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CHARACTERIZATION OF THE CARBOHYDRATE COMPONENTS OF *Taenia solium* ONCOSPHERE PROTEINS AND THEIR ROLE IN THE ANTIGENICITY

Yanina Arana[1], **Manuela Verastegui**[1], **Iskra Tuero**[2], **Louis Grandjean**[1,3], **Hector H. Garcia**[1,4], and **Robert H. Gilman**[1,5,6]

[1]Department of Cellular and Molecular Sciences, Faculty of Sciences and Philosophy, Universidad Peruana Cayetano Heredia, PO Box 5045, Lima, Peru

[2]Vaccine Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892–5065, USA

[3]Wellcome Centre for Clinical Tropical Medicine, Imperial College St. Mary's Campus, Praed Street, London, W2 1PG

[4]Cysticercosis Unit, Instituto de Ciencias Neurologicas. Jr. Ancash 1271, Barrios Altos, Lima, Peru

[5]Department of International Health, Johns Hopkins University, Bloomberg School of Hygiene and Public Health, Baltimore, 615 N. Wolfe Street Room W#5515, Baltimore, MD 21205, USA

[6]AB PRISMA, Calle Carlos Gonzales 251, San Miguel, Lima 32 – Peru

Abstract

This study examines the carbohydrate composition of *Taenia solium* whole oncosphere antigens (WOAs), in order to improve the understanding of the antigenicity of the *T. solium*. Better knowledge of oncosphere antigens is crucial to accurately diagnose previous exposure to *T. solium* eggs and thus predict the development of neurocysticercosis. A set of seven lectins conjugates with wide carbohydrate specificity were used on parasite fixations and somatic extracts. Lectin fluorescence revealed that D-mannose, D-glucose, D-galactose and N-acetyl-D-galactosamine residues were the most abundant constituents of carbohydrate chains on the surface of *T. solium* oncosphere. Lectin blotting showed that post-translational modification with N-glycosylation was abundant while little evidence of O-linked carbohydrates was observed.

Chemical oxidation and enzymatic deglycosylation *in situ* were performed to investigate the immunoreactivity of the carbohydrate moieties. Linearizing or removing the carbohydrate moieties from the protein backbones did not diminish the immunoreactivity of these antigens, suggesting that a substantial part of the host immune response against *T. solium* oncosphere is directed against the peptide epitopes on the parasite antigens. Finally, using carbohydrate probes, we demonstrated for the first time that the presence of several lectins on the surface of the

Corresponding Author: Manuela Verastegui, PhD. Department of Microbiology, Faculty of Sciences and Philosophy, Universidad Peruana Cayetano Heredia, PO Box 5045, Lima, Peru. Phone (511) 4832942, mveraste@jhsph.edu.

oncosphere was specific to carbohydrates found in intestinal mucus, suggesting a possible role in initial attachment of the parasite to host cells.

Keywords

Taenia solium; oncosphere; lectins; carbohydrates; deglycosylation; periodate oxidation; glycosidases

Introduction

Worldwide, parasitic cestodes, such as *Taenia solium, Taenia saginata, Echinococcus granulosus* and others are the causative agents of several diseases in animals and humans. The life cycle of *T. solium* includes the pig as the normal intermediate host, harboring the larval vesicles or cysticerci; and humans as the definitive host, harboring the adult tapeworm. Humans can also serve as the intermediate host and develop the cystic form after ingesting *T. solium* eggs found in food or water contaminated with feces (Verastegui et al. 2003; Verastegui et al. 2002). Human cysticercosis is an important contributor to neuro-pathology in endemic areas, while porcine cysticercosis is an important disease present in 30–60% of free range pigs in Peru and other Latin American countries, and is responsible for widespread economic losses among farmers (Flisser et al. 2004; Flisser et al. 2003; Garcia et al. 2010; Verastegui et al. 2003).

Recent studies on infections produced by helminthic parasites as *Trichinella spiralis*, *Schistosoma mansoni* or *E. multilocularis* have shown that the immune response in infected humans and animals is targeted toward carbohydrate determinants on their outer surface and secreted glycoconjugates (Alvarez et al. 2008; Gruden et al. 2002; Hulsmeier et al. 2010; Kouguchi et al. 2011; Miguez et al. 1996; Nyame et al. 2004). In *T. solium* cysticercosis, the oncosphere (hexacanth larva) and its secretions are a potent source of immunogens (Pathak and Gaur 1990; Plancarte et al. 1999; Verastegui et al. 2003; Verastegui et al. 2002; Zimic et al. 2007). Indeed, several studies have demonstrated that vaccination with oncospheral antigens provides a high degree of protection in pigs (Jayashi et al. 2012; Flisser et al. 2004; Lightowlers et al. 2003; Plancarte et al. 1999; Verastegui et al. 2003; Verastegui et al. 2002). In *T. solium* studies, nearly 100% of all human *T. solium* tapeworm carriers have antibodies to one or both oncospheral antigens of 22.5 and 31.3 kDa (OAs) (Verastegui et al. 2003; Verastegui et al. 2002). This finding highlights the potential for using both OAs as predictors of exposure to *T. solium* eggs and to demonstrate which patients may develop cysticercosis in the future. The 31.3 kDa oncospheral antigen has been cloned (Tso31 immunogen) and tested as a vaccine but failed to confer significant protection against cysticercosis in pigs (Mayta et al. 2007), while the protective role of the 22.5 kDa oncospheral antigen remains unknown. However, the immunity against *T. solium* oncosphere was evaluated by *in vitro* oncosphere-killing assays where activated oncospheres were incubated with sera from pigs vaccinated with the recombinant *T. solium* oncosphere protein TSOL18 and a source of complement. After 10 days of culture the oncospheres were killed in comparison to the control demonstrating that the immunity was mediated by the joint action of antibodies and complement (Kyngdon et al. 2006).

In this study we evaluated the carbohydrate composition on *T. solium* whole oncosphere antigens (WOAs) and its possible role in antigenicity. Detailed knowledge of sugar composition of the glycocalix of *T. solium* oncosphere is not available. To improve the understanding of the function of WOAs it is important to know if antigenicity is conferred by their carbohydrate or peptide composition. Furthermore, using carbohydrate probes we describe for the first time the presence of lectins on the *T. solium* oncospheral surface that could be involved in the host-parasite interaction.

To study the composition and distribution of carbohydrates on *T. solium* oncosphere, a set of lectin conjugates with wide carbohydrate specificity were used on parasites, which were fixed on the slides by fluorescent histochemistry and their somatic extract by the lectin blot assay. The relative contribution of carbohydrates to the antigenicity of WOAs was addressed by chemical oxidation with sodium periodate, and by enzymatic deglycosylation. The changes in antigenicity following these two approaches were analyzed by Western blot employing sera from pigs immunized with crude extract of *T. solium* oncospheres.

Materials and methods

Oncosphere preparation

Adult tapeworms of *T. solium* were collected as described before in Verastegui et al. (2003). Briefly, the gravid proglottides were collected by sieving fecal samples collected after treatment and washed thoroughly with distilled water and stored in 25% glycerol supplemented with penicillin (1000 UI/mL), gentamicin (100 μg/mL), amphotericin B (0.02 mg/mL), and streptomycin (1 mg/mL) at 4°C until used. Species were differentiated by histopathology and polymerase chain reaction (PCR) - restriction enzyme analysis as previously described in Mayta et al. (2000). Eggs were obtained from gravid proglottides in a 2.5% potassium dichromate solution (SIGMA, St. Louis, MO), incubated for 48 hours at 4°C and subsequently stored in 25% glycerol until used. Eggs were washed three times in distilled water prior to hatching. *In vitro* hatching of oncospheres was performed using 0.75% sodium hypochlorite for 10 minutes at 4°C. Oncospheres were washed three times and resuspended in RPMI 1640 media (SIGMA) supplemented with penicillin (1,000 IU/mL) and gentamicin (100 g/mL). Oncospheres were counted using a Neubauer chamber.

Characterization of carbohydrates on the surface *T. solium* oncosphere using lectins labeled with FITC

After washing with RPMI media, oncospheres were activated by incubation for 1 h at 37°C in artificial intestinal fluid (AIF, pancreatin 1% (SIGMA), fresh porcine bile 1%, $NaHCO_3$ 0.2% in medium RPMI-1640, pH 8.04). Activated oncospheres were washed three times with RPMI media and resuspended in phosphate buffered saline (PBS, 0.02 M Na_2HPO_4 / NaH_2PO, 0.15 M NaCl, pH 7.2). Well slides (MP Biomedicals, Cleveland, OH) were covered with poly-L-lysine (SIGMA) following the manufacturer's instructions. Activated oncospheres (300 oncospheres / 15 uL / well) were allowed to rest on the poly-L-lysine-coated slides at room temperature until dry. Attached activated oncospheres were incubated for 2 h at room temperature with 0.05% PBS-Tween 20 (PBS-T) containing different concentrations of each of the following biotinylated lectins (VECTOR LABORATORIES,

Burlingame, CA): *Concanavalia ensiformis*-Con A (α-D-mannose / α-D-glucose), 20 μg/mL; *Lens culinaris* agglutinin-LCA (α-D-mannose), 40 μg/mL; *Ricinus communis* agglutinin I-RCA I (α-D-galactose), 40 μg/mL; *Glycine maximus* (soybean) agglutinin SBA (α-galNAc), 40 μg/mL; *Ulex europaeus* agglutinin I-UEA I (α-L-fucose), 100 μg/mL; *Triticum vulgaris*-Wheat germ agglutinin WGA (GlcNAc), 40 μg/mL and *Maackia amurensis* lectin II-MAL II (sialic acid / Neu5Ac), 100 μg/mL. Activated oncospheres incubated with 0.05% PBS-T were used as the negative control. Slides were then washed four times with PBS and incubated 1 h at room temperature with a 1:50 dilution of fluorescein isothiocyanate (FITC)-conjugated Streptavidin (VECTOR LABORATORIES), in PBS containing 0.005% of Evan's blue (SIGMA). Slides were finally washed five times with PBS, mounted in 90% glycerin in PBS, and read using a Nikon fluorescent microscope at 40 x magnifications.

Characterization of lectins on the surface *T. solium* oncosphere using fluorescence carbohydrate probes

Characterization of surface *T. solium* oncosphere lectins was also accomplished using carbohydrate probes labeled with FITC-"Glyc-PAA-fluor", (Multivalent fluoresceinated polymer-monosaccharide) (GLYCO-TECH, Gaithersburg, MD). Activated oncospheres were attached to immunofluorescence slides and incubated with each one of the following carbohydrate probes: α-D-Glucose-PAA-fluor, α-D-Mannose-PAA-fluor, α-D-Galactose-PAA-fluor, α-L-Fucose-PAA-fluor, α-Neu5Ac-PAA-fluor, α-GalNAc-PAA-fluor, β-GalNAc-PAA-fluor, and β-GlcNAc-PAA-fluor. The probes were diluted at 200 μg/mL in buffer A (20 mM HEPES, 150 mM NaCl and 2 mM CaCl. pH 7.4) containing 0.1% of ovoalbumin (SIGMA), then incubated for 1 hour at 4°C. The activated control oncospheres, non-treated with carbohydrate probes, were incubated with buffer A and 0.1% ovoalbumin. Slides were washed three times with buffer A, mounted with buffered glycerin (pH 7.2), and then evaluated using a Nikon fluorescent microscope at 40x.

Extraction of whole oncosphere antigens (WOAs)

WOAs for Western and lectin blot analyses were obtained as described previously Verastegui et al. (2003). Briefly, oncospheres were suspended in RPMI 1640 media (40,000 oncospheres / mL) and sonicated at 4°C three times for 1 min. (1 min. on and 1 min pause) at 70 Hz. The somatic extract was centrifuged at 28,000 x g for 30 min at 4°C. The supernatant was collected and supplemented with proteases inhibitors, Pefabloc SC (AEBSF, - Boehringer Mannheim Biochemicals, Indianapolis, IN) 1mM, Leupeptin (SIGMA) 0.001ug/uL and Pepstatin A (EMD Chemicals Inc, Savannah, GA) 0.001 ug/uL and stored at −70 °C until used.

SDS-PAGE and Western blot

Somatic extract containing the *T. solium* WOAs was analyzed by SDS-PAGE under non-reducing conditions using the Protean II System (BIO-RAD, Hercules, CA) and gradient polyacrylamide gels (5–22.5%) as described previously Verastegui et al. (2003). The electrophoresis was performed using 0.028 ug / uL / mm of WOAs protein. Western blots were performed using pooled positive control sera – protective sera (sera from pigs

vaccinated with WOAs that no developed cysticercosis after experimental infection with *T. solium* eggs) and pooled negative control sera – negative sera (sera from known negative pigs) (Verastegui et al. 2002).

Lectin blot assay

T. solium WOAs were separated by SDS-PAGE under non-reducing conditions using the Protean II System (BIO-RAD, Hercules, CA). The strips were blocked with a blocking solution containing 0.2% PBS-T at 4°C overnight. After washing, each strip was incubated with a different biotinylated lectin (VECTOR LABORATORIES): Con A (2.5 ug/mL), LCA (20 ug/mL), RCA I (20 ug/mL), SBA (20 ug/mL), UEA I (20 ug/mL), WGA (20 ug/mL), and MAL II (20 ug/mL) in 0.05% PBS-T for 2 h at room temperature with gentle rocking, washed five times, and then incubated for 2 h at room temperature with Streptavidin horseradish peroxidase conjugate (Roche Applied Science, Indianapolis, IN) 1:375 diluted in 0.05% PBS-T. After washing, the strips were incubated in 0.06% diaminobenzidine tetrahydrochloride-DAB (SIGMA) (w/v) in 50 mM of Tris-HCl (MP Biomedicals) (pH 7.6) and 0.1% of H_2O_2 (v/v). Blots were developed until a dark brown band appeared. A strip that reacted without biotinylated lectin was used as negative control. Reactions were stopped after 1–2 min by transferring the strips to distilled water.

To exclude non-specific lectin binding, each lectin was incubated in the presence of the appropriate competitive carbohydrate diluted in 0.05% PBS-T at 4°C. The following concentrations were used: α-methyl-glucose, 0.2 M; α-methyl-mannose, 0.2 M; galactose, 0.2 M; α-L-fucose, 0.1 M; N-acetyl-neuraminic acid or NANA, 0.4 M; N–acetyl-galactosamine, 0.2 M and N-acetyl-glucosamine, 0.5 M (Fig. 3).

Antigenicity evaluation

For *in situ* chemical oxidation, the nitrocellulose strips containing *T. solium* WOAs were treated with sodium periodate according to Woodward et al. (1985). Prior to exposure to periodate, the strips were washed with 50 mM sodium acetate buffer (SIGMA) pH 4.6. Strips were then exposed for 30 min at room temperature in the dark to varying concentrations of sodium periodate (SIGMA) 5, 10, 20, 40, 80, 100 and 120 mM in 50 mM sodium acetate buffer. Following a brief rinse with 50 mM sodium acetate, the strips were incubated with 50 mM sodium periodate (SIGMA) in PBS for 30 min at room temperature. Following five washes with PBS-T, the Western and lectin blot were carried out as described above for the lectin binding assays. The serum samples were tested in parallel against treated and untreated periodate nitrocellulose strips. The changes in antigenicity or lectin affinity were evaluated by Western and lectin blot, respectively. The success of the sodium periodate treatment was assessed by the loss of binding of the WOAs to the *Lens culinaris* lectin.

For *in situ* deglycosylation, N-glycosidase F (PNGase F) from *Flavobacterium meningosepticum* (QA-Bio, LLC, Palm Desert, CA) and O-glycosidase from *Streptococcus pneumoniae* (QA-Bio, LLC) were used in enzymatic deglycosylation experiments. To ensure maximum effectiveness of O-glycosidase treatment, a previous *in situ* Sialidase Au (QA-Bio, LLC, Palm Desert, CA) treatment was developed. The somatic antigens were run

in a polyacrylamide gel, blotted onto nitrocellulose membranes, and the strips containing the glycoproteins were blocked for 1 h with 5 mg/mL of BSA in TBS (100 mM Tris-HCl pH 7.5, NaCl 150 mM). The deglycosylation conditions for these enzymes were adopted from manufacturer's instructions. Western blots were then performed to assess any changes in antigenicity. With the use of Con A, lectin blots were done to determine the efficiency of the deglycosylation. Control membranes were submitted to identical conditions except for the absence of enzyme.

Results

Characterization of carbohydrates on *T. solium* oncosphere by lectin fluorescence assay

The lectin binding distribution of *T. solium* oncosphere was studied by fluorescent histochemistry on fixations of activated oncospheres. The results are summarized in Table 1. Reactive carbohydrate molecules were present both at the surface and the internal structures. ConA, and LCA, lectins produced a bright and wide staining which was distributed throughout the oncosphere, with a very strong binding to the surface (Table 1, Fig. 1B). RCA-I and SBA lectins showed selective and very strong binding to internal structures as penetration glands (Table 1, Fig. 1C), and strong binding to the oncospheral surface. WGA showed moderate binding to both the oncospheral surface and internal structures. No specific binding of UEA-I and MAL-II lectins was observed. These lectins were all specifically inhibited from binding to the oncosphere when they were pre-incubated with their specific sugars, suggesting that the binding of the lectins to oncosphere was specific.

Detection of lectins on *T. solium* oncospheral surface

The reactivity of carbohydrate probes is summarized in Table 2. Carbohydrate probes α-D-Mannose-PAA-fluor and α-Neu5Ac-PAA-fluor reacted strongly with activated oncospheres (Table 2 and Fig. 2B) while all other sugars probes had moderate and weak reactions (Table 2), with the exception of α-L-Fucose-PAA-fluor, which did not react at all (Fig. 2C). These results suggest that the activated oncosphere had lectins that reacted mainly with mannose and sialic acid but not with fucose residues.

Lectin-binding studies

Western blot, using positive control sera, showed the antigenic reactivity of eleven bands between 56.23 – 14.44 kDa, that we have described as WOAs (Fig. 3, lane P). The sugar composition of the WOAs was examined by lectin blot using lectins with different monosaccharide specificities for mannose/glucose (Con A, LCA), N-acetylglucosamine (WGA), galactose / N-acetylgalactosamine (RCA-I, SBA), N-acetyl neuraminic acid or NANA or sialic acid (MAL-II) and fucose (UEA-I). Of the lectins used in the present study, UEA-I and MAL-II did not bind to any WOAs bands. Most of WOAs bands (44.65, 40.61, 36.49, 33.86, 22.5, and 14.4 kDa) reacted with Con A, LCA, RCA, SBA, and WGA with different intensity. The band of 56.23 kDa reacted with RCA, SBA, and WGA, but there was no reaction with Con A and LCA was present while 31.3 kDa reacted with Con A, RCA, SBA and WGA (Fig. 3). The specificity of the lectin binding was demonstrated by the decreased intensity of lectins blots after pre-incubation with their specific sugar (Fig. 3). Post-translational modification with N-glycosylation seems to be abundant for WOAs

molecules based on their binding to Con A, LCA, and WGA. These lectins are specific for high mannose-, hybrid-, and complex-type N- linked oligosaccharides with core α1–6 fucosylation, respectively.

Chemical oxidation with sodium periodate

To investigate the antigenic role of carbohydrate moieties on WOAs, changes in the antigenicity of these proteins were evaluated by Western blot after *in situ* sodium periodate treatment of blotted antigens. The treatment with sodium periodate linearizes the hexose ring and destroys any immunological reactivity that carbohydrate may possess, but it does not alter the structure of the polypeptide chain (Chung et al. 2004; Woodward et al. 1985). Although lectin binding (RCA-I and LCA) was shown to be completely abolished at concentrations greater 20 mM (Fig. 4a), the reaction is still even at concentration of 120 mM of the sodium periodate reaction with our positive control sera (Fig. 4b, lanes 1–6). The positive sera bound with equal intensity to treated and control strips; there was no reduction of antigenic reactivity in any of WOAs bands as represented by Western blot (Fig. 4b, lanes P, 1–7). These results demonstrated that the disruption of carbohydrate side-chains by sodium periodate treatment does not affect any of the WOAs antigenicity.

Enzymatic digestion

Based on the N-type glycosylation suggested by the lectins blots, deglycosylation *in situ* with PNGase F was carried out to confirm the presence of these types of carbohydrates on WOAs. Lectin and Western blots were developed to determine the efficacy of deglycosylation, and the changes in antigenicity, respectively. After PNGase F treatment, Western blots did not show any reduction of antigenic reactivity on WOAs bands (Fig. 5, lane 2). Furthermore, the *in situ* deglycosylation was 100% efficient based on the total loss of affinity for biotinylated ConA (Fig. 5, lane 3) and LCA (data not shown) for N- linked carbohydrates on WOAs bands.

O-glycosidase deglycosylation was used to confirm the presence of O-linked carbohydrates and *Arachis hypogaea*-PNA lectin (O-glycan-specific lectin) was used as control to discriminate between N- and O-glycans (Restrepo et al. 2000; Schallig and van Leeuwen 1996; Tantrawatpan et al. 2003; Tian et al. 2004). A unique WOAs band of 56.23 kDa was stained with PNA lectin (Fig. 6, lane 1). This result might indicate that O-linked oligosaccharides are present on *T. solium* oncosphere glycoproteins.

After O-glycosidase treatment, Western blot did not show any reduction of antigenic reactivity on WOAs bands (data no shown). Furthermore, the *in situ* deglycosylation with O-glycosidase was 100% efficient for the 56.23 kDa band based on the total loss of its affinity for biotinylated PNA (Fig. 6, lane 2).

Discussion

In this study, we report the first analysis of the carbohydrate composition and type of glycosylation present in the *T. solium* oncosphere. The lectin binding in fluorescent histochemistry studies on *T. solium* oncosphere fixations (Table 1, Fig. 1) and lectin blot on somatic extract (Fig. 3) suggest that D-glucose, D-mannose, D-galactose and N-acetyl-D-

galactosamine residues are the major glycan constituents on the oncospheral glycoproteins, while N-acetyl-D-glucosamine residues were found in smaller proportions. This carbohydrate composition has been reported in the tegument of *T. solium* metacestode (Alvarez et al. 2008), the adult worm, and protoscolex of *Echinococcus multilocularis* and *E. granulosus*, *Taenia saginata* metacestode, *T. taeniaeformis* and *Hymenolepis diminuta* tapeworms (Georgieva et al. 1999; Hulsmeier et al. 2010; Kouguchi et al. 2011). Our results confirm the presence of all of these carbohydrates as part of the cestode glycocalyx of *T. solium* oncosphere.

In addition to these carbohydrates, an additional residue, sialic acid, has been reported on membrane surface in *T. solium* cysticerci (Landa et al. 2010). In contrast to the strong reaction for sialic acid described in the cysticerci surface, we found on the oncosphere that this carbohydrate, although present, only had a weak reactivity. We also noted the absence of fucose residues on the oncospheral stage, while its presence in the cysticerci stage has not yet been reported. The results for both these residues could be temporal and their appearance conditioned to a change of stage or host as this had been reported for other cestodes (Alvarez et al. 2008; Georgieva et al. 1999; Hammerschmidt and Kurtz 2005). The sugars identified in this study could play an important role in host-parasite interactions.

The lectin binding patterns indicated the presence of extensive N-linked glycosylation (high mannose, hybrid and complex type). The results reaffirmed the findings reported in other studies using neurocysticercosis diagnostic glycoproteins from cysticerci where those studies highlighted the extent of N-linked glycosylation (Haslam et al. 2003; Obregon-Henao et al. 2001; Restrepo et al. 2000). It has been reported that N-linked oligosaccharides are important in protein folding, conformational maturation, intracellular targeting, and cell-cell recognition in eukaryotic cells (Freire 2002; Helenius 1994). In *Entamoeba hystolitica* when N-glycosylation is inhibited the Gal/GalNAc lectin of this amoeba is unable to the target cell, emphasizing the importance of this glycosylation in lectin structure (Petri et al. 2002). Extensive N-glycosylation of the *T. solium* oncosphere could be associated with an effective mechanism of protection against immune system since N-linked oligosaccharides cover large areas of a glycoprotein's surface (Helenius 1994). Several working groups have reported that peptide epitopes are important for the induction of protective immunity in helminth infections and that these epitopes might be masked by carbohydrate epitopes (Georgieva 1999; Hammerschmidt and Kurtz 2005; Schallig and van Leeuwen 1996). N-linked glycosylation seems to be abundant in *T. solium* parasite. This kind of glycosylation had also been reported in a novel *T. solium* oncosphere antigen, Tso31 protein (Mayta et al. 2007) however the biological significance of the N-glycans structures in the *T. solium* oncosphere is unknown.

The oxidation of carbohydrates with sodium periodate is frequently used to assess carbohydrate antigenicity (Jarvis and Pritchard 1992; Obregon-Henao et al. 2001; Schallig and van Leeuwen 1996; Woodward et al. 1985). In this study, the *in situ* oxidation resulted in a strong reduction in lectin binding, which is indicative of terminal carbohydrate disruption; however, the intensity of antigen recognition by positive control sera did not show a significant decrease in WOAs bands as demonstrated by Western blot. Similar results were obtained with PNGase F treatment where this endoglycosidase was employed to

remove N-linked carbohydrates. These results suggest that a substantial portion of the humoral immune response of the host is directed against peptide epitopes from *T. solium* oncosphere. This finding is supported by the studies undertaken by Mayta et. al (2007) where sera from pigs protected against cysticercosis vaccinated with crude oncosphere antigens recognized native Tso31 and Tso31 recombinant, protein exclusive to *Taenia solium* onccosphere and Kingdon et al. (2006) which demonstrated that both the antibodies produced against recombinant *T. solium* oncosphere antigens and complement work in combination to kill of oncopheres *in vitro*. Although carbohydrate moieties may elicit a proportion of the immune system response in *T. solium*, as suggested studies developed for cysticerci antigens (Haslam et al. 2003; Obregon-Henao et al. 2001; Restrepo et al. 2000), they are not likely to be an absolute prerequisite for protection. Nevertheless, vaccination studies involving purified sodium periodate treated WOAs or crude extract must be tested to confirm that peptide portions from these glycoproteins are single-handedly capable of inducing protection against challenge infections.

Conversely to that reported in studies developed in *T. solium* metacestode glycoproteins (Haslam et al. 2003; Restrepo et al. 2000), we found evidence of O-linked glycosylation on *T. solium* oncosphere. O-glycosylation is the most notable molecular alteration in cancer cells, and in recent years these tumor-associated structures have been proven in different species of parasites (Freire 2002). The presence of this kind of glycosylation on one of WOAs bands (56.23 kDa) was screened by using *Arachis hypogaea* – PNA (O-glycan-specific lectin). We also demonstrated its loss of affinity after deglycosylation with O-glycosidase. This study shows that O-linked glycosylation is present on *T. solium* oncosphere glycoproteins but is less dominant when compared to N-linked glycosylation. The role of O-linked oligosaccharides in *T. solium* oncosphere is unknown. In other helminth infections such as *Echinoccocus granulosus* and *Fasciola hepatica*, their presence has been associated with host interaction (Freire T, 2002).

Finally, using carbohydrate probes we reported for the first time the presence of lectins on the *T. solium* oncospheral surface. This study demonstrated that oncospheres of *T. solium* mainly express sialic acid and mannose-specific surface lectins. Gal/GalNac, GlcNAc and Glc specific-lectins were found to be less extensive on the oncospheral surface (Table 2). The presence of these lectins could be related to the mucus adhesion of the parasite during the infection. Mucus is a highly hydrated aggregate secretion that forms a protective barrier at mucosal surfaces (intestinal and respiratory epithelia). Their sugar composition is comprised of Gal, GalNAc, GlcNAc, and fucose, which usually have terminal charged residues, such as sialic acids and sulphate (Hicks et al. 2000). The presence of several specific lectins to carbohydrate components of mucus on *T. solium* oncospheral surface suggests a possible role in initial attachment of the parasite to host intestinal cells as reported in *Pseudomonas aeruginosa* (Woodward et al. 1985), *Cryptosporidium parvum* (Hicks et al. 2000; Joe et al. 1998), Trichomonads (Bonilha et al. 1995; Hicks et al. 2000) and, *Entamoeba histolytica* (Hicks et al. 2000; McCoy et al. 1994; Petri et al. 2002; Ravdin et al. 1989). Mannose residues have not been described as components of intestinal mucus, however the presence of mannose-specific ligand on *T. solium* oncosphere could be involved

in the adherence of a parasite to mannose residues on the surfaces of epithelial cells as described for *Escherichia coli* bacteria (Sharon 2006).

Limitations of the current work include the difficulty in obtaining sufficient sample, the cost related to sample collection, transport and processing, and the lack of access to mass spectrometry and liquid chromatography to further characterize carbohydrate composition in-depth. However, despite these limitations, this study reports the first evidence of the presence of carbohydrates and lectins on *Taenia solium* oncospheres and their role in antigenicity. Further studies with increased amount of whole extract and more sophisticated techniques are warranted.

In summary, the presence of several specific lectins to carbohydrate components of mucus on *T. solium* oncospheral surface suggests a possible role in the initial attachment of the parasite to host cells. Characterization of carbohydrate components using lectins demonstrated that N-linked glycosylation is widespread on the *T. solium* WOAs. Modification of carbohydrates by chemical oxidation or enzymatic deglycosylation did not abolish the reaction of WOAs with protective sera suggesting that glycan composition does not play a significant role in oncosphere's antigenicity. Therefore, the dominant epitopes of the WOAs to stimulate immune response in porcine cysticercosis were found to be mainly peptide. The precise role of carbohydrate and peptide epitopes in protective immunity against *T. solium* cysticercosis remains to be established. Finally, this data suggests a higher probability of success in the production of the WOAs in an *E. coli* recombinant expression system.

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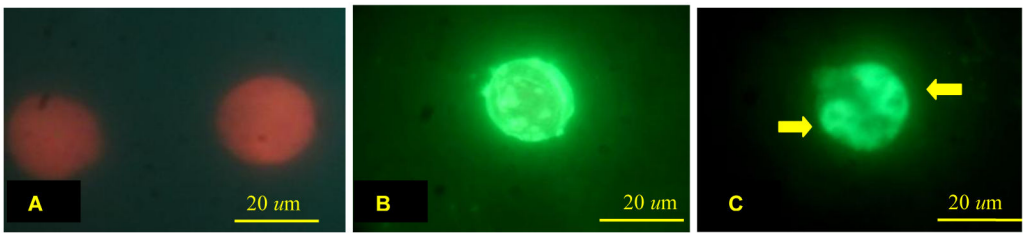

Fig. 1. Lectin fluorescence microscopy on *T. solium* oncosphere fixations shows the presence of carbohydrates on the surface and internal structures

A. Negative control (no lectin), B. LCA binding to oncospheral surface. C. RCA-I binding to internal structures (arrows show the penetration glands).

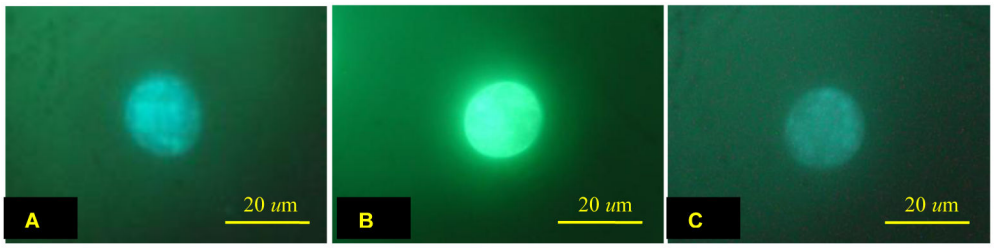

Fig. 2. Carbohydrate probes fluorescence microscopy on *T. solium* oncosphere fixations shows the presence of lectins

A. Negative control (no probe), B. α-D-Mannose-PAA-fluor (positive reaction), C. α-L-Fucose-PAA-fluor (negative reaction).

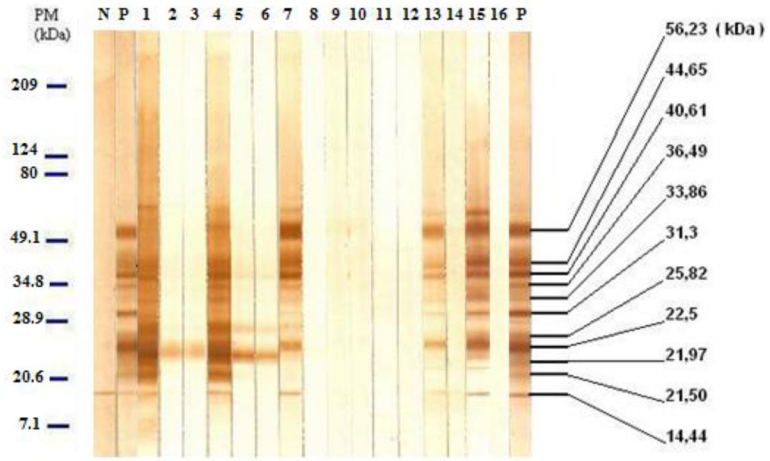

Fig. 3. Characterization of carbohydrate components on whole oncosphere antigens of *T. solium* (WOAs) by lectin blot and evaluation of the specificity of lectins by carbohydrate inhibition

Lane N.- Western blot developed with negative control sera. Lane P.- Western blot developed with positive control sera. Lane 1.- *Concanavalia ensiformis*-Con A control. Lane 2.- ConA incubated with α-D-mannose (0.2M). Lane 3.- ConA incubated with α-D-glucose (0.2M). Lane 4.- *Lens culinaris* agglutinin-LCA control. Lane 5.- LCA incubated with α-D-mannose (0.2M). Lane 6.- LCA incubated with α-D-glucose (0.2M). Lane 7.- R*icinus communis* agglutinin I-RCA I control. Lane 8.- RCA incubated with galactose (0.2M). Lane 9.- *Ulex europaeus* – UEA-I control. Lane 10.- UEA-I incubated with α-L-fucose (0.1M). Lane 11.- *Maackia amurensis* – MAL II control. Lane 12.- MAL II incubated with N-acetyl-neuraminic acid or NANA (0.4M). Lane 13.- *Glycine maximus* (soybean) agglutinin SBA control. Lane 14.- SBA incubated with N–acetyl-galactosamine (0.2 M). 15.-*Triticum vulgaris* – WGA control. Lane 16.- WGA incubated with N-acetyl-glucosamine, 0.5 M. Lane PM.- Molecular weight marker (BIO-RAD).

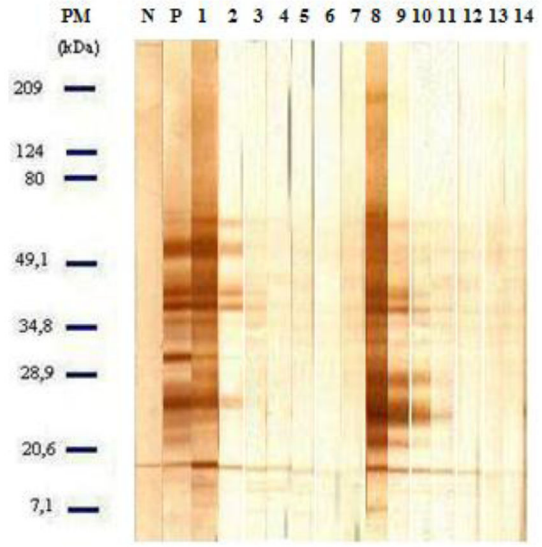

Fig. 4a.

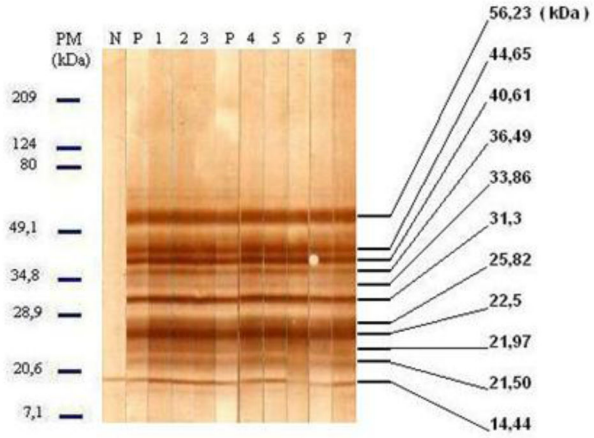

Fig. 4b.

Fig. 4.

Fig. 4a. Effect of treatment with increasing molarities of sodium periodate on the glycosylation of WOAs, measured by RCA and LCA lectin binding. Lane N.- Negative control sera, Lane P.- Positive control sera, Lane 1.- LCA control. Lanes 2, 3, 4, 5, 6 and 7.- LCA binding to WOAs with 5, 10, 20, 40, 80, and 120 mM sodium periodate pre-treatment respectively. Lane 8.- RCA control. Lanes 9, 10, 11, 12, 13 and 14.- RCA binding to WOAs with 5, 10, 20, 40, 80, and 120 mM sodium periodate pre-treatment. PM.- Molecular weight marker (BIO-RAD).

Fig. 4b. Effect of treatment with sodium periodate in the antigenicity of WOAs. Lane N.- Negative control sera. Lane P.- Positive control sera. Lanes 1 to 6.- WOAs pre-incubated with several concentrations of sodium periodate (5, 10, 20, 40, 80, and 120 mM respectively) and developed with positive control sera showed that oxidation of the carbohydrates did not affect the antigenicity of WOAs bands. Lane 7.- WOAs incubated with acetate buffer without periodate and developed with positive sera. Lane PM.- Molecular weight marker (BIO-RAD).

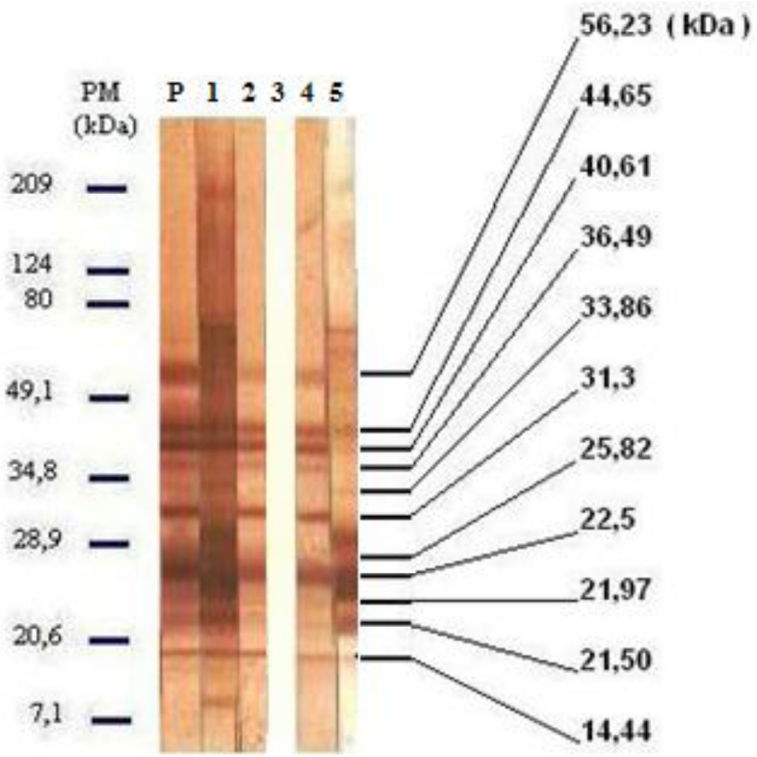

Fig. 5. ***In situ*** **deglycosylation of WOAs using PNGase F**

Lane P.- Positive control sera. Lane 1.- Con A control. Lane 2.- WOAs incubated with PNGase F endoglycosidase and developed with positive control sera indicated that the removal of N-linked oligosaccharides did not affect the antigenicity of WOAs bands. Lane 3.- WOAs incubated with PNGase F endoglycosidase and developed with ConA showed the complete removal of N-linked oligosaccharides. Lane 4.- WOAs incubated with deglycosylation buffer only and developed with positive control sera. Lane 5.- WOAs incubated with deglycosylation buffer only and developed with ConA. Lane PM.- Molecular weight marker (BIO-RAD).

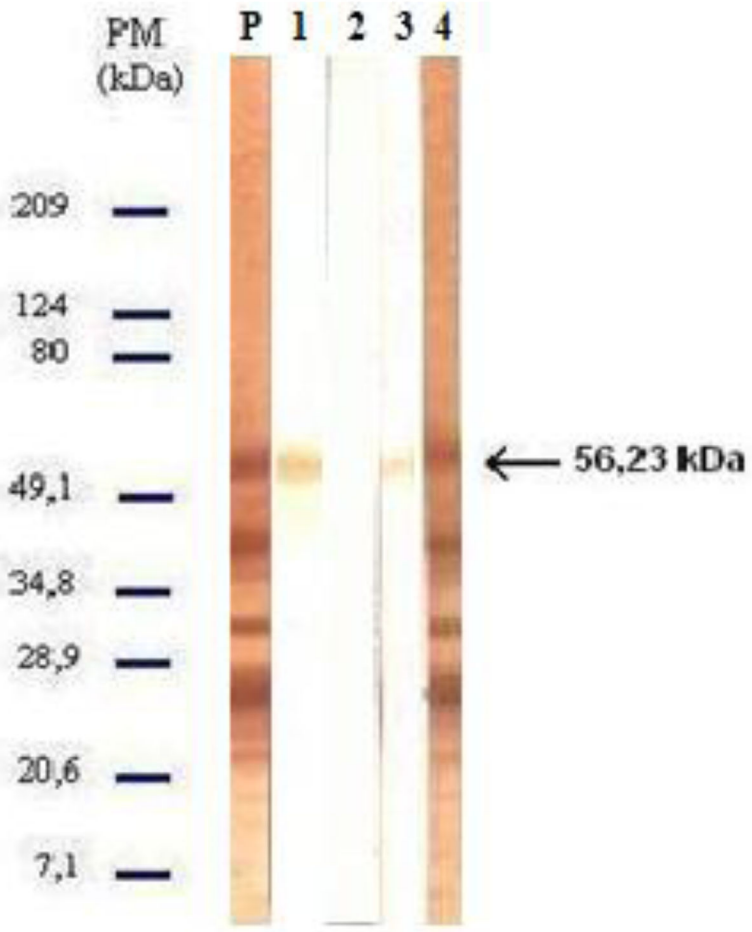

Fig. 6. ***In situ*** **deglycosylation of WOAs using O-glycosidase**

Lane P.- Positive control sera. Lane 1.- PNA lectin control showed an unique band of 56.23 kDa. Lane 2.- WOAs incubated with O-glycosidase and developed with PNA showed the complete removal of O-linked glycosylation. Lane 3.- WOAs incubated with deglycosylation buffer only and developed with PNA. Lane 4.- WOAs incubated with deglycosylation buffer only and developed with positive control sera. Lane PM.- Molecular weight marker (BIO-RAD).

Table 1

Reactivity of lectins with activated *T. solium* oncospheres.

Lectins	**Monosaccharide specificity**	**Activated *Taenia solium***	
		Oncosphere	
		Surface	**Internal structures**
Canavalia ensiformis (ConA)	α-D-mannose / α-D-glucose	+++	−
Lens culinaris (LCA)	α-D-mannose	+++	−
Ricinus communis (RCA I)	α-D-galactose	++	+++
Glycine max (SBA)	α-galNAc	++	+++
Ulex europaeus (UEA-I)	α-L-fucose	−	−
Triticum vulgaris (WGA)	GlcNAc	+	+
Maackia amurensis (MAL-II)	sialic acid / Neu5Ac	+/−	−

Lectins were tested by immunofluorescent microscopy on *T. solium* oncospheres. Relative intensities of the fluorescent stain are indicated as (−) negative; (+/−) weak; (+) moderate; (++) strong; (+++) very strong.

Table 2

Reactivity of carbohydrate probes with activated *T. solium* oncospheres.

Carbohydrate – probes	***T. solium* oncospheral surface**
α-D-Glucose-PAA-fluor	+/−
α-D-Mannose-PAA-fluor	+++
α-D-Galactose-PAA-fluor	+
α-GalNAc-PAA-fluor	+/−
β-GalNAc-PAA-fluor	+
β-GlcNAc-PAA-fluor	+/−
α-L-Fucose-PAA-fluor	−
α-Neu5Ac-PAA-fluor	+++

Carbohydrate probes were tested by immunofluorescent microscopy on *T. solium* oncospheres. Relative intensities of the fluorescent stain are indicated as (−) negative; (+/−) weak; (+) moderate; (++) strong; (+++) very strong.