An experimental model of neosporosis in mice

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SUMMARY. The study presents an experimental *Neospora caninum* infection in health and immunosuppressed mice. Mice were clinically observed during the experiment and they have been morphopathological and histopathological examined. Clinical signs appeared first in the mice of immunosuppressed group after the treatment with corticosteroids. In all inoculated mice histopathological examined central nervous lesions were characterized by multifocal nonsuppurative infiltration represented by focus of mononuclear cell infiltration around a central area of necrosis. No cysts or tachisoites were found.

Key words: neospora, mice, experimental infection.

Introduction

*Neospora caninum* is a recently recognized protozoan parasite of animals which until 1988 was misidentified as *T. gondii* (Dubey at al., 1988). Neosporosis is a major cause of abortion in cattle in many countries. It has a wide host range, but its zoonotic potential is unknown. Mice and rats may be used for experimental infections (Lindsay and Dubey, 1990). *N. caninum* is infective to mice and the mouse model is useful to study biology, histopathology, immunology and chemotherapy.

The aims of the study were:

- The study of clinical aspects during the experimental infection with *N. caninum* tachizoites in all 4 mice groups.
- The study of morphopathological and histopathological aspects of nervous substance from brain mice infected with *N. caninum* infection.

Material and method

During the experiment we used 4 groups as following:

**Group number 1:** 4 mice males, NMRI strain, inoculated intramuscular with *N. caninum* tachizoites;

**Group number 2:** 4 mice male, NMRI strain; inoculated intramuscular with *N. caninum* tachizoites and immunosuppressed with Dexamethasone (Dexamethasone 2mg/ml – Alfasan Woerden-Holland);

**Group number 3:** 4 mice male, NMRI strain; inoculated subcutaneously with *N. caninum* tachizoites;

**Group number 4 (reference group):** 4 mice male, NMRI strain, nonimmunosuppressed but inoculated intramuscular with NaCl 4% as a referent suspension.

At the start of the experiment the age of the mice was eight weeks.

The strain of *N. caninum* tachizoites was N.C.1 (imported from Belgium).

The inoculation suspension contained free fresh *N. caninum* tachizoites obtained with cell culture cultivation method (Suteu si col. 2004) (fig. 1).

The tachizoites were counted in Thoma counting camera and they were examined in optical microscope for observing their shape and mobility (fig. 2).
The dose of inoculation suspension was 1000 tachizoites/animal suspended in 0.33 ml PBS supplied with antibiotics (penicillin and streptomycin 1:10).

For immunosuppression treatment we used an unique dose of 0.5 ml of dexamethasone (s.c.) (Dexamethasone 2mg/ml – Alfasan Woerden-Holland).
Results and discussions

The study presents for the first time in Romania an experimental model of infection with *N. caninum*.

Daily clinical exam revealed:

- Days 1-4 post inoculation:
  
  We observed no pathological clinical signs in all 4 mice groups.

- Days 4-7 p.i.:
  
  In mice from group 2 pathological clinical signs appeared: anorexia, ataxia the lack of mobility.

- Days 7-9 p.i.:
  
  In mice from groups number 1 pathological clinical signs appeared: anorexia, ataxia the lack of mobility.

- Days 9-12 p.i.:
  
  The mice from groups 1 and 2 did not fed at all, with no mobility and fotofobia.

- Days 12-16 p.i.:
  
  The same as previously for groups number 1 and 2.

In group number 3 appeared the first clinical sign: anorexia and prostration.

- Day 13 p.i.:
  
  2 mice from group 1 died and because of brain lysis this was unpropite for histopathological examination.

In all these days in the mice from group number 4 (reference group) clinical exam revealed no pathological signs.

- 14 day p.i.:
  
  3 of the mice from group number 2, 1 mouse from group number 3 and all mice from reference group were found dead (we suspect they were killed by an rat). The brain of these mice was also damaged because of the protein lysis and unfortunately was not histopathological examined.

The rest of the mice were killed and morphopathological and histopathological examined. In all inoculated mice histopathological lesions were characterized by multifocal nonsupurative infiltration represented by focus of mononuclear cell infiltration around a central area of necrosis (fig. 3).

![Figure 3](image-url)

Mononuclear cell infiltration around a central area of necrosis
No cysts or tachyzoites were found.

The development of clinical neosporosis depends on the isolate of the mouse (Dubey 1996). Nude mice developed clinical neosporosis after inoculation with N.C 1 isolate tachyzoites (Yammage at all., 1996). Our results shows that clinical neosporosis appeared in experimental infection of NMRI mice with N.C 1 protozoan parasite strain.

Balb/c mice infected with *N. caninum* develop encephalomyelitis (Lindsay at all., 1995). Outbred adult mice do not develop clinical neosporosis, but *N. caninum* can encyst in them (Dubey at all., 1998b). The absence of the cysts may be explained because the period from inoculation to histological examination was too short or because maybe the mice strain NMRI does not develop *N. caninum* cysts.

Another aspect which must be presented is the fact that because the reference group was enable to be histopathological examined and compared and we found no parasitic forms we cannot be sure that the lesions we found have been produced by the *N. caninum* infection. This kind of lesions are characteristic for *N. caninum* infection but also for viruses infection. For all that, clinical aspects appeared only after the inoculation of *N. caninum* strain, so we may consider that *N. caninum* is involved in the brain lesions.

**Conclusions**

Some of the conclusions are:

- NMRI strain mice developed clinical neosporosis after inoculation with N.C 1 isolate tachyzoites.
- Clinical signs appeared first in the mice from imunosupressed group after the treatment with corticosteroids.
- In all inoculated mice histopathological examined lesions were characterized by multifocal nonsuppurative infiltration represented by focus of mononuclear cell infiltration around a central area of necrosis.
- No cysts or tachyzoites were found.

**Bibliography**