EXPERIMENTAL TOXOPLASMOSIS IN PREGNANT MARES: CLINICAL SIGNS, PARASITEMIA AND IMMUNOLOGICAL OBSERVATIONS

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ABSTRACT: *Nine pregnant mares were orally inoculated with sporulated* Toxoplasma gondii *oocysts and three other were maintained as controls. The oocysts used were obtained by infecting susceptible cats, which received viable cysts from chronic infected mice with the* Toxoplasma gondii *"P" strain. The clinical signs, observed in experimentally infected mares, consisted of hyperthermia, lack of appetite, prostration, diarrhea, ocular mucous discharge and nasal serous discharge. At three days post inoculation leucopenia was observed. Parasitemia of* Toxoplasma gondii *was detected from two to 47 DPI. The humoral immune response appeared at the 10th DPI and its highest average level (1:16384) occurred after the 20th DPI.*

KEY WORDS: Toxoplasma gondii, *Toxoplasmosis, Equine toxoplasmosis.*

1. INTRODUCTION

Toxoplasma gondii is a coccidian parasite of cats with many mammals and birds as intermediate hosts (Dubey et al., 1970; Frenkel et al., 1970; Miller et al., 1972). The agent can be transmitted by ingestion oocysts and by ingestion of meat infected with cysts. *T. gondii* may also be transmitted congenitally and causes abortion and death of newborn human beings, sheep, swine, goat and several other species of domestic and wild animals (Hartley and Marshall, 1957; Wong, 1974; Dubey, 1977; Roperto et al., 1983; Vidotto et al., 1987; Vitor et al., 1992).

Information concerning clinical toxoplasmosis in horses is rare and few studies about equine toxoplasmosis have been conduced (Cusick et al., 1974).

The purpose of this report is to describe the clinical signs, parasitemia and serological observations in pregnant mares orally infected with *T. gondii* sporulated oocysts.

2. MATERIAL AND METHODS

T. gondii oocysts were collected from faeces of two susceptible cats that had been fed about 1,2 x 10^3 cysts of *T. gondii* from the brain of mice chronically infected with the "P" strain. Oocysts were sporulated in 2% H_2SO_4 (Dubey et al., 1972), and stored at 4°C by thirty days. Definitive identification of the oocysts was made by morphological criteria (Zaman, 1970) and by intraperitoneal inoculation in mice (Dubey et al., 1972).

The selected pregnant mares were distributed into four different groups according to their pregnancy stage and each group received the inoculum by mouth as shown on Table 1.

The animals were clinically examined twice a day, at morning and afternoon. After one month, the animals were observed daily until parturition.

Parasitemia was determined by the technique of Remington et al. (1961), every two days until the 32nd DPI. After the 32nd day, parasitemia was determined on days 39, 47, 54, 61, 68, 75, 81, 88. The humoral immune response of the inoculated and control mares was tested by Indirect Fluorescent Antibody Test (IFAT). Before inoculation none of the pregnant mares presented any anti-*T. gondii* antibodies.

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Table 1. Outline of the procedure used in the experiment, Jaboticabal, 1988.

Group	Mare number	Days of pregnancy	Number of sporulated oocysts, oral route, from each animal.
I	01	122	1.5×10^4
	03	132	1.5×10^4
	06	130	1.5×10^4
II	05	182	1.5×10^4
	11	172	1.5×10^4
	12	167	1.5×10^4
III	04	220	1.5×10^4
	07	217	1.5×10^4
	09	216	1.5×10^4
IV	02	30	Control
	08	211	Control
	10	192	Control

3. RESULTS

All animals inoculated with sporulated *T. gondii* oocysts presented hypertermia between the 2_{nd} and 4_{th} DPI. The inoculated mares presented other clinical signs as follows: lack of appetite, prostration, diarrhea, lacrimation and ocular mucous discharge. No clinical abnormality was observed in the mares of control group.

The results of the parasitemic diagnosis are shown on Table 2. The evolutive humoral responses of each inoculated mare and of the control mare, determined by IFAT are presented on Table 3.

Table 2. Parasitemia in pregnant mares inoculated with $1,5 \times 10^4$ sporulated oocysts of *Toxoplasma gondii* and control group, Jaboticabal, 1988.

Mare no.	Group	Parasitemia (DPI)											
		02	04	06	08	10	12	18	22	26	28	30	47
01	I	-	-	+	-	-	-	-	-	-	-	-	-
03		-	-	-	-	-	-	-	+*	-	-	-	+
06		-	-	+*	+	-	-	+*	+	+	-	-	-
05	II	-	-	-	-	+	-	-	-	-	+	-	-
11		-	-	-	-	-	-	-	-	-	+	-	-
12		+	-	-	-	-	-	-	+*	-	-	-	-
04	III	-	-	-	-	-	-	-	-	-	-	-	-
07		-	+	-	+	-	-	+	-	-	-	+	-
09		-	-	+	+	-	+	+*	-	+*	-	-	-
02	IV	-	-	-	-	-	-	-	-	-	-	-	-
08		-	-	-	-	-	-	-	-	-	-	-	-
10		-	-	-	-	-	-	-	-	-	-	-	-

+ - Mice which were inoculated with a leucocyte mass from pregnant mare and showed positive serology (>1:64 titer) for the presence of anti-*Toxoplasma* antibodies when tested by indirect immunofluorescence

+* - Tachyzoites of *Toxoplasma gondii* in peritoneal exudate of mice

Table 3. IFAT in sera of pregnant mares experimentally inoculated with 1,5 X 10^4 sporulated oocysts of *T. gondii* and control group, Jaboticabal, 1988.

	Group I			Group II			Group III			Group IV		
DPI	01	03	06	05	11	12	04	07	09	02	08	10
0**	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	16	16	0	0	16	0	0	0
10	64	0	64	64	256	256	0	64	64	0	0	0
11	2048	0	256	4096	4096	2048	0	256	2048	0	0	0
12	4096	64	64	1024	1024	2048	64	2048	1024	0	0	0
13	4096	256	1024	2048	2048	1024	256	1024	2048	0	0	0
14	2048	256	1024	4096	2048	2048	1024	1024	1024	0	0	0
15	2048	1024	2048	2048	4096	1024	1024	1024	1024	0	0	0
16	1024	256	1024	256	2048	256	1024	1024	1024	0	0	0
17	1024	256	1024	256	2048	1024	1024	1024	256	0	0	0
18	2048	256	1024	1024	4096	1024	256	256	256	0	0	0
19	4096	1024	2048	256	16384	256	256	1024	1024	0	0	0
20	2048	1024	2048	1024	4096	256	256	1024	1024	0	0	0
21	4096	2048	4096	2048	4096	256	256	4096	4096	0	0	0
22	2048	1024	4096	2048	4096	1024	256	4096	2048	0	0	0
23	2048	256	16384	2048	2048	4096	256	16384	1024	0	0	0
24	4096	1024	4096	2048	2048	4096	256	16384	1024	0	0	0
25	16384	2048	2048	1024	1024	4096	256	4096	2048	0	0	0
26	16384	2048	2048	1024	1024	4096	256	2048	2048	0	0	0
27	16384	2048	1024	1024	2048	16384	1024	1024	4096	0	0	0
28	4096	1024	2048	256	2048	4096	256	1024	1024	0	0	0
29	2048	256	1024	1024	2048	1024	256	1024	256	0	0	0
30	1024	256	2048	256	2048	256	256	1024	256	0	0	0
45	2048	1024	2048	1024	2048	1024	256	2048	1024	0	0	0
60	4096	1024	16384	2048	4096	256	1024	2048	16384	0	0	0
75	1024	256	1024	1024	1024	256	256	2048	2048	0	0	0
90	4096	2048	256	256	1024	256	256	256	256	0	0	0
110*	-	-	-	-	-	-	256	-	-	-	-	-
114*	-	-	-	-	-	-		-	1024	-	-	-
119*	-	-	-	-	-	-		-	-	-	-	-
124*	-	-	-	-	-	-	-	256	-	-	0	-
142*	-	-	-	256	-	-		-	-	-	-	-
147*	-	-	-	-	-	256		-	-	-	-	-
148*	-	-	-	-	1024	-		-	-	-	-	0
188*	-	-	256	-	-	-		-	-	-	-	-
198*	-	1024	-	-	-	-		-	-	-	-	-
205*	1024	-	-	-	-	-		-	-	-	-	-
299*	-	-	-	-	-	-		-	-	0	-	-

* Day of parturition.
** Day of inoculation.

4. DISCUSSION

Considering the clinical normality presented, during the whole observation period, in the control group, the symptoms observed in the inoculated mares (hypertermia, diarrhea and ocular mucous discharge) may be considered the most frequent ones for pregnant mares carrying toxoplasmosis. There were no difference in the clinical symptoms among the inoculated groups. The thermal data obtained by Al-Khalid et al. (1980) are very similar to those achieved in this experiment. In the inoculated equines with *T. gondii* a sligh increase in the temperature was observed between the seventh and eighth DPI by Dubey (1985) and the fourth and eigth DPI by Sposito Filha et al., (1992). Altan et al. (1977) did not perform clinical observations in the animals inoculated with *T. gondii* oocysts.

It must be emphasized that the clinical signs observed in the mares were similar to those in other species experimentaly infected by *T. gondii*, as swine (Beverley and Henry, 1978; Vidotto et al., 1987), ovine (Marques and Costa, 1985) and bovine (Costa et al., 1977; Stalhein et al., 1980). On the other hand, symptoms like uncoordinated movements, ataxia, facial paralisis, recumbency, blindness, walking in circles and deaths, described on equines naturally infected by *T. gondii* (McDonald and Cleary, 1970; Beach and Dodd, 1974; Macruz et al., 1974; Clark et al., 1981; Dorr et al., 1984) could not be confirmed.

No reproductive abnormalities were observed in all mares of this experiment. Macruz et al. (1974), Aleandri et al. (1978) and Roperto et al. (1983) observed reproductive abnormalities in mares infected naturally by *T. gondii*. Nevertheless, it should not be discarded the possibility that *T. gondii* may cause disturbances during the mare gestation, similar to what happens in ovines (Beverley et al., 1975; Dubey and Sharma, 1980), goats (Dubey et al., 1980; Dubey, 1981), sows (Vidotto

et al, 1987) and cows (Stanlhein et al., 1980) when infected during the initial gestation phases.

Parasitemia was detected in eight of the nine pregnant mares inoculated with *T. gondii* sporulated oocysts (Table 2). The parasitemic evidence occurred between the 2nd and the 47th days after inoculation. These findings are similar to results using several other species of domestic animals (Costa et al., 1977; Dubey and Sharma, 1980; Dubey, 1981).

All the pregnant mares inoculated with *T. gondii* oocysts presented humoral immunization response to IFAT. The responses started around the 10th DPI in mares that received oocysts (Table 3). The highest titer observed was 1:16384 and occurred, on the average, after the 20th DPI. Data reported by Al-Khalid et al. (1980) mentioned that the highest Dye Test antibodies titer observed in ponies was that of 1:16384 beginning on the 21st DPI, and Dubey (1987) indicate that equids can develop high *T. gondii* antibody titres without clinical signs. The comparison between both tests (IFAT and Dye Test) by sorological investigation of *T. gondii* antibodies in equids demonstrate similar sensitivity (Ishizuka et al., 1975).

In view of our findings, it is suggested that T. gondii should be not considered as a possible etiological factor causing abortions in mares.

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RESUMO: *Nove éguas gestantes foram inoculadas, via oral, com oocistos esporulados de* T. gondii, *e três outras foram mantidas como controles. Os oocistos utilizados foram obtidos de fezes de gatos experimentalmente infectados com cistos oriundos de camundongos cronicamente infectados com a "cepa P". Os sinais clínicos observados nas éguas experimentalmente infectadas foram hipertermia, perda de apetite, prostração, diarréia, secreção ocular mucosa e corrimento nasal seroso. No 3º e 18º dias após inoculação detectou-se leucopenia e aumento dos níveis séricos de aspartato aminotransferase, respectivamente. Parasitemia foi detectada entre os dias 2 e 47 após inoculação. A resposta imunitária humoral iniciou-se por volta do 10º DPI e os maiores títulos (1:16384) ocorreram após o 20º DPI.*

PALAVRAS-CHAVE: Toxoplasma gondii, *Toxoplasmosis, Toxoplasmose em eqüinos.*

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