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# EXPERIMENTAL TOXOPLASMOSIS IN PREGNANT MARES: A STUDY OF FETUSES AND PLACENTAS

*TOXOPLASMOSE EXPERIMENTAL EM ÉGUAS GESTANTES: ESTUDO DOS FETOS E PLACENTAS*

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## SUMMARY

Nine pregnant mares were orally infected with sporulated ***T. gondii*** oocysts. Three additional, pregnant, uninfected mares were used as control. ***T. gondii*** were found in the placenta, retina, esophagus, liver, diaphragm, brain, spinal cord, skeletal muscles, heart, lung and tongue of newborn foals from experimentally infected mares. The finding of ***T. gondii*** in the foals reinforced the hupothesis of transplacentary transmission of this protozoa in equines.

**UNITERMS**: ***Toxoplasma gondii***; Toxoplasmosis; Mares

## INTRODUCTION

In order to evaluate gestational and fetal alterations, studies on experimental infection with T.G. have been performed in pregnant cows[5,7,16], sheep[2,8], goats [6,9], and sows[19]. In mares, the effect of toxoplasmosis on pregnancy and fetal development has been studied only in a small number of spontaneous cases[1,12,15,17,18].

The main objective of the present investigation was to study the transplacental transmission of ***I. gondii*** in equines.

## MATERIAL AND METHODS

Nine pregnant mares of undefined race and serologically negative for toxoplasmosis, as determined by the indirect immunofluorescence reaction (IFAT), were infected by oral route with sporulated ***I. gondii*** oocysts of P strain according to the schedule presented in Tab. 1. There additional, uninfected, pregnant mares were used as control.

Soon after parturition, each foal was weighed and submitted to detailed clinical examination. Blood was also collected from each foal the determination of anti-***T. gondii*** antibodies by the IFAT[4].

All foals were sacrificed and submitted to autopsy on the second day of life. Fragments of placenta, skeletal muscle, lungs, heart, liver, spleen, lymph nodes (mesenteric, precrural, prescapular and submandibular), diaphragm, brain, spinal medulla (thoracic, cervical and lumbar portions), cerebellum, kidneys, intestines, and esophagus were collected at random during autopsy, stained with hematoxylin-eosin, and submitted to histopathologic examination by using the technique of Michalany[13].

Fragments of approximately 50g from the placenta, spleen, brain, spinal medulla, liver, skeletal muscle, heart, lung, kidneys, tongue, esophagus, intestine, diaphragm, retina, and prescapular lymph nodes were tested for the presence of ***T. gondii***. Spleen, liver, brain, spinal medulla, and retina were ground in a mortar and suspended in 0.9% sterile saline conteining 2.000 IU/ml penicillin and 200 mg/ml streptomycin. The remaining fragments were ground individually and submitted to artificial peptic digestion[10]. The material from each digested tissue was submitted to several washings by centrifugation for the removal of pepsin and hydrochloric acid and resuspended in the same antibiotic solution.

Each of the preparations thus obtained was inoculated intraperitoneally into three adult albino mice, in two 1 ml doses separated by a 12-hour interval. The animals that died during this observation period or that presented increased abdominal volume, or any other sign suggesting ***T. gondii*** infection, were examined for the possible presence of the parasite. After six weeks of observation, the

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surviving mice were bled and the possible presence of anti- ***T. gondii*** antibodies in serum was determined by IFAT.

## RESULTS

When the foals were examined on the day of birth, only nº 11, son of mare nº 11 belonging to group II, presented an exposed penis. The organ was fully outside the preputial bag, slightly curved and bad the glans partially turned towards the caudal region. No congenital abnormality was detected in any of the other foals.

Tab. 2 presents foals' weight at birth, sex and period of mare observation from the day of inoculation to parturition. Results of the serological tests carried out on each foal on the day of birth are listed in Tab. 3. No post-mortem alteration was detected in the foals from inoculated mares or control ones.

Histopathologic examination revealed the following abnormalities:

Foal nº 3 (Group I) - hepatocytes with increased volume and with vacuolization. The vacuoles had inclear limits, suggesting a picture of cellular tumefaction.

Foal nº 6 (Group I) - hepatic granuloma characterized by a central region of necrotic tissue surrounded by a collar of epithelioid cells followed in turn by mononuclear cells, some Langhans-type giant cells, macrophages, and rare eosinophils. All of these structures were surrounded by connective tissue, fibroblasts and fibrin.

Foal nº 4 (Group III) - hepatocytes with increased volume and with vacuolization. The vacuoles were large and had clear limits, and the cells' nuclei were shifted to the periphery, suggesting a picture of diffuse and moderate steatosis.

Tissue parasitism by ***T. gondii*** was demonstrated in two placentas and in different organs of eight foals from inoculated mares (Tab. 4). Tissue parasitism was not observed in the placentas or in the organs of foals from control mares.

## DISCUSSION

Clinical examination of the foals on the day of birth did not show any congenital abnormality, except for foal 11 (group II) wich presented an exposed penis. In view of the frequent occurrence of a prolapsed penis in newborn foals[3], the congenital anomaly detected here could not be attributed to ***T. gondii***. The mean weight of control foals did not differ from that of the animals from mares of all other groups (Tab. 2).

Serologic examinations performed on foals by IFAT, on the day of birth, demostrated the presence of anti- ***T. gondii*** antibodies in all animals from inoculated mares. These antibodies may not have been transferred through the placenta, but due the immune response of the foals, since no correlation was detected between foal titers and those of the respective mares on the day of parturition. Mare titers were relatively higher than those of the respective foals. Ontogeny of immunocompetence may start from the very beginning of embryo development in all mammals. For this reason, the fetus inside the uterus is not passive when confronted by parasites or their antigens. Protection of the fetus against intrauterine infection is largely mediated by the transfer of maternal antibodies to the uterus, but we do not know whether this protection is extended to the fetus. The fetuses own immune responses contribute to their protection[11].

Histopathologic alterations observed in the livers of foals nºs. 1 and 6 (group II), and 4 (group III) may not possibly be attributed to ***T. gondii***, since the parasite was not detected in the histologic sections studied. On the other hand, the lack of alterations in the organs of foals from the control group does not permit us to rule out the participation of ***T. gondii*** in the etiology of the lesions observed. Hepatic disorders, similar to those detected in these foals (steatosis, cellular tumefaction and granuloma), were detected by VIDOTTO et al[20] (1987) piglets with toxoplasmic infection acquired in utero. Granulomatous lesions in swine with chronic toxoplasmosis have also been observed by MOLLER et al[14], (1970).

The finding of ***T. gondii*** in various organs of 8 of the 9 newborn foals from mares inoculated with ***T. gondii*** oocysts (Tab. 3) proves, for the first time, the transplacental transmission of experimental toxoplasmic infection in equines .

Frequently parasitized organs were the retina and espohagus.

These results agree in part with those reported by ALEANDRI et al.[1] (1978); ROBERTO et al.[15] (1983) and TURNER; SAVVA[18], (1992), who reported the presence of ***T. gondii*** in equine fetuses aborted.

Thus, it was clearly shown that ***T. gondii*** is likely to be transmitted by the transplacental rout in mares, but the importance of this disease regarding horse reproduction should be better evaluated in further, more in-depth studies.

## TABLE 1

Outline of the procedure used in the experiment, Jaboticabal - SP, 1992.

| Group | Mare number | Days of pregnancy | Nº. of infecting forms/ animal/ oral route |
|---|---|---|---|
| I | 01 | 122 | $1,5 \times 10^5$ |
| | 03 | 132 | $1,5 \times 10^5$ |
| | 06 | 130 | $1,5 \times 10^5$ |
| II | 05 | 182 | $1,5 \times 10^5$ |
| | 11 | 172 | $1,5 \times 10^5$ |
| | 12 | 167 | $1,5 \times 10^5$ |
| III | 04 | 220 | $1,5 \times 10^5$ |
| | 07 | 217 | $1,5 \times 10^5$ |
| | 09 | 216 | $1,5 \times 10^5$ |
| IV | 02 | 30 | Control |
| | 08 | 211 | Control |
| | 10 | 192 | Control |

## TABLE 2

Table Foal sex, weight at birth, and duration - in days - of the period of mare observation, from inoculation with *T. gondii* to parturition. Jaboticabal - SP, 1992.

| Group | Foal Nº | Weight at Birth | Sex | Period - In days - of observation of each mare, from inoculation to parturition |
|---|---|---|---|---|
| I | 01 | 34 | M | 205 |
| | 03 | 33 | M | 198 |
| | 06 | 32 | F | 188 |
| II | 05 | 33 | F | 142 |
| | 11 | 40 | M | 148 |
| | 12 | 28 | F | 147 |
| III | 04 | 35 | F | 110 |
| | 07 | 30 | M | 124 |
| | 09 | 36 | F | 114 |
| IV | 02 | 30 | F | 299 |
| | 08 | 35 | F | 119 |
| | 10 | 36 | M | 148 |

## TABLE 3

Reciprocal antibody titers obtained by indirect immunofluorescence reaction, in sera of newborn foals, from mares orally inoculated with $1,5 \times 10^5$ sporulated oocysts of *T. gondii*, Jaboticabal - SP, 1992.

| Group | Foal/ Mare Nº | Reciprocal Titer Foals | Mares |
|---|---|---|---|
| I | 01 | 64 | 1024 |
| | 03 | 16 | 1024 |
| | 06 | 16 | 256 |
| II | 05 | 16 | 256 |
| | 11 | 16 | 1024 |
| | 12 | 64 | 256 |
| III | 04 | 16 | 256 |
| | 07 | 16 | 256 |
| | 09 | 16 | 1024 |
| IV | 02 | 00 | 00 |
| | 08 | 00 | 00 |
| | 10 | 00 | 00 |

## TABLE 4

Presence of ***T. gondii*** parasitizing the tissue of placenta and other organs of foals from mares inoculated with ***T. gondii*** oocysts. Jaboticabal - SP, 1992.

| Foal Group | N | Placenta Diaphragm | Brain | Medula | Retinas | Liver | Organ Skeletal | Heart | Lung | Tongue | Esophagus | Muscle |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 01 | - | - | + | + | + | - | - | - | - | + | + |
| | 03 | - | - | - | + | - | - | + | + | - | - | - |
| | 06 | - | - | - | + | + | - | - | - | - | + | |
| II | 05 | + | - | - | + | - | - | - | - | - | - | - |
| | 11 | - | - | - | - | - | + | - | - | - | - | - |
| | 12 | + | + | + | - | - | - | - | - | - | + | + |
| III | 04 | - | - | - | - | - | - | - | - | - | - | - |
| | 07 | - | + | - | + | + | + | - | - | - | + | - |
| | 09 | - | - | - | + | - | - | + | - | + | + | + |
| IV | 02 | - | - | - | - | - | - | - | - | - | - | - |
| | 08 | - | - | - | - | - | - | - | - | - | - | - |
| | 10 | - | - | - | - | - | - | - | - | - | - | - |

+ = Parasitism demonstrated by the positivity (>1:64 titer) to the IFAT in mice inoculated with digest or ground tissue.

**RESUMO**

Nove éguas prenhes foram inoculadas, via oral, com oocistos esporulados de *T. gondii*. Três éguas prenhes, não infectadas, foram mantidas como testemunhas. O *T. gondii* foi encontrado na placenta, retina, esôfago, fígado, diafragma, cérebro, medula espinhal, músculo esquelético, coração, pulmão e língua de potros nascidos de éguas inoculadas. A obtenção e *T. gondii* em diferentes tecidos, desses potros reforça a hipótese da transmissão transplacentária deste protozoário em eqüinos.

**UNITERMOS**: *Toxoplasma gondii*; Toxoplasmose; Éguas.

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