

# GASTRIC NODULES WITH *Neospora caninum* ZOOITES IN A CONFIRMED CLINICAL NEOSPORA CANINUM INFECTION IN A DOG

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**ABSTRACT:** *Generalized neosporosis was diagnosed in a two year old rottweiler. The dog had a history of tetraparesis, muscle atrophy, regurgitation and vomiting. Serological analysis by indirect fluorescent antibody test was 1/400 for Neospora caninum. Contrasted radiological surveys showed megaesophagus and during ultrasonografic examination an 8.2 mm diameter mural nodule was observed at the pyloric region. Citologic evaluation revealed highly number of zooites. Microscopically, severe myositis were observed in histologic muscle sections and several groups of tachyzoites and tissue cysts were associated with these lesions. The result of the immunohistochemical staining was: protozoa in muscle sections of the dog stained strongly using 5.2.15 monoclonal antibodies against N. caninum tachyzoites. This paper describes a clinical case of canine neosporosis with vomiting associated to Neospora gastric nodule.*

**Keywords :** Neosporosis- polimiositis- dog- gastric nodules

## NÓDULOS GÁSTRICOS CON NEOSPORA EN UN CANINO

**RESUMEN:** *Se realizó el diagnóstico de neosporosis generalizada en un canino rottweiler de dos años de edad. El paciente presentaba las siguientes alteraciones: tetraparesia, atrofia muscular, regurgitación y vómito. El resultado del análisis serológico por prueba de inmunofluorescencia indirecta fue 1/400 para Neospora caninum. Los estudios radiológicos contrastados mostraron megaesófago y durante el examen ultrasonográfico se observó un nódulo mural de 8,2 mm de diámetro en la región pilórica. La evaluación citológica reveló numerosos zooitos. En las secciones histológicas de músculo se observaron varios grupos de taquizoitos y quistes asociados a miositis severa. El resultado de las técnicas de inmunohistoquímica fue: protozoos en secciones de músculo que se tiñeron fuertemente con 5.2.15 anticuerpos monoclonales contra taquizoitos de N. caninum. Este reporte describe un caso clínico de neosporosis canina con vómitos asociados a nódulo gástrico de Neospora.*

**Palabras clave:** Neosporosis- polimiositis- perro- nódulos gástricos.

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## INTRODUCTION

*Neospora caninum* is a coccidian parasite of animals. Dogs and coyotes are both the intermediate and definitive hosts for *N. caninum*. Infection may occur transplacentally or vertically in dogs fed with uncooked beef.

The most severe cases of neosporosis occur in young, congenitally infected puppies. Young dogs develop hind limb paresis that turns into a progressive paralysis. Neurologic signs are depend on the site parasitized. The hind limbs are more severely affected than the fore limbs and often in rigid hyperextension (Dubey Korean). Adult dogs more often present multifocal encefalomyelitis and polymyositis. Other systemic signs are dysphagia, regurgitation due to megaesophagus, fever, vomiting, icterus (associated to pancreatitis or hepatitis).

This paper describes a clinical case of canine neosporosis with vomiting associated to neospora gastric nodules. The patient also presented regurgitation due to megaesophagus and tetraparesis because of generalized polymyositis.

## CASE REPORT

A two-year-old female rottweiler (22 kg) was presented with one month history of lameness, tetraparesis, inappetence, regurgitation and vomiting. It had been treated with corticosteroids a month before consultation.

On physical examination, the dog was in lateral recumbence with normal mental status. It exhibited generalized muscular pain, decreased muscle tone and atrophy. Spinal reflexes were alternatively normal and diminished. Sensitive evaluation was normal.

During internation it was observed regurgitation and intermittent bilious vomiting.

A CBC and serum biochemical analyses were performed. Moderated neutrophilic leukocytosis (22, 500 neutrophils/ $\mu$ l) was found. Biochemical abnormalities included: hyperglobulinemia (6 g/l) and increasing of creatine kinase (400 u/l U/L. Serological analysis by indirect fluorescent antibody test (IFAT) was negative for *Toxoplasma gondii* and 1/400 for *Neospora caninum*.

Contrasted radiological surveys showed megaesophagus and showed intestinal gas during abdominal survey. Ultrasonographic examination showed gastric wall thickening due to a thicker mucous layer. An 8,2 mm diameter mural nodule was also observed at the piloric region (Figure 1).

Within the first 24 hours of hospitalization, a gastric tube for enteral feeding was introduced. During the proceeding several smaller nodules were seen in the gastric fundus. A fine-needle aspirate was performed of the gastric nodule and

mesenteric lymph nodes. Cytologic evaluation of the gastric nodule revealed highly number of zoites and a few neutrophils (Figure 2). High count of lymphocytes (70%), neutrophils (20 %) and lymphoblasts (10%) were seen on lymph nodes smears.

Samples of apendicular muscle were taken for histopathologic and immunohistochemical evaluation by an open surgical biopsy procedure.

Histological examination revealed myositis with an abundant and predominantly histiocytic and plasmacytic infiltration, separates individual fibers, myofibers necrosis and atrophy. Tachyzoites were associated with these lesions, some of them were free, while others formed cystic. Segmental muscle fiber degeneration was observed.

Polymerase chain reaction (PCR). DNA was isolated from a paraffin-embedded muscle sample taken by biopsy using phenol extraction and ethanol precipitation as described by Moore (1992) with previous steps of xylol-ethanol treatment (2-times xylol 5 min, 2-times ethanol 100° C 5 min, ethanol 96° C 5 min, ethanol 70° C 5 min, ethanol 50° C 5 min and distilled water 5 min). The amplification was performed with the specific primer pair for *Neospora caninum* Np6+/Np21+ (Müller et al, 1996). The primers were used at a final concentration of 0.5  $\mu$ M and dNTP's at a final concentration of 250  $\mu$ M each (GE Healthcare, München, Germany). DyNAzyme II DNA polymerase, (Finnzymes, Espoo, Finland) was used as DNA polymerase at 1 U/25  $\mu$ l with the provided buffer. The PCR cycling conditions were 94° C for 5 min, followed by 10 cycles of 56° C (with a 0.5° C decrement per cycle after the first cycle) for 1 min, 72° C for 1 min and 94° C for 1 min, followed by 40 cycles of 51° C for 1 min, 72° C for 1 min and 94° C for 1 min. The PCR ended with 51° C for 1 min and a final extension at 72° C for 5 min. The amplification was performed in a thermal cycler (Personal Thermal Cycler, Eppendorf, Hamburg, Germany). The amplicons were analysed in 1.5% agarose gels using a 100 bp DNA ladder (Invitrogen, Carlsbad, USA) as reference. DNA isolated from NC 1- *N. caninum* tachyzoites and sterile RNase-free water (Sigma) were used as positive and negative controls, respectively.

## IMMUNOHISTOCHEMICAL STAINING

Sections of muscle were immunohistochemically labelled with an anti *N. caninum* with the ABC test using a commercial kit (Vectastain Elite ABC Kit Mouse IgG, Vector Laboratories Inc., Peterborough, UK). Primary antibody was a monoclonal undiluted antibody 5.2.15, specific for *N. caninum* tachyzoites (Schaes et al., 1999). Horse anti-mouse IgG biotinylated antiserum was used as the secondary antibody. Incubation with

the primary antibody was performed overnight at room temperature. Positive controls were heart sections of a *N. caninum*-naturally infected calf and negative controls, lung and liver sections of a naturally infected meerkat (*Suricata suricatta*). The reaction was visualized using a 0.05 % diaminobenzidine solution in imidazol-buffer and hydrogen peroxide and Papanicolau solution as a contrast staining.

**RESULTS:**

**PCR:** After electrophoresis, a positive result (337 bp amplicons) was observed in the samples corresponding to the muscle biopsy and to the positive control in the *N. caninum* specific PCR.

**Immunohistochemical staining:** Protozoa in muscle sections of the dog stained strongly using 5.2.15 monoclonal antibodies against *N. caninum* tachyzoites (Figure 3).

Lactated Ringer’s solution was administered throughout hospitalization. The therapy used for neosporosis was clindamycin (20 mg/kg IM twice daily) until dog dyed, three days later. Owner refused necropsy.



Figure 1. Ultrasonographic examination showing gastric wall thickening and nodule

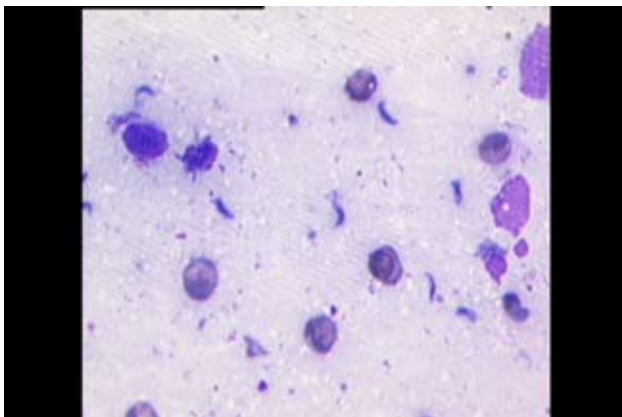


Figure 2. Cytologic evaluation revealing high number of zoites and a few neutrophils.

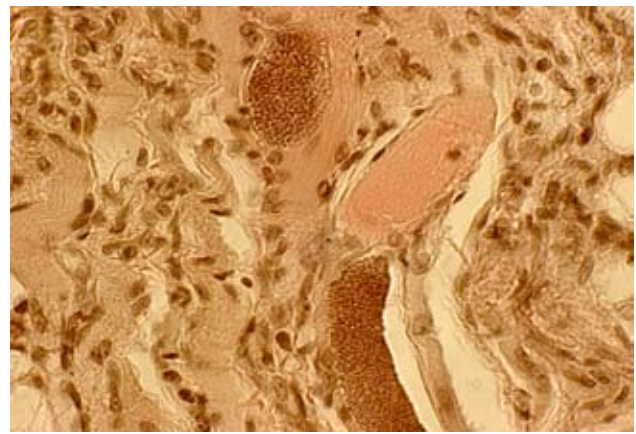


Figure 3. Protozoa in muscle section stained strongly using 5.2.15 monoclonal antibodies against *N. caninum* tachyzoites.

**DISCUSSION**

The combination of generalized lower motor neuron paralysis, muscle pain, high serum creatine kinase and lesions in muscle biopsy ensure that the dog fulfills the diagnostic criteria of polymyositis. Histological examination revealed myositis with tachyzoites associated, some of them free and others in cystic form. Polymyositis due to *Neospora caninum* has been documented by numerous authors (Podell, 2002, Green, 2006, Basso, 2005). The presence of megaesophagus associated with *N. caninum* infection has been reported before by Barber and Trees (1996) in 2 of 27 dogs. In this case such lesion was attributed to neosporosis. Several digestive signs have been described associated to canine neosporosis. They are regurgitation due to megaesophagus and vomiting, icterus and fever associated to pancreatitis and hepatitis (Green, 2006). The dog in the present case had gastric nodules with tachyzoites inside and vomiting. There are no previous reports of gastric affection in canine neosporosis infections. This is the first report of gastric nodules with tachyzoites in this disease. We assume that the stages observed in the muscle sections are tachyzoites because monoclonal antibodies 5.2.15 recognize only *N. caninum* tachyzoites and failed to detect tissue cysts in brain sections (Schaes et al, 1999). These antibodies react with a 38 kDa surface antigen that seems to be restricted to the tachyzoite stage and absent in bradyzoites (Schaes et al, 1999). Serological, histopathological and immunohistochemical studies allowed a final diagnosis of generalized neosporosis. The lesions and the presence of large numbers of tachyzoites suggested that the severity of the clinical signs observed were due to a *N. caninum* in

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