ANTIGENIC COMPARISON BETWEEN TRITRICHOMONAS SUIS, T. FOETUS, TRICHOMONAS VAGINALIS AND T. GALLINAE BY GEL IMMUNODIFFUSION*

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Summary

Twelve rabbits were used for the production of the immune sera against *T. suis*, *T. foetus*, *T. vaginalis* and *T. gallinae* antigens. Three animals were injected with each species of *Tritrichomonas* and *Trichomonas*. In the reactions between these immune sera and antigens of each of special the largest number of precipitating lines were observed between homologous systems using gel immunodiffusion. Using the Outcherlony technique the antigens of *T. suis*, *T. foetus*, *T. vaginalis* and *T. gallinae* showed 1, 2, 3 or 4 lines in the A antigenic group and 1 and 2 precipitating lines in group B. The results obtained with the immune sera of *T. suis*, *T. foetus*, *T. vaginalis* and *T. gallinae* indicated that the four trichomonads have identical antigens, or at least a closely related antigenic structure.

Resumo

Comparação antigênica entre Tritrichomonas suis, T. foetus, Trichomonas vaginalis e T. gallinae pela imunodifusão em gel

Doze coelhos foram usados para a produção dos imuno-soros contra antígenos de *Tritrichomonas suis*, *T. foetus*, *Trichomonas vaginalis* e *T. gallinae*. Três animais foram injetados com cada espécie de *Tritrichomonas* e *Trichomonas*. Nas reações entre estes imuno-soros e os antígenos de cada uma das espécies, o maior número de linhas de precipitação foi observado, entre os imuno-soros e antígenos homólogos na difusão em gel. Usando-se a técnica de Outcherlony, os antígenos de *T. suis*, *T. foetus*, *T. vaginalis* e *T. gallinae* mostraram 1, 2, 3, ou 4 linhas no grupo antigênico A e 1 e 2 linhas de precipitação no grupo B. Os resultados obtidos com os imuno-soros de *T. suis*, *T. foetus*, *T. vaginalis* e *T. gallinae* indicaram que os quatro tricomonas têm antígenos idênticos, ou pelo menos com estrutura antigênica relacionada muito próxima.

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Introduction

Studies of the antigenic comparison between *Tritrichomonas suis* and *T. foetus* have been performed using the indirect immunofluorescence (4), gel immunodiffusion (5) and immunoelectrophoresis techniques (6). The results obtained indicated the existence of antigens that are common to both species or perhaps associated with a generic antigenic identity. In the present investigation the antigenic relation between *T. suis*, *T. foetus*, *T. vaginalis* and *T. gallinae* was studied through gel immunodiffusion with the purpose of obtaining new information on the antigenic afinity of the four species.

Material and Methods

Protozoan species - Tritrichomonas suis - This strain has been described previously (3). Cultures of the protozoa were maintained in axenic cultures in modified Diamond medium (3) at 35,5ºC and transferred every 48 hours.

Tritrichomonas foetus - The history of this specie was given in a previous report (4). At first the sample was spread in Feinberg & Whittington medium and transferred every 72 hours (8). After that the axenic culture was maintained in modified Diamond medium at 35,5ºC and transferred every 48 hours.

Trichomonas vaginalis - The sample TV 1 was isolated from the vaginal secretion from a woman, patient in a gynecological clinic in Porto Alegre, RS. This strain has been kept in axenic culture in modified Diamond medium since 1973 (2). The cultures were incubated at 35,5ºC and transferred every 48 hours.

Trichomonas gallinae - The methods of isolation, cultivation and preservation of the TG 1 isolated by De Carli, Pansera & Guerrero from upper digestive tract of a domestic pigeon were described previously (7). The culture of the parasites were maintained in modified Diamond medium at 35,5ºC and transferred every 48 hours.

Preparation of antigens for immunization - Antigens of each species were extracted from each organism as previously described (4). The strains of *T. vaginalis* and *T. gallinae* which had the same treatment during the preparation of the antigens, were included. All disrupted antigens were stored at -20ºC.

Concentration of total nitrogen and protein of the antigens - The concentration of total nitrogen was determined by technique of Kjeldhal modified by Taveira & Bethelem (21) and total protein was determined by the method of Foling-Ciocoalteau as described by Lowry (16). The results of these analyses are given in Table 1.

Immunization of rabbits and immune sera - Twelve rabbits were used for the production of the immune sera against each of the four species. The method of preparation was as previously described (4). The immune sera and the rabbit normal sera (NRS), prepared for the indirect immunofluorescence study (4) were used in the present investigation.

Gel immunodifusion technique - The technique of immunodifusion described by Outcherlony (18) was used for the identification of *Tritrichomonas* and *Trichomonas* antigens. Glass slides (75 x 50mm) were used for this technique. The gel layer was prepared by addition of 5ml of liquid purified agar (BBL) at 1% over the glass slides. Sodium azide

Table 1 - Protein and total nitrogen of the antigens used in the gel diffusion precipitin reactions

Antigens		Protein mg/ml	Total N mg/ml
T. suis	TS	1,8	0,37
T. foetus	TF	16,0	2,61
T. vaginalis	TV	5,0	0,8
T. gallinae	TG	6,0	0,96

(Difco) was used as a preservative of the gel in a final concentration of 0,1%. A seven wells pattern cutter used being the internal diammeter of the wells of 5mm with a distance of 5mm between them, or five wells pattern cutter was used being the internal diammeter of the wall of 5mm with distance of 10mm between them. The external wells of the perforated gel were used for the immune sera and the internal well was used for the antigens. For all reactions, the wells were loaded with 20µl of antigens and 50µl of undiluted antiserum. The antigen antibody reactions were processed in humid chamber at room temperature (22ºC). The readings were made 48 hours after the beginning of the diffusion. Obliquous lighting against a dark field was used for reading the reactions. The precipitating lines were stained by Ponceau fixing stain. All of the reactions were photographed as previously described (22). All of the reactions were photographed 48 hours after the incubation using the technique described by Williams & Chase (22).

Results and Discussion

Negative results were found in the reactions that involved normal rabbit serum (NRS) and four protozoan antigens studied. No precipitating lines were formed when the four antisera reacted with the sterile culture medium, sterile components of these media, horse sterile components of these media, house sterile and inactivated horse serum diluted and not diluted. No precipitin lines were formed in the slides containing sterile distilled water, sterile physiological saline solution. Negative reactions among baseline bleeding serum and *Tritrichomonas* and *Trichomonas* antigens proved that the rabbits utilizes in this study had not a previous contact with these antigens. In each series of experiments the specific immune serum reacted against the homologous and heterologous antigens. The immune reactions between the immune sera of both systems were represented by two groups of precipitation lines or bands.

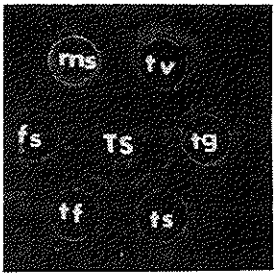
According to Goldman & Honigberg (11) these group were arbitrarily designated group A and group B based on their position. Group A of precipitation lines always developed closer to the serum wells, and were concave to their side. Group B lines, were located closer to the antigen wells and concave to its side. The precipitationg lines obtained in the heterologous systems were classified according to Wilson & Pringle (23); Outcherlony (18) and Crowle (9, 10). The tests performed with each antiserum show reactions comparing the immune sera and NRS with the heterologous and homologous antigens as follows; one-well reactions with the homologous antigens and three-well reactions with the heterologous antigens.

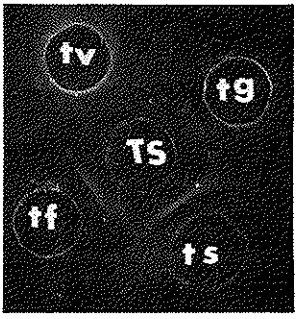
Anti-Tritrichomonas suis serum - When *T. suis* (TS) antigen reacted against its homologous antiserum it showed the strongest reaction. A great number of lines from groups A and B were formed in the reaction among TS antigen and their homologous antiserum. No reactions occured between TS antigen and *T. vaginalis* antiserum (tv) (Fig. 1 and 2). When the antigen TS reacted against homologous and heterologous sera it showed two, three and four lines in group A and one and two lines in group B (Fig. 1, 2 and 3). When the TS antigen reacted against *T. foetus* (tf) and *T. gallinae* (tg) immune sera showed lines with total identity (Fig. 1 and 3). Most of these lines from both homologous and heterologous systems showed total identity (Fig. 1 and 3) and partial identity (Fig. 6). No conclusion can be described about any lines of group B antigenic systems. The results of these experiments with anti-*T. suis* serum indicated that both *T. foetus* and *T. gallinae* have antigen which are identical or at least very closely structurally related or identical antigen in the A and B group.

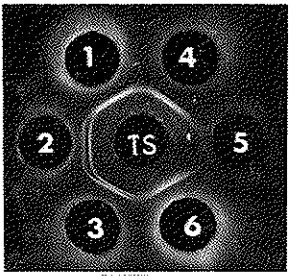
Anti-Tritrichomonas foetus serum - This serum gave the largest number of lines with its homologous antigen. When the *T. foetus* (TF) antigen reacted against the homologous and heterologous antisera one to three lines in group A and two lines in group B were formed (Fig. 5 and 7). When TF antigen reacted against the immune sera of *T. suis* (ts) and *T. gallinae* (tg) only lines with partial identity were developed (Fig. 4 and 6). Most of these lines from both homologous and heterologous

system showed total identity (Fig. 4) and partial identity (Fig. 6). No reaction occured between TF antigen and *T. vaginalis* (tv) antiserum (Fig. 5). It is evident from the results that either *T. foetus*, *T. suis* and *T. gallinae* have identical antigens, or are very closely related in the group A. The *T. foetus* aparently appear to share one of the group B antigen of *T. gallinae* (Fig. 5).

Figures 1, 2, 3 - Precipitation lines of reaction between *T. suis* antigen (TS) and anti-*T. vaginalis* antisera (tv), anti-*T. gallinae* antisera (tg), anti-*T. foetus* antisera (tf and wells 4, 5 and 6) and anti-*T. suis* antisera (ts wells 1, 2 and 3)

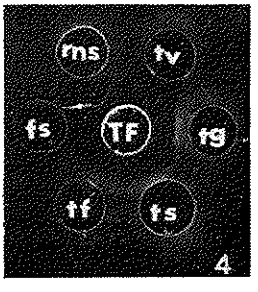


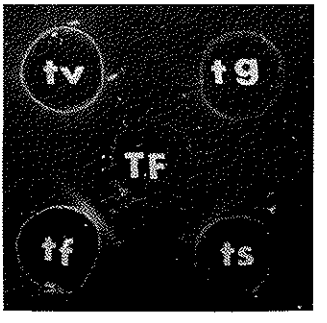


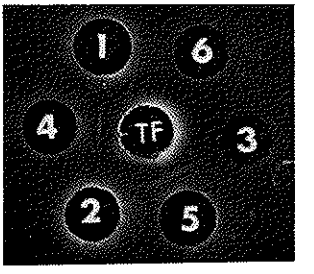


Anti-Trichomonas vaginalis serum - Reactions between *T. vaginalis* (tv) antiserum and its homologous antigen resulted in the formation of one and two lines on the group A (Fig. 8). When *T. vaginalis* (tv) antigen reacted against *T. gallinae* (tg) antiserum, there was one line with total identity in group A (Fig. 9). No reactions occured between TV antigen and *T. suis* (ts) and *T. foetus* (tf) antiserum (Fig. 8 and 9). When TV antigen reacted against homologous and heterologous sera, these were one and two lines in A group alone, and not lines in B group. The results indicated a very close antigenic relation of superficial antigens between the two species of *Trichomonas*.

Figures 4, 5, 6 - Precipitation lines of reactions between *T. foetus* antigen (TF) and anti-*T. vaginalis* antisera (tv), anti-*T. gallinae* antisera (tg), anti-*T. foetus* antisera (tf and wells 4, 5 and 6) and anti-*T. suis* antisera (ts wells 1, 2 and 3)

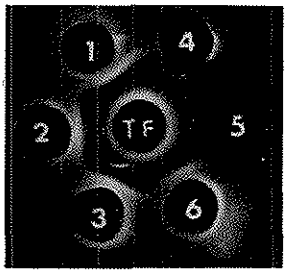






Anti-Trichomonas gallinae serum - The strongest reactions was observed between *T. gallinae* (TG) antigen and its homologous antiserum (Fig. 10). When the TG antigen reacted against homologous and heterologous sera it showed one and two lines in the A group (Fig. 10). The reactions between TG antigen against *T. vaginalis* (tv) serum showed two lines in group A with partial identity (Fig. 11). The precipitin lines observed in reaction involving TG antigen and *T. suis* (ts) and *T. foetus* (tf) immune sera showed one line on the A group with total identity. The results of experiments with *T. gallinae* (TG) antigen against *T. suis* (ts), *T. foetus* (tf) and *T. vaginalis* (tv) antiserum showed evidence that this trichomonads have identical antigens or very closely related antigenic structure.

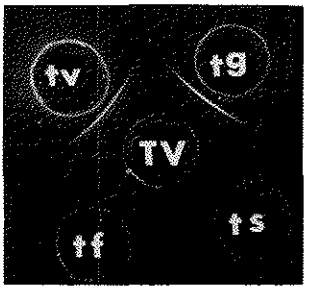
Figure 7 - Precipitation lines of reactions between *T. foetus* antigen (TF) and anti-*T. vaginalis* antisera (tv), anti-*T. gallinae* antisera (tg), anti-*T. foetus* antisera (tf and wells 4, 5 and 6) and anti-*T. suis* antisera (ts and wells 1, 2 and 3)



Conclusions

The results of previous study on indirect immunofluorescence (4), gel immunodiffusion (5) and immunoelectrophoresis (6) suggested the close antigenic relationships between *T. suis* and *T. foetus*. Recent light and electronic observations provided a similar morphology in the two *Tritrichomonas* and two *Trichomonas* species (7, 13, 15, 17, 19). The close antigenic relationships between these two genera could reveal the existence of cross reacting antigens but did not give information concerning the number of common antigens responsible for these reactions (12, 14, 20). They indicated, also that *T. suis*, *T. foetus* and *T. gallinae* were antigenically more closely related to each other. It has been proved through gel immunodiffusion that *T. vaginalis*, *T. gallinae* and *T. foetus* share structurally identical or closely related antigens, confirming the results suggested previously through studies with trichomonads of genera *Tritrichomonas*.

Figures 8, 9 - Precipitation lines of reactions between *T. vaginalis* antigen (TV) and anti-*T. vaginalis* antisera (tv), anti-*T. gallinae* antisera (tg), anti-*T. foetus* antisera (tf), and anti-*T. suis* antisera (ts)



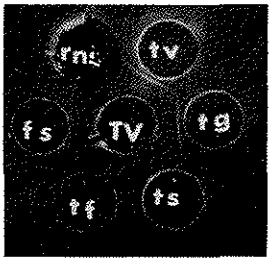


Figure 10 - Precipitation lines of reactions between *T. gallinae* antigen (TG) and anti-*T.vaginalis* antisera (tv), anti-*T. gallinae* antisera (tg), anti-*T. foetus* antisera (tf), and anti-*T. suis* antisera (ts)

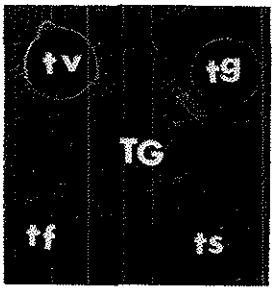
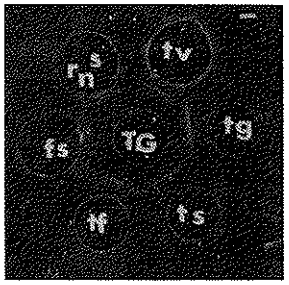


Figure 11 - Precipitation lines of reactions between *T. gallinae* antigen (TG) and anti-*T.vaginalis* antisera (tv), anti-*T. gallinae* antisera (tg), anti-*T. foetus* antisera (tf), and anti-*T. suis* antisera (ts)



Abbreviations

TS - *T. suis* antigen; TF - *T. foetus* antigen; TV - *T. vaginalis* antigen; TG - *T. gallinae* antigen.
ts - *T. suis* antisera; tf - *T. foetus* antisera; tv - *T. vaginalis* antisera; tg - *T. gallinae* antisera.
1,2 and 3 - antisera of *T. suis*; 4, 5 and 6 - antisera of *T. foetus*.

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