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Neospora caninum and *Toxoplasma gondii*: Relationship between hepatic lesions, cytological and biochemical analysis of the cavitary liquid during the acute phase of the diseases in experimental models

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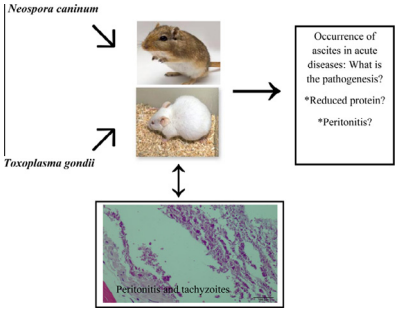
HIGHLIGHTS

- Ascites in acute toxoplasmosis (mice) and neosporse (gerbils).
- There is a reduction of the protein levels in serum and associated with hepatic lesion.
- Ascites is related to the occurrence of peritonitis, the presence of tachyzoites multiplying the peritoneum.
- In peritoneal fluid, presence of high levels of proteins and inflammatory cells, as well as increase of oxidant molecules.

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this study was to evaluate the pathogenesis of ascites in mice infected with *Toxoplasma gondii* and gerbils infected with *Neospora caninum* during the acute phase disease. For that, 12 gerbils [Experiment I: not infected/control ($n = 6$) and infected ($n = 6$)] and 12 mice [Experiment II: control ($n = 6$) and infected ($n = 6$)] were used. Infected gerbils and mice showed marked ascites on days 5–7 post-infection (PI), while the not-infected animals had not ascites. Peritoneal liquid was collected from the all mice with uninfected animals receiving 1.5 mL of saline solution into their abdominal cavity, allowing the recovery of cavity liquid. As a result, it was possible to observe differences in physics, chemistry and cytological analysis of the fluid cavity of animals infected with *N. caninum* and *T. gondii*, when they were compared with uninfected animals, as well as between animals experimentally infected. Additionally both, *N. caninum* and *T gondii*, caused an increase in the levels of nitric oxide (NO_x–nitrate/nitrite), protein oxidation (AOPP) and lipid peroxidation (TBARS), while serum total protein and albumin were reduced in infected gerbils and mice. Gerbils infected with *N. caninum* showed multiple large cells with multilobulated nucleus, lytic necrosis and abundant amount of eosinophilic cytoplasm into the hepatic parenchyma. By the other hand, mice infected with *T. gondii* developed myriad foci of lytic necrosis combined with tachyzoites and cysts containing bradyzoites in liver. Both experimental models for

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N. caninum and *T. gondii* showed inflammatory foci and tachyzoites the peritoneum, which could be a major cause of ascites. Toxoplasmosis and neosporosis were able to cause clinical signs in experimental models with similar alterations in peritoneal fluid; however the toxoplasmosis histological changes were much more evident. Therefore, the pathogenesis of ascites appears to be directly related to liver damage, which strongly suggests alteration in the normal production of proteins as observed in this study, along with peritonitis.

1. Introduction

Neosporosis and toxoplasmosis are caused by the protozoan parasite, *Neospora caninum* and *Toxoplasma gondii,* respectively. The *N. caninum* was confused with *T. gondii* for many years, according to the literature (Dubey and Beattie, 1988; Dubey et al., 1988). Both parasites have similar biological cycle, i.e. tachyzoites, tissue cysts, and oocysts (Dubey and Lindsay, 1996). The major difference between the parasites related to this definitive host, i.e. felines and canines that release *T. gondii* and *N. caninum* oocysts in the feces, respectively. The neosporosis is considered as a serious problem for cattle, which can cause miscarriage, as well as fetuses may die in uterus, be resorbed, mummified, autolyzed, stillborn, born alive with clinical signs, or born clinically normal but chronically infected (Dubey and Lindsay, 1996). *T gondii* is capable of causing severe disease in human, as well as other animals. In sheep and goats the toxoplasmosis may cause embryonic death and resorption, fetal death and mummification, abortion, stillbirth, and neonatal death. In young pigs can occur mortality, as well as pneumonia, myocarditis, encephalitis, and placental necrosis. Already cattle and horses are more resistant to clinical toxoplasmosis than are other species of livestock (Dubey and Carpenter, 1993; Dubey and Lindsay, 1996).

Several animal models have been used in study the pathology of the infection, testing the efficacy of vaccines and new drugs for the treatment of neosporosis and toxoplasmosis. Gerbils (*Meriones unguiculatus*) are a logical candidate for a model of acute neosporosis because they are more susceptible to neosporosis than mice (Ramamoorthy et al., 2005). Already, the mice have been found to be the most susceptible to *T. gondii* and are particularly interesting model to study the toxoplasmosis pathology (Fux et al., 2000). In gerbils and mice, signs of these illness include walking difficulties, hunched appearance and evidence of emaciation and dehydration, as well as mortality (Nicoll et al., 1997; Ramamoorthy et al., 2005).

In neosporosis and toxoplasmosis, a fairly common pathological finding is the accumulation of fluid in the abdominal cavity, composed by high amount of tachyzoites. However, its pathogenesis still not well understood, and therefore, the objective of this study was to evaluate the formation process of ascites during the acute phase of both diseases, in mice and gerbils infected with *T. gondii* and *N. caninum*, respectively.

2. Materials and methods

2.1. T. gondii and N. caninum strains and inoculum preparation

For this study, a strain of *T. gondii* (RH) and *N. caninum* (Nc-1) were used, initially kept in liquid nitrogen in laboratory environment (Laboratory of Parasitic Diseases, Federal University of Santa Maria). *T. gondii* was reactivated and replicated in mice, allowing tachyzoites recoveries that were used later for the inoculation of the experimental group. *N. caninum* strain was inoculated in Vero cell cultures for propagation, generating infective doses that were used in the infection of the experimental group. The quantification of tachyzoites of *T. gondii* and *N. caninum* was carried out in a *Neubauer* chamber in order to set the infective dose.

2.2. Experimental models

Specific experimental models accepted by the scientific community for both diseases were used in the present study. Thus, *N. caninum* and *T. gondii* were inoculated in gerbils (*Meriones unguiculatus*) and BALB/c mice (*Mus musculus*), allowing us to perform two different experiments named as Experiment I (EI) and Experiment II (EII). EI was composed by 12 male gerbils, 60 days-old, while EII was formed by twelve 70 days-old male mice. Animals were kept in cages housed in an experimental room with controlled temperature and humidity (25 °C; 70%), fed with commercial ration and received water *ad libitum*. All animals had a period of 10 days for acclimatization and considered as clinically healthy at the beginning of experiment. The procedure was approved by the Animal Welfare Committee of Centro Universitário Franciscano, under identification number 02/2012.

2.2.1. Experiment I (EI)

The 12 gerbils of EI were divided into two groups: one group ($n = 6$) represented the negative control, or uninfected, and a second group ($n = 6$) was composed by gerbils infected with *N. caninum*. In order to reach the acute neosporosis, the gerbils were inoculated intraperitoneally with 0.1 mL of saline solution containing 2×10^6 tachyzoites. Samples of blood, tissue and peritoneal fluid of both groups were collected at day 7 post-infection (PI).

2.2.2. Experiment II (EII)

The 12 mice of EII were divided in two groups with six animals each. Thus, control group was represented by uninfected mice; other group of mice was experimentally infected by *T. gondii*. The infection was performed by intraperitoneal via with 0.1 mL of peritoneal fluid containing 2×10^6 tachyzoites. Samples of blood, tissue and peritoneal fluid of both groups were collected on day 5 PI. Uninfected animals of EI and EII also received, intraperitoneally, 0.1 mL of saline solution.

2.3. Samples collection

The establishment of the periods of collection was based on clinical signs, since some animals were too debilitated, representing to be in a terminal phase of life. For this reason days 5 and 7 PI were set as the data collection for mice and gerbils, respectively.

The animals of this experiment were anesthetized with isoflurane in order to perform the blood collection through intra-cardiac puncture. Then, serum was separated by centrifugation at 3500*g* for 10 min and stored at −20 °C for analysis. Finally, cervical dislocation of gerbils and mice was carried out as recommendations of the Ethics Committee. After this last procedure all the animals were prepared for the collection of other samples. Briefly, the skin of abdominal region was mechanically removed and 1.5 mL of saline solution was administered into the abdominal cavity, followed by an external and mechanical homogenization. Then, using syringe and needle (3 mL and 25 × 7, respectively), abdominal liquid collection was performed. Part of it was stored in anticoagulant tube for biochemical and cytological analysis, while the remaining cavity liquid was stored in tubes without anticoagulant, centrifuged at 5400*g* for 15 min. The supernatant was collected and frozen (−20 °C) for analysis of oxidative parameters.