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Quantitative-PCR Assessment of *Cryptosporidium parvum* Cell Culture Infection

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A quantitative TaqMan PCR method was developed for assessing the *Cryptosporidium parvum* infection of in vitro cultivated human ileocecal adenocarcinoma (HCT-8) cell cultures. This method, termed cell culture quantitative sequence detection (CC-QSD), has numerous applications, several of which are presented. CC-QSD was used to investigate parasite infection in cell culture over time, the effects of oocyst treatment on infectivity and infectivity assessment of different *C. parvum* isolates. CC-QSD revealed that cell culture infection at 24 and 48 h postinoculation was approximately 20 and 60%, respectively, of the endpoint 72-h postinoculation infection. Evaluation of three different lots of *C. parvum* Iowa isolate oocysts revealed that the mean infection of 0.1 N HCl-treated oocysts was only 36% of the infection obtained with oocysts treated with acidified Hanks' balanced salt solution containing 1% trypsin. CC-QSD comparison of the *C. parvum* Iowa and TAMU isolates revealed significantly higher levels of infection for the TAMU isolate, which agrees with and supports previous human, animal, and cell culture studies. CC-QSD has the potential to aid in the optimization of *Cryptosporidium* cell culture methods and facilitate quantitative evaluation of cell culture infectivity experiments.

Ongoing challenges for the water industry are *Cryptosporidium* detection and infectivity determination. Mouse models have frequently been used to determine the infectivity of *Cryptosporidium* oocysts (2, 10, 18, 20). However, animal infectivity determination is costly and labor-intensive and requires as long as 2 weeks to complete. Furthermore, it is not feasible for the analysis of environmental samples. In an attempt to simplify infectivity determination, several in vitro methods have been developed, such as in vitro excystation and microscopic assays evaluating the uptake or exclusion of fluorogenic dyes (3, 4) and fluorescence in situ hybridization techniques (35). Various PCR strategies have also been developed to assess the viability of *Cryptosporidium parvum* oocysts, including the integration of in vitro excystation (5, 9, 36) and the use of reverse transcription-PCR for the detection of mRNA transcripts found only in viable oocysts (24, 30).

Strategies using in vitro cell culture of *C. parvum* are attractive alternatives to animal infectivity testing. In most of these assays, oocysts are stimulated to excyst by exposure to sodium hypochlorite (bleach), bile enzymes, and/or acidic pH (7, 23, 24, 32) and are then inoculated onto in vitro-cultivated cell culture monolayers. Sporozoites released from oocysts invade and replicate within the intracellular environment of the cells. After incubation, parasites are detected within the cell cultures by a variety of techniques, including immunofluorescence microscopy (28), reverse transcription-PCR (24), and standard PCR (7). Quantification of cell culture infection for pharmaceutical and disinfection studies has been achieved with an enzyme-linked immunosorbent assay (38). Most-probable-number formats using immunofluorescence microscopy (27, 29), colorimetric in situ hybridization (25, 37), and reverse transcription-PCR (26) have also been described. However, most of these methods were developed for disinfection or inactivation studies and are used to assess differences in oocyst infectivity over several orders of magnitude. Intrinsically, these assays require high numbers of oocysts and labor-intensive microscopic techniques or numerous nucleic acid extractions. Methods that are less labor-intensive and that have greater flexibility in the numbers of oocysts required are needed for other types of cell culture studies.

TaqMan probe chemistry is commonly used for real-time quantitative PCR. TaqMan quantitative PCR requires the use of primers similar to those used in a conventional PCR; however, unlike a conventional PCR, TaqMan quantitative PCR also requires an oligonucleotide probe labeled with 5′ reporter and 3′ quencher fluorescent dyes and a thermal cycler equipped with a fluorometer (13). During each cycle of the PCR, if the target of interest is present, the probe specifically anneals to the target amplicon between the forward and reverse primer sites. Because of the 5′ nuclease activity of *Taq* DNA polymerase, the probe is cleaved during the polymerization step (PCR product formation) of the PCR, resulting in an increase in the reporter fluorescence detected by the instrument. The target signal increases in direct proportion to the concentration of the PCR product being formed. The threshold cycle (C_T) is the fractional PCR cycle number at which a significant increase in target signal fluorescence above the baseline is first detected for a sample. By using amplification standards consisting of known quantities of target nucleic acid or organisms to generate a standard curve, the starting copy number of nucleic acid targets or target organisms for each sample can be estimated.

Recently, several real-time qualitative and quantitative PCR methods for the detection of *C. parvum* oocysts have been

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described (6, 11, 12, 14). This report describes an integrated cell culture quantitative PCR method to quantify *C. parvum* cell culture infection and provides several examples of its application to this field of research. The method developed, referred to as cell culture quantitative sequence detection (CC-QSD), uses HCT-8 human ileocecal adenocarcinoma in vitro cell culture in a 96-well plate format and TaqMan PCR targeting the heat shock protein 70 gene (*hsp70*) (17) of *C. parvum*. For relative comparison of samples, a CC-QSD standard curve was generated by inoculating HCT-8 monolayers with different numbers of *C. parvum* oocysts and performing linear regression of sample mean C_T values plotted against numbers of inoculated oocysts. Applications of the CC-QSD method presented in this paper include evaluation of the effects of incubation time on cell culture development of *C. parvum*, evaluation of the effects of oocyst pretreatment on cell culture infectivity, and comparison of different *C. parvum* isolates. The CC-QSD method represents a less labor-intensive, more flexible alternative for these types of *C. parvum* cell culture infection studies, as opposed to the use of techniques developed for disinfection studies.

MATERIALS AND METHODS

Oocysts and microscopy. Purified viable *C. parvum* oocysts (Iowa, Harley Moon isolate) were obtained from Waterborne, Inc. (New Orleans, La.) and the Sterling Parasitology Laboratory (University of Arizona, Tucson). Oocysts of the *C. parvum* TAMU isolate were kindly provided by Cynthia Chappell, University of Texas Health Science Center, Houston, and propagated by the Sterling Parasitology Laboratory. Oocyst stocks were enumerated by immunofluorescence assay microscopy with Dynal Spot-On-treated well slides (Dynal, A.S., Oslo, Norway) or three-well slides (Meridian Diagnostics, Inc., Cincinnati, Ohio) and fluorescein isothiocyanate-labeled anti-*Cryptosporidium* monoclonal antibody (Waterborne, Inc.) as previously described (7).

In vitro cell culturing of *C. parvum* and oocyst treatment. Human ileocecal adenocarcinoma (HCT-8; ATCC CCL-244) cells were cultivated in 96-well cell culture microplates as previously described (7, 38). The cell monolayer in each well of the microplate was considered a single replicate. Cell culture maintenance medium consisted of RPMI 1640 medium with Glutamax (Invitrogen, Carlsbad, Calif.), 5% fetal bovine serum (pH 7.2; HyClone, Logan, Utah), 15 mM HEPES buffer, 100,000 U of penicillin G liter^{-1}, 100 mg of streptomycin liter^{-1}, 700 μg of amphotericin B liter^{-1}, and 12.5 mg of tetracycline liter^{-1}. Growth medium used for the in vitro development of *C. parvum* contained 10% fetal bovine serum, 15 mM HEPES buffer, 50 mM glucose, 35 mg of ascorbic acid liter^{-1}, 1.0 mg of folic acid liter^{-1}, 4.0 mg of 4-aminobenzoic acid liter^{-1}, 2.0 mg of calcium pantothenate liter^{-1}, 100,000 U of penicillin G liter^{-1}, 100 mg of streptomycin liter^{-1}, 700 μg of amphotericin B liter^{-1}, and 12.5 mg of tetracycline liter^{-1}. Twenty-four hours prior to inoculation, each microplate well was seeded with 100 μl of maintenance medium containing 5×10^4 HCT-8 cells. Microplates were incubated at 37°C in a 5% CO_2 humidified incubator to allow the development of 90 to 100% confluent monolayers.

Oocysts were pretreated for cell culture by suspension in acidified (pH 2.0) Hanks' balanced salt solution supplemented with trypsin (type II-S porcine pancreas; Sigma Chemical Co., St. Louis, Mo.) to a final sample concentration of 1% (wt/vol; AHBSS-trypsin) and then incubated at 37°C for 1 h with 10 s of vortexing every 15 min (7). After incubation, an equal volume of growth medium was added and samples were centrifuged at maximum speed in a microcentrifuge for 2 min without the brake. Samples were then immediately and carefully aspirated down to 50 μl; 500 μl of growth medium was added, and samples were centrifuged as described above. Samples were carefully aspirated to 20 μl and resuspended in 80 μl of prewarmed (37°C) growth medium containing 0.05% trypsin. This allowed precise control over the trypsin concentration in the sample and cell culture.

Just prior to inoculation of monolayers, 50 μl of maintenance medium was removed. Monolayers were inoculated and then incubated at 37°C in a 5% CO_2 humidified incubator for 72 h prior to DNA extraction. AHBSS-trypsin-treated viable *C. parvum* oocysts were used as CC-QSD positive controls (approximately 5 to 20 oocysts per cell monolayer, depending on the infectivity of the oocyst stock), and single-cycle freeze-thaw-killed oocysts were used as CC-QSD negative controls. In addition, intact oocyst no-infection controls (IONICs) were used to evaluate the potential background CC-QSD signals due to intact oocysts remaining on monolayers. IONICs consisted of 5 to 250 intact viable oocysts added to previously washed, uninoculated monolayers just prior to DNA extraction.

DNA extraction from oocysts and cell culture monolayers. After incubation, the cell monolayers were washed five times with 200 μl of PBS to remove unexcysted oocysts. DNA was purified from HCT-8 cells with the QIAamp DNA mini kit (QIAGEN, Valencia, Calif.) and a modified extraction protocol. Modifications included incubation of the cell monolayers with lysis buffer for 15 min at 70 to 75°C and elution of DNA from the QIAamp DNA columns with 50 μl of 0.01× Tris-EDTA (TE), pH 8.0, at 70 to 75°C for 5 min. This method of cell culture DNA purification was used, rather than our previous crude freeze-thaw lysis method (7), because it provided samples with the purity required for performing TaqMan quantitative PCR.

Oocyst DNA was prepared by a rapid and simple freeze-thaw extraction procedure. Biotechnology grade Chelex 100 resin (catalog no. 143-2832; Bio-Rad, Hercules, Calif.) was prepared as an overnight-equilibrated 1:1 volume-to-volume slurry of resin and 1× TE buffer (pH 8.0). An equal volume of prepared Chelex resin slurry was added to each sample with a micropipettor fitted with large-bore (genomic) tips. For each sample, the Chelex slurry was thoroughly resuspended and aliquots were quickly withdrawn from the reagent tube to prevent settling of the resin and maintain a 1:1 volume-to-volume ratio of resin and 1× TE buffer. Oocysts were lysed by eight cycles, 1 min each, of freezing in liquid nitrogen and thawing at 94 to 98°C. Total sample volumes were 200 μl or less to ensure complete freezing and thawing during 1-min cycles.

CC-QSD. CC-QSD was performed with the Applied Biosystems 7700 or 7000 Sequence Detector (Applied Biosystems, Foster City, Calif.). PCR primers and a TaqMan probe specific for the *C. parvum hsp70* gene were used, resulting in a 346-bp product. The primer and probe sequences were as follows: forward primer CPHSPT2F, 5′ TCCTCTGCCGTACAGGATCTCTTA 3′; reverse primer CPHSPT2R, 5′ TGCTGCTCTTACCAGTACTCTTATCA 3′; TaqMan probe CPHSP2P2, 5′ 6-carboxyfluorescein TGTTGCTCCATTATCACTCGGT TTAGA 6-carboxytetramethylrhodamine 3′. Each 100-μl CC-QSD mixture contained 10.0 μl of 10× TaqMan A amplification buffer (Applied Biosystems); 1.5 mM $MgCl_2$; 200 μM each dATP, dTTP, dCTP, and dGTP (Amersham Pharmacia Biotech, Piscataway, N.J.); 200 nM each forward and reverse CPHSPT2 primer and CPHSP2P2 TaqMan probe (Synthegen, Houston, Tex.); 5.0 μl of 30-mg ml^{-1} bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.); and 5.0 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). Amplification conditions were as follows: initial denaturation at 95°C for 10 min and 45 cycles of denaturation at 95°C for 30 s and annealing at 60°C for 1 min, followed by a final extension at 72°C for 10 min.

Purified cell culture DNA for each sample (50 μl) or aliquots of Chelex-treated oocyst DNA controls (up to 10 μl) were used as templates. Molecular-grade water was used to prepare no-template negative controls. A *C. parvum* CC-QSD standard curve and equation for relative quantitation of cell culture infection was generated on the basis of the mean background (IONICs)-subtracted C_T values of samples plotted against the log number of oocysts inoculated per cell monolayer.

Data analysis and statistics. C_T data for experiments were analyzed and expressed as the percent mean and standard deviation of replicates adjusted to experimental controls. To determine the statistical significance of results, data were analyzed by one-way analysis of variance and post hoc Bonferroni pairwise mean comparisons with SYSTAT 9 (SPSS Inc., Chicago, Ill.). An alpha level of 0.05 was used for all statistical tests.

RESULTS

CC-QSD quantitation of *C. parvum* infection in HCT-8 cell culture. CC-QSD analysis of DNA extracted from *C. parvum*-inoculated cell monolayers revealed an increase in infection with increasing numbers of oocysts inoculated per monolayer (Fig. 1). In most cases, analysis of variance with post hoc Bonferroni test analysis of data revealed that CC-QSD values were only statistically significantly different for monolayers inoculated with 10-fold differences in oocyst density. For example, CC-QSD quantitation of cell culture infection differed for monolayers inoculated with 250 and 25 oocysts ($P = 0.001$) and

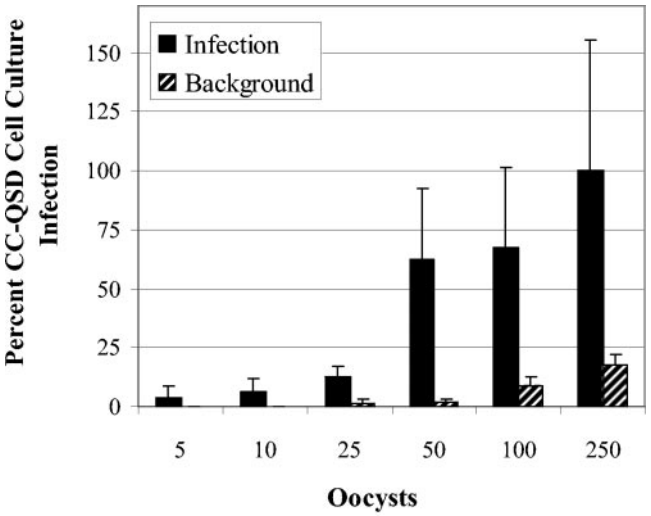

FIG. 1. CC-QSD quantitation of *C. parvum* Iowa isolate infection in HCT-8 cell culture. Cell monolayers were inoculated with 5 to 250 AHBSS-trypsin-treated oocysts, and DNA was extracted at 72 h postinoculation. To evaluate the background from intact oocysts remaining on monolayers, 5 to 250 viable oocysts were added to washed, previously uninoculated monolayers just prior to DNA extraction. Data are means and standard deviations of five replicates.

100 and 10 oocysts ($P = 0.047$), but not for monolayers inoculated with 250 and 100 oocysts ($P > 0.05$) or 25 and 5 oocysts ($P = 0.064$).

IONICs were used to investigate false CC-QSD infection signal background that could potentially arise from intact oocysts remaining on cell monolayers. A comparison of the number of CC-QSD-positive samples for AHBSS-trypsin-treated oocysts, IONICs, and CC-QSD background signals of IONICs is presented in Table 1. At 5 and 10 oocysts per monolayer, 3 (60%) of 5 and 4 (80%) of 5 replicates of AHBSS-trypsin-treated oocysts tested positive by CC-QSD, respectively, whereas all of the corresponding IONICs tested negative. At inoculum levels of 25 to 250, all replicates of AHBSS-trypsin-treated oocysts tested positive by CC-QSD, while the number of IONIC replicates testing positive increased from 2 to 5 (40 to 100%), respectively. Background CC-QSD signals of IONICs were low and ranged from 0 to 18% for inoculum densities of 5 to 250 oocysts per monolayer, respectively. A CC-QSD

TABLE 1. Background CC-QSD signal due to intact oocysts remaining on cell monolayers compared to AHBSS-trypsin-treated positive infection controls

No. of oocysts/ monolayer	% of CC-QSD-positive monolayers ($n = 5$)		Mean % CC-QSD background signal[a]
	AHBSS-trypsin-treated oocysts	IONICs	
5	60	0	0
10	80	0	0
25	100	40	8
50	100	40	3
100	100	100	13
250	100	100	18

[a] Values derived from Fig. 1 quantitation data.

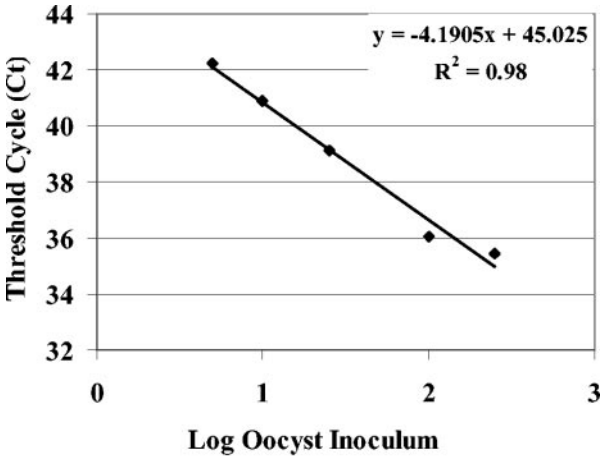

FIG. 2. CC-QSD standard curve for *C. parvum* Iowa isolate infection of HCT-8 cell culture monolayers. The curve shown is based on mean C_T values of five replicates at each oocyst inoculum level.

standard curve was generated by linear regression of mean background-subtracted CC-QSD C_T values plotted against the number of AHBSS-trypsin-treated oocysts inoculated per cell monolayer (Fig. 2, $R^2 = 0.98$).

Development of *C. parvum* infection in HCT-8 cell culture. CC-QSD was used to evaluate the increase in *C. parvum* infection in HCT-8 cell culture from 24 to 72 h postinoculation. CC-QSD results indicated that *C. parvum* infection increased significantly over the 72-h incubation period ($P < 0.001$). As determined by CC-QSD, *C. parvum* infection at 24 and 48 h postinoculation was approximately 20 and 60%, respectively, of the endpoint 72 h postinoculation infection (Fig. 3). Monolayers inoculated with oocysts and immediately analyzed (infection-negative controls) tested negative by CC-QSD.

Effects of oocyst treatment on cell culture infectivity. U.S. Environmental Protection Agency (USEPA) methods 1622 and 1623 for the detection of *Cryptosporidium* in water (33, 34) require oocysts recovered from surface water samples to be exposed to 0.1 N HCl during the immunomagnetic separation

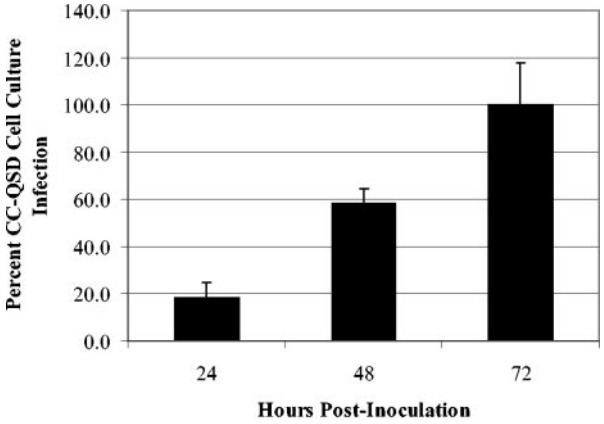

FIG. 3. Development of *C. parvum* infection in HCT-8 cell culture from 24 to 72 h postinoculation. Each cell monolayer was inoculated with approximately 100 oocysts, and data are means and standard deviations of three replicates and are adjusted to 100%.

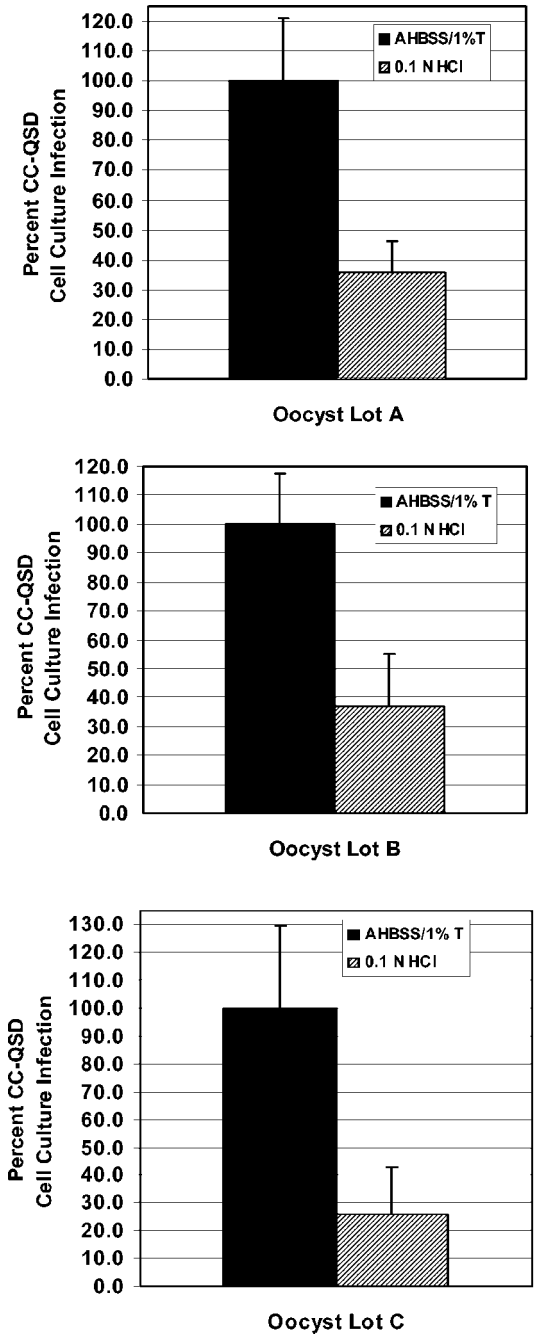

FIG. 4. Effects of 0.1 N HCl and AHBSS-trypsin treatment of *C. parvum* Iowa isolate oocysts on cell culture infectivity. HCT-8 cell monolayers were inoculated with approximately 300 oocysts, and three different lots of oocysts (A, B, and C) were evaluated. Data are means and standard deviations of three replicates and are adjusted to 100%.

(IMS) dissociation step. To evaluate the potential effects of the method 1622 and 1623 IMS dissociation step on *C. parvum* infectivity, oocysts were resuspended in equivalent volumes of either 0.1 N HCl or AHBSS-trypsin. The 0.1 N HCl treatment was for 10 min at room temperature, while the AHBSS-trypsin treatment was for 1 h at 37°C. Samples were neutralized with 1.0 NaOH, centrifuged, and resuspended in cell culture growth medium, and cell monolayers were inoculated. CC-QSD evaluation of three different lots of oocysts (Fig. 4) revealed that 0.1 N HCl-treated oocysts had consistently lower HCT-8 cell culture infectivity compared to AHBSS-trypsin-treated oocysts ($P = 0.005$, 0.026, and 0.024, respectively). Results indicated that for the *C. parvum* Iowa isolate the mean infection obtained with 0.1 N HCl-treated oocysts was only 36% of that obtained with AHBSS-trypsin-treated oocysts.

Comparison of cell culture infectivities of different *C. parvum* isolates. The CC-QSD technique may also be useful for the comparison of cell culture infectivity of different *C. parvum* isolates. To investigate the potential application of CC-QSD and to provide additional data on the *C. parvum* Iowa and TAMU isolates previously compared for their cell culture and mouse (26) and human infectivity (21), we compared these isolates by CC-QSD. Lots of *C. parvum* Iowa and TAMU isolate oocysts were similar in age postshedding (4 to 6 weeks) and were propagated by the same laboratory. Repeat trials revealed consistent and highly significant differences in the infectivity of the isolates (Fig. 5) ($P = 0.003$ and 0.001, respectively), with the *C. parvum* Iowa isolate having an average of only 33% of the *C. parvum* TAMU isolate cell culture infectivity.

DISCUSSION

This report describes the development of a quantitative TaqMan PCR method (CC-QSD) for assessing the infection of an HCT-8 human cell culture by *C. parvum*. Various applications of this method were investigated, including the development of parasite infection in cell culture over time, evaluation of the effects of oocyst treatment on infectivity, and assessment of the infectivity of different *C. parvum* isolates. The CC-QSD method represents a less labor-intensive, more flexible alternative for these types of *C. parvum* cell culture infection studies, as opposed to the use of techniques developed for disinfection studies. CC-QSD is not recommended for disinfection studies because of the likely background signal obtained at the high oocyst inoculum densities typically used. In addition to revealing the versatility of the CC-QSD method, results of this study have important implications for the application and interpretation of *C. parvum* cell culture infectivity assays.

In this study, we were interested in the relative comparison of cell culture infection between treatment groups given inocula with similar numbers of oocyst rather than absolute quantitation of the number of infective oocysts per sample. Therefore, the CC-QSD standard curve generated by linear regression of mean background-subtracted CC-QSD C_T values plotted against the log number of AHBSS-trypsin-treated oocysts inoculated per cell monolayer was sufficient. A similar relative quantitation approach using a DNA standard curve generated with only oocyst DNA, instead of infected cell monolayers, was recently reported for a disinfection study (16). The development of a universal standard curve for the absolute quantitation of infective *C. parvum* oocysts is a significant challenge. This is due to several factors, including (i) the variability in the actual numbers of oocysts inoculated per monolayer introduced by pipetting and dilution effects, (ii) the fact that not all oocysts are infective, and (iii) the inherent biological variability in the number and type of parasite intracellular developmental stages and consequently the copy number of

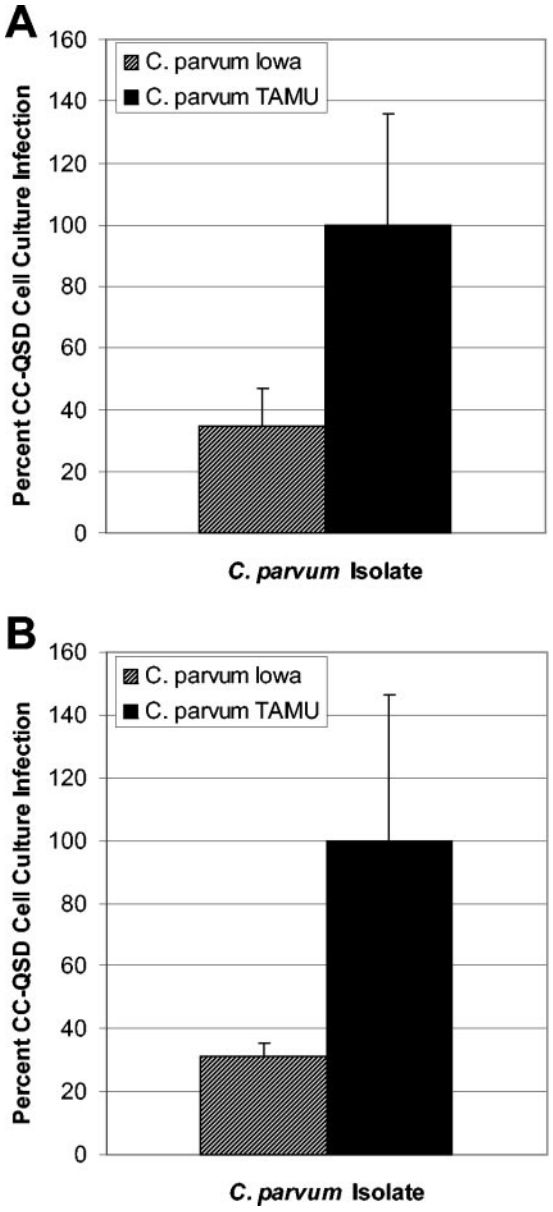

FIG. 5. Comparison of *C. parvum* Iowa and TAMU isolate cell culture infectivities. Cell monolayers were inoculated with approximately 300 oocysts, and duplicate experiments were performed (A and B). Data are means and standard deviations of five replicates and are adjusted to 100%.

genomic targets. Despite the challenges facing absolute quantitation, our results show promise for the use of CC-QSD for the quantitation of low numbers of infective oocysts, since infection in monolayers inoculated with 5 and 25 oocysts differed, although not significantly ($P = 0.064$). Greater numbers of replicates and more precise numbers of oocysts may allow further discrimination of inoculum levels. Our nonquantitative cell culture PCR (CC-PCR) method has been compared to the conventional USEPA method 1623 immunofluorescence assay microscopy detection of naturally occurring oocysts in surface water (19). In that study, the two methods were found to have comparable recoveries and the data suggested that approximately 37% of the environmental oocysts detected by microscopy were infectious. However, because of the biological variability described above, the potential application of the CC-QSD method presented in this paper should be limited to qualitative (presence or absence) real-time PCR detection rather than quantitative detection of infectious oocysts in environmental samples until further research has been performed. Additional research with flow cytometry-counted oocyst inocula and paired CC-QSD and microscopic detection of infectivity is planned to better quantify *Cryptosporidium* cell culture infection.

Differences in postinoculation incubation time prior to assessment of infection may have a significant impact on the amount of observed infection. Many *C. parvum* cell culture studies have used 48 h postinoculation as the endpoint of infection (16, 24, 26, 28, 29, 31). In this study, CC-QSD revealed that infection at 48 h postinoculation was only 60% of the amount of infection at 72 h postinoculation. Therefore, incubation periods may have an effect on the ability to detect infection, particularly with very low numbers of infectious oocysts. In our previous studies using conventional CC-PCR to assay environmental samples, an incubation period of 72 h was used, which may have aided the detection of low numbers of naturally occurring *C. parvum* oocysts (1, 7, 19). Therefore, on the basis of our successful CC-PCR analysis of environmental water samples and the CC-QSD data presented here, we recommend a 72-h incubation period for the CC-QSD method. Another study reported the maintenance of *C. parvum* infection in HCT-8 cell culture for up to 25 days by subculturing (15). Clearly, this is an area that deserves more investigation for optimization of cell culture infection and detection strategies.

It was previously reported that 0.1 N HCl-treated *C. parvum* oocysts retained their in vitro cell culture infectivity (23), although high numbers of fresh oocysts were used and infections were not quantified. A subsequent study (22) by the same laboratory evaluating the infectivity of oocysts by USEPA method 1622 used in vitro excystation with bile salts rather than the 0.1 N HCl dissociation step of method 1622. Therefore, the effects of the 0.1 N HCl dissociation step on *C. parvum* cell culture were uncertain. In the present study, the mean infection of 0.1 N HCl-treated oocysts was only 36% of that obtained with AHBSS-trypsin-treated oocysts. Additional research is needed to determine whether the HCl exposure damages oocysts or is simply an insufficient stimulus for cell culture. However, if the observed low cell culture infectivity of 0.1 N HCl-treated oocysts is due to an adverse effect, this would likely be more pronounced with environmentally stressed oocysts. Therefore, the AHBSS-trypsin method is recommended over 0.1 N HCl treatment for *C. parvum* cell culture-based assays that rely upon IMS purification of naturally occurring oocysts from environmental water samples.

A recent study established in vitro cell culture as an equivalent to the "gold standard" mouse infectivity assay for measuring the infectivity of *C. parvum* (26). In that study, the authors also reported that the *C. parvum* TAMU isolate was more infectious than the *C. parvum* Iowa isolate in HCT-8 and Caco-2 cell culture and CD-1 mouse infectivity assays. The authors found that the TAMU isolate had a 3.9-fold lower 50% infectious dose (ID_{50}) than the Iowa isolate on the basis of an

HCT-8 cell culture reverse transcription-PCR technique. Human volunteer infectivity trials have reported an ID_{50} of 132 oocysts for the Iowa isolate (8), compared to an ID_{50} of 9 oocysts for the TAMU isolate (21). In our study, CC-QSD revealed a threefold higher level of cell culture infection for the TAMU isolate compared to the Iowa isolate, which confirms and extends these previous findings. Assessment of the infectivities of different human and animal *C. parvum* isolates is critical to our understanding of *Cryptosporidium* biology and ecology, and the CC-QSD method may be a useful tool for these types of studies.

Current research efforts are aimed at the use of CC-QSD for the optimization of oocyst pretreatment for cell culture, optimization of cell culture conditions to maximize *C. parvum* infection, and the use of CC-QSD for the quantitative detection of infectious *C. parvum* oocysts in environmental water samples.

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