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STANDARDIZATION OF A FLUORESCENT-BASED QUANTITATIVE ADHESION ASSAY TO STUDY ATTACHMENT OF *Taenia solium* ONCOSPHERE TO EPITHELIAL CELLS *In Vitro*

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Abstract

To fully understand the preliminary stages of *Taenia solium* oncosphere attachment in the gut, adequate tools and assays are necessary to observe and quantify this event that leads to infection. A fluorescent-based quantitative adhesion assay, using biotinylated activated-oncospheres and monolayers of Chinese hamster ovary cells (CHO-K1) or human intestinal monolayer cells (INT-407, HCT-8 or HT-29), was developed to study initial events during the infection of target cells and to rapidly quantify the *in vitro* adhesion of *T. solium* oncospheres. Fluorescein streptavidin was used to identify biotinylated activated-oncospheres adhered to cells. This adherence was quantified using an automated fluorescence plate reader, and the results were expressed as fluorescence intensity values. A series of three assays were performed. The first was to identify the optimum number of biotinylated activated-oncospheres to be used in the adhesion assay. The goal of the second assay was to validate this novel method with the established oncosphere-binding system using the immunofluorescent-antibody assay (IFA) method to quantify oncosphere adhesion. A total of 10,000 biotinylated activated-oncospheres were utilized to assess the role of sera and laminin (LM) in oncosphere adherence to a CHO-K1 cell monolayer. The findings that sera and LM increase the adhesion of oncospheres to monolayer cells were similar to

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results that were previously obtained using the IFA method. The third assay compared the adherence of biotinylated activated-oncospheres to different types of human intestinal monolayer cells. In this case, the fluorescence intensity was greatest when using the INT-407 cell monolayer. We believe this new method of quantification offers the potential for rapid, large-scale screening to study and elucidate specific molecules and mechanisms involved in oncosphere-host cell attachment.

Keywords

Taenia solium oncosphere; biotinylation; quantitative adhesion assay; epithelial cells

Introduction

Human and Porcine cysticercosis is a disease caused by the larval cestode *Taenia solium*. The adult tapeworm lives exclusively in the small intestines of humans. Both humans and pigs can acquire cysticercosis, the larval form of the infection, by ingesting *T. solium* eggs released by the adult tapeworm through fecal-oral contamination. This parasitic disease is a major public health problem in developing countries and causes great economic loss to farmers in developing countries. It is strongly correlated with poor sanitary conditions and a deficient sanitation infrastructure in regions where pig rearing is common (Garcia et al., 1993; Willingham and Engels, 2006, Budke et al., 2009)

When cysticercosis involves the central nervous system, it is called neurocysticercosis. Neurocysticercosis is common throughout Latin America, most of Asia, Sub-Saharan Africa, and parts of Oceania. Human neurocysticercosis is believed to be the leading cause of acquired epilepsy worldwide (Garcia et al., 1993; Garcia et al., 2005; Mahanty et al., 2010).

The eggs of *T. solium* contain a multinucleated embryo called an oncosphere. This oncosphere is released into the intestine when the egg hatches. Oncospheres become activated, cross the intestinal wall and migrate to the muscles, eyes and central nervous system. Once in the tissue the oncosphere can transform into a cysticercus, a larval stage where the parasite is invaginated in a cyst filled sac.

The exact mechanism of how *T. solium* oncospheres infect the host and develop into cysticerci is still not known. Other microorganisms often initiate infection by adhering to host cells, and for this reason our research on *T. solium* focuses on host cell adherence. While it has been shown that *T. solium* oncospheres can adhere to host cells (Verastegui et al., 2007), it is still unclear which parasite or host proteins are involved in this mechanism. An understanding of the adherence proteins could allow for the development of various preventive interventions, including the development of vaccines that could inhibit parasite adherence and consequently infection.

It is important to establish a reliable and efficient *in vitro* model in order to investigate the role of proteins in adherence. We previously reported an *in vitro* model in Chinese Hamster Ovary cells (CHO-K1) to study the adherence of *T. solium* oncospheres to host cells. In this model, an immunofluorescent-antibody assay (IFA) was used to quantify oncosphere attachment to CHO-K1 cells by direct visualization of attachment (Verastegui et al., 2007). The IFA proved to be useful for direct visualization of attachment, but is time-consuming (Joe et al., 1998).

While CHO-K1 cells have been used in various adhesion assays for studies on other microorganisms, these cells are not of intestinal origin and therefore probably do not possess the same receptors as intestinal cells. Using cells that more closely resemble *in vivo* conditions would enhance the applicability of these *in vitro* studies. For this reason, we developed a Fluorescent-based quantitative adhesion assay using biotinylated activated-oncospheres with CHO-K1 cells (as a control cell line) or alternatively with Human intestinal cells (INT-407 cells (jejunum-ileum), HCT-8 cells (ileum-cecum) or HT-29 cells (colon)) as comparison cell lines.

Materials and methods

Taenia solium oncosphere preparation

The hatching of eggs and the activation of *T. solium* oncospheres were performed as described by Verastegui et al. (2007). Adult *T. solium* tapeworms were collected from newly diagnosed patients. The eggs were obtained from gravid proglottids of adult tapeworms by gentle homogenization in a 2.5 % potassium dichromate solution (Sigma, St. Louis, Missouri). Eggs were then washed three times in distilled water by centrifugation prior to hatching. The eggs were hatched and oncospheres were released with a solution of 0.75% sodium hypochlorite (Mallinckrodt Baker, Inc, Phillipsburg, NJ) in water for 10 min at 4°C. Oncospheres were then washed three times in RPMI medium (Sigma Aldrich, St. Louis, MO). They were activated by incubation at 37°C for 45 min with artificial intestinal fluid (1 g pancreatin [Sigma Aldrich, St. Louis, MO], 200 mg Na₂CO₃, and 1 ml. pig bile, with the volume adjusted to 100 ml with RPMI 1640 medium [pH 8.04]) followed by another incubation for 1 hour at 37°C in binding medium (RPMI medium containing 2% fetal bovine serum (FBS)). After activation, the oncospheres were washed three times in prewarmed (37°C) phosphate-buffered saline (PBS), pH 7.5. The oncospheres were then resuspended in this buffer and counted using a Neubauer chamber (Fisher Scientific, Atlanta, Georgia).

Biotinylation of *T. solium* activated oncospheres

We tested different concentrations of NHS-LC-Biotin (Thermo Scientific, Rockford, IL) in order to find the optimum concentration for labeling activated oncospheres. The activated oncospheres were washed and resuspended in prewarmed (37°C) PBS and a solution of 0.5 mM, 1mM or 2 mM of biotin in dimethyl sulfoxide was added and incubated for 25 min at -37°C. The reaction was stopped by adding 10 mM Tris/HCl, pH 8. The oncospheres were then washed 3 times in TBS (10 mM Tris/140 mM NaCl, pH 8.06) and resuspended in binding medium. To ensure that the oncospheres had been correctly labeled with biotin, the oncospheres were fixed on a slide and were then incubated with 20µg/ml of fluorescein streptavidin (Vector laboratories, Inc.Burlingame, CA) for 1 hour at 37°C and then were analyzed by direct visualization with fluorescent microscopy. The trypan blue exclusion method was also used to assess oncosphere viability before and after biotin treatment.

Cell culture

Established lines of adult Chinese Hamster Ovary cells (CHO-K1), Caucasian intestine embryonic cells (INT-407), Human colorectal adenocarcinoma cell line (HCT-8), and Human colon adenocarcinoma grade II cell line (HT-29) were obtained from ATCC (Manassas, VA). Cultures were routinely maintained in Ham's F-12K medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% FBS and 40 µg/ml gentamicin for CHO-K1 cells, BMEN medium supplemented with 10 % FBS for INT-407 and HT-29 cells, and RPMI medium supplemented with 10% of FBS for HCT-8 cells. Cells were incubated under 5% CO₂ in the ambient environment at 37°C. The medium was changed every 2 days and cells were passaged when a monolayer reached confluence. Cells were harvested using a

trypsin-EDTA treatment (Sigma Aldrich, St. Louis, MO) after a complete monolayer was formed in a culture flask. The cells were then seeded into a 96 Well Black w/Clear Flat Bottom Cellstar Plate (Phenix Research Products, Candler, NC). Adherence assays were performed only when after the cells formed a monolayer.

Fluorescent-Based quantitative adhesion assays

In order to develop a Fluorescent-based quantitative adhesion assay, biotinylated activated-oncospheres and monolayer CHO-K1 cells were used. Viable monolayer cells were used immediately after monolayer formation in 96 Well Black w/Clear Flat Bottom Cellstar Plates. The viable monolayer cells were first blocked with 1% of Bovine serum albumin (BSA) in PBS for 1 h at 37°C. They were then washed with PBS and incubated with biotinylated activated-oncospheres in a binding medium at 37°C for 90 minutes. After the incubation, the unbound oncospheres were rinsed three times with RPMI, and the oncospheres bound to the cells were fixed with methanol-acetone (1:1) for 10 min at -20°C. After two washes with PBS-Tween 20 (0.05 % v/v) the wells were incubated for 1 h at 37°C with 20ug/ml of fluorescein streptavidin. After three washes with PBS, attached oncospheres were quantified as fluorescence intensity using a Plate Chamaleon V Microplate Reader (485 nm excitation and 535 nm emission) (Hidex, Turku, Finland).

Standardization of the number of activated oncospheres to be used in the Fluorescent-Based quantitative adhesion assay

In order to identify the optimum number of parasites needed for subsequent adhesion assays, we conducted the fluorescent-based quantitative adhesion assays adhesion with CHO-K1 cells using a variable number of biotinylated activated-oncospheres (0 – 15,000). The fluorescent-based quantitative adhesion assay was performed as described above.

Validation of the Fluorescent-based quantitative adhesion assay in CHO-K1 cells

Role of sera in oncosphere adhesion in CHO-K1 cells—In a previous study, our laboratory showed that *T. solium* oncosphere adhesion increased linearly as the serum concentration of FBS in the binding medium increased (Verastegui et al., 2007). We assessed our Fluorescent-based quantitative adhesion assay by reproducing these assays, measuring adherence with increasing concentrations of FBS. The viable monolayer CHO-K1 cells were blocked with 1% BSA in PBS and then incubated with 10,000 biotinylated *T. solium* activated oncospheres (which is the optimum number of biotinylated oncospheres used in our new method as described above) in RPMI medium with 0, 2.5, 5, 7.5 and 10% of FBS at 37°C with 5% CO₂. The biotinylated activated-oncospheres bound to monolayer CHO-K1 cells were quantified using a plate reader as described above.

Role of laminin (LM) in oncosphere adhesion in CHO-K1 cells—We compared the adhesion assay in presence of LM and anti-LM using our fluorescent-based quantitative adhesion assay and the IFA method (Verastegui et al., 2007) in order to validate our new method and to determine the role of laminin in the adherence mechanism of *T. solium* oncosphere to CHO-K1 cells.

Fluorescent-Based quantitative adhesion assay: Three wells with CHO-K1 monolayer cells were incubated with 10,000 biotinylated activated-oncospheres in the presence of 90 µg/ml of Natural Mouse LM (Invitrogen life technologies, Carlsbad, CA) diluted in RPMI media. Three wells with CHO-K1 monolayer cells were incubated with 10,000 biotinylated activated-oncospheres in the presence of 90 µg/ml of LM plus anti-LM rabbit serum (1:1) diluted in RPMI media. Finally, three wells with CHO-K1 cell monolayers were incubated with 10,000 biotinylated activated-oncospheres in the presence of 90 µg/ml of LM plus

preimmune rabbit serum (1:1) diluted in RPMI media. The biotinylated activated-oncospheres bound to monolayer CHO-K1 cells were quantified by the use of a plate reader as described above. Controls consisted of biotinylated activated-oncospheres incubated with monolayer CHO K-1 cells and RPMI media alone.

Immunofluorescent-antibody assay (IFA) method: This *in vitro* model was used as previously described (Verastegui et al., 2007). The *T. solium* oncosphere-binding system was performed over confluent CHO-K1 cell monolayers in 8-well chamber slides system with 0.7 cm² well areas (Nalgene-Nunc, Rochester, NY). Three wells with CHO-K1 monolayer cells were incubated with 5,000 activated oncospheres in the presence of 90 µg/ml of LM diluted in RPMI media. Three wells with CHO-K1 monolayer cells were incubated with 5,000 activated oncospheres in the presence of 90 µg/ml of LM plus anti-LM rabbit serum (1:1) diluted in RPMI media. Three wells with CHO-K1 cell monolayers were incubated with 5,000 activated oncospheres in the presence of 90 µg/ml of LM plus preimmune rabbit serum (1:1) diluted in RPMI media. Controls consisted of activated oncospheres incubated with monolayer CHO K-1 cells and RPMI media alone. They were then washed with RPMI to remove the non-adherent oncospheres and subsequently fixed with methanol-acetone (1:1). The activated oncospheres bound to monolayer CHO-K1 cells were stained by indirect immunofluorescence using anti-oncosphere rabbit hyperimmune serum and goat anti-rabbit immunoglobulin G labeled with fluorescein isothiocyanate (Vector laboratories, Inc. Burlingame, CA) to visualize and quantify oncosphere adherence to monolayer cells (Verastegui et al., 2007). Only activated oncospheres without oncospherical membranes were counted.

Fluorescent-Based quantitative adhesion assay in human intestinal epithelial cells

Parallel *in vitro* adhesion assays with INT-407, HCT-8 and HT-29 cell monolayers using 10,000 biotinylated activated-oncospheres were conducted in order to compare characteristic adhesion of *T. solium* oncosphere to different epithelial cells. The previously described fluorescent-based quantitative adhesion assay with CHO-K1 cells served as controls for the assays using intestinal cell lines.

Data analysis

Every assay was performed in triplicate, and each set of assays was independently replicated at least 2 times. Results were expressed as fluorescence intensity mean values, with 95% confidence intervals (CI). The Student's t-test was also performed using the software Stata 10.0, with a significance level of 0.05.

Results

Biotinylation of *Taenia solium* activated oncospheres

As seen in Fig.1 there is a strong and clear fluorescent signal in the activated oncosphere when we used 0.5 mM of biotin, whereas the non activated-oncospheres were not labeled with this concentration. The percentage viability of activated-oncospheres before and after the biotinylation was 97.24% and 97.51% respectively. These differences were not statistically significant ($p = 0.8659$) which indicates that the biotin does not affect the viability of activated oncospheres.

Standardization of the number of biotinylated activated oncospheres to be used in the Fluorescence-Based quantitative adhesion assay

We labeled the activated oncospheres with biotin and then performed the fluorescent *in vitro* adhesion assay. A plate fluorescent reader quantified the biotinylated activated-oncospheres

attached to the CHO-K1 cell monolayer and the results were expressed as fluorescence intensity. Fig.2 shows that the fluorescence intensity was proportional to the number of biotinylated activated-oncospheres added to each well containing the CHO-K1 cells monolayer. From 2,500 biotinylated activated-oncospheres we obtained fluorescence intensity significantly higher than using monolayer cells alone. Nevertheless, we chose to use 10,000 biotinylated activated-oncospheres per well for all subsequent studies for two reason: 1) Increase the sensitivity in order to detect the minimal difference when doing different assay of oncosphere adhesion. 2) Due to a limited number of parasites available to the laboratory, we could not exceed the number of 10,000 parasites per well.

Validation of the Fluorescent-Based quantitative adhesion assay

Role of sera in the adhesion assay in CHO-K1 cells monolayer—In the previous study using the IFA to quantify the *T. solium* oncosphere adhesion to CHO-K1 cells (Verastegui et al 2007), it was determined that RPMI media plus fetal bovine serum (FBS) increased oncosphere adherence to viable CHO-K1 cell monolayers. We found similar results with our new fluorescent-based quantitative adhesion assay compared to the previously reported method of assessment. We observed that the fluorescence intensity increased in the presence of FBS, and also reached a plateau when over 7.5% of FBS.

Role of LM in the adhesion assay in CHO-K1 cell monolayers—We used 90 µg/ml of LM and a solution of 1:1 anti-LM in RPMI in the current Fluorescent-based quantitative adhesion assay to compare these results with the established IFA method. Fig.3 (A) shows that, in the presence of LM, the fluorescence intensity of oncospheres adhered to CHO-K1 cells significantly increased ($p = 0.036$) (this condition was compared to adhesion with RPMI alone), indicating that LM increased oncosphere adhesion. When this assay was conducted in the presence of LM plus anti-LM, we observed a significant decrease in the fluorescence intensity of oncospheres adhered to CHO-K1 cells (this condition was compared with RPMI plus LM alone ($p = 0.0098$) and RPMI plus LM plus pre-immune serum alone ($p = 0.0001$)). This supports the hypothesis that LM is important in the mechanism of adhesion of *T. solium* oncospheres. These results are similar to those using the IFA method as seen in the Fig.3 (B). On the other hand, Fig.4 also shows that LM plus pre-immune serum greatly increased the fluorescence intensity or the number of oncospheres adhered to CHO-K1 cells.

Fluorescent-based quantitative adhesion assay in human intestinal epithelial cells

Fig.4 shows that the observed fluorescence intensity was proportional to the number of biotinylated activated-oncospheres added to each well containing the intestinal epithelial cell monolayer. This indicates that these cells are functional and could be utilized within this model. Also it shows that the oncospheres displayed the highest adhesion to INT-407 cells. The level of fluorescence intensity, which corresponds to the amount of oncosphere adhesion, was significantly different from the other intestinal cell lines (HCT-8 ($p < 0.01$) and HT-29 ($p < 0.001$)) when we used 5,000 to 15,000 oncospheres. No statistical difference in the fluorescence intensity values was observed between HCT-8 and HT-29 cells. In developing this fluorescent-based quantitative adhesion assay, the use of a cell line that exhibits high oncosphere adherence will allow for ease and efficiency in studying the oncosphere attachment to intestinal cells, indicating that INT-407 should be used in future experiments rather than other types of epithelial cells.

Discussion

This study describes a new method to quantify oncosphere adhesion to epithelial cells based on the use of biotinylated activated-oncospheres and their subsequent detection by

fluorescein streptavidin. When we tested different concentrations of biotin, we found that 0.5mM of biotin was ideal to label activated oncospheres. We standardized our fluorescent-based quantitative adhesion assay in CHO-K1 cells and we found that FBS and LM increase the *in vitro* adhesion of *Taenia solium* oncospheres to CHO-K1 cells. In this study we also standardized our fluorescent-based quantitative adhesion assay in different types of human intestinal epithelial cells, where *T. solium* oncosphere has higher adhesion to cell lines originating from the upper part of the small intestine. This method does not require counting of individual oncospheres by eye thus lending itself to high through put, increased objectivity and decreased labor costs.

We chose to label the oncospheres with biotin because it can subsequently be conjugated to many proteins without altering its biological activities. Also, due to its small size (244 Da), biotin labeling of adherent cells (Pearce-Pratt et al., 1991; Mendis et al., 2001; De Almeida Dias et al., 2007), bacterium (Ofek et al., 1986; Fallgren, et al., 2001; Yurdusev, 2001) and parasites (Flores-Romo et al., 1993; Moreno et al., 2005) has been successfully performed without interfering with the binding process. In the adhesion assay it is important to differentiate between activated and non-activated oncospheres because *in vivo* only the activated oncosphere can penetrate the host intestinal wall, and therefore, we only considered and quantified activated oncospheres in the adhesion assay (Verastegui et al, 2007). For this reason, we tested different concentrations of biotin in order to label only activated oncospheres. The difference in labeling between activated and non-activated oncospheres occurs because activated oncospheres lose their oncospherical membrane during the activation process (Verastegui et al., 2007). The activated oncosphere has a free oncospherical tegument, which is rich in proteins and after activation, granules and proteins localized exclusively in the penetration gland cells of the oncosphere become distributed in the oncospherical tegument (Jabbar et al., 2010). This would make it easier for the biotin to access oncosphere proteins and consequently label them without altering the viability of the activated oncosphere. In contrast, non-activated oncospheres, that still retain their oncospherical membrane, offer an additional barrier for biotin labeling oncosphere proteins, because the oncosphere membrane has a high concentration of lipids (Smyth and McManus, 1989) which makes the biotin does not label the oncospherical membrane.

In this study, we standardized our fluorescent-based quantitative adhesion assay in CHO-K1 cells. This line cell was used previously in developing the IFA method of quantification, and consequently we can compare our results with those obtained using the IFA method (Verastegui et al., 2007). In our novel fluorescent-based quantitative adhesion assay, the biotinylated activated oncospheres adhering to CHO-K1 cells were detected by fluorescent streptavidin. Biotin binds with high affinity to streptavidin proteins (Weber et al., 1989; Sélo et al., 1996) and the interaction between biotin and fluorescent streptavidin can be detected using a fluorescence reader. In many studies, the interaction between biotin and streptavidin has been used to quantify parasite adherence to cells *in vitro*. The biotinylation offers the possibility of using various second-step labeling reagents such as fluorescein (Yurdusev, 2001) as used in our study, or alternatively, peroxidase that can be used in an ELISA-plate reader (Ofek et al., 1986; Fallgren et al., 2001; De Almeida Dias et al., 2007). In this work, we also used peroxidase streptavidin (*data not shown*), however, we obtained a high background on monolayer cells alone, likely due to the presence of endogenous peroxidase present in the cells. Furthermore, using peroxidase involves an additional step, thus making the assay more laborious. For these reasons we chose to use fluorescein streptavidin.

Using our fluorescent-based quantitative adhesion assay, we found that the presence of FBS in binding medium increases *T. solium* oncosphere adhesion to CHO-K1 cells. This result is similar to that reported before using the IFA quantification (Verastegui et al., 2007). By independently reaching the same conclusion, we were able to validate both methods of

quantification. These findings are corroborated by other studies which have demonstrated that sera increase the adhesion of *Echinococcus multilocularis* and *E. granulosus* protoscoleces to human endothelial cell monolayers (Kirkpatrick and Svilenov, 1987). Serum components, such as fibronectin, laminin, collagen, and vitronectin, may play an important role in oncosphere-host cell interactions (Pearlstein et al., 1980; Ruoslahti et al., 1985; Hogemann et al., 1986). Using the IFA method, we found that laminin and fibronectin increase oncosphere adherence (data not shown) which may explain why serum increases parasite adherence, however, it will be important to evaluate the probable role of other serum components as well.

Also, using our fluorescent-based quantitative adhesion assay, we demonstrated that LM is able to increase *T. solium* oncosphere adhesion to monolayer CHO-K1 cells, results similar to using the IFA quantification. These results suggest that LM is an important mediator of *T. solium* oncosphere adhesion to host cells. The specificity of the LM binding was demonstrated when we conducted an inhibition assay of oncosphere adhesion with hyperimmune sera against LM. These findings are corroborated by other studies which have demonstrated that LM, an extracellular matrix glycoprotein, provides a mechanism of adherence for several human pathogens like *Toxoplasma gondii* (Furtado et al., 1992), *Trichomonas vaginalis* (Crouch and Alderete, 1999), and *Leishmania donovani* (Ghosh et al., 1999). These pathogens express laminin-binding protein which mediates cellular attachment to eukaryotic host cells. In cestodes it has also been reported that *Echinococcus granulosus* expresses a laminin binding protein (Zhang et al., 1997). In addition, oncospheres incubated with laminin substrate showed an increase in the expression of elongated surface microvilli (data not shown), which have been shown to be important in oncosphere attachment to host cells (Verastegui et al., 2007). This could explain why laminin increases oncosphere adherence. The activated oncosphere of *T. solium* probably has a laminin binding protein that mediates oncosphere adhesion. It will be important to identify this laminin binding protein in the oncosphere surface, because the action of these *T. solium* oncosphere proteins could potentially be inhibited through vaccination.

On the other hand, LM plus pre immune sera showed a high percentage of adhesion of oncospheres to CHO-K1 cells. This is likely because serum contains other proteins such as fibronectin and other factors which can increase adherence (Curtis and Forrester, 1984), indicating that there are also other proteins important in the mechanism of *T. solium* oncosphere adherence that can be studied.

It is important to note, however, that CHO-K1 cells are a cell line derived from the ovaries of a Chinese hamster. This cell line has been used in many *in vitro* assays to study adhesion mechanisms of other human pathogens such as *Entamoeba histolytica* (Ravdin and Guerrant, 1981) and *Trichomonas* (Bonilha et al., 1995). However, these cells are not representative of typical intestinal epithelium. Because of this they probably do not present specific receptors that may be present in host intestinal epithelial cells. For this reason we also conducted the fluorescent-based quantitative adhesion assay using different types of human intestinal epithelial cells such as INT-407 cells (jejunum-ileum), HCT-8 cells (ileum-cecum) and HT-29 cells (colon). Adhesion analysis revealed that the *T. solium* oncospheres, in general, showed higher adhesion to cell-lines originating from the upper part of the small intestine (jejunum-ileum, INT-407) than those from the lower part of the small intestine (ileum to cecum, HCT-8) or the upper part of the large intestine (colon, HT-29). The INT-407 cell line was derived from nonmalignant jejunum and ileum of a 2-month-old human embryo, and it has a complex ultrastructural fimbrial extracellular matrix. Recently, this cell line has been utilized to study the intestinal attachment of *Giardia lamblia* (Sousa et al., 2001). Therefore, the human INT-407 cell line represents an *in vitro* model to investigate the adhesion of *T. solium* oncospheres with intestinal cells.

This fluorescent-based quantitative adhesion assay for assaying oncosphere adherence to epithelial monolayer cells has many advantages. First, we have reduced the time to perform the assay. The process of making biotinylated activated-oncospheres involves only one step for labeling with fluorescein streptavidin, whereas in the previous IFA method two steps were required to prepare the oncospheres. Second, a fluorescence reader was used instead of visual observation to quantify bound oncospheres. This method of automated data collection reduces elements of human variability and error. Third, this new method of quantification allows for a higher number of samples to be processed at one time, as a 96 well plate was utilized in this new method instead of the 8 well chamber slides that was previously used in the IFA method. An accurate and efficient in vitro adhesion model offers a powerful tool for researchers to further elucidate and understand the mechanisms involved in *T. solium* adherence.

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Abbreviations

CHO-K1	Chinese hamster ovary cells
IFA	immunofluorescent-antibody assay
LM	laminin
FBS	fetal bovine serum
BSA	Bovine serum albumin
PBS	phosphate-buffered saline
INT-407	Caucasian intestine embryonic cells
HCT-8	Human colorectal adenocarcinoma cell line
HT-29	Human colon adenocarcinoma grade II cell line

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Highlights

We described a new method to quantify oncosphere adhesion to epithelial cells. We used biotinylated oncospheres and CHO-K1 or human intestinal monolayer cells. Sera and laminin increased the *in vitro* adhesion of oncospheres to CHO-K1 cells. Oncosphere has higher adhesion to cell from the upper part of the small intestine. It offers a rapid, large-scale screening to study the oncosphere-host cell adhesion.

Fig. 1. Illustration of biotinylated oncospheres incubated with 20 $\mu\text{g/ml}$ of fluorescein streptavidin. Light microscopy photographs with Evan's blue (A) and ultraviolet microscopy photographs (B) show the same field of view (Magnification 40X). We also show the difference in the fluorescence intensity between a biotinylated activated-oncosphere (thick arrow) and a biotinylated non activated-oncosphere (thin arrow).

Fig. 2. Measures of fluorescence intensity when using varying numbers of biotinylated activated-oncospheres per well (from 2500 up to 15000). 95% Confidence intervals for the mean values of fluorescence intensity of biotinylated activated-oncospheres adhered to CHO-K1 monolayer cells.

Fig. 3. Comparison of Fluorescent-based quantitative adhesion assay (A) and IFA method (B) in presence of LM, LM plus anti-LM and LM plus Preimmune sera. 95% Confidence intervals for the mean values of fluorescence intensity (A) or number (B) of oncospheres adhered to CHO-K1 monolayer B cells.

Fig. 4. Measures of fluorescence intensity when using varying numbers of biotinylated activated-oncospheres per well (from 2500 up to 15000). Mean and standard deviations of fluorescent intensity of biotinylated activated-oncospheres adhered to different intestinal monolayer cells.