromised hosts.

*Cryptosporidium parvum* is transmitted in susceptible populations via ingestion of infective oocysts in feces or water. The role of fomites in the risk of transmission of the disease also has been reported. Similar routes of transmission have been postulated for *Giardia* spp. Although *C parvum* is accepted as a zoonotic organism, the status of *Giardia* spp is less clearly defined, with some investigators reporting that transmission of infections between humans and other animals is infrequent, whereas others classify giardiosis as a zoonothropic disease. By virtue of the fact that they live in watersheds, infected dairy animals are incriminated as sources of water contamination.

*Cryptosporidium parvum* often is implicated as being transmitted from ruminants to humans through agricultural effluent, although there is little conclusive evidence to document the link between infection in dairy cattle and humans via agricultural run-off that contaminates municipal water supplies. Two cycles persist in nature, 1 anthroponotic and 1 zoonotic, as characterized by genotyping. Of 12 outbreaks that have reported genotype data, 4 had genotypes consistent with the zoonotic cycle. Thus, an infected animal that is shedding organisms is a potential source of infection to other animals on farms and to the water- and food-consuming public. Lack of approved and consistently effective chemotherapeutics make prevention the key for managing *C parvum*. Although there are recognized treatments for humans and other animals with giardiosis, it is often most economically efficient to prevent infection.

Fayer et al reported the shedding pattern of 26 calves experimentally infected with *C parvum* oocysts, and O’Handley et al described the pattern of shedding for *Giardia* spp and *C parvum* in 20 naturally infected calves from a single dairy farm. Data regarding the total number of oocysts or cysts shed by infected animals from a diverse population under natural conditions is lacking. Such data are necessary to enable investigators to understand the dynamics of shedding for these 2 organisms in animal populations so that cost-effective strategies can be designed to control and manage the risk they pose. This information will be beneficial to dairy producers, veterinarians, public health officials, and risk assessors. Therefore, objectives of the study reported here were to estimate the number of *C parvum* oocysts and *Giardia* spp cysts shed by dairy calves dur-
ing the period when they were most at risk and to estimate these numbers for various durations of shedding and various quantities of feces excreted.

**Materials and Methods**

**Study Population**—The target population of animals was young dairy cattle in the Catskill-Delaware portion of the New York City Watershed in southeastern New York. The study population used in the investigation was recruited from the target population, using a longitudinal study that included cattle on 37 dairy farms. Inclusion criteria for selection of the study population for the *C parvum* model were that the calves had to be shedding oocysts and had to be between 4 and 21 days old. To be included in the *Giardia* spp model, calves or heifers had to be shedding cysts and had to be between 4 and 180 days old. These age ranges represent the period when calves are most at risk for shedding, as determined on the basis of a cross-sectional study performed in this population.

**Sample collection and diagnostic procedures**—Fecal samples were collected per rectum from each calf in the study, using latex gloves. Age of each calf was recorded at time of fecal collection. Color and consistency of feces were scored at the time of collection. Samples were refrigerated and transported to our laboratory for examination. Laboratory analysis of fecal samples was conducted, using a standard quantitative centrifugation-concentration flotation technique with bright-field and phase microscopy. Zinc oxide (specific gravity, 1.18) was used as the flotation medium for *Giardia* spp, and sugar solutions (specific gravity, 1.33 and 1.20) were used as flotation media for *C parvum*. One gram of each fecal specimen was added to 15 ml of each of the 3 flotation solutions described. The sugar solution with specific gravity of 1.20 was used to quantify number of oocysts or cysts per gram of each fecal specimen. Samples from calves < 1 month old were cleaned with a solution of polyoxyethylene sorbitan monopalmitate before addition of flotation medium.

**Data analysis**—A systematic conceptual approach was used to estimate the number of oocysts or cysts shed during a specified period. First, the mathematic relationship between the number of oocysts or cysts per gram of feces that was shed by calves of specific ages was described. Second, the function that described the pattern of shedding was integrated to determine the number of oocysts or cysts per gram of feces. Third, the total number of oocysts or cysts excreted for a specific shedding period, adjusted for total amount of feces, was computed.

To describe the mathematic relationship, distribution of the number of oocysts or cysts per gram of feces by age of each animal was plotted. Visual inspection of the distribution was used to develop hypotheses as to the best-fit model. Models were considered and tested. Analyses were performed, using a computer software program. Decision criteria for goodness of fit of the model were based on the significance of the regression coefficients and the coefficient of determination. Appropriate transformations were considered and tested. Analyses were performed, using the following general regression model:

\[ \text{Oocysts or cysts per gram of feces} = f(\text{age}) \]

All variables of the equation were evaluated at a value of \( \alpha < 0.05 \).

To determine the number of oocysts or cysts shed per gram of feces for a specified period, the area under the curve, as described by the best-fitting function, was calculated by numeric integration. The lower limit of integration was the age at onset of shedding, and the upper limit of integration was the age at which shedding terminates. For example, the total concentration of oocysts shed between 5 and 10 days of age would be estimated by use of the following equation:

\[ \left( \frac{1}{x} \right) \int f(\text{age}) \, dx \]

To compute the total number of oocysts or cysts shed, the resulting concentration from the integration was multiplied by the expected mass of feces excreted during the corresponding period, as determined on the basis of a report of the amount of fecal output measured directly from healthy calves and calves with diarrhea.

**Results**

*Cryptosporidium parvum*—A total of 478 calves met the inclusion criteria for the *C parvum* study population. Fecal oocyst counts in calves 4 to 21 days old varied from 1 to 847,152 oocysts/g of feces. Arithmetic mean for the calves in this age range was 90,867 oocysts/g of feces.

The best-fit relationship between the number of oocysts per gram of feces and age of each calf was calculated (Fig 1). This relationship was described by the following second-order polynomial function:

\[ \text{No. of oocysts shed per gram of feces} = (32,105.2 \times \text{age}) – \left(1,398.6 \times \text{age}^2\right) \]

The function reveals that the number of oocysts shed increased with the age of the calves until approximately day 11 or 12, after which it decreased.

To determine the number of oocysts shed per gram of feces, area under the curve was calculated by use of polynomial integration. For example, shedding that commences on day 6 and lasts until day 12 will yield 1,028,786 oocysts/g of feces (Fig 1). Total number of oocysts can then be computed by multiplying the concentration that resulted from the integration by the likely mass of feces excreted for the period. For example, if a calf with diarrhea produces 5.4 kg of feces/day, then that calf could shed 3.89 × 10^8 oocysts into the environment for the period between 6 and 12 days of age.

*Giardia spp*—A total of 1,016 calves met the inclusion criteria for the *Giardia* spp study population. Distribution of the data dictated that 2 functions best described the shedding pattern for cysts. Cyst counts of calves that were 4 to 14 days old varied from 1 to 175,824 cysts/g of feces. The arithmetic mean was 16,084 cysts/g of feces for the period of 4 to 14 days. For calves that were 15 days to 6 months old, cyst counts varied from 1 to 197,120 cysts/g of feces. For this age range, mean cyst count was 7,431 cysts/g of feces.

Figure 1—Graph of the function that describes the relationship between age of a calf and the number of *Cryptosporidium parvum* oocysts shed by dairy calves that are 4 to 21 days old. Total number of oocysts per gram of feces shed by a calf between 6 and 12 days of age is indicated (shaded area).
understand the economic effect of needed on oocyst output by livestock to more fully environment for the period between 50 and 56 days of age.

One investigator recently indicated that data are used by researchers or the amount of feces expected to be excreted, a large number of oocysts or cysts can be shed into the environment, which places susceptible domestic animals or humans at risk. The number of C. parvum oocysts predicted by the model reported here is particularly staggering if one considers that the median infective dose for humans without IgG antibodies to C. parvum is 132 oocysts, whereas the median infective dose for seropositive humans is 1,880 oocysts.

Clinical disease (ie, diarrhea in calves) becomes apparent when the loading dose and frequency of exposure override the host’s immunity. Factors that affect host immunity (eg, failure of passive transfer, concurrent infection, immunosuppressive drugs) can result in the dose that causes disease or shedding to differ. Thus, this model only directly accounts for the variability in shedding attributable to age. A multivari-able model that accounts for other biological and management factors could provide a more accurate assess-
tween 40. Sigma Chemical Co, St. Louis, Mo.

SAS, version 7.0, SAS Institute Inc, Cary, NC.


References


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