ELSEVIER

Veterinary Parasitology 99 (2001) 73–78

veterinary parasitology

www.elsevier.com/locate/vetpar

Short communication

Experimental infection with *Tritrichomonas suis* in heifers

Eduardo Rubén Cobo [a], Dora Cano [b], Carlos Manuel Campero [b,*]

[a] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
[b] *Estacion Experimental Agropecuaria, Patología Veterinaria, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 276, 7620 Balcarce, Argentina*

Received 8 December 2000; received in revised form 24 April 2001; accepted 3 May 2001

Abstract

Nine heifers were intravaginally challenged with 9.3×10^6 *Tritrichomonas suis* reference strains. Vaginal mucus and serum samples were collected weekly 4 weeks post-inoculation. Vaginal mucus was cultured for *T. suis* and sera was tested by ELISA against whole cell antigens for *T. suis* and *Tritrichomonas foetus*. All vaginal mucus cultures were *T. suis*-negative during the experiment. ELISA values for both antigens were similar and differences were not significant ($P > 0.05$). Positive control serum samples from one heifer vaccinated against *T. foetus* showed anti-*T. suis* ELISA values. We concluded that *T. suis* intravaginal inoculation induced a low level of serum immune response in heifers measured by ELISA and both protozoa probably share a common antigen. However, under the experimental conditions of this trial, colonization of the heifers' genital tract was not possible in any of the nine animals.

Keywords: *Tritrichomonas suis*; *Tritrichomonas foetus*; Cattle-protozoa; ELISA

1. Introduction

Tritrichomonas foetus is a flagellated protozoan parasite that is found in the bovine genital tract and is transmitted by venereal contact causing severe economic losses (Skirrow and BonDurant, 1988; Felleisen, 1999). The natural host is *Bos taurus* and *Bos indicus* cattle, but occasionally it may be isolated from swine, horses, and deer (Skirrow and BonDurant, 1988). In bulls, infection persists for years without clinical symptoms and no detrimental effects on libido and sperm quality were mentioned (Skirrow and BonDurant, 1988;

* Corresponding author. Tel.: +54-2266-439100/439120; fax: +54-2266-439101.
E-mail address: ccampero@balcarce.inta.gov.ar (C.M. Campero).

0304-4017/01/$ – see front matter
PII: S0304-4017(01)00444-7

Felleisen, 1999). In infected cows, *T. foetus* produces infertility and reproductive losses due to vaginitis, endometritis, and salpingitis with early abortion (Skirrow and BonDurant, 1988). *Tritrichomonas suis* is found in the nasal cavity and digestive tract of swine (De Carli and Guerrero Ramirez, 1975; Felleisen, 1999) without clinical signs or only with occasional mild rhinitis (De Carli and Guerrero Ramirez, 1975; Felleisen, 1999).

Remarkable similarities between *T. foetus* and *T. suis* such as morphology, in vitro cultivation, antigens, DNA sequences, chromosome numbers, and morphology were found by different researchers (Kerr, 1958; De Carli and Guerrero Ramirez, 1975; Mattos et al., 1997; Felleisen et al., 1997; Xu et al., 1998). Recently, by comparative analysis of the rRNA gene unit sequence and using the random amplified polymorphic DNA (RAPD) technique, it was postulated that both the microorganisms would be strains of the same species characterized by differences in the host and pathogenicity range (Felleisen, 1998). However, only a few reports on cross-infection between *T. foetus* and *T. suis* in swine and cattle were made around the 1950s, and not too much new information about the immune response in female cattle is available (Fitzgerald et al., 1955, 1958; Kerr, 1958). The knowledge about *T. foetus* and *T. suis* pathogenicity in non-common hosts and the immune response generated in such hosts would enable the evaluation of the antigenic expression of both trichomonads as well as the host–parasite interaction. The purpose of the present study was to induce an experimental infection in heifers with *T. suis* and provide information to evaluate the systemic immune response in the host.

2. Materials and methods

Nine virgin heifers, Aberdeen Angus, Hereford and their crossbreeds, approximately 27 months old, with palpably normal reproductive tracts were used in the present study. They came from a herd free of venereal diseases. To verify that all heifers were free of *T. foetus* and *T. suis*, two weekly cultures of vaginal mucus were made before the experiment.

Heifers were challenged with a *T. suis* strain kindly provided by Dr. BonDurant, University of California, Davis, CA, USA (American Type Culture Collection No. 30169, ATCC, Rockville, MD, USA) grown on liver infusion medium with 10% of horse serum and antibiotics and incubated at 37°C (Campero et al., 1986). Heifers were inoculated by deep intravaginal infusion of 9.3×10^6 *T. suis* suspended in 3 ml of phosphate-buffered saline solution (PBSS) (Campero et al., 1993). Weekly collection of blood from the jugular vein and vaginal mucus secretions were performed with Vacutainer tube and Cassou artificial insemination pipette from the cranial regions of the vagina next to the cervix (Campero et al., 1993; Corbeil et al., 1998), respectively. Samples were collected since inoculation day (day 0) until 29 days after inoculation. Mucus was cultured onto the same liver infusion medium as was mentioned (Campero et al., 1986) and was examined daily with the microscope during a week after collection (Campero et al., 1986).

An ELISA test was used to detect anti-*T. foetus* and anti-*T. suis* immunoglobulins in the serum as described by Skirrow and BonDurant (1990) for *T. foetus,* with minor corrections. Briefly, optimal serum and rabbit anti-bovine whole immunoglobulins conjugated with peroxidase (Sigma, St. Louis, MO, USA) dilutions were 1:1000 and 1:5000, respectively, diluted with PBSS plus 0.05% (v/v) Tween 20 and 0.2% gelatin (PBSS Tw-g). For color

development, 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) (Sigma, St. Louis, MO, USA) was used. Stock solution of 40 mM ABTS (2194 mg/ml bidistilled water) was diluted $\frac{1}{4}$ in 0.05 M citric acid, pH 4.5, containing 0.006% hydrogen peroxide. After 4 min, the reaction was stopped with 2 M sulfuric acid and the optical densities (OD) were read on a microplate reader (Multiskan EX, Labsystems, Helsinki, Finland) at 205 nm. A high titer serum obtained from a cow immunized eight times subcutaneously with 10^8 formalized *T. foetus* whole cells were used as a positive control on each plate. Negative control serum from non-infected and non-exposed heifers was included on each plate. Each sample was tested in quadruplicate. OD were corrected according to the following formula (Hum et al., 1991):

$$\text{ELISA values (EV)} = \left[\frac{(\text{mean sample OD} - \text{mean OD of negative control on the same plate})}{(\text{mean OD of positive control on the same plate} - \text{mean OD of negative control on the same plate})} \right] \times 100.$$

The EV mean of anti-*T. foetus* and anti-*T. suis* antibody was analyzed for each post inoculation day by the Mixed SAS test for repeated measures. For the comparisons between the EV of both antigens in each post inoculation day, a two-tailed *t*-test was used and the level of significance was set at $P < 0.05$.

3. Results

Cultures of *T. suis* from vaginal mucus samples showed that infection was not established in any heifer after inoculation.

Serum samples from heifers, vaccinated against *T. foetus*, used as a positive control had an OD mean of 0.22 for *T. foetus* and an OD mean of 0.20 for *T. suis*. Serum samples used as a negative control had an OD mean of 0.01 for *T. foetus* and *T. suis*.

Table 1
Serum ELISA values anti-*T. foetus* of heifers inoculated with *T. suis*

Heifers No.	Post-inoculation days				
	0	8	15	22	29
1	−1.09	5.49	12.63	2.19	4.39
2	9.89	1.64	12.63	4.94	5.49
3	−2.74	2.19	0	−6.04	9.34
4	7.69	7.69	1.64	−0.54	1.09
5	2.19	8.79	4.94	24.17	42.85
6	6.04	−2.74	−3.84	−4.39	0
7	−6.04	2.19	2.74	4.94	−1.64
8	5.49	1.09	6.59	0.54	1.64
9	−6.04	1.09	3.29	−0.54	2.19
Means	1.70	3.05	4.51	2.80	7.26
Standard deviation	5.95	3.62	5.47	8.83	13.73

Table 2
Serum ELISA values anti-*T. suis* of heifers inoculated with *T. suis*

Heifers No.	Post-inoculation days				
	0	8	15	22	29
1	−0.98	1.96	1.96	2.45	2.94
2	5.39	−3.43	−0.49	6.37	0.98
3	−3.92	−2.45	−2.94	0.98	5.88
4	5.39	4.41	3.92	6.86	4.41
5	−2.45	2.94	8.82	24.01	37.74
6	1.47	3.92	−0.98	0.98	1.96
7	−4.41	2.45	5.39	7.35	0.49
8	1.47	8.33	14.70	11.27	5.88
9	−5.88	2.94	−0.49	0.49	−4.90
Means	−0.43	2.34	3.32	6.75	6.15
Standard deviation	4.14	3.53	5.62	7.44	12.29

Specific immune responses to *T. foetus* and *T. suis* antigens were evaluated in serum by ELISA (Tables 1 and 2, respectively). Both EV levels of anti-*T. foetus* and anti-*T. suis* antibody were not statistically significant in serum samples in any post inoculation day (p 0.14) and (p 0.37), respectively. Heifer No. 5 showed higher EV anti-*T. foetus* and anti-*T. suis* antigens at days 22 and 29 post-inoculation (Tables 1 and 2).

4. Discussion

Experimental vaginal infection of beef heifers was not achieved in the present work, although the *T. suis* reference strain was similar to that used in the previous works on molecular characterization (Felleisen, 1997, 1998; Felleisen et al., 1997; Mattos et al., 1997). Viability of the *T. suis* strain was proved in liver broth infusion after vaginal inoculation of the heifers. Experimental intravaginal inoculation of the organism was performed in agreement with previous reports on inoculation of heifers with *T. foetus* (Campero et al., 1993; Corbeil et al., 1998). The *T. suis* inoculum used in the present work had a higher number of microorganisms than that used in experimental infections with *T. foetus* (Skirrow and BonDurant, 1990; Corbeil et al., 1998). Nevertheless, research conducted in the 1950s found similar patterns of genital infection with *T. suis* and *T. foetus* (Fitzgerald et al., 1955, 1958; Kerr, 1958). However, details about inoculation dose, strain origin, and other procedures were not mentioned (Fitzgerald et al., 1958; Kerr, 1958). More work should be done to establish virulence factors and the effect of the *T. suis* strain on heifers.

In the present work, the humoral immune response was evaluated by ELISA comparing the EV mean levels during post inoculation days. A significant increase in the EV level for both antigens was not seen. However, a tendency to a very low increase in systemic IgG was noted during the assay. This circumstance suggests very poor antigen recognition of the host immune system as in naturally and experimentally *T. foetus* infected heifers (Skirrow and BonDurant, 1990). Only heifer No. 5 showed an immune response against *T. suis* and possibly it was a particular case where the animal could also share

antigens with other organism from the reproductive or digest tracts (Skirrow and BonDurant, 1990).

On the other hand, IgG serum levels for *T. suis* and *T. foetus* antigens from heifers vaccinated against *T. foetus* used as a positive control measured by ELISA were similar. These results demonstrated that *T. foetus* and *T. suis* probably share common antigens. In its turn, there was morphological similarity between *T. foetus* and *T. suis* under optical (Kerr, 1958; De Carli and Guerrero Ramirez, 1975) and ultrastructural microscopy (Mattos et al., 1997). It was also mentioned that a high degree of isozymic homogeneity in electrophoretic analysis (Mattos et al., 1997) exists between *T. foetus* and *T. suis* and that they share the same number of chromosomes ($2n = 10$) with identical morphology (Xu et al., 1998). Besides, recent reports showed genetic identity evidenced by RAPD technique (Felleisen, 1998) and the same 5.8S rRNA sequence and internally transcribed spacer (Felleisen, 1997). However, osmotic differences between cell membranes of both the trichomonads (Xu et al., 1998) and of some of their isozymes (Mattos et al., 1997) are mentioned. In its turn, similarity in the bovine vaginal immune response between *T. suis* and *T. foetus* var. Belfast but different from *T. foetus* var. Manley was reported (Kerr, 1958). We concluded that *T. foetus* and *T. suis* would be the same species, but with variations in their antigenic expressions. Such variations could limit the colonization of the female genital tract, expressing different mechanisms of pathogenicity and virulence. Further studies are necessary to understand the host–parasite interaction and to elucidate the relationship between *T. suis* and *T. foetus*.

References

Campero, C.M., Catena, M., Medina, D., 1986. Caldo infusión hígado para el cultivo de *Tritrichomonas foetus*. Vet. Arg. 3, 80–81.

Campero, C.M., Patitucci, A., Medina, D., 1993. Tricomoniasis bovina: infección experimental y natural en hembras. Vet. Arg. 10, 662–670.

Corbeil, L., Anderson, M., Corbeil, R., Eddow, J., BonDurant, R., 1998. Female reproductive tract immunity in bovine trichomoniasis. Am. J. Reprod. Immunol. 39, 189–198.

De Carli, G., Guerrero Ramirez, J., 1975. *Tritrichomonas suis*: isolamento, morfologia e incidéncia na cavdade nasal de porcos domésticos do Rio Grande do Sul. Rev. Fac. Med. Vet. Zootec. Univ. S. Paulo 12, 269–276.

Felleisen, R., 1997. Comparative sequence analysis of 5.8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. Parasitol. 115, 111–119.

Felleisen, R., 1998. Comparative genetic analysis of tritrichomonadid protozoa by the random amplified polymorphic DNA technique. Parasitol. Res. 84, 153–156.

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

Felleisen, R., Schimid-Lambelet, N., Walubengo, J., 1997. Comparative evaluation of methods for the diagnosis of bovine *Tritrichomonas foetus* infection. Protozoo. Res. 7, 90–101.

Fitzgerald, P.R., Johnson, A.E., Thorne, J.L., Hammond, D.M., 1955. Experimental infections of the bovine genital tract with trichomonads from swine. J. Parasitol. 41 (Suppl.), 17.

Fitzgerald, P.R., Johnson, A.E., Hammond, D.M., Thorne, J.L., Hibler, C.P., 1958. Experimental infections of young pigs following intranasal inoculation with nasal, gastric, or cecal trichomonads from swine or with *Tritrichomonas foetus*. J. Parasitol. 44, 597–602.

Hum, S., Stephens, L., Quinn, C., 1991. Diagnosis by ELISA of bovine abortion due to *Campylobacter fetus*. Aust. Vet. J. 68, 272–275.

Kerr, W.R., 1958. Experiments in cattle with *Trichomonas suis*. Vet. Rec. 70, 613–615.

Mattos, A., Solé-Cava, A.M., DeCarli, G., Benchimol, M., 1997. Fine structure and isozymic characterization of trichomonadid protozoa. Parasitol. Res. 83, 290–295.

Skirrow, S., BonDurant, R., 1988. Bovine trichomoniasis. Vet. Bulletin 58, 591–603.
Skirrow, S., BonDurant, R., 1990. Immunoglobulin isotype of specific antibodies in reproductive tract secretions and sera in *Tritrichomonas foetus*-infected heifers. Am. J. Vet. Res. 51, 645–653.
Xu, W.-D., Lun, Z., Gajadhar, A., 1998. Chromosome numbers of *Tritrichomonas foetus* and *Tritrichomonas suis*. Vet. Parasitol. 78 (4), 247–254.