Letter to the Editor

Viability of Cryptosporidium parvum Oocysts: Assessment by the Dye Permeability Assay

The paper by Jenkins et al. (6) provides interesting data on Cryptosporidium parvum oocyst permeability and survival. The vital dye assay (4) relies upon oocyst permeability and exhibits limitations, especially in assessment of disinfectant efficacy (3). However, it is rewarding to note that it is considered to have utility and can provide pertinent data.

Although we agree with many points addressed by Jenkins et al. (6), we feel we should make the following three comments which, in part, reiterate conclusions from our previous work (4, 5, 7, 8). These points were perhaps overlooked by Jenkins et al. (6). However, it is rewarding to note that it is considered to have utility and can provide pertinent data.

(i) In our paper describing the vital dye assay (4), impermeable oocysts (DAPI− PI−) are not described as dead (not viable), as suggested by Jenkins et al. (6). Rather, we concluded (4, 8) that a further “trigger” was required to increase oocyst permeability and thus excystation capability (see Table 1 in reference 4). Intriguingly, in an earlier paper by this research group (1) it appears that they did appreciate that such oocysts (DAPI− PI−) were capable of becoming viable. In this paper (1) they write, with reference to our original paper (4), that “PI-negative, DAPI-negative oocysts are also considered viable, but with the caveat that some treatment, e.g., acidification, is required before excystation will occur.”

We consider oocyst permeability to be a dynamic situation (up until death or excystation), which is reduced by incubation with saliva (8) and storage in cow feces (7) and increased by acidic incubation (8). Rather than simply describing oocysts as alive (viable) and dead (nonviable), our data revealed an additional oocyst state in which the oocysts were impermeable to both dyes and became viable (able to excyst under defined conditions) after a further trigger. Such “quiescent” oocysts could enter either stage, becoming viable or nonviable depending upon environmental factors.

(ii) During correlation of in vitro excystation and the dye permeability assay results, we ensured that any pretreatment was performed on both oocysts to be excysted and oocysts to be subjected to the assay (4, 8). Jenkins et al. (6) used our recommended pretreatment only for oocysts to be excysted and apparently not for oocysts to be subjected to the dye assay; we are therefore not surprised that their results differ from ours. Although we demonstrated a strong positive correlation between in vitro excystation and DAPI+ PI− oocysts, Jenkins et al. (6) did not observe this correlation. Indeed, they report a correlation between DAPI− PI+ oocysts and excystation. If the pretreatment used for in vitro excystation by Jenkins et al. had also been used for the dye permeability assay, we would predict that they would have observed a correlation similar to that noted by us (4) and others (2).

Furthermore, if both DAPI+ PI− and DAPI− PI+ oocysts are considered to be viable, reductive arithmetic argument shows that PI alone is being used as the indicator of viability. Here DAPI provides no information on oocyst viability, although it may provide some information on alteration of permeability of oocysts to this dye.

(iii) Addition of FITC-conjugated antibody to the assay to assist in oocyst detection during survival studies is pertinent and has been used by us (7) and others (1) as well as by Jenkins et al. (6). However, due to the additional manipulative steps required when DAPI and PI are used, it should be noted that, when viability assessment is conducted simultaneously with detection, oocyst recovery may be reduced. Simultaneous detection and viability assessment should therefore be treated cautiously for environmental monitoring in which detection of oocysts is of primary importance.

REFERENCES


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Authors’ Reply

In their Letter to the Editor, Robertson, Campbell, and Smith raise the issue that we (5) may have overlooked some aspects of their work on Cryptosporidium parvum oocyst viability (3, 4, 7, 8). First, they point out that we suggested (1, 5) that they described impermeable oocysts (i.e., intact oocysts that do not take up the two fluorogenic dyes DAPI and PI [DAPI− PI−]) as not viable. We were, in fact, confused by some of the terminology used in their papers (3, 4). Our initial understand-
ing from those papers was that the DAPI− PI− oocysts were considered to be “viable” only “after further trigger” (3) or “viable after further stimulus” (4), the trigger or stimulus being acid treatment. These locations imply that the impermeable oocysts were thought to be something other than viable (i.e., not viable) before the “further trigger” or “further stimulus” was applied. Furthermore, viability was defined in their work as “the ability of an oocyst to excyst in a described excystation protocol” (4). “Viable” (from the Old French vie, life, derived from the Latin vita) is generally defined as “capable of reproducing under appropriate conditions” (9). To say that something is not viable tacitly implies that it is dead and not capable of reproducing, even if the conditions are appropriate. From these statements we concluded originally that Robertson, Campbell, and Smith thought that oocysts were not viable if they were impermeable to DAPI and PI and did not excyst. We now recognize that that was not their interpretation. We are grateful for their efforts to clarify their earlier work.

Our papers (1, 5) offer a further attempt to clarify the viability issue. Our experiments (5) comparing oocyst dye permeability with in vitro excystation and mouse infectivity demonstrate that, under the conditions of the assay, impermeable (DAPI− PI−) oocysts are, in fact, excystable and infective and therefore represent potentially infective oocysts.

In the Letter to the Editor, such phrases as “capable of becoming viable” in reference to DAPI− PI− oocysts are still somewhat confusing, especially with regard to the viability status of the impermeable DAPI− PI− oocysts. We think that a term such as “quiescent” or “dormant” would better describe these impermeable, but still potentially infective, oocysts.

It is important to note that, in our work, our goal was to assess oocyst survival in naturally contaminated calf feces, soil, and sediment. In our experimental design, we explicitly treated the dye permeability assay, which gives an indirect assessment of viability or infectivity, and the in vitro excystation assay, which directly tests excystability as two independent assays. Although our results did not indicate a correlation between the DAPI+ PI− oocysts and the excystable oocysts in the in vitro excystation assay, they did demonstrate a correlation between the potential for viability as determined by the sum of DAPI− PI− and DAPI+ PI− oocysts in the dye permeability assay and the excystable oocysts in the in vitro excystation assay. Therefore, our results are in concurrence with those of Campbell et al. (3) and Black et al. (2). By using the dye permeability assay (5), we demonstrated that a majority of oocysts in contaminated feces as well as in fresh sucrose-purified oocyst suspensions were impermeable to DAPI and PI (i.e., potentially infective) and that, over time, especially at elevated temperatures, there was an increase in the number of the more permeable, but still potentially infective, DAPI+ PI− oocysts. Eventually, the latter were replaced by the very permeable DAPI+ PI+ (i.e., dead) oocysts. We consider this time-dependent change from impermeable (DAPI− PI−) to more permeable (DAPI+ PI−) to very permeable (DAPI+ PI+) oocysts to indicate a sequence of events leading to the ultimate inactivation of oocysts. The time- and temperature-dependent increases in oocyst wall permeability show that oocysts can become increasingly susceptible to detrimental environmental factors such as temperature (5) and NH₃ (6), and these factors can increase oocyst inactivation rates, as indicated by increased permeability under certain manure storage conditions. Therefore, for our purposes, the inclusion of DAPI in the dye-permeability assay yields significantly more information on the changes in oocysts that occur as they become inactivated.

The third issue raised by Robertson, Campbell, and Smith concerns the simultaneous use of the immunofluorescence assay and the dye permeability assay. We found this combination of assays to be particularly useful for identifying potentially infective and dead oocysts in semiopaque environmental matrices such as soil, sediment, and feces (1). It should be remembered that we were investigating oocyst survival under various environmental conditions in which oocyst identification was difficult because of the presence of the opaque material in the samples. We certainly agree with Robertson et al. that, for routine environmental monitoring purposes, such simultaneous detection and viability assessment should be approached with caution.

**REFERENCES**


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