Experimental Parasitology 147 (2014) 48–53

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Full length article

Variation in the saccharide lectin binding pattern from different isolates of *Tritrichomonas foetus*

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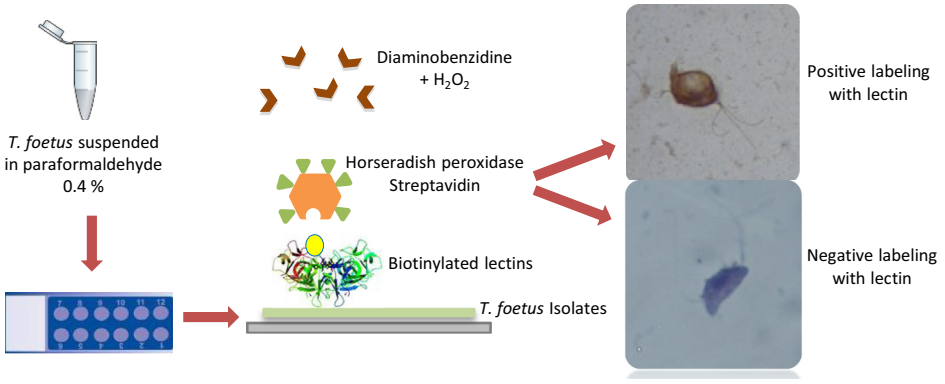
HIGHLIGHTS

- Saccharide patterns of 28 *T. foetus* isolates were analyzed by lectincytochemistry.
- D-mannose, D-glucose, N-acetylglucosamine and sialic acid residues were predominant.
- A low concentration of N-acetylgalactosamine, L-fucose and galactose was observed.
- Labeling variations could be related to differences in the isolates pathogenicity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 May 2014
Received in revised form 15 September 2014
Accepted 19 September 2014
Available online 13 October 2014

Keywords:
Tritrichomonas foetus
Saccharide pattern
Lectincytochemistry

ABSTRACT

Tritrichomonas foetus (*T. foetus*) is the causal agent of bovine tritrichomonosis (BT), a venereal disease that causes significant economic losses in the bovine livestock industry. The structural organization of *T. foetus* presents a cell membrane, an undulating membrane which extends along the parasite, three anterior flagella and a recurrent posterior flagellum. The interaction between the superficial glycoconjugates of the parasite and the host cell is one of the most relevant pathogenic mechanisms. In the present study, we analyzed the saccharide pattern through lectincytochemistry of the cell membrane, undulating membrane, cytoplasm and flagella of 28 isolates of *T. foetus*. Lectins that labeled most of the isolates were WGA, Con-A, RCA-I, LCA, GS-II and PHA-E showing the presence of D-mannose, D-glucose, N-acetylglucosamine and sialic acid. On the other hand, no labeling was observed in any of the structures with VVA, STA, LEA, Jacalin, GS-I, SJA, PHA-L, DSA, and weak labeling was observed with DBA, PNA, SBA and UEA I, showing therefore a low expression of N-acetylgalactosamine, L-fucose and galactose. In addition, GS II labeled in a granular pattern when lectincytochemistry was positive, whereas LCA strongly labeled the membranes and weakly the cytoplasms. The labeling variations observed among the isolates analyzed in the present work, could be related to differences in the pathogenic behavior of the isolates.

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http://dx.doi.org/10.1016/j.exppara.2014.09.009
0014-4894/

1. Introduction

Bovine tritrichomonosis (BT) is a venereal disease that causes significant economic losses in the bovine livestock industry. The causal agent is a non-invasive flagellated protozoan, *Tritrichomonas foetus* (*T. foetus*) (Parsonson et al., 1976; Riedmüller, 1928).

The structural organization of *T. foetus* presents a cell membrane, an undulating membrane extending along the parasite following the trail of the costa, which is the cytoskeleton of the protozoan. Besides, *T. foetus* possesses three anterior flagella and a recurrent posterior flagellum that accompanies the undulating membrane (BonDurant, 1997; Honigberg, 1978).

The interaction of *T. foetus* with the host cell is indeed the initial and crucial step for the establishment of BT. The most important mechanism in this interaction is the adhesion between the cells of the endometrial epithelium and the protozoan, where superficial molecules that contain saccharides are involved (Bonilha et al., 1995; Singh et al., 1999). *T. foetus* presents adhesion molecules, as a lectin capable of attaching the sialic acid of the epithelium and numerous adhesins which include Tf 1.17, Tf 190, lipophosphoglycan and soluble glycosylated antigens (SGA). These molecules located in the cell membrane of *T. foetus* contain saccharides such as glucose, D-mannose, xylose, D-galactose, N-acetylgalactosamine, N-acetylglucosamine, sialic acid, rhamnose and fucose. (Babál and Russell, 1999; Benchimol et al., 1981; Shaia et al., 1998; Singh et al., 2001). It has been shown that some of these glycoconjugates are involved in processes related with pathogenesis, for example, the adhesins are essential for adhesion to host tissues (Burgess and McDonald, 1992; Felleisen, 1999).

On the other hand, *T. foetus* produces glycosidases that may modify the saccharide pattern of the host cell (Cifali and Dias Filho, 1999). These changes in the saccharide pattern were observed by lectinhistochemistry in the infection with *T. foetus* in heifers (Cobo et al., 2004), unpregnant mice (Monteavaro et al., 2008), and pregnant mice (Woudwyk et al., 2013). There are scarce studies about the saccharide pattern of *T. foetus.* One study has used the agglutination of lectins (Benchimol et al., 1981), and another has used gold-labeled and fluorescent lectins, both techniques used for the localization of glycoconjugates on the cell surface and internal cell compartments using electron microscopy (Benchimol and Bernardino, 2002). These works only study one strain of the protozoan and therefore do not consider possible variations within isolates.

Because glycoconjugates are involved in the pathogenicity of BT, the aim of this work was to analyze the saccharide lectin binding pattern from different *T. foetus* isolates by lectincytochemistry.

2. Materials and methods

2.1. T. foetus *isolates*

Twenty eight isolates of *T. foetus* obtained from bovine preputial samples of different herds in the province of Buenos Aires (Argentina) were used. Afterwards, the isolates were cryopreserved in liquid nitrogen until further use, in order to avoid long time under in vitro conditions. After thawing the isolates were cultivated in Diamond's medium (Diamond, 1983) with tryptose, yeast and maltose (TYM) agar for 24–48 h at 37 °C, which corresponds to logarithmic growth. Afterwards, each isolate was suspended in paraformaldehyde saline solution (PSS) 0.4 % and then stored at 4 °C.

2.2. Lectins

The biotinylated lectins used were:

Concanavalin A *Concanavalia ensiformis* (Con-A); Horse gramagglutinin *Dolichos biflorous* (DBA); *Datura stramonium* agglutinin (DSA); *Griffonia* (bandeiraea) *simplicifolia* (GS-II); *Griffonia* (bandeiraea) *simplicifolia* (GS-I); Jacalin; *Lens culinaris* agglutinin (LCA); *Lycopersicon esculentum* aglutinin (LEA); *Phaseolus vulgaris* Erythroagglutinin (PHA-E); *Phaseolus vulgaris* Leucoagglutinin (PHA-L); Peanut agglutinin *Arachishy pogaea* (PNA); Castor bean *Ricinus communis* agglutinin I (RCA I); Soybean agglutinin *Glycine max* (SBA); *Sophora japonica* agglutinin (SJA); *Solanum tuberosum* (STA); *Ulex europaeus* agglutinin-1 (UEA-I); *Vicia villosa* aglutinin (VVA); Wheat germ agglutinin *Triticum vulgaris* (WGA) (Vector Laboratories, Burlingame, CA, USA).

Table 1
Specificity of lectins used in the lectincytochemistry assay. The binding characteristics of the lectins are shown. Information was obtained from http://www.vectorlabs.com.

Lectins	Specificity
Con-A (Concavalin A)	α Man, α Glc
DBA (*Dolichos biflorus)*	α GalNAc
DSA (*Datura stramonium*)	$(GlcNac)_{2-4}$
GS-II (*Griffonia* (Bandeiraea) *simplicifolia* II)	α or β GlcNAc
GS-I (*Griffonia* (Bandeiraea) *simplicifolia* I)	αGal, αGalNAc
Jacalin	Galβ3GalNAc
LCA (*Lens culinaris*)	α Man, α Glc
LEA (*Lycopersicon esculentum*)	$(GlcNac)_{2-4}$
PHA-E (*Phaseolus vulgaris* Erythroagglutinin)	Galβ4GlcNAcβ2Manα6(GlcNAcβ4), (GlcNAcβ4Manα3)Manβ4
PHA-L (*Phaseolus vulgaris* Leucoagglutinin)	Galβ4GlcNAcβ6(GlcNAcβ2Manα3)Manα3
PNA (*Peanut* agglutinin)	Galβ3GalNAc
RCA-I (*Ricinus communis* I)	Gal
SBA (*Soybean* agglutinin)	α > βGalNAc
SJA (*Sophora japonica*)	βGalNAc
STA (*Solanum tuberosum*)	$(GlcNac)_{2-4}$
UEA-I (*Ulex europaeus* I)	αFuc
VVA (*Vicia villosa*)	GalNAc
WGA (wheat germ agglutinin)	GlcNac, NeuNac

Saccharide abbreviations: Fuc, L-fucose; Gal, D-galactose; GalNAc, N-acetylgalactosamine; Glc, D-glucose; GlcNAc, N-acetylglucosamine; Man, mannose; NeuNAc, N-acetylneuraminicacid (sialic acid).

These lectins possess different carbohydrate specificity (resumed in Table 1).

2.3. Lectincytochemistry

The isolates of *T. foetus* were previously allowed to adhere to coverslips with silane (γ-Methacryloxypropyl-Trimethoxysilane). Afterwards, they were incubated with hydrogen peroxide 0.03% in methanol for 15 min at room temperature to inhibit endogenous peroxidase activity and were rinsed in deionized water and then subsequently rinsed in PBS. Coverslips were initially incubated with bovine serum albumin 1% in PBS for 15 min and then incubated with biotinylated lectins for 2 h at room temperature. Following the manufacturer instructions, the concentration used for all lectins was 30 μg/ml in PBS, except for PNA which was applied at 10 μg/ml.

Following incubation with biotinylated lectins, coverslips were subsequently rinsed in PBS and incubated with streptavidin-peroxidase for 30 min (1.0 μl/ml active conjugate, ready to use [Vector Laboratories]). The binding was visualized by applying of substrate working solution (20 μl 3,3'-diaminobenzidine (DAB) per ml of Substrate Buffer containing Imidazole-HCl buffer pH 7.5, hydrogen peroxide and an anti-microbial agent [Dako Laboratories Carpinteria, CA, USA]) for 3–5 min. Coverslips were rinsed in deionized water, and later counterstained with haematoxilin for 20 s (ready to use, Modified Mayer's formula [Vector Laboratories]). Finally the coverslips were rinsed with running tap water until rinse water was colorless, rinsed in deionized water, dehydrated with graded ethanol solutions, cleared in xylene and mounted in

Table 2
Lectincytochemistry results for different isolates.

Isolate no.	DBA			PHA-E			UEA I			WGA			PNA			Con A			RCA I			GS II			SBA			LCA		
	M	C	m	M	C	m	M	C	m	M	C	m	M	C	m	M	C	m	M	C	m	M	C	m	M	C	m	M	C	m
C 1	0	1	0	1	1	0	0	0	0	2	1	0	2	2	0	3	1	3	0	0	0	3	3	2	0	1	0	0	0	0
C 2	0	1	0	0	0	0	1	1	0	3	1	0	1	1	0	2	1	1	0	0	0	2	2	0	0	0	0	0	0	0
C 5	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	3	3	2	0	1	0	0	1	0	0	0	0	0	0	0
C 9	0	0	0	0	1	0	1	1	0	1	2	0	0	2	0	3	3	2	0	1	0	0	1	0	0	0	0	0	0	0
C 10	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0
C 11	0	0	0	0	0	0	2	1	0	0	1	0	0	0	0	3	1	2	0	0	0	1	1	0	0	0	0	0	3	0
C 37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	2	0	0	0	0	2	2	0
C 38	0	0	0	0	0	0	0	0	0	1	1	0	2	2	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0
C 41	0	0	0	0	0	0	1	0	0	0	1	0	0	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
C 50	0	0	0	0	0	0	0	0	0	2	1	0	0	2	0	1	1	1	0	0	0	1	2	0	0	0	0	0	0	0
TR 48	0	0	0	1	1	3	0	0	0	3	3	2	0	0	0	2	2	3	0	0	0	1	2	1	0	0	0	2	2	2
TR 52	0	0	0	2	2	2	0	0	0	2	1	2	0	0	0	0	0	0	2	2	2	0	0	0	0	0	0	3	1	3
TR 57	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	2	1	2	2	1	2	1	3	2	0	0	0	1	0	1
TR 60	0	0	0	1	1	2	0	0	0	3	2	2	0	0	0	2	2	3	3	3	3	2	3	2	0	0	0	2	1	3
TR 62	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	1	0	2	1	2	2	2	2	0	0	0	0	0	0
TR 63	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0	0	0	0	3	3	2	0	0	0	0	0	0	0	0	0
TR 65	0	1	0	2	1	1	0	0	0	1	1	1	0	0	0	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0
TR 67	0	0	0	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	0	1	0	0	0	0	3	2	2
TR 69	1	1	0	3	1	2	2	1	1	1	1	1	0	0	0	3	3	3	3	1	3	0	0	0	0	0	0	3	1	1
TR 72	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	2	1	1	2	2	2	0	0	0	0	0	0	1	1	0
TR 82	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	1	2	0	1	0	0	0	0	0	0	0
TR 85	0	0	0	0	0	0	1	0	0	2	1	2	0	0	0	0	0	0	2	2	3	0	1	0	0	0	0	0	0	0
TR 96	0	0	0	2	1	1	0	0	0	0	1	1	0	0	0	1	1	1	2	2	3	0	1	0	0	0	0	1	0	0
TR 97	0	0	0	2	1	1	0	0	0	1	1	0	0	0	0	1	1	0	2	1	2	0	0	0	0	0	0	0	0	0
TR 98	0	0	0	2	1	0	0	0	0	2	1	0	0	0	0	2	2	2	2	2	2	0	0	0	0	0	0	2	0	0
TR99	0	0	0	2	1	1	0	0	0	1	1	1	0	0	0	3	2	3	2	2	2	2	3	2	0	0	0	1	0	0
TR108	0	0	0	0	0	0	0	0	0	2	2	2	0	0	0	2	2	2	0	0	0	0	2	1	0	0	0	1	0	2
TR109	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	1	2	0	1	0	2	0	0	0	0	2	0	2

Reference: 0, no labeling; 1, weak labeling; 2, moderate labeling; 3, strongly positive; M, cell membrane; C, cytoplasm; m, undulating membrane.

Permount (Fisher Scientific International Inc., Liberty Lane Hampton, NH, USA).

Controls for lectin labeling included: exposure to horseradish-peroxidase and substrate medium without lectin, and blocking by pre-incubation with the appropriate blocking sugars (0.1–0.2 M in PBS) for 1 h at room temperature before applying lectin to the sections. The intensity of lectin binding was subjectively scored from (0) (none) to 3 (strongly positive). At least 20 protozoans from each isolate for each lectin were observed, determining the following structures: cell membrane, undulating membrane, flagellum and cytoplasm.

3. Results

The results show important differences between isolates. Sixteen of the 28 *T. foetus* isolates analyzed were strongly labeled by some of the lectins used.

The labeling with VVA, STA, LEA, Jacalin, GS-I, SJA, PHA-L and DSA was negative in all the structures from all the analyzed isolates.

DBA, PNA, SBA and UEA-I weakly labeled some of the samples. GS-II marked some isolates with a granular pattern in the cytoplasm, and LCA labeled intensively all the membranes and weakly the cytoplasms. The lectins that labeled the higher number of isolates and with a remarkable intensity were WGA, Con-A, RCA-I, PHA-E, GS-II and LCA. The lectins WGA and GS-II showed a high heterogeneity of cell labeling, which was detected in the same isolates.

The structure that showed the highest reactivity was the cell membrane whereas the flagellum was the structure that showed the lowest reactivity. This structure was only found to be positive for WGA, Con-A, RCA-I and only one isolate with UEA-I (data not shown in Table 2). The results from labeling the cytoplasm, cell membrane and undulating membrane are shown in Table 2 and Fig. 1. Lectins that did not mark the analyzed structures in any of the isolates are excluded from the Table 2.

In all cases the controls for exposure to horseradish-peroxidase and substrate medium without lectin were negative, showing no reactivity with DAB. The blocking with the appropriate blocking sugars before applying lectin showed no labeled in all the analyzed isolates (Fig. 2).

4. Discussion

Superficial molecules that include saccharides are involved in the processes of adhesion and colonization of *T. foetus* (Bonilha et al., 1995). The importance of these saccharides is recognized by the changes generated by *T. foetus* over the genital epithelium of heifers (Cobo et al., 2004) and mice (Monteavaro et al., 2008; Woudwyk et al., 2013) that could facilitate the infection. Regarding the saccharides that are found in *T. foetus*, several studies have revealed a great diversity forming part of glycoproteins and lipophosphoglycan-like molecules (Benchimol and Bernardino, 2002; Bonilha et al., 1995; Singh et al., 1999, 2001). However, according to the reviewed bibliography the superficial saccharide pattern of *T. foetus* was studied in two cases where one single isolate was used (Benchimol and Bernardino, 2002; Benchimol et al., 1981).

Some of the isolates analyzed in our work show similar results to those found by Benchimol and Bernardino (2002), i.e. the high labeling produced by LCA and WGA lectins in the cell membrane. There were also agreements with SBA, which showed negative labeling for the membrane in the mentioned study, and did only label weakly one isolate in our work. In addition, this lectin showed a weak cytoplasmatic pattern in some of the isolates, similar to what was previously described by these authors.

Besides, another agreement was observed with Con-A, which marked all the structures in most of the isolates of our work, and in the work of Benchimol and Bernardino (2002) showed great af-

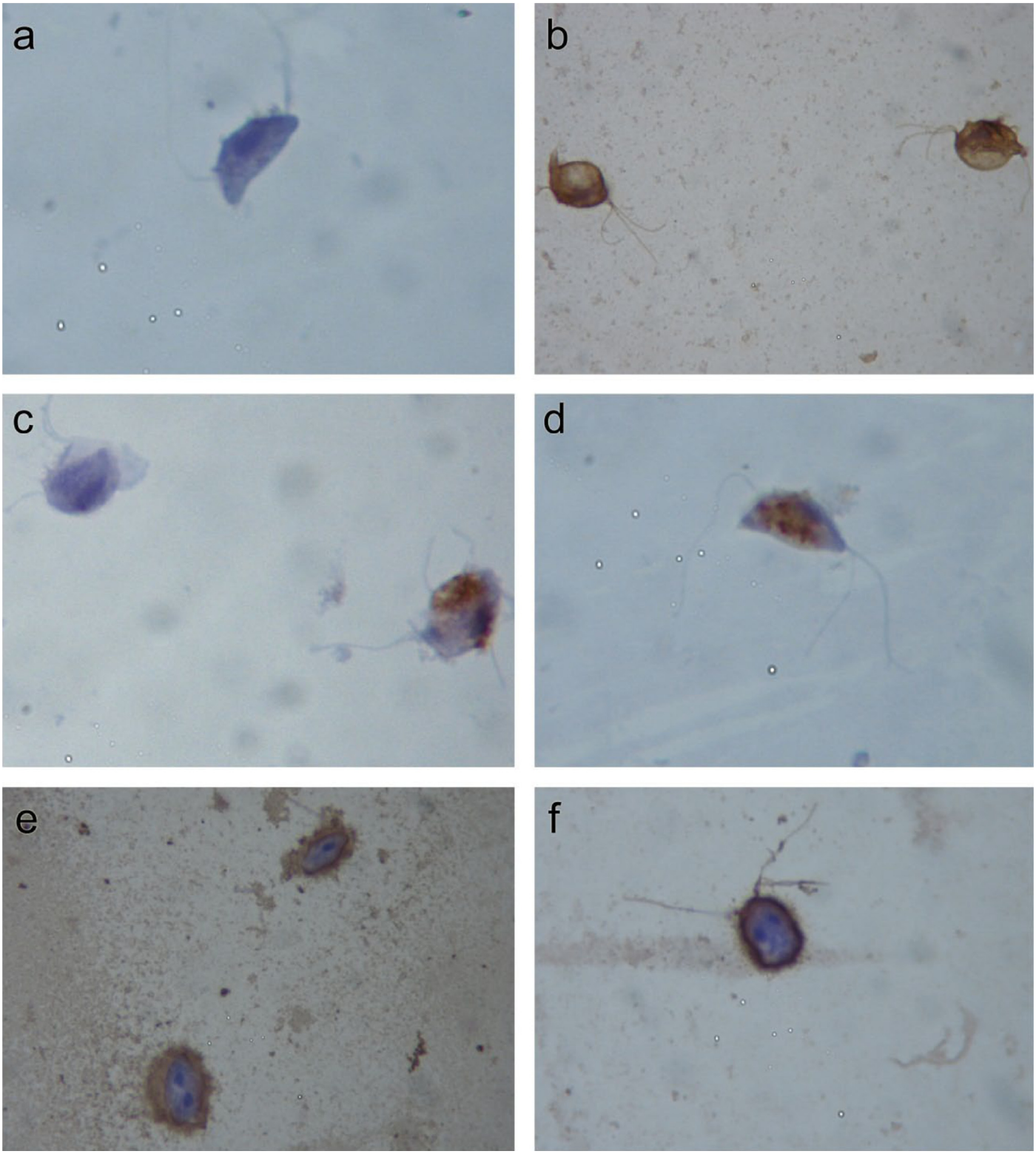

Fig. 1. Lectincytochemistry of different *T. foetus* isolates. 1000×, DAB chromogen and hematoxylin contrast. (a) Shows the negative labeling with RCA-1 lectin in TR 48 isolate, as an example of the lectin that showed no affinity for protozoan structures. (b) Shows strong labeling for RCA-1 in all the structures analyzed in TR 60 isolate. (c) Shows the heterogeneity to GS II labeling of TR 99 isolate among the protozoans in the same sample. (d) Shows the granular cytoplasmatic pattern and weak membrane labeling with GS II in TR 57 isolate. (e) Shows the strongly affinity for the membrane and undulating membrane, and the weak affinity for the cytoplasm observed with Con A in TR 69 isolate. (f) Shows the strong labeling of the membrane, the moderate labeling of the undulating membrane and the weak affinity for the cytoplasm in TR 69 isolate with PHA-E lectin.

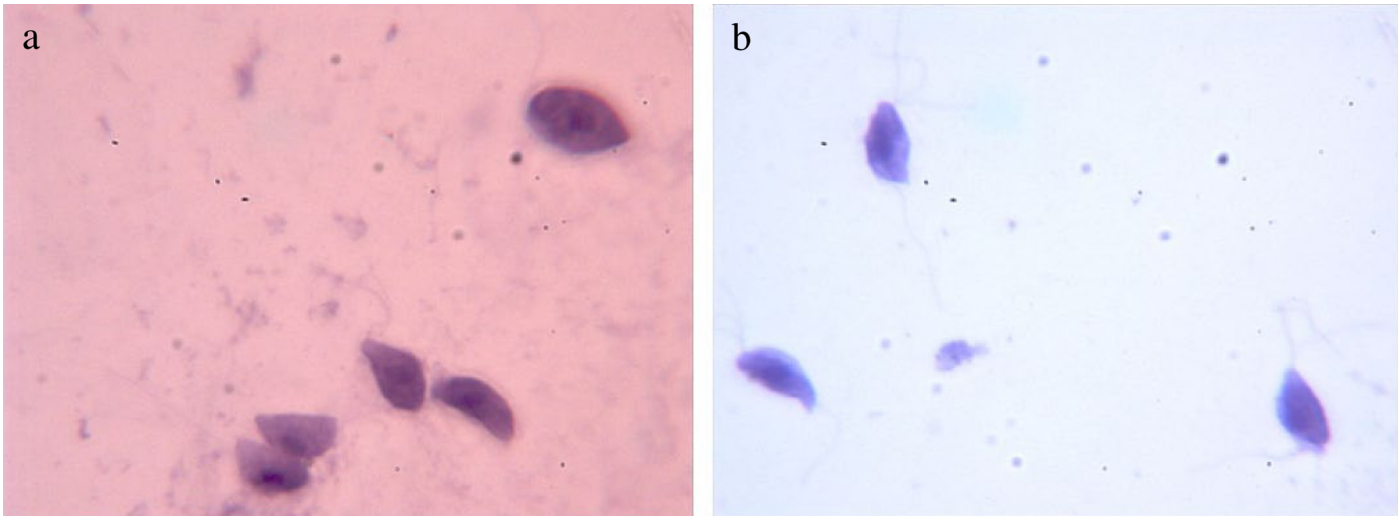

Fig. 2. Controls for lectincytochemestry, 1000×. (a) Shows the negative labeling for the exposure to horseradish-peroxidase and substrate medium without lectin in TR 69 isolate. (b) Shows the negative labeling in TR 69 isolate with PHA-E previously blocked by pre-incubation with mannose.

finity for the nuclear envelope, endoplasmatic reticulum, Golgi complex, vacuole and cell surface of *T. foetus*.

A particular case was observed with lectin GS-II, which mainly showed a cytoplasmic granular labeling pattern in our isolates, whereas in the work from Benchimol and Bernardino (2002) labeled positively the cytoplasmic vesicles.

Nevertheless, other lectins such as GS-I and UEA-I did not label, or weakly labeled our isolates, whereas in the previously mentioned work a strong labeling was observed.

Benchimol et al. (1981) also studied the carbohydrates expression pattern. However, they only studied the parasite surface, using an agglutination technique. Regarding this, the agreements include a high affinity for WGA, Con-A, and low affinities for SJA and PNA. The lectins DBA, PNA, PHA-E and RCA-I require higher concentrations in order to produce agglutination, which shows a low expression of the saccharide. In our case these lectins labeled the membrane and the cytoplasm in some of the isolates, therefore showing a remarkable interisolate variation. In this context, it should be considered that in our studies, we analyzed a large number of isolates and observed important differences between the results of the different isolates. Therefore, differences could be expected between our isolates and the single strain analyzed by these authors (Benchimol and Bernardino, 2002; Benchimol et al., 1981).

The labeling heterogeneity found within the same isolate using the same lectin, particularly with WGA and GS-II, is one of the important results of the present work. Similar findings were previously described by Benchimol and Bernardino (2002). The differences found within the same group could be the effect of temporal variations in the metabolic activity of the protozoans, and not due to differences in their genome.

Considering previous studies where the affinities of different lectins were published (Damjanov, 1987; Jones et al., 2003; Roth, 2011), the absent or weak labeling of SBA, DBA, VVA, SJA, GS-I and PNA in most of our isolates indicates a low concentration of N-acetylgalactosamine. The remarkable labeling observed with Con-A, LCA and PHA-E shows the high expression of mannose residues. On the other hand, we observed variations with GlcNAc. Lectins that recognized $(GlcNAc)_{2-4}$ as LEA, STA and DSA showed negative labeling, whereas GS-II and WGA, which label GlcNAc, were positive for many isolates. In addition, some isolates were positive for WGA and negative for GS II, hence indicating the presence of sialic acid, the other carbohydrates to which WGA presents affinity.

Singh et al. (2001) found adhesins with glucosamine, galactose and glucose in *T. foetus*. These residues were recognized by RCA-I, GS-II, WGA and LCA in the present work. The low labeling observed with lectins that recognize GalNAc in the majority of our isolates is agreement with a low concentration of this residue in the lipophosphoglycan and glycosylated surface antigens studied in *T. foetus* by these authors. However, the weak reaction observed with UEA-I was especially remarkable since *T. foetus* adhesins showed numerous fucose residues that were recognized by this lectin (Singh et al., 2001). Fucose probably forms oligosaccharides with low affinity for UEA-I and, in the present work, no other lectins recognizing this monosaccharide were used.

Some lectins that recognize glucose and mannose, including PHA-E and especially Con-A, showed a high labeling of the cytoplasm in many isolates. This result correlates with the DNases composition of *T. foetus* purified by Greenwell et al. (2008), who used Con A and determined the abundance of glucose and mannose residues in these glycoproteic enzymes.

On the other hand, Kneipp et al. (1998) determined the presence of chitin in the cell surface of *T. foetus*. Chitin is a linear polysaccharide polymer containing N-acetyl-D-glucosamine units. This residue can be labeled with WGA and GS-II. These lectins showed a high affinity for the membrane in many of our isolates.

Lectins were used to differentiate strains of different pathogenicity in bacteria as *Neisseria gonorrhoea, Campylobacter jejuni* and *Campylobacter coli*. This technique was used previously in both pathogenic and not pathogenic species of flagellated protozoan (Ghosh et al., 1988), including strains of different pathogenicity of *Leishmania enrietti* (Schottellius, 1990) and *Trypanosoma cruzi* (Schottellius, 1982).

In *T. foetus* similar studies with different strains have not been performed, while in *Trichomonas vaginalis*, a related protozoan, studies have shown variations in the lectin binding pattern within strains. This variation could be related to the strains pathogenicity (Kon et al., 1988; Warton and Honigberg, 1983). Our study demonstrates a high variation in the saccharides pattern found in different *T. foetus* isolates. In order to correlate the variation in the saccharides pattern and the pathogenicity of *T. foetus* strains, further studies should be carried out. This correlation may be useful in future assays to differentiate strains of different pathogenicity, antigenic analyses and for the formulation of immunogens.

Acknowledgments

E.J.G. and C.G.B. are Researchers and M.L.D. is a postgraduate student of CONICET (the National Scientific Research Council). P.S. and C.E.M are researchers of CIVETAN-CONICET. The authors thank Mr. Claudio Cacciato for his careful technical assistance. The investigation was partially funded by grants 11/V193, 11/V/191 (National University of La Plata) and 03 / H253 (National University of the Center of the Buenos Aires Province).

References

Babál, P., Russell, L., 1999. Sialic acid-specific lectin mediated adhesion of *Tritrichomonas foetus* and *Tritrichomonas mobilensis*. J. Parasitol. 85, 33–40.

Benchimol, M., Bernardino, M.V., 2002. Ultrastructural localization of glycoconjugates in *Tritrichomonas foetus*. Parasitol. Res. 88, 134–143.

Benchimol, M., Pereira, M.E., Elias, C.A., De Souza, W., 1981. Cell surface carbohydrates in *Tritrichomonas foetus*. J. Protozool. 28, 337–341.

BonDurant, R.H., 1997. Pathogenesis, diagnosis and management of trichomoniasis in cattle. Vet. Clin. North Am. Food Anim. Pract. 13, 345–361.

Bonilha, V.L., Ciavaglia, M.C., De Souza, W., Silva Filho, F.C., 1995. The involvement of terminal carbohydrates of the mammalian cell surface in the cytoadhesion of trichomonads. Parasitol. Res. 8, 121–126.

Burgess, D.E., McDonald, C.M., 1992. Analysis of adhesion and cytotoxicity of *Tritrichomonas foetus* towards mammalian cells with monoclonal antibodies. Infect. Immun. 60, 4253–4259.

Cifali, A.P., Dias Filho, B.P., 1999. Purification and partial characterization of N-acetyl-beta-D-glucosaminidase from *Tritrichomonas foetus*. Parasitol. Res. 85, 256–262.

Cobo, E., Campero, C.M., Gimeno, E.J., Barbeito, C.G., 2004. Lectin binding patterns and immunohistochemical antigen detection in the genitalia of *Tritrichomonas foetus*-infected heifers. J. Comp. Pathol. 131, 127–134.

Damjanov, I., 1987. Lectin cytochemestry and histochemistry. Lab. Invest. 57, 5–20.

Diamond, L.S., 1983. Lumen dwelling protozoa: Entamoeba, Trichomonads, and Giardia. In: Jensen, J.B. (Ed.), In Vitro Cultivation of Protozoan Parasites. CRC Press, Boca Raton, FL, pp. 67–109.

Felleisen, R.S., 1999. Host parasite interaction in bovine infection with *Tritrichomonas foetus*. Microbes Infect. 1, 807–816.

Ghosh, D.K., Ghosh, A.K., De, A., Bhattachayra, A., 1988. Differentiation of pathogenic and non-pathogenic kinetoplastid flagellates by lectins. In: Bog-Hansen, T.C. (Ed.), Lectins: Biology, Biochemistry, Clinical Biochemistry. Sigma Chemical Company, St Louis, MO, pp. 559–564.

Greenwell, P., Younes, M., Rughooputh, S., 2008. Purification and analysis of DNases of *Tritrichomonas foetus*: evidence that these enzymes are glycoproteins. Int. J. Parasitol. 38, 749–756.

Honigberg, B.M., 1978. Trichomonads of veterinary importance. In: Kreier, J.P. (Ed.), Parasitic Protozoa. Academic Press, New York, pp. 163–273.

Jones, C.J.P., Cateni, C., Guarino, F.M., Paulesu, L.R., 2003. Glycosylation of the materno-foetal interface in the pregnant viviparous placentotrophic lizard Chalcides chalcides: a lectin histochemical study. Placenta 24, 489–500.

Kneipp, L.F., Andrade, A.F.B., de Souza, W., Angluster, J., Alviano, C.S., Travassos, L.R., 1998. *Trichomonas vaginalis* and *Tritrichomonas foetus*: expression of chitin at the cell surface. Exp. Parasitol. 89, 95–204.

Kon, V.B., Papadimitriou, J.M., Robertson, T.A., Warton, A., 1988. Quantitation of concanavalin A and wheat germ agglutinin binding by two strains of *Trichomonas vaginalis* of differing pathogenicity using gold particle-conjugated lectins. Parasitol. Res. 75, 7–13.

Monteavaro, C., Soto, P., Gimeno, E.J., Echevarría, H.M., Catena, M., Portiansky, E., et al., 2008. Histological and lectin binding changes in the genital tract of mice infected with *Tritrichomonas foetus*. J. Comp. Pathol. 138, 40–45.

Parsonson, I.M., Clark, B.L., Dufty, J.H., 1976. Early pathogenesis and pathology of *Tritrichomonas foetus* infection in virgin heifers. J. Comp. Pathol. 86, 59–66.

Riedmüller, L., 1928. Uber die morphologie, übertragungsversuche und klinische. Bedeutung der beim sporadischen abortus des rindes vorkommenden trichomonoden, Zentralbl. Bakteriol. Orig. 108, 103–118.

Roth, J., 2011. Lectins for histochemical demonstration of glycans. Histochem. Cell Biol. 136, 117–130.

Schottellius, J., 1982. Lectin typing of Leishmania-strains from the new and old wor. In: Bog-Hausen, T.C. (Ed.), Lectins: Biology, Biochemistry, Clinical Biochemistry. Sigma Chemical Company, St Louis, MO, p. 531.

Schottellius, J., 1990. New world trypanosomatidae: The importance of lectins for their differentiation with special emphasis on Trypanosoma cruzi, the agent of Chagas disease. In: Bog-Hansen, T.C. (Ed.), Lectins: Biology, Biochemistry, Clinical Biochemistry. Sigma Chemical Company, St Louis, MO, pp. 321–327.

Shaia, C.I., Voyich, S., Gillis, S.J., Singh, B.N., Burgess, D.E., 1998. Purification and expression of the Tf190 adhesin in *Tritrichomonas foetus*. Infect. Immun. 66, 1100–1105.

Singh, B.N., Lucas, J.J., Beach, D.H., Shin, S.T., Gilbert, R.O., 1999. Adhesion of *Tritrichomonas foetus* to bovine vaginal epithelial cells. Infect. Immun. 67, 3847–3854.

Singh, B.N., BonDurant, R.H., Campero, C.M., Corbeil, L.B., 2001. Immunological and biochemical analysis of glycosylated surface antigens and lipophosphoglycan of *Tritrichomonas foetus*. J. Parasitol. 87, 770–777.

Warton, A., Honigberg, B.M., 1983. Analysis of surface saccharides in *Trichomonasvaginalis* strains with various pathogenicity levels by fluorescein-conjugated plant lectins. Z. Parasitenkd 69, 149–159.

Woudwyk, M.A., Gimeno, E.J., Soto, P., Barbeito, C.G., Monteavaro, C.E., 2013. Lectin binding pattern in the uterus of pregnant mice infected with *Tritrichomonas foetus*. J. Comp. Pathol. 149, 341–345.