Environmental Load of Cryptosporidium parvum Oocysts from Cattle Manure in Feedlots from the Central and Western United States


ABSTRACT

The first step in assessing the risk of water contamination by Cryptosporidium parvum oocysts from feedlot cattle (Bos taurus) production systems is to quantify the number of C. parvum oocysts present in the fecal material deposited by feedlot cattle. Our primary objective for this project was to estimate the daily environmental load of C. parvum oocysts in fecal material deposited by feedlot cattle from across the central and western USA. Our secondary goal was to genotype isolates of C. parvum from feedlot cattle to help facilitate proper identification of mammalian sources of waterborne C. parvum. Based on 5274 fecal samples from 22 feedlots in seven states (California, Washington, Colorado, Oklahoma, Texas, Nebraska, and South Dakota), we estimated a point prevalence of C. parvum of 0.99 to 1.08% in fecal material from feedlot pens from a wide range of climates and a diverse range of feedlot management systems. On average, fresh fecal material from throughout feedlot systems (recent arrivals to nearing slaughter) contained about 1.3 to 3.6 oocysts/g feces, which roughly translates to about 2.8 × 10³ to 1.4 × 10⁴ oocysts/animal per day.

Cryptosporidium parvum is a ubiquitous waterborne protozoal pathogen, with specific genotypes readily transmitted between livestock and humans (Peng et al., 1997; Spano et al., 1997; Awad-El-Kariem et al., 1998; Okhuysen et al., 1999; Xiao et al., 2004). One of the first steps in designing watershed management plans for minimizing the occurrence of C. parvum in drinking water supplies is to identify major sources of this parasite (Atwill, 1996), followed by the implementation of appropriate management practices to mitigate this prioritized load (Walker et al., 1998; Rosen et al., 2000). Adult cattle are often considered to be potential sources of environmental contamination for C. parvum, but there is disagreement over the relative importance of adult cattle in loading watersheds with significant quantities of C. parvum oocysts. In particular, the reported prevalence of fecal shedding of C. parvum for adult beef and dairy cattle ranges from lows of 0 to 10% (Villacorta et al., 1991; Atwill et al., 1998a, 1998b, 2003; Fayer et al., 2000; Hoar et al., 2000; Sischo et al., 2000; Wade et al., 2000; Huetink et al., 2001; Atwill and Pereira, 2003) to highs of 20 to 70% in groups of clinically healthy adult cattle (Lorenzo et al., 1993; Scott et al., 1995; Quilez et al., 1996; Grazyck et al., 2000).

Some of the variation in the observed prevalence of fecal shedding can be explained by use of diagnostic assays of differing sensitivity and specificity, but much of the variation appears to be the result of differences in populations of cattle studied, including beef vs. dairy operation type, age distribution, and variation in management practices (Atwill et al., 2003). With respect to both beef and dairy cattle, the rate of environmental loading of C. parvum per animal unit or mass of fecal material is conditional on the underlying age distribution of the herd (e.g., Atwill et al., 1998a,b, 2003; Kuczensa and Shelton, 1999; Fayer et al., 2000; Grazyck et al., 2000; Huetnik et al., 2001; Uga et al., 2000; Wade et al., 2000; Santin et al., 2004); the prevalence of infection is associated with factors including calf management, farm hygiene, and the duration of the calving season (Maldonado-Camargo et al., 1998; Atwill et al., 1999; Mohammed et al., 1999; Hoar et al., 2001).

Presently, little is known about C. parvum loading rates from cattle in commercial feedlots. Large numbers of cattle are concentrated in feedlots across the USA, leading to high volume point sources of fecal waste. To properly assess the risk of water contamination with C. parvum from such operations, the first step is to generate a valid estimate of the number of C. parvum oocysts present in the fecal material deposited by feedlot cattle. Whether designing a waste management system or calculating a total maximum daily load for C. parvum on a watershed, reliable estimates of quantitative loading of C. parvum from biological sources are needed if management objectives for reducing this waterborne hazard are to be achieved. Therefore, the primary objective for this project was to quantify the environmental load of C. parvum oocysts in fecal material deposited by feedlot cattle from across the central and western USA during a 22-mo period. A secondary objective of this project was to genotype isolates of C. parvum from feedlot cattle to help facilitate proper identification of mammalian sources of waterborne C. parvum.

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Abbreviations: DFA, direct immunofluorescent microscopy; IMS-DFA, immunomagnetic bead separation followed by direct immunofluorescent microscopy.
**MATERIALS AND METHODS**

**Feedlot Selection and Fecal Sampling**

Veterinary Medical Officers of the USDA, Animal and Plant Health Inspection Services, Veterinary Services (Elmi, Brewer, Riggs, and Carpenter), and academic faculty (Epperson and Smith) identified one to four cattle feedlots in their respective states for cooperation on this project (Table 1), for a total of 22 feedlots in 7 states (California, Washington, Colorado, Oklahoma, Texas, Nebraska, and South Dakota). Different feedlots from different states were sampled in sequence so that fecal samples were collected at different climate regimes and from a wide variety of animal husbandry practices both within and across states. For each feedlot, approximately 240 fecal samples were collected from between four and six pens of cattle, with one pen of cattle being recent arrivals to the feedlot, one pen of cattle nearing their harvest date (nutrition primarily forages), one pen of cattle being recent arrivals to the feedlot, one pen of cattle nearing their harvest date (nutrition primarily forages), one pen of cattle nearing their harvest date (nutrition primarily forages), and one pen of cattle nearing their harvest date (nutrition primarily forages). Presumptive Cryptosporidium andersoni was distinguished from C. parvum by measuring the length and width axes and was considered an incidental finding (data not reported). Presumptive C. parvum isolates were subjected to DNA confirmation as described below.

In addition to DFA for routine screening of fecal samples, immunomagnetic separation of oocysts coupled with direct immunofluorescent microscopy (IMS-DFA) was performed on 10 randomly chosen DFA-negative samples per feedlot to quantify the prevalence of DFA false-negative samples, if present. This procedure is one of the most sensitive methods for detecting C. parvum oocysts in adult bovine feces, with a probability of 50 and 90% for detecting 0.68 and 2.4 oocysts/g, respectively (Atwill and Pereira, 2003). Briefly, 2 g of feces was resuspended in 40 mL of double distilled sterile water, strained through a folded 2-ply gauze, and centrifuged for 10 min at 1000 x g. The supernatant was discarded, pellet resuspended in 10 mL sterile distilled water, and transferred to Leighton tubes for immunomagnetic separation using the Dynabeads anti-Cryptosporidium assay as described by the manufacturer (Dynal, Lake Success, NY), with oocysts labeled using a fluorescein isothiocyanate-labeled anti-Cryptosporidium monoclonal antibody assay (Waterborne, New Orleans, LA).

**Enumeration of Cryptosporidium parvum Oocysts**

Direct immunofluorescent microscopy (DFA) was used as the primary diagnostic procedure for detecting and enumerating C. parvum oocysts in fecal samples (Pereira et al., 1999). About 5 g of each sample was mixed with 40 mL of deionized water and washed through folded 2-ply gauze. Fecal suspensions were centrifuged at 1000 x g for 10 min, supernatants aspirated, and the residual fecal sediments resuspended 1:1 (v/v) in deionized water to a final volume of 3 to 5 mL. Ten μL of fecal suspension that weighed on average 11.7 mg was smeared onto glass slides and dried overnight for immunofluorescent microscopy. Detection of oocysts was performed using the direct immunofluorescent assay Merifluor Cryptosporidium/Giardia detection kit (Meridian Diagnostic, Cincinnati, OH). The total number of C. parvum oocysts was recorded for each smear at x200 magnification; it was recorded as zero if no oocysts were observed. Cryptosporidium andersoni was distinguished from C. parvum by measuring the length and width axes and was considered an incidental finding (data not reported). Presumptive C. parvum isolates were subjected to DNA confirmation as described below.

**Table 1. Number of fecal samples deposited by cattle from 22 different feedlots in central and western USA that contained detectable levels of Cryptosporidium parvum oocysts, August 2000 to January 2002.**

<table>
<thead>
<tr>
<th>State/month/year of feedlot visit</th>
<th>No. of fecal samples with C. parvum†</th>
<th>No. of oocysts/g feces in positive samples</th>
<th>No. of false negative cattle‡</th>
<th>C. parvum genotype§</th>
</tr>
</thead>
<tbody>
<tr>
<td>California</td>
<td>0/245</td>
<td>203, 203</td>
<td>0/10</td>
<td>Bovine A</td>
</tr>
<tr>
<td>September 2000</td>
<td>0/240</td>
<td>203, 406, 3242, 7702</td>
<td>0/10</td>
<td>ND</td>
</tr>
<tr>
<td>Florida</td>
<td>0/240</td>
<td>203, 203</td>
<td>0/10</td>
<td>ND</td>
</tr>
<tr>
<td>New Mexico</td>
<td>0/240</td>
<td>203, 203</td>
<td>0/10</td>
<td>ND</td>
</tr>
<tr>
<td>South Dakota</td>
<td>0/240</td>
<td>203, 203</td>
<td>0/10</td>
<td>ND</td>
</tr>
<tr>
<td>East Carolina</td>
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<td>Washington</td>
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<td>ND</td>
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<tr>
<td>Total</td>
<td>2/240</td>
<td>203, 203</td>
<td>0/10</td>
<td>ND</td>
</tr>
</tbody>
</table>

† Direct immunofluorescent microscopy (DFA) used as the diagnostic procedure (Pereira et al., 1999).
‡ Ten fecal samples per feedlot that were negative on DFA were reexamined using immunomagnetic separation followed by DFA (Pereira et al., 1999).
§ Based on DNA sequencing and/or restriction fragment length polymorphism of a nested polymerase chain reaction product of the 18S SSU rRNA gene (Xiao et al., 1999). ND = not determined due to failure of PCR amplification.
Estimating Diagnostic Test Sensitivity

Naturally infected dairy calves from a local commercial dairy were the source of bovine genotype A Cryptosporidium parvum oocysts (Xiao et al., 1999). Using an acid fast procedure for oocyst detection (Harp et al., 1996), fecal samples having more than 25 oocysts per ×400 microscope field were washed through a series of 40, 100, 200, and 270 mesh sieves. The resulting suspension was decanted off and centrifuged at 1000 × g for 10 min. Supernatant was discarded and the pellet washed in Tween water (0.01% Tween 80 in deionized water, v/v). Discontinuous sucrose gradient was used to purify Cryptosporidium parvum oocysts from fecal suspensions (Arrowood and Sterling, 1987). The concentration of purified oocysts was determined as the arithmetic mean of six separate counts using a phase contrast hemacytometer (Bright-Line Hemacytometer; Hauser Scientific, Horsham, PA).

The sensitivity of DFA for detecting Cryptosporidium parvum oocysts was evaluated for every other feedlot sample. For this sensitivity evaluation, fecal samples that tested negative for Cryptosporidium parvum were randomly selected from two different pens. Aliquots of 4.5 g of fecal sample were combined with 500 μL of Cryptosporidium parvum oocyst suspension to yield final concentrations of 1000 and 10 000 oocysts/g feces. Four replicates of each concentration from each fecal sample were then subjected to DFA as performed for the project survey, resulting in 176 sensitivity evaluations per feedlot.

Percentage recovery (r) was defined as \( r = x/k \), where \( x \) was the observed number of oocysts counted for each fecal sample using DFA and \( k \) was the total number of oocysts spiked into the sample. The variable, \( k \), was further decomposed into \( eW \), where \( e \) was the number of oocysts/g feces and \( W \) was the mass of fecal material examined per assay (e.g., weight of the smear on a slide) (Atwill et al., 2003).

Negative binomial regression, derived as a Poisson-γ mixture distribution (Hardin and Hilbe, 2001), was selected as the model to characterize the variance of \( S \) and allow for covariates and clustering effects to enter the model. With the observed number of oocysts per assay set as the outcome variable, oocyst concentration was modeled as a continuous variable, number of spiked oocysts per fecal sample set as the exposure variable, weight of smear was a covariate, and cow identification (source of feces) was set as a cluster variable to adjust the standard errors for any intra-cow effects on oocyst recovery.

Test sensitivity for a randomly selected sample with \( e \) oocysts/g feces, \( S(e) \), was then defined as the probability of detecting one or more oocysts per assay given that oocysts were present. This is equivalent to saying that sensitivity is equal to 1 minus the probability that zero oocysts were detected as follows:

\[
S(e) = P(X > 0) = 1 - P(X = 0)
\]

where \( X \) is the number of oocysts observed per assay. For the negative binomial regression model under consideration (Hardin and Hilbe, 2001), the sensitivity equation is as follows:

\[
S(c_i) = 1 - \left( 1 + \alpha c_i W e^{e+b} \right)^{1/c_i} \quad [1]
\]

whereby the mean of the negative binomial is given by \( c_i W e^{e+b} \); the overdispersion is \( 1 + \alpha c_i W e^{e+b} \), \( c_i \), and \( W \), defined above, \( e+b \) the percentage recovery of the diagnostic assay as a function of the covariates, and \( \alpha \) an ancillary parameter for modeling dispersion.

Genotype Determination of Cryptosporidium parvum

Two grams of each sample containing oocysts was mixed with 40 mL of double distilled sterile water and strained through folded 2-ply gauze. The suspension was centrifuged for 10 min at 1000 × g, supernatant removed, and the pellet resuspended in double distilled sterile water to a final volume of 10 mL. Oocysts were purified by immunomagnetic separation using the same protocol as described above, except that the bead-oocyst complex was dissociated by adding 100 μL of 0.1 M HCl. Purified oocysts were subjected to three freeze-thaw cycles (−76°C and +80°C) and then incubated overnight with 1 mg/mL of proteinase K (Life Technologies, Rockville, MD) at 65°C. Oocyst DNA was extracted by Phenol-Chloroform-Isomyl alcohol (USB, Cleveland, OH) or by using QiAmp DNA Mini isolate columns (Qiagen, Valencia, CA).

Amplification of the 18S SSU rRNA gene locus was performed according to the methodology described by Xiao et al. (1999), except that 3 mM MgCl2 was used for both primary and secondary PCR reaction. Expected base pair length for primary and nested PCR was 1325 bp for the outer primers and 831 to 834 bp for the internal nested primers. The PCR products were separated on 1.0% agarose gel and visualized with ethidium bromide. The nested PCR fragment was sequenced in both directions (forward and reverse sequences) using an ABI 3730 Capillary Electrophoresis Genetic Analyzer (Applied Biosystems, Foster City, CA). A positive control was obtained from an infected dairy calf from Pixley, CA; the negative control was sterile water.

Multiple alignment of the DNA reverse and forward sequences was done with the Vector NTI Advance package (InforMax, Frederick, MD). The aligned sequence was compared with other sequences present in GenBank database and to the sequence obtained from Cryptosporidium parvum isolated from an infected dairy calf in California.

RESULTS AND DISCUSSION

Quantitative estimates of the environmental load of Cryptosporidium parvum in bovine fecal material require both the prevalence and intensity of Cryptosporidium parvum oocysts/g feces be generated (Hoar et al., 2000; Atwill et al., 2003). With respect to the prevalence of Cryptosporidium parvum in fecal material from feedlot pens, 5274 fresh fecal samples were collected off the pen floor from 22 feedlots from seven different states beginning 10 Aug. 2000 and ending 3 Jan. 2002 (Table 1). Among these 5274 samples, 9 (0.17%) were found to contain detectable levels of oocysts as determined by DFA (Table 1). This low apparent prevalence of Cryptosporidium parvum in fecal samples collected off the ground is similar to a previous study by Hoar et al. (1999) in which 1/558 (0.18%) fecal pats collected from 25 different cow-calf herds throughout California had detectable levels of oocysts. Interestingly, this earlier study found a sixfold higher prevalence of Cryptosporidium parvum in fecal samples collected per rectum from the adult cattle (1.1%) compared with the very low prevalence of Cryptosporidium parvum (0.18%) in fecal pats collected off the ground from the same herds on the same day. This suggests that once fecal material is deposited in the environment, Cryptosporidium parvum oocysts are subjected to one or more environmental stressors (e.g., thermal) that function to reduce the effective environmental load of this protozoal parasite (Walker et al., 2001). For example, we have shown that seasonal temperature fluctuations common to California’s agricultural regions induce rapid inactivation via premature excystation of Cryptosporidium parvum oocysts once the daily maximum air temperature
exceeds 25°C (Li et al., 2005). It may be possible that similar processes were occurring within the accumulated fecal load on feedlot pens during spring through fall, resulting in the very low apparent prevalence of *C. parvum*.

To better interpret the validity of this low apparent prevalence of *C. parvum* among fecal pats deposited by central and western U.S. feedlot cattle, the sensitivity of DFA for detecting oocysts was evaluated across the duration of the project. Based on 176 sensitivity evaluations and using a negative binomial regression model (Hardin and Hilbe, 2001), the percentage recovery for DFA as practiced in our laboratory was 42.7% (95% CI, 40.0–45.6%) for a mean smear weight of 11.7 mg. Inserting these values into Eq. [1] to estimate $S(c)$, the concentration of oocysts detected with a 90% probability ($DT_{90}$) was 472 oocysts/g feces and the $DT_{50}$ was 140 oocysts/g feces (Fig. 1). Therefore, fecal samples containing in excess of 400 oocysts/g were reliably detected with our DFA assay, while samples with <100 oocysts/g were likely missed by DFA (false negatives).

Given our previous work on *C. parvum* infections in beef cattle (Atwill et al., 1999, 2003; Hoar et al., 2001), we anticipate that infected cattle in feedlots might shed very low concentrations of oocysts, making detection difficult. To estimate the number of false negatives in our data either due to infected cattle shedding very low concentrations of oocysts, making detection difficult. To estimate the number of false negatives in our data either due to infected cattle shedding very low concentrations of oocysts (Atwill et al., 2003) and/or rapid environmental reductions of *C. parvum* once fecal material was excreted (Hoar et al., 1999), 10 randomly chosen DFA-negative fecal samples from each feedlot ($n = 219$) were screened for *C. parvum* using IMS-DFA (Pereira et al., 1999; Atwill and Pereira, 2003). To our knowledge this assay is the most sensitive method published to date for detecting *C. parvum* oocysts in adult bovine fecal material. The $DT_{90}$ was 2.4 oocysts/g feces, $DT_{50}$ was 0.68 oocysts/g feces, and there was a 64% probability of detecting 1 oocyst/g feces (Fig. 1). Only 2 out of 219 (0.91%) DFA-negative fecal samples contained detectable levels of oocysts using this highly sensitive assay, suggesting a low false negative proportion in our data (Table 1). Nevertheless, future studies of *C. parvum* shedding among feedlot cattle may benefit from using diagnostic assays with a higher sensitivity than DFA alone, given this ~1% false negative proportion.

Using these diagnostic results and the sensitivity function, $S(c)$, for DFA and IMS-DFA (as shown in Fig. 1), we estimated the true point prevalence of *C. parvum* oocysts in fecal material deposited by feedlot cattle from the central and western USA. The apparent point prevalence based on DFA was 0.17%. Given that the false negative proportion for DFA (using IMS-DFA as the gold standard) was 0.91%, the estimated total number of false negatives in our sample of 5264 DFA-negative fecal pats would be $48 (0.0091 \times 5264)$. Finally, four of the nine DFA-positive fecal samples identified in this survey had sufficient numbers of *C. parvum* oocysts to allow molecular confirmation as *C. parvum*, as discussed below. This leaves five DFA-positive samples without molecular confirmation and therefore possible false positives (e.g., DFA positive yet truly negative). At one extreme we can assume that all five were false positives. This results in the specificity being 99.9% (5217/5222) and the estimated true number of fecal pats containing detectable numbers of *C. parvum* oocysts as 52 ($4 + 48$), leading to a true point prevalence of 0.986% (52/5274). Alternatively, we could assume all five of these DFA-positive samples without molecular confirmation were true positives. This results in the specificity being 100% (5217/5217) and the estimated true number of

![Fig. 1. Probability of detecting one or more Cryptosporidium parvum oocysts in fecal material deposited by feedlot cattle using direct immunofluorescent microscopy or immunomagnetic separation coupled with direct immunofluorescent microscopy.](reproducedfromjournale nviro ENVQALITY.PUB.ISHEDbYASA, CSSA, AND SSSA. ALLC)
fecal pats containing detectable numbers of *C. parvum* oocysts as 57 (9 + 48), leading to a true point prevalence of 1.08% (57/5274). It should be noted that either of these two estimates for the true point prevalence (0.986 and 1.08%) are based in large part on the IMS-DFA results that were generated from only 219, with 9 to 10 samples per feedlot.

Establishing the true point prevalence as 0.99 to 1.08% and the percent recovery of the DFA and IMS-DFA assays as shown above, we estimated the overall environmental load of *C. parvum* oocysts/kg of fresh fecal material deposited by feedlot cattle. Among the 9 DFA-positive fecal samples, six had ~203 oocysts/g feces (four of these six were DNA-confirmed as *C. parvum*), one had ~406 oocysts/g feces, one had ~3242 oocysts/g feces, and one had ~7702 oocysts/g feces. The arithmetic and geometric mean concentration of *C. parvum* oocysts for these nine DFA-positive samples was 1396 and 446 oocysts/g feces, respectively. If we exclude non-DNA-confirmed samples from this estimate, the arithmetic and geometric mean both have the value of 203 oocysts/g feces. Among the two IMS-DFA positive fecal samples, one had 1.1 oocysts/g feces and the other had 256 oocysts/g feces. The arithmetic mean concentration of *C. parvum* oocysts for these IMS-DFA positive samples was 129 and 16.9 oocysts/g feces, respectively. Previous studies of oocyst shedding among noncalf populations of beef cattle have ranged from 70 to 900 oocyst/g feces (Faubert and Litvinsky, 2000; Grazycz et al., 2000; Hoar et al., 2000; Scott et al., 1995), and 100 to 999 oocyst/g feces (Grazycz et al., 2000; Hoar et al., 2000; Scott et al., 1995), consistent with the range of estimates we observed in this study.

The above values for the true point prevalence and the shedding intensity can be used to roughly estimate the mean intensity of oocysts/g feces for all 5274 fecal samples evaluated in the study, using the following equation:

\[
I_{\text{overall}} = \left( \frac{1}{\alpha + \beta} \right) (I_{\text{DFA+}}) + \left( \frac{\beta}{\alpha + \beta} \right) (I_{\text{IMS+}}) \cdot P \quad [2]
\]

whereby \(I_{\text{overall}}\) represents the weighted mean intensity of oocysts/g feces for all 5274 fecal samples evaluated in the study (our estimate for the population average), \(\alpha\) is the number of observed DFA+ samples (\(\alpha\) is either 9 or 4 given that specificity is 100 or 99.9%, respectively), \(\beta\) is the number of false negatives in our sample (~48) as estimated by the proportion of IMS-DFA positive samples among DFA negative samples multiplied by the total number of DFA negative samples in the study [(2/219)(5265 or 5270 given that specificity is 100 or 99.9%, respectively), \(I_{\text{DFA+}}\) is the mean intensity of the DFA+ samples, \(I_{\text{IMS+}}\) is the mean intensity of the IMS-DFA positive samples, and \(P = (\alpha + \beta)/5274\) is the true point prevalence of fecal samples containing *C. parvum* oocysts (\(P = 1.08 \text{ or } 0.986\%\) given that specificity is 100 or 99.9%, respectively). Therefore, assuming that the specificity of DFA for these feedlot fecal samples was either 100 or 99.9%, the arithmetic mean intensity of oocysts/g feces for all 5274 fecal samples can be calculated as \([(9/57)(1396 \text{ oocysts/g}) + (48/57)(128.7 \text{ oocysts/g})]/((9 + 48)) = 3.6 \text{ oocysts/g and }([(45/52)(202.7 \text{ oocysts/g}) + (48/52)(128.7 \text{ oocysts/g})]/(45 + 48)) = 1.3 \text{ oocysts/g feces, respectively. Likewise, the geometric mean intensity of oocysts/g feces for all 5274 fecal samples was 0.92 and 0.31 oocysts/g feces, respectively.}

If we assume that feedlot steers produce between 20 and 40 kg of feces per day (ASAE, 1992), then the arithmetic mean environmental loading rate of *C. parvum* oocysts by feedlot steers in this seven-state study would range from 2.8 × 10^4 to 1.4 × 10^5 oocysts/steer per day, depending on the value for the specificity of DFA we use for these calculations (Table 2). The geometric mean environmental loading rate of *C. parvum* oocysts by feedlot steers would likewise range from 9.1 × 10^3 to 3.7 × 10^4 oocysts/steer per day. Given that we measured the *C. parvum* load in fresh fecal pats rather than samples collected per rectum, this *C. parvum* loading rate per steer is an indication of the amount of *C. parvum* present in fecal material on the pen floor that is 1 to 2 d post-defecation. It is important to note that confined animal feeding operations such as feedlots do not allow their livestock to directly defecate into surface water supplies such as rivers and lakes. The mechanism for waterborne contamination from confined animal operations like feedlots is therefore mediated by such processes as runoff from the pens containing fecal material or from discharges from the waste management system, and not from direct fecal deposition by cattle themselves. As such, estimates of the actual environmental microbial load for feedlot cattle fecal material could be upwardly biased if such an estimate was based on samples taken per rectum, as we found in a previous study on beef cows (Hoar et al., 1999). With these caveats in mind, the arithmetic mean environmental loading rate of *C. parvum* oocysts by feedlot steers in this 7-state study (2.8 × 10^4 to 1.4 × 10^5 oocysts/animal per day) was 0.5 to 1.5 log_{10} more than the 3.9 × 10^3 to 9.2 × 10^3 oocysts/animal per day estimated for periparturient beef cows on California rangeland (At-

<table>
<thead>
<tr>
<th>Estimated mean</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arithmetic mean</td>
<td>Sp = 99.9%</td>
<td>27,687</td>
<td>41,531</td>
</tr>
<tr>
<td></td>
<td>Sp = 100%</td>
<td>71,085</td>
<td>106,628</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>Sp = 99.9%</td>
<td>9,119</td>
<td>13,679</td>
</tr>
<tr>
<td></td>
<td>Sp = 100%</td>
<td>18,309</td>
<td>27,464</td>
</tr>
</tbody>
</table>

†Environmental loading rate (Hoar et al., 2000; Atwill et al., 2003) calculated as the mean concentration of oocysts observed in fecal samples positive for *C. parvum* (no. oocysts/g feces), adjusted for the percentage recovery of the assay, multiplied by the true point prevalence of *C. parvum* (using immunomagnetic separation coupled with direct immunofluorescent microscopy as the gold standard for detecting *C. parvum*), multiplied by the estimated daily production of feces per feedlot steer (kg feces produced/animal per day) (ASAE, 1992).

‡Test specificity (probability of testing negative given a negative sample) of direct immunofluorescent microscopy (DFA). Values of 100% = (5217/5217) or 99.9% = (5217/5222) are generated when we assume either 0 or 5 of the 9 DFA+ samples shown in Table 1 are *C. parvum* negative. Four of the 9 DFA+ had sufficient numbers of oocysts for molecular confirmation as *C. parvum* via PCR and DNA sequencing.

Table 2. Estimated mean environmental loading rate† of Cryptosporidium parvum oocysts in fresh feces deposited by feedlot cattle in central and western USA. 

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will et al., 2003) and 0.8 to 2.0 log$_{10}$ more than the 1.3 × 10$^3$ to 4.1 × 10$^3$ oocysts/animal per day we estimated for periparturient dairy cows from large commercial dairies in the southern San Joaquin Valley, California (values extrapolated from Atwill and Pereira, 2003). Once such estimates of the environmental load of C. parvum are generated, management practices can be appropriately designed to attenuate the quantified microbial load (Atwill et al., 2002).

Of the nine positive DFA and two positive IMS-DFA samples, four isolates had sufficient numbers of oocysts to allow molecular characterization of Cryptosporidium. The species of these isolates was confirmed to be C. parvum, based on sequencing the 834 amplicon of a nested PCR procedure (Xiao et al., 1999) and a BLAST search at NCBI. For three of these four isolates, restriction sites for VspI and SspI were identified along the DNA sequences that generated the 11, 12, 108, 254, and 449 pattern of fragments characteristic of bovine genotype A C. parvum (Xiao et al., 1999).

CONCLUSIONS

Our primary objective for this project was to quantify the environmental load of C. parvum oocysts in fecal material deposited by feedlot cattle from across the central and western USA. Such an estimate would support the proper design of a fecal waste management system and the valid calculation of the total maximum daily load for C. parvum on a watershed containing one or more feedlots. We found a point prevalence of C. parvum of 0.99 to 1.08% in fecal samples from the pens of feedlot cattle from a wide range of climates and a diverse range of feedlot management systems. On average, using the arithmetic mean as our measure of central tendency, fresh fecal material from throughout feedlot systems (recent arrivals to nearing slaughter) contained 1.3 to 3.6 oocysts/g feces, which translates to 2.8 × 10$^3$ to 1.4 × 10$^5$ oocysts/animal per day. It can take <100 C. parvum oocysts of bovine origin to infect a healthy human (Okhuysen et al., 1999), so it is important to minimize contact between fresh bovine feces from feedlot operations and water supplies used for municipal, recreational, or irrigation purposes. Offsetting this risk of waterborne contamination from accumulated feces from feedlot cattle are a variety of processes (e.g., fecal material incorporation into soil via hoof action) and beneficial management practices such as composting or drying of fecal material that can substantially reduce the infectivity of C. parvum oocysts in bovine manure. In addition, beneficial management practices exist that minimize off-site transport of C. parvum oocysts, such as the strategic use of vegetated buffer strips between sources of fecal material and adjacent water supplies of concern (Atwill et al., 2002; Davies et al., 2004; Tate et al., 2004; Trask et al., 2004). Central to the success of such beneficial management practices that target waterborne C. parvum is an accurate measure of the environmental load of C. parvum, which this project has provided for feedlot production systems located across the central and western USA.

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REFERENCES


