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

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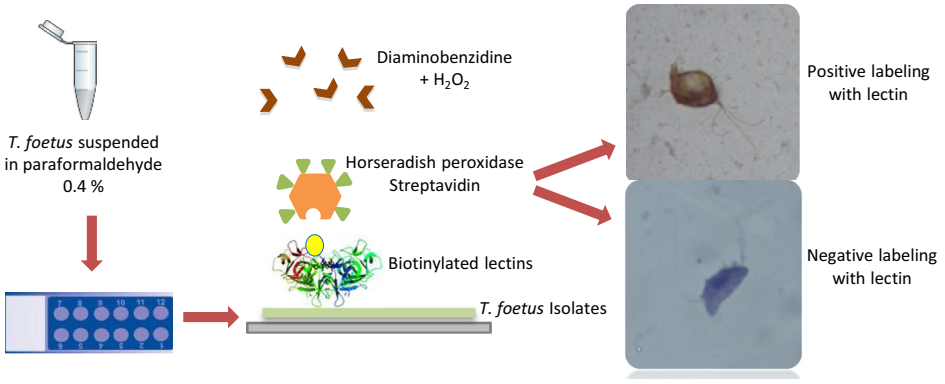
HIGHLIGHTS

- 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



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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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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