Toxoplasma gondii in experimentally infected *Bos taurus* and *Bos indicus* semen and tissues[1]

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ABSTRACT.- Scarpelli L., Lopes W.D.Z., Migani M., Bresciani K.D.S. & Costa A.J. 2009. ***Toxoplasma gondii* in experimentally infected *Bos taurus* and *Bos indicus* semen and tissues.** *Pesquisa Veterinária Brasileira 29(1):59-64.* Departamento de Medicina Veterinária Preventiva, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Via de Acesso Prof. Paulo Donatto Castellani s/n, Jaboticabal, SP 14884-900, Brazil. E-mail: wdzlopes@hotmail.com

Eighteen young steers were inoculated with *Toxoplasma gondii* and randomly distributed into three groups of six animals each: GI, $2.5x10^5$ "P" strain oocysts, GII, $5.0x10^6$ "RH" strain tachyzoites, and GIII (Control). Clinical, serological and parasitemia exams were realized. Parasite investigation by bioassay and PCR was realized on semen and fragments of skeletal musculature, lymph nodes, brain, retina, spleen, liver, lung, testicle, epididymis and seminal vesicle. Blood and semen samples were collected on days -2, -1, 1, 3, 5, 7, 14 and weekly thereafter, up to postinfection day (PID) 84. The inoculated steers (GI and GII) presented hyperthermia from PID 3 to 16. Antibodies against *T. gondii* were detected through the indirect fluorescence antibody test (IFAT) on PID 5 (1:16) in both inoculated groups (oocysts and tachyzoites), reaching peaks of 1:4096 on PID 7. Parasitemia outbursts occurred in all infected bovines, principally from PID 7 to 28, independent of the strain and inoculate used. Bioassays revealed the presence of parasites in semen samples of animals infected with oocysts (GI) and tachyzoites (GII) on several experimental days between PID 7 and 84. Tissue parasitism by *T. gondii* was diagnosed by bioassay and the PCR technique in several organ and tissue fragments. These findings suggest the possibility of sexual transmission of *T. gondii* in the bovine species.

INDEX TERMS: Cattle, PCR, semen, *Toxoplasma gondii,* Apicomplexa.

RESUMO.- [***Toxoplasma gondii* em semen e tecidos de *Bos taurus* and *Bos indicus* experimentalmente infectados.**] Dezoito bovinos foram inoculados com *Toxoplasma gondii* e distribuídos aleatoriamente em três grupos de seis bovinos cada: GI ($2,5x10^5$ oocistos da cepa "P"), GII ($5,0x10^6$ taquizoítos da cepa "RH") e GIII (controle). Exames clínicos, sorológicos e parasitêmicos foram realizados. Pesquisas do parasito, por meio da bioprova e pela técnica de Reação em Cadeia pela Polimerase (PCR), foram realizadas no sêmen e em fragmentos de musculatura esquelética, linfonodos, cérebro, retina, baço, fígado, pulmão, testículo, epidídimo e vesícula seminal. Amostras de sangue e sêmen foram colhidas nos dias -2, -1, 1, 3, 5, 7, 14 e, semanalmente, até o 84º dia pós-infecção (DPI). Os bovinos inoculados (GI e GII) apresentaram hipertermia do 3º ao 16º DPI. Anticorpos contra *T. gondii* foram detectados (IFI) no 5º DPI (1:16), em ambos grupos inoculados (oocistos e taquizoítos), atingindo picos de 1:4096 no 7º DPI. Surtos parasitêmicos ocorreram em todos os bovinos infectados, principalmente do 7º ao 28º DPI, independente da cepa e inóculo utilizados. O bioensaio revelou a presença do parasito em amostras seminais dos bovinos infectados com oocistos (GI) e taquizoítos (GII), em diversas datas experimentais, entre o 7º e 84º DPI. Parasitismo tissular por *T. gondii* foi diag-

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nosticado por meio da bioprova e pela técnica da PCR, em vários fragmentos de tecidos e/ou órgãos. Os achados sugerem a possibilidade da ocorrência da transmissão sexual do *T. gondii* na espécie bovina.

TERMOS DE INDEXAÇÃO: Bovinos, PCR, sêmen, *Toxoplasma gondii,* Apicomplexa.

INTRODUCTION

Toxoplasma gondii (Nicolle & Manceaux 1909), an intracellular parasite presenting a complex biological cycle, attacks practically all warm-blooded species (Dubey & Beattie 1988), with felines being the only hosts that eliminate oocysts through the feces. All other mammals maintain the asexual biological phase and perform the role of intermediate hosts (Dubey 1995).

Natural infection in cattle was first diagnosed in Germany by Houersdorf & Holtz (1952) and quoted by Sanger et al. (1953). Although oocyst ingestion is the most frequent infection via for *T. gondii*, other possible disseminating vias for this zoonosis were studied by Dubey (1999).

Data concerning infection by *T. gondii* in cattle is limited to seroprevalence studies and parasite isolates in tissue, certifying its possible transmission to humans (Dubey & Thulliez, 1993).

Since it is a practically asymptomatic infectious disease, diagnosis is difficult. Besides the conventional laboratory techniques used to detect parasite and antibody presence (bioassay, IFAT and ELISA), Polymerase Chain Reaction (PCR) can significantly contribute to toxoplasmic research, especially in association with the above mentioned techniques (Esteban Redondo et al. 1999).

Although the isolation of *T. gondii* in the semen of sheep (Spence et al., 1978), goats (Dubey & Sharma, 1980) and swine (Moura et al., 2007) has been successfully realized, there are no studies in the literature that prove the isolation of this protozoan in the semen of cattle presenting toxoplasmic infection. The present study aimed to diagnose the eventual presence of *T. gondii* in semen samples of experimentally infected steers.

MATERIALS AND METHODS

Toxoplasma gondii strains

Toxoplasma gondii "P" (Jamara & Vieira 1991) and "RH" strains (Sabin 1941) were used in this study.

The inoculants were obtained by means of periodic inoculations of brain cysts ("P" strain) and/or tachyzoites ("RH") in albino mice. *T. gondii* oocysts were obtained according Dubey et al. (1970).

Eighteen 11 to 12-month-old cattle, 9 *Bos taurus* and 9 *Bos indicus*, serologically negative for *T. gondii*, were selected, identified, randomized and inoculated according to the experimental outline (Table 1). All the animals were maintained in individual bays, where they received water and feed *ad libitum*.

Serological tests

Serological exams to detect antibodies against other infectious diseases that could provoke reproductive disorders, such as brucellosis, neosporosis and leptospirosis, were realized on all the experimental bovine, both pre and pos inoculation.

Table 1. Experimental outline of bovine inoculated with *Toxoplasma gondii*

Group	Bovine number	Breed	*Toxoplasma gondii* - inoculation via
I	9, 28, 32	*B. taurus*	1x 10^6 oocysts -oral
	681, 687, 700	*B. indicus*	1x 10^6 oocysts - oral
II	5, 8, 33	*B. taurus*	5 x 10^6 tachyzoites - subcutaneous
	683, 689, 693	*B. indicus*	5 x 10^6 tachyzoites - subcutaneous
III	6, 7, 241	*B. taurus*	Placebo
	685, 691, 735	*B. indicus*	Placebo

Parasitemia was determined by inoculation of the leukocyte layer in mice, in accordance with the technique described by Oliveira et al. (2001).

Blood samples were collected two days prior the inoculation and on 1, 3, 5 and 7, pos inoculation (DPI) then weekly up to 84 (DPI), to obtain serum and investigate the presence of antibodies against *T. gondii*, using indirect fluorescence antibody test (IFAT) according Camargo (1964).

Bioassay and Polymerase chain reaction (semen and tissues of cattle)

Concurrently, semen samples of the 18 steers were obtained by electroejaculator. Investigation of *T. gondii* in bovine semen was realized by bioassay, in mice (Teale et al. 1982) and by PCR (Fuents et al., 1996). For the bioassay, 1.0mL aliquot of each semen sample was inoculated into five mice that were examined in accordance with the criteria adopted by Oliveira et al. (2001). The inoculated mice that survived until the 42nd DPI, were euthanized to research *T. gondii* cysts.

After the experimental period of the semen collection (84 post inoculation days), all of the cattle (inoculated groups and control) were necropsied, in order to carry out research by bioassay (Dubey 1998) and Polymerase chain reaction (Fuents et al. 1996), on tissue parasitism of these animals.

DNA from the semen and tissue (skeletal musculature, lymph nodes, brain, retina, spleen, liver, lung, testicle, epididymis and seminal vesicle) of cattle and positive control samples ("RH") was extracted in accordance the methodology described by Sambrook & Russell (2001). For detection of *T. gondii* in these samples, a 194 base pair (bp) fragment of the *T. gondii* B_1 gene was amplified using the primers 5'-GGAACTGCATCCGT TCATGAG-3' ($B1_1$) and 5'TCTTTAAAGCGTTCGTGGTC -3' ($B1_2$) described by Fuentes et al. (1996). PCR was realized by addition of 500ng of DNA in a reaction medium containing 2mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 9), 0.01% Triton X-100, 0.2mM dNTPs, 10pmoles of initiator and 5.0 units of TaqDNA polymerase.

Analysis of the amplified products was performed by electrophorese on 2% agarose gel containing restriction fragments separated by electrophorese, stained in 0.5µg/mL ethidium bromide solution dissolved in water for 20 min and observed in transilluminator UV.

Tissue parasitism, in cattle, by *T. gondii* was also investigated by bioassay (Dubey 1998) and PCR (Fuentes et al. 1996) in fragments of skeletal musculature, lymph nodes, brain, retina, spleen, liver, lung, testicle, epididymis and seminal vesicle.

Statistical analysis of the post-inoculation *Bos taurus* and *Bos indicus* were evaluated using Split Plot in Time analysis, considering the inoculated groups (GI and GII). All statistical analyses were conducted using SAS software (2001).

Table 2. Antibodies anti-*T. gondii* serological titer obtained by the indirect fluorescence antibody test in cattle inoculated with 1x 106 oocysts (GI), 5x106 tachyzoites (GII) and noninoculated (GIII)

Post inovulation day	Serological titer values																	
	GI						GII						GIII					
	Bos taurus			*Bos indicus*			*Bos taurus*			*Bos indicus*			*Bos taurus*			*Bos indicus*		
	9	28	32	681	687	700	5	8	33	683	689	693	6	7	241	685	691	735
-2	-[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	16	-	-	16	-	-	-	-	-	-	-	-	-
7	64	64	16	4096	64	64	4096	256	256	64	16	1024	-	-	-	-	-	-
14	256	64	64	256	64	64	256	256	256	256	256	256	-	-	-	-	-	-
21	256	256	64	256	64	64	256	256	256	256	256	256	-	-	-	-	-	-
28	256	64	64	256	64	64	256	256	256	256	256	256	-	-	-	-	-	-
35	16	64	64	256	256	64	256	256	256	256	64	64	-	-	-	-	-	-
42	256	16	16	256	64	64	64	64	256	64	64	64	-	-	-	-	-	-
49	+[b]	16	64	256	64	16	256	256	256	64	64	64	-	-	-	-	-	-
56	+	16	16	256	64	16	64	64	256	256	64	256	-	-	-	-	-	-
63	+	16	16	256	64	16	64	64	256	256	64	256	-	-	-	-	-	-
70	+	16	16	256	64	64	64	64	64	256	64	256	-	-	-	-	-	-
77	+	16	16	256	16	64	64	64	16	64	64	64	-	-	-	-	-	-
84	+	16	16	64	16	16	64	64	16	64	64	64	-	-	-	-	-	-

[a]- Negative serology.
[b]+ Cattle died.

Table 3. Parasitemia outbursts detected in cattle (*Bos taurus* and *Bos indicus*) inoculated with 1x 106 oocysts (GI), 5 x 106 tachyzoites (GII) and noninoculated (GIII)

Bovine number	Group	Bioassay/postinoculation days																
		-2	-1	3	5	7	14	21	28	35	42	49	56	63	70	77	84	Total
9[a]	I	-[c]	-	-	-	Positive	Positive	-	-	-	+[e]	+	+	+	+	+	+	2
28[a]		-	-	Positive[d]	-	Positive	Positive	-	-	-	-	-	Positive	-	-	Positive	-	5
32[a]		-	-	Positive	-	-	-	-	Positive	-	-	-	-	-	Positive	-	Positive	4
681[b]		-	-	-	-	Positive	Positive	Positive	-	Positive	-	-	Positive	Positive	-	-	-	6
687[b]		-	-	-	-	-	-	-	Positive	-	-	-	-	-	Positive	-	Positive	3
700[b]		-	-	Positive	-	Positive	-	-	-	-	-	Positive	-	-	-	-	Positive	4
Total		0	0	3	0	4	3	1	2	1	0	1	2	1	2	1	3	24
5[a]	II	-	-	-	Positive	Positive	-	Positive	-	-	-	-	Positive	-	-	-	-	4
8[a]		-	-	Positive	-	Positive	-	-	-	Positive	Positive	-	-	-	-	Positive	Positive	6
33[a]		-	-	-	Positive	Positive	-	-	-	-	Positive	-	Positive	-	-	-	-	4
683[b]		-	-	Positive	-	-	Positive	-	Positive	-	-	Positive	Positive	Positive	-	Positive	-	7
689[b]		-	-	-	-	Positive	-	-	-	-	-	-	-	-	-	Positive	-	2
693[b]		-	-	-	-	Positive	-	-	-	-	-	-	Positive	-	-	-	-	2
Total		0	0	2	2	5	1	1	1	1	2	1	4	1	0	3	1	25
6[a]	III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
7[a]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
241[a]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
685[b]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
691[b]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
735[b]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Total		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]*Bos taurus,* [b]*Bos indicus.*
[c]Negative serology (mice).
[d]IFAT (≥1:64).
[e]+ Cattle died.

RESULTS AND DISCUSSION

Experimental toxoplasmic infection of cattle, after inoculation with *T. gondii* oocysts or tachyzoites, was confirmed by parasitemia and by seroconversion of the animals inoculated with the different strains. Based on the vital signs analyzed, observation showed that animals of GI (oocysts) and GII (tachyzoites) presented hyperthermia from day to 16 PI, independent of the strain and form of the inoculums. These results are similar to those obtained by Esteban Redondo et al. (1999) and Dubey (1983), who reported hyperthermia in cattle experimentally infected with *T. gondii* from day 5 to 8 PI and 2 to 7 PI, respectively.

Table 4. Presence of *Toxoplasma gondii* in semen samples from reproductive cattle (*Bos taurus* and *Bos indicus*) inoculated with 1x 106 oocysts (GI), 5 x 106 tachyzoites (GII) and noninoculated (GIII) by bioassay in mice

Bovine number	Group	Bioassay/postinoculation days																
		-2	-1	3	5	7	14	21	28	35	42	49	56	63	70	77	84	Total
9[a]	I	-[c]	-	-	-	-	Positive	-	-	-	+[e]	+	+	+	+	+	+	1
28[a]		-	-	-	-	-	Positive	Positive	-	-	Positive	Positive	-	-	-	-	-	4
32[a]		-	-	-	-	-	-	Positive	-	-	-	-	-	-	-	-	-	1
681[b]		-	-	-	-	-	-	Positive	-	-	-	-	-	Positive	-	Positive	Positive	4
687[b]		-	-	-	-	-	-	Positive	-	-	-	-	-	-	-	-	-	1
700[b]		-	-	-	-	-	-	Positive	-	Positive	Positive	Positive	-	Positive	-	-	-	5
Total		0	0	0	0	0	2	5	0	1	2	2	0	2	0	1	1	16
5[a]	II	-	-	-	-	Positive[d]	-	Positive	-	-	-	Positive	-	Positive	-	-	-	4
8[a]		-	-	-	-	Positive	-	-	-	-	-	Positive	-	Positive	-	-	-	3
33[a]		-	-	-	-	-	-	-	Positive	-	-	-	-	-	-	-	-	1
683[b]		-	-	-	-	-	-	Positive	-	-	Positive	-	-	-	-	-	-	2
689[b]		-	-	-	-	-	Positive	Positive	-	-	Positive	-	-	-	-	Positive	Positive	5
693[b]		-	-	-	-	Positive	Positive	Positive	-	-	-	-	Positive	-	-	-	-	4
Total		0	0	0	0	3	2	4	1	0	2	2	1	2	0	1	1	19
6[a]	III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
7[a]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
241[a]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
685[b]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
691[b]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
735[b]		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Total		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]*Bos taurus,* [b]*Bos indicus.*
[c]- Negative serology (mice).
[d]IFAT (≥1:64) and presence of brain cysts in inoculated mice with cattle semen.
[e]+ Cattle died.

Table 5. Presence of *Toxoplasma gondii* in organs of cattle (*Bos taurus* and *Bos indicus*) inoculated with 1x 106 oocysts (GI), 5 x 106 tachyzoites (GII) and noninoculated (GIII)

Bovine number	Group	Bioassay/PCR/Organs										
		Skeletal musculature	Lymph nodes	Brain	Retina	Spleen	Liver	Lung	Testicle	Epididymis	Seminal vesicle	Total Bioassay/ PCR
9[a]	I	-[c]	Positive	-	-	Positive	-	-	-	-	-	-
28[a]		Positive[d]	-	Positive	-	Positive	Positive	-	Positive	-	-	-
32[a]		-	-	Positive[d]	-	Positive[d]	Positive	Positive	-	-	-	-
681[b]		-	Positive	Positive[d]	Positive	Positive	Positive[d]	Positive	Positive	Positive	-	-
687[b]		-	-	Positive[d]	-	Positive	Positive[d]	-	Positive	-	Positive[d]	-
700[b]		-	Positive	-	Positive	Positive[d]	-	Positive	-	-	Positive[d]	-
Total Bioprova		1	3	4	2	6	4	3	3	1	2	29
Total PCR		1	0	3	0	2	2	0	0	0	2	10
5[a]	II	Positive[e]	Positive	Positive	-	Positive[d]	Positive[d]	Positive	Positive	Positive	-	-
8[a]		Positive[d]	Positive	-	Positive	-	Positive[d]	-	-	Positive	-	-
33[a]		-	-	Positive	Positive	-	Positive[d]	Positive[d]	-	-	-	-
683[b]		-	-	-	Positive	-	-	Positive[d]	-	-	-	-
689[b]		Positive[d]	Positive[d]	Positive	Positive	Positive	Positive	Positive	Positive	-	Positive	-
693[b]		-	-	-	Positive	-	-	-	Positive	-	Positive	-
Total Bioprova		3	3	3	5	2	4	4	3	2	2	31
Total PCR		2	1	0	0	1	2	2	0	0	0	8
6[a]	III	-	-	-	-	-	-	-	-	-	-	-
7[a]		-	-	-	-	-	-	-	-	-	-	-
241[a]		-	-	-	-	-	-	-	-	-	-	-
685[b]		-	-	-	-	-	-	-	-	-	-	-
691[b]		-	-	-	-	-	-	-	-	-	-	-
735[b]		-	-	-	-	-	-	-	-	-	-	-
Total		0	0	0	0	0	0	0	0	0	0	0

[a]*Bos taurus,* [b]*Bos indicus.*
[c]Negative serology (mice).
[d]Presence of brain cysts in inoculated mice with cattle tissues and by PCR technique.
[e]IFAT (≥1:64) and presence of brain cysts in inoculated mice with cattle tissues.

Experimental infection triggered a rapid immunological response, with antibody detection occurring from day 5 PI (Table 2). This early humoral immune response in experimental infections of *T. gondii* was also detected by Berverley & Waston (1971) in sheep and by Moura et al. (2007) in swine. In this study, antibody were firstly detected on day 5 PI and remained at high levels from 7 to 35 DPI, when a gradual reduction in humoral immune response occurred; however, none of the steers were sero-negative by the end of the experimental period.

T. gondii detection in the blood stream, by means of parasitemia, was verified by the seroconversion of mice inoculated with leukocyte layers. Parasitemia outbursts occurred in all the inoculated bovines (Table 3). It should be highlighted that all the cattle of both subspecies studied presented parasitemia (*T. gondii*), mainly from 7 to 28 PI, independent of the form of infection used. Esteban Redondo et al. (1999) also in cattle, detected parasitemia outbursts similar to those identified in the present research.

While working with calves and cows experimentally infected with *T. gondii* oocysts, Dubey (1983) detected no parasitemia through the use of mice.

The first report regarding the isolation of *T. gondii* in semen was a reference cited by Spence et al. (1978), in which Disko et al. (1971) were successful in their attempt to recuperating this agent from semen samples in three out of 125 men who contracted toxoplasmic infection naturally. Later, numerous authors obtained positive results using experimentally infected animal semen samples: Spence et al. (1978), Teale et al. (1982) and Aganga et al. (1988) studying sheep, Dubey & Sharma (1980) studying goats, and Moura et al. (2007), studying swine.

The results of this study report the first description of *T. gondii* isolated from semen samples of experimentally infected bovines, detected both indirectly (IFAT) and directly (*T. gondii* brain cysts) in mice inoculated with aliquots of semen from diverse bovine ejaculates collected between 7 and 84 DPI (Table 4). It should be noted that the results obtained by PCR did not fully agree with the bioassays, which presented much greater sensitivity, in comparison with PCR, regarding *T. gondii* isolation in the experimentally infected bovine reproducers. Hitt & Filice (1992) affirmed that despite presenting minimal practicability in the laboratory, the efficacy of the bioassay at isolating *T. gondii* is far greater in relation to the PCR technique. This conclusion was also ratified by Esteban Redondo et al. (1999) regarding the isolation of this protozoan in sheep and bovine.

Tissue parasitism by *T. gondii* was diagnosed in all the bovines using the bioassay and in 11 out of 12 inoculated animals by the PCR technique. The organs diagnosed as infected by bioassay in decreasing order were the liver and spleen, retina, brain, lung, lymph nodes, testicles, skeletal musculature and seminal vesicle (Table 5). Similar results in cattle were found by Sanger et al. (1953), Jacob et al. (1960), Work (1967), Cata et al. (1969), and Oliveira et al. (2001). In this study the two breeds (*Bos taurus* and *Bos indicus*) evaluated showed, in inoculated groups, no statistical difference (P>0,05) between diagnostic (serological tests, bioassay and PCR).

In the present study, a 194 bp segment of the *T. gondii* B_1 gene was amplified, since this gene is present on at least 35 loci in the *T. gondii* genome (Fuentes et al. 1996, Burg et al. 1989). Verification regarding the most affected organs by means of the PCR technique, in decreasing order, revealed: liver, spleen, brain, skeletal musculature, lung, seminal vesicle and lymph nodes (Table 5).

The absence of positivity for parasitism by PCR, found in genomic samples of semen and certain tissues, does not discard the possibility that the parasitic agent is present. In some of these samples, DNA could have been lost during the extraction technique; also the 500ng of genomic DNA (host + parasite) per reaction, could contain minimal quantities of parasite DNA, insufficient to visualize the amplification of the 194 bp segment of the B_1 gene on 2% agarose gel (Aquizerate et al. 1993, Dubey & Thulliez 1993, Esteban Redondo et al. 1999).

Some authors affirm that the PCR technique can be a favorable method when associated with other diagnostic methods (Steuber et al. 1995, Ellis 1998).

CONCLUSION

The isolation of *T. gondii* in bovine semen associated with tissue parasitism in fragments parts of the reproductive system observed in the present work suggests the viability of sexual transmission of this protozoan in the bovine species.

REFERENCES

Aganga A.O., Umoh J.U., Kyewalabye E.K. & Ekwempu C.C. 1988. Comparative experimental transmission studies with Nigerian isolates and TS-I strain of *Toxoplasma gondii* in sheep. J. Anim. Prod. Res. 8(1):104-120.

Aquizerate F., Cazenave J., Poirier L., Verin P.H., Cheyrou A., Begueret J. & Lagoutte F. 1993. Detection of *Toxoplasma gondii* in aqueous humour by the polymerase chain reaction. Brit. J. Ophthalmol. 77(1):107-109.

Beverley J.K.A. & Waston W.A. 1971. Prevention of experimental and of naturally occurring ovine abortion due to toxoplasmosis. Vet. Res. 88(1):39-44.

Burg J.L., Grover C.M., Pouletty P. & Boothroyd J. 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii* by Polymerase Chain Reaction. J. Clin. Microbiol. 27(5):1787-1792.

Camargo M.E. 1964. Improvised technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. Revta Inst. Med. Trop. São Paulo 6(3):117-118.

Cata G., Bergendi L. & Halkova R. 1969. Isolation of *Toxoplasma gondii* from swine and cattle. J. Parasitol. 55(9):952-955.

Dubey J.P., Miller N.L. & Frenkel J.K.A. 1970. Characterization of the new fecal from of *Toxoplasma gondii*. J. Parasitol. 56(4):447-450.

Dubey J.P. & Sharma S.P. 1980. Parasitemia and tissue infection in sheep fed *Toxoplasma gondii* oocysts. J. Parasitol. 66(1):111-119.

Dubey J.P. 1983. Distribution of cysts and tachyzoites in calves and pregnant cows inoculated with *Toxoplasma gondii* oocysts. Vet. Parasitol. 13(1):199-201.

Dubey J.P. & Beattie C.P. 1988. Toxoplasmosis of Animal and Man. CRC Press, Boca Raton. 200p.

Dubey J.P. & Thulliez P. 1993. Persistence of tissue cysts in edible tissues of cattle fed *Toxoplasma gondii* oocysts. Am. J. Vet. Res. 54(2): 270-273.

Dubey J.P. 1995. Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. J. Parasitol. 81(3):410-415.

Dubey J.P. 1998. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. Vet. Parasitol. 74(1):75-77.

Dubey J.P., Shen S.K., Kwow O.C.H. & Frenkel J.K. 1999. Infection and immunity with the RH strain of *Toxoplasma gondii* in rats and mice. J. Parasitol. 85(5):657-662.

Ellis J.T. 1998. Polymerase chain reaction approaches for the detection of *Neospora caninum* and *Toxoplasma gondii*. Int. J. Parasitol. 28(9): 1053-1060.

Esteban Redondo, Maley S.W., Thomson K., Nicoli S., Wright S., Buxton D. & Innes E.A. 1999. Detection of *Toxoplasma gondii* in tissues of sheep and cattle following oral infection. Vet. Parasitol. 86(1):155-157.

Fuents S.I., Rodriguez M., Domingo C.J., Fernando C.C., Juncosa T. & Alvar J. 1996. Urine sample used for congenital toxoplasmosis diagnosis by PCR. J. Clin. Microbiol. 3(10):2368-2372.

Hitt J. & Filice G. 1992. Detection of *Toxoplasma gondii* parasitemia by gene amplification cell culture and mouse-inoculation. J. Clin. Microbiol. 30(10):3181-3184.

Jacobs L., Remington J.S. & Mecton M.L. 1960. A survey of meat samples from swine, cattle and sheep for the presence of encysted *Toxoplasma.* J. Parasitol. 46(1):23-28.

Jamara L.M.F. & Vieira M.P.L. 1991. Isolamento do *Toxopalsma gondii* de exudato peritoneal e órgãos de camundongos com infecção experimental. Revta Inst. Med. Trop. São Paulo 33(4):435-441.

Moura A.B., Costa A.J., Filho J.S., Paim B.B., Pinto F.R. & Di Mauro D.C. 2007. *Toxoplasma gondii* in semen of experimentally infected swine. Pesq. Vet. Bras. 27(10):430-434.

Nicolle C. & Manceaux L. 1909. Sur un protozoaire nouveau du *gondii*. Acad. Sci. 148:369.

Oliveira F.C.R., Costa A.J., Bechara G.H. & Sabatini G.A. 2001. Distribuição e viabilidade de cistos de *Toxoplasma gondii* (Apicomplexa: Toxoplasmatinae) em tecidos de *Bos indicus, Bos taurus* and *Bubalus bubalis* infectados com oocistos de. Revta Bras. Med. Vet. 23(1):28-34.

Sas, Institute. 2001. SAS® User's Guide: Estatistics. SAS Institute Inc., Cary, NC, USA.

Sabin A.B. 1941. Toxoplasmic encephalitis in children. J. Am. Med. Assoc. 116(5):801-807.

Sambrook J. & Russell D.W. 2001. Molecular Cloning. 3rd ed. Cold Spring Harbor Press, New York.

Sanger V.L., Chamberlani K.W., Cole C.R. & Farrell R.L. 1953. Toxoplasmosis isolation of *Toxoplasma gondii* from cattle. J. Am. Vet. Med. Assoc. 123(1):87-91.

Spence J.B., Beattie J., Faulkner L. & Watson W.A. 1978. *Toxoplasma gondii* in the semen of rams. Vet. Rec. 102(1):38-39.

Steuber V., Niu A., Bauer C., Reetz J., Roth A. & Janitschike K. 1995. Detection of *Toxoplasma gondii* in tissues of ovine abortions using the polymerase chain reaction. Dtsch. Tierärztl. Wochenschr. 102(1):91-96.

Teale A.J., Blewett D.A., Miller J.K. & Buxton D. 1982. Experimentally induced toxoplasmosis in young rams: The clinical syndrome and semen secretion of Toxoplasma. Vet. Rec. 111(1):53-55.

Work K. 1967. Isolation of *Toxoplasma gondii* from the flesh of sheep, swine and cattle. Acta Pathol. Microbiol. Scand. 71(2):296-306.