

## CLINICAL, PARASITOLOGICAL AND OBSTETRIC OBSERVATIONS IN PREGNANT BITCHES WITH EXPERIMENTAL TOXOPLASMOSIS

### TOXOPLASMOSE EXPERIMENTAL EM CADELAS GESTANTES - OBSERVAÇÕES CLÍNICAS, PARASITOLÓGICAS E OBSTÉTRICAS

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#### SUMMARY

Eight pregnant mixed breed bitches, serologically negative for *Toxoplasma gondii*, were divided into three groups. Group I: bitches 01 and 02 ( $1.0 \times 10^7$  tachyzoites of *Toxoplasma gondii*, subcutaneous route); Group II: bitches 03, 04 and 05 ( $1.5 \times 10^4$  oocysts, oral route); Group III: bitches 06, 07 and 08 (as control). Clinical and obstetric examinations were conducted twice a day on each bitch, and weekly ultra-sonographic evaluations were performed until the end of pregnancy. From the 2<sup>nd</sup> day the bitches presented clinical alterations such as fever, nasal flowing, lachrymation, prostration, lymphadenopathy, premature parturition, abortion and fetal death. In Group I, *Toxoplasma gondii* was isolated from the bitches urine (day 10) and saliva (day 18). The bitches inoculated with oocysts showed milk samples collected on day 07 and day 16 after inoculation positive for *Toxoplasma gondii*. Saliva collected on day 13 and urine samples collected on days 1, 3, 7 and 16 from these bitches also showed positive. All inoculated bitches reacted positively to antigenic stimulus, with production of *Toxoplasma gondii* antibodies from day 3 on, with highest titers detected on day 11. Antibodies were detected by Indirect fluorescent antibody test (IFAT) and Enzyme linked immunosorbent assay (ELISA) tests.

**Key words:** toxoplasmosis, *Toxoplasma gondii*; pregnant bitches, pathology.

#### RESUMO

Oito cadelas gestantes, sem raça definida, clínica e sorologicamente negativas para *Toxoplasma gondii*, foram

distribuídas em três grupos, de acordo com o inóculo: Grupo I – cadelas 01 e 02 ( $1 \times 10^7$  taquizoítos, por via subcutânea); Grupo II – cadelas 03, 04 e 05 ( $1,5 \times 10^4$  oocistos esporulados de *Toxoplasma gondii*, por via oral) e Grupo III – cadelas 06, 07 e 08 (controle/placebo). Monitoramento clínico e obstétrico diários e acompanhamento ultra-sonográfico semanal foram realizados até o final da gestação. Os principais sinais clínicos evidenciados a partir do 2º dia pós-inoculação (DPI) foram: febre, corrimento nasal, lacrimejamento, prostração, linfadenopatia, parto prematuro, abortamento e morte fetal. No Grupo I, *Toxoplasma gondii*, foi isolado da urina no 10º dia e da saliva no 18º dia. As amostras de leite colhidas de cadelas infectadas com oocistos (Grupo II), foram positivas para a presença do parasito no 7º e 16º DPI. No Grupo II, *Toxoplasma gondii* também foi isolado de amostras de urina colhidas nos dias 01, 03, 07 e 16 DPI e na saliva no dia 13 DPI. Todas as cadelas reagiram positivamente ao estímulo antigênico, produzindo anticorpos anti- *Toxoplasma gondii*, através do teste de Imunofluorescência indireta (IFI) e ensaio imunoenzimático indireto (ELISA), a partir do 3º DPI, alcançando títulos máximos no 11º dia pós- inoculação.

**Palavras-chave:** toxoplasmoze; *Toxoplasma gondii*; cadelas gestantes; patologia.

#### INTRODUCTION

In the last decade, toxoplasmosis in dogs has been detected in many countries (DUBEY &

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BEATTLE, 1988; LINDSAY *et al.*, 1990; GUIMARÃES *et al.*, 1992). Concerning epidemiological, parasitological and clinical aspects of this infection, clinical alterations such as hypothermia, lymphadenopathy, respiratory and digestive disorders were described in dogs experimentally infected with *Toxoplasma gondii* by OPPERMAN (1971) and SHARMA *et al.* (1973).

Natural toxoplasmosis was showed in a Fox Hound dog in Jaboticabal, S.P. Brazil (COSTA *et al.*, 1978) during which clinical, serological, histopathological and parasitological alterations were observed.

Congenital *Toxoplasma gondii* infection has been reported, but no case has been well documented in dogs, reviewed by DUBEY (1985), DUBEY & BEATTLE, 1988). BRESCIANI *et al.* (1999), first showed that dogs could be congenitally infected with *Toxoplasma gondii* after oral inoculation of bitches with oocysts. The main purpose of this study was to collect more information on toxoplasmosis in pregnant bitches since current information on the subject was sparse.

## MATERIAL AND METHODS

A inoculum of *Toxoplasma gondii*, P strain, maintained at the "Center of Research in Small Animal Health"- CPPAR/UNESP, was employed. Identification of sporulated forms was made using morphological criteria (ZAMAN, 1970) and intraperitoneal inoculation in mice, according to DUBEY *et al.* (1972).

Eight mixed breed pregnant bitches were used, all serologically negative for *Toxoplasma gondii*. Three of these were kept as controls inoculated with a placebo. In order to confirm pregnancy, an ultra-sonography apparatus was used (Kontron Sigma 21 transducer wobbler 7.5mhz and linear transducer 5mhz). Bitches were kept in individual cages, with free access to commercial dog food and water. Clinical and laboratorial testings were done for blood, feces and urine samples, for assessment of overall good health of animals. Before inoculation bitches were distributed as shown in table 1.

Complete clinical examinations were conducted on each dog twice a day, in the morning and afternoon up, until end of the pregnancy. Weekly ultra-sonography evaluations were performed.

Hematological examinations were performed as recommended by SCHALM *et al.* (1986). Determinations of alanine amine transferase (ALT), aspartate amine transferase (AST), lactic

Table 1 - Experimental inoculation of *Toxoplasma gondii* in pregnant bitches : bitches 01 and 02, subcutaneously inoculated with tachyzoites; bitches 03, 04 and 05 inoculated orally with oocysts; and bitches 06, 07 and 08 given placebo subcutaneously and orally (control group).

Groups	Number	Stage of pregnancy of bitch (days)	<i>Toxoplasma gondii</i> inoculum		
			Evolutive Form	Quantity	Route
I	1	52	Tachyzoites	$1.0 \times 10^7$	Subcutaneous
	2	40	Tachyzoites	$1.0 \times 10^7$	Subcutaneous
II	3	56	Oocysts	$1.5 \times 10^4$	Oral
	4	40	Oocysts	$1.5 \times 10^4$	Oral
	5	32	Oocysts	$1.5 \times 10^4$	Oral
III	6	52	Saline	Control	Subcutaneous
	7	55	Saline	Control	Oral
	8	51	Saline	Control	Oral

dehydrogenase (LDH), creatinine phosphokinase (CPK), alkaline phosphatase, albumine, total proteins, glucose, direct and indirect bilirubin, amylase and lipase in biochemical analysis of blood serum, according to customary techniques (KANEKO & CORNELIUS, 1989), using photometric readings (COLLEMAN 295 - CELM - São Paulo, SP) were assessed, every week, for all dogs until the end of the experiment.

Urine samples were collected from the bitches one week before inoculation, and at seven day intervals after inoculations until the end of the experimental period. Attempts with the same periodicity at the exams urine, were collected for the parasite isolation.

Urine, saliva and milk samples were taken from each pregnant bitch (VITOR & PINTO, 1991) and inoculated by the peritoneal route in groups of three albino mice, weighing 18 to 25 grams, both sexes being represented. After 6 weeks the surviving mice were bled for the obtention of sera in which anti *Toxoplasma gondii* antibodies were searched by IFAT (CAMARGO, 1964) and the mice encephales were examined for the eventual presence of cysts.

Saliva samples were taken immediately before death through endovenous administration of a pilocarpine solution. *Toxoplasma gondii* isolation from milk, was attempted mostly in the postparturition, after giving each bitch oxytocin endovenously (VITOR & PINTO, 1991). All bitches were euthanased immediately after parturition, through painless process (thiopental sodic + potassium chloride).

In the sera collected daily during the first fortnight and at every three days until euthanasia, screening for *Toxoplasma gondii* antibodies was done using an indirect immunofluorescence test (IIFAT), as described by CAMARGO (1964) and an enzyme linked immunosorbent assay test (ELISA), as used by DOMINGUES *et al.* (1998).

The soluble antigen was prepared from purified parasite suspension. The protein concentration was determined by the method of HARTREE (1972). The IFAT a FITC labelled rabbit anti-dog IgG (SIGMA IMMUNO CHEMICALS) diluted 1/100 and ELISA tested an alkaline phosphatase conjugated polyclonal rabbit anti-dog (SIGMA IMMUNO CHEMICALS) diluted 1/200, were used. Secondary antibodies were used, respectively.

On the day of birth blood samples were taken from all pups born alive and screened for presence of *Toxoplasma gondii* antibodies (IFAT and ELISA).

## RESULTS AND DISCUSSION

Two days after inoculation all bitches showed alteration at clinical signs (Table 2) consisting mainly of fever, increase in respiratory resonance, respiratory stertors, nasal flowing, eye secretion and lymphadenopathy. These were similar to findings of OPPERMAN (1971), SHARMA *et al.* (1973) and COSTA *et al.* (1977; 1978). In opposition to the observations made by AVERIL & de LAHUNTA (1971), NESBIT *et al.* (1981) and SUTER *et al.* (1984) no neurologic abnormalities were observed in the infected bitches. The non appearance of nervous manifestations inoculated bitches may be due to the limited period of observation.

Lymphadenopathy was frequent in infected bitches. All of those infected with oocysts

presented hyperthermia. Clinical signs presented were similar to those described by VIDOTTO & COSTA (1987) in infected pregnant sows. The reproductive disorders observed on inoculated bitches were abortion, fetal death and premature parturition (Table 3).

The decrease in number of red blood cells, of hemoglobin content and in hematocrit values may possibly be attributed to an immunity response. Responses to inoculation according to type of inoculum used. Due to this variation, the determination of leucocytary response was not made possible.

All bitches infected with *Toxoplasma gondii* showed an increase of AST, direct and indirect bilirubin and ALT levels, besides hypoalbuminemia. All these signs indicated a possible pathology of the liver (DUNCAN & PRASSE, 1982). The determinations of lactic dehydrogenase (LDH), creatinine phosphokinase (CPK), alkaline phosphatase, albumin, total proteins, glucose, direct and indirect bilirubin, amylase and lipase didn't demonstrate alterations.

Concerning the milk samples, *Toxoplasma gondii* could be detected only in those collected from bitches inoculated with oocysts (Table 4). The isolation of *Toxoplasma gondii* from urine and saliva (Table 4), similarly to that observed in goats by VITOR & PINTO (1991), emphasizes the possible importance of those organic liquids on the dissemination of zoonosis. Presence of tachyzoites in urine, saliva or milk was shown only in the acute stage of infection. The largest period of observation was 16 days. Since the dogs were euthanased immediately after parturition, it was not possible to collect samples from the chronic phase of infection that would occur afterwards.

All the infected bitches reacted to the antigenic stimuli at inoculation with production of *Toxoplasma gondii* antibodies after 3 days and the highest titer values occurred about day 15. These results are in accordance to those found by VIDOTTO & COSTA, (1987). It is interesting to mention that one of the main clinical signs observed – lymphadenopathy – coincided with the beginning of the humoral response. Serological titers obtained by IFAT and ELISA were in accordance.

Table 2 - Clinical signs in pregnant bitches either from the control group (6, 7 and 8) or inoculated subcutaneously with *Toxoplasma gondii* tachyzoites (1 and 2) or orally with *Toxoplasma gondii* oocysts (3, 4 and 5).

Bitch N°	H <sup>1</sup>	Tc <sup>2</sup>	Tp <sup>3</sup>	IRR <sup>4</sup>	PS <sup>5</sup>	A <sup>6</sup>	Ap <sup>7</sup>	ONS <sup>8</sup>	L <sup>9</sup>
01	+(2-3)	+(3)	+(3)	+(3)	-	-	-	+(2-3)	+(2-3)
02	-	-	+(6-7)	+(1-7)	+(8-18)	-	-	+(2-18)	+++ (1-18)
03	+(6-7)	-	+(6-7)	+(6-7)	-	-	-	+(2-7)	+(1-7)
04	+(11-12)	+(9-10)	+(2-6)	+(6-16)	+(6-16)	+(9-10)	+(6-10)	+(3-16)	++ (6-16)
05	+(2-3)	-	-	-	-	-	-	-	++ (2-7)
06 <sup>t</sup>	-	-	-	-	-	-	-	-	-
07 <sup>t</sup>	-	-	-	-	-	-	-	-	-
08 <sup>t</sup>	-	-	-	-	-	-	-	-	-

<sup>1</sup>: H=Hyperthermia; <sup>2</sup>:Tc=Tachycardia; <sup>3</sup>:Tp=Tachypnea; <sup>4</sup>: IRR=Increase respiratory resonance;

<sup>5</sup>:PS =Pulmonary stertors; <sup>6</sup>:A=anorexia; <sup>7</sup>:AP=apathy; <sup>8</sup>:ONS=Ocular and nasal secretion;

<sup>9</sup>:L=Lymphadenopathy; + =clinical alterations; ( )=day after inoculation

Table 3 - Obstetric signs in pregnant bitches either from the control group (6, 7 and 8) or inoculated subcutaneously with *Toxoplasma gondii* tachyzoites (1 and 2) or orally with *Toxoplasma gondii* oocysts (3, 4 and 5).

Bitch Number	Premature birth	Abortion	Fetal death	Normal parturition
01	(3)*	-	-	-
02	-	-	(18)*	-
03	-	-	-	(7)*
04	-	-	(16)*	-
05	-	(7)*	-	-
06	-	-	-	(10)*
07	-	-	-	(7)*
08	-	-	-	(14)*

\* Day of necropsy  
( ) Day after inoculation

Among the bitches that received tachyzoites and aborted, the highest titer observed was 1:256 (IFAT) and level 05 (ELISA). Those of Group II, inoculated with oocysts, and newborn pups alive from Groups I and II, showed a serological peak of 1: 1024 and level 04, for IFAT and ELISA, respectively. The differences in the inocula (type, dosage and route of administration) may perhaps justify this immunitary discrepancy. Therefore, these results confirm high sensibility on the pregnant bitches to the oocysts of *Toxoplasma gondii* as well as their importance in public health.

Table 4 - Results of screening for *Toxoplasma gondii* in milk, saliva and urina of pregnant bitches either from the control group (6, 7 and 8) or inoculated subcutaneously with tachyzoites (1 and 2) or orally with oocysts (3, 4 and 5) in days after inoculation .

Bitch N°	Days after inoculation							
	01	03	07	10	13	15	16	18
01		*						
02				U				S*
03	U		M*					
04		U			S		MU*	
05			MU*					
06				*				
07			*					
08					✱			

\* Day of necropsy  
✱ Day before necropsy  
M - Presence of *Toxoplasma gondii* antibodies (IIF, 1: 64) in inoculated mice with milk pregnant bitches.  
U - Presence of *Toxoplasma gondii* antibodies (IIF, 1: 64) in inoculated mice with urina pregnant bitches.  
S - Presence of *Toxoplasma gondii* antibodies (IIF, 1: 64) in inoculated mice with saliva pregnant bitches.

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