A Neurological Presentation of Neosporosis in Calves

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**Abstract:** Neospora caninum is an important cause worldwide of abortion, stillbirth and calves born weak. Abortion is the major clinical sign associated to Neospora caninum infection, it typically occurs between months four to seven of gestation, but may occur from three months to term. Abortions tend to occur during mid-gestation and it appears that foetuses exposed in early gestation are more likely to abort and that foetuses exposed later are more likely to survive. Although most calves born to infected cows will appear to be clinically normal, there may be some neurological disease that is not readily apparent. However we present here an unusual presentation of the disease with recurrent neurological disease in the newborn calves.

**Key words:** Animal parasitic diseases, ELISA, neospora, Neospora caninum, calf

**INTRODUCTION**

Neosporosis in cattle is an abortifacent parasitariy disease caused by *Neospora caninum*. This protozoan parasite has an ubiquitous distribution and is a well known cause of abortion in cattle. Congenital disease has been described in association to *Neospora caninum* too\(^1\). The parasite has a two-host life cycle, with the dog as definitive host\(^1\). *Neospora caninum*, was first described a little over a decade ago and has since been shown to be a major cause of bovine abortion throughout the world. It appears that vertical transmission (through the placenta) is the main means of disease transmission. Infection can thus be maintained on farms even without the definitive host.

Neosporosis can be diagnosed by serology and/or histopathology. Important organs from which to identify the organism via histopathology include the brain, heart, liver, and skeletal muscle. The most characteristic lesion in the foetus is focal encephalitis, characterized by necrosis and nonsuppurative inflammation\(^1,3\). Although most aborted foetuses are autolyzed, *Neospora* organisms and/or the characteristic lesions may be found in the brain. A herd diagnosis can be achieved by serology. By comparing the *Neospora* status of aborting versus nonaborting cows, it will become clear if seropositivity correlates with the tendency to abort.

**MATERIALS AND METHODS**

**Animal Samples:** Here we report the clinical and pathological findings in a 15 days old Limousine calf referred to the Veterinary Teaching Hospital of the University of Extremadura in Cáceres, Spain. The calf came from a group of 80 adults farmed. The owner requested necropsy of the animal. Specimens were collected for histological, microbiological and parasitological examination. Representative tissue samples were collected for histological evaluation and preserved in 10% buffered formalin. The specimens were transported to the Laboratory Department, Veterinary Teaching Hospital, University of Extremadura in Cáceres, Spain.

**Sampling and Culture Techniques:** Samples of lung, digestive content, central nervous system, liver, spleen and kidney were cultured for aerobic and microaerophilic organism. Based on clinical sings and history, differential diagnosis was set up. Animals were seronegative in two previous controls (internal controls in the farm) to bovine rhinotracheitis (IBR) virus and bovine viral diarrhea (BVD) virus. No evidences of *Salmonella* spp grown or isolation were found, and in the same way no isolation of *Listeria* spp from brain samples could be attempted. Orine samples obtained by cystocentesis were negative to *Leptospira* spp based on dark field microscopy technique. No attempt was made...
to isolate mycoplasmas, chlamydas, campylobacter or mycotic infections due to absence of pathological sings.

**Histopathology:** Tissue simples obtained from the brain (cortex, midbrain, medulla and cerebellum), heart, lung, liver, spleen, kidney and digestive were fixed in neutral-buffered 10% formalin, routinely embedded in paraffin and stained with haematoxylin and eosin.

**Haematological and Biochemical Blood Analysis:**
We checked changes of the following parameters: blood cells count, hematocrit, creatinine phosphokinase levels, blood urea nitrogen, creatinin levels, aspartate aminotransferase, total blood protein, lactate dehydrogenase, albumin, alkaline phosphatase, alanine transaminase and gamma-glutamyl transpeptidase. The blood cell count was performed by ABCvet analyzer\(^1\) (ABX hematology, France) and biochemical parameters by Reflovet\(^2\) (Scil Animal Care, Germany).

**ELISA:** Serological detection of specific antibodies to *Neospora caninum* by ELISA method was performed with POURQUIER\(^3\) ELISA *Neospora caninum* (Institut Pourquier, France). Validation criteria of the test, and interpretation of the results was according with manufacturers instructions. Sera from clinical case and three adults (including the mother) were tested.

**RESULTS AND DISCUSSION**

The calf showed evident neurological sings including incoordination and paralysis of the limbs; however the animal had been being breeding by the owner without complications. The results of haematological and biochemical analysis revealed just a moderate leucopenia (3.2 x 10\(^3\) leucocytes/µL, reference range 4.0 x 10\(^3\) to 12.0 x 10\(^3\) leucocytes/µL) and elevated creatine phosphokinase levels (176 UI/L, reference range < 110 UI/L) showing the presence of skeletal muscle disease. No significant alterations were found in other haematological values such hematocrit or erythrocyte count (40.0%, reference range 24% to 46% and 9.18 x 10\(^9\), reference range 5.0 x10\(^9\) to 10.0 x10\(^9\)/µL respectively). The study of the biochemical parameters revealed no especial alterations in the values of blood urea nitrogen (25 mg/dl, reference range 20.0 to 30.0 mg/dl), creatinin levels (1.1 mg/dl, reference value 1.0 to 2.0 mg/dl), aspartate aminotransferase (54 UI/L, reference value 78.0 to 132.0 UI/L) and total blood protein (5.9 g/dl, reference value 5.7 to 8.1 g/dl). Other parameters such lactate dehydrogenase, albumin, alkaline phosphatase, alanine transaminase and gamma-glutamyl transpeptidase were measured not registering alterations out of the physiological values (data not shown).

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Fig. 1: Cortex. Evident signs of apoptosis were found in neural cells. HE stain, X40 objective.

Fig. 2: Cortex. Diffuse non-suppurative leukocytic infiltration of the meninges and eosinophilic infiltration. HE stain, X40 objective.
necrosis. As previously reported, multifocal encephalitis is quite distinctive and in combination with myocarditis is strongly suggestive of neosporosis[3, 5].

With the purpose of clarify the role of *Neospora caninum* in this case, serological assay for detecting antibodies to *Neospora caninum* were performed. Sera from the necropsied calf and from his mother as well as from other adult cows from the farm were analyzed. In calves or adult cattle, a few days after primary infection specific IgM and IgG antibodies appear. IgG levels increase during the first weeks up to 3-6 months after primary infection. Animals that transplacentally transmit de infection to their calves often have high *Neospora caninum*-specific antibodies[4]. In this way, a negative serological test makes it unlikely that *Neospora caninum* was involved in neonatal mortality or most common in abortion. ELISA assay was performed in order to detect specific antibodies against *Neospora caninum*. Manufacturer cut-offs indicate that sera from the calf and from his mother were considered coming from animals that carried antibodies against *Neospora caninum* and then positive. The rest of samples analyzed were considered as coming from animals that did not carry antibodies against *Neospora caninum* and therefore negative. Intra-uterine infection with *Neospora caninum* seems to provoke the development of specific antibodies against *Neospora caninum* in the majority of infected calves[5], being the absence of antibodies against the parasite in stillborn or newborn calves infected by *Neospora caninum rare*[6].

The clinical, histological and serological findings in this case are consistent with Neospora-associated neonatal infection in calves. However no abortions, the major clinical sign associated with *Neospora sp* were observed. Here were report a rare presentation that may lead to an erroneous diagnostic. To our knowledge, this was the first known instance of infection of cattle with the neosporan protozoa associated to recurrent neurological presentation in Extremadura, Spain. Although this organism has been identified in several cases of bovine abortion in our area, this is, in addition, the first report in which this infection has been successfully diagnosed in a newborn calf. The diagnosis of *Neospora sp* in most clinical cases analyzed in our laboratory have been widely associated to abortion, this is the first time that we report a rare neurological presentation that do not.

REFERENCES


Fig 3: Myocardium. Image from the myocardium showing interstitial myocarditis plus degeneration and necrosis of myocardial cells and leukocytic infiltration. HE stain, X40 objective.