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Case Report

Transplacental transmission of *Neospora caninum* in moose (*Alces alces*)

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ABSTRACT

A 5-years-old moose (*Alces alces*) cow kept in a zoo in the German Federal State of Brandenburg aborted a female foetus of 44 cm crown rump length (CRL). Pathohistological analysis revealed several *Neospora (N.) caninum* infected cells and cysts, as well as multifocal gliosis, necrosis, haemorrhages, dystrophic mineralisation and haemosiderosis in the brain, predominantly in cerebrum and brainstem. In addition, mild lymphocytic meningitis was present. Together with the fresh foetus, a mummified foetus of 16 cm CRL was expelled. Neither focal necrosis, nor inflammation was detected in the brain of the mummified foetus. By two polymerase chain reactions (PCR) targeting the *pNc5* gene of *N. caninum* (i.e. an end point PCR and a real-time PCR), by two serological methods (immunofluorescence test and immunoblot), by histological and immunohistochemical analyses, transplacental *N. caninum* infection was confirmed in the fresh foetus and interpreted as possible cause of abortion. Infection with other agents causing abortion including Bovine Herpesvirus 1 (BHV1), Bluetongue Virus (BTV), Bovine Virus Diarrhoea Virus (BVDV), *Brucella* spp., *Chlamydia* spp., *Coxiella burnetii* and *Toxoplasma gondii* were excluded. Our findings show that control measures may be necessary to protect captive moose against accidental *N. caninum* infection. Further studies are needed to explore the importance of neosporosis in wild and captive moose.

1. Introduction

Neospora (N.) caninum is regarded as an important cause of abortion, stillbirth and perinatal death in cattle (Dubey et al. 2007). Infections and *N. caninum* associated abortions also occur in other domestic ruminants including sheep and goats but also wild ruminants in captivity (Donahoe et al. 2015; Dubey et al. 2007). Cases of neosporosis were reported in a full-term stillborn Eld's deer (*Cervus eldi siamensis*) in a zoo in France (Dubey et al. 1996), two full-term twin antelope calves (*Tragelaphus imberbis*) in a zoo in Germany (Peters et al. 2001), a 3-week-old fallow deer (*Dama dama*) from a captive fallow deer group in Switzerland (Soldati et al. 2004) and in a herd of axis deer (*Axis axis*) from a zoo in Argentina, in which perinatal mortality associated with *N. caninum* infections was reported (Basso et al. 2014). Reports on clinical neosporosis in non-captive ruminants are rare; the authors are aware of only one report from a 2-months-old black-tailed deer (*Odocoileus hemionus columbianus*) in California, USA, with a generalized *N. caninum* infection (Woods et al. 1994).

Domestic dogs and wild living canids such as coyotes (Gondim et al. 2004b), Australian dingoes (King et al. 2010) and gray wolves (Dubey et al. 2011) are definitive hosts of *N. caninum*. Ruminants can be postnatally infected by the ingestion of food or water contaminated with oocysts shed by definitive hosts (Dubey et al. 2007). Consequently, it is assumed that a sylvatic cycle exists between wild ruminants as intermediate hosts and wolves or coyotes as definitive hosts (Dubey et al. 2011; Gondim 2006; Rosypal and Lindsay 2005).

In addition to postnatal infection of intermediate hosts via ingestion of oocysts, transplacental transmission from an infected dam to the foetus during gestation represents the predominant route of infection in many animal species (Dubey et al. 2007). Transplacental transmission due to postnatal infection by ingestion of oocysts during gestation is regarded as exogenous transplacental transmission while foetal infection due to recrudescence in a chronically infected dam is regarded as endogenous transplacental transmission (Trees and Williams 2005). While endogenous transplacental transmission seems to be the predominant cause of foetal infection in European cattle, the relative importance of endogenous and exogenous transplacental transmission in other animal species including wild and captive ruminants is

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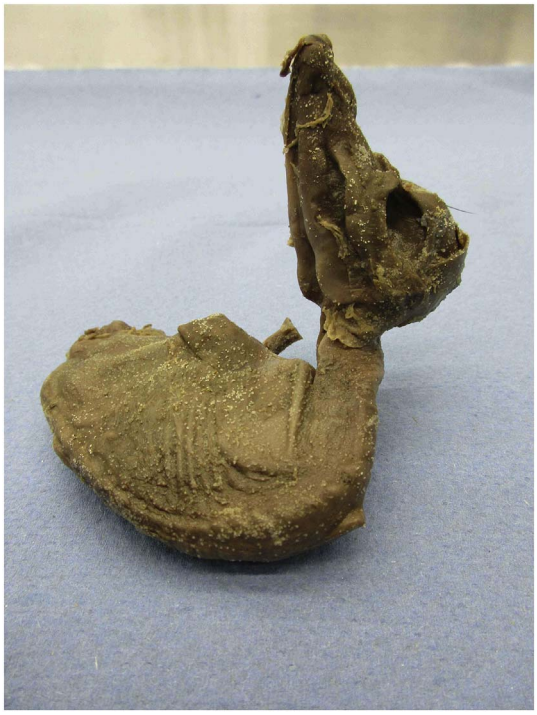

Fig. 1. Mummified moose foetus of 16 cm crown-rump length (CRL). This foetus was expelled together with a fresh foetus of 44 cm CRL.

unknown (Dubey and Schares 2011; Dubey et al. 2007).

Antibodies against *N. caninum* have been detected in wild living moose from North-Eastern Poland by an agglutination test (NAT), a commercial competitive screening enzyme-linked immunosorbent assay, an enzyme-linked in-house immunoassay and an immunoblot (Moskwa et al. 2014). In contrast, an examination for antibodies against *N. caninum* in Swedish moose using an ISCOM Neospora ELISA (that is an ELISA making use of antigens incorporated into immunostimulating complexes) revealed no seropositive animals (Malmsten et al. 2011).

To the best of our knowledge, this case report is the first describing transplacental transmission of *N. caninum* in a moose worldwide.

2. Material and methods

2.1. Abortion in a 5-years-old moose cow

A 5-years-old moose cow kept in a small zoo in the Federal State of Brandenburg, Germany, aborted during its first gestation in June 2016. One freshly dead female foetus of 44 cm crown-rump length (CRL) was expelled together with a mummified foetus of 16 cm CRL (Fig. 1). The moose cow was bred in another zoo in the Federal State of Schleswig-Holstein, Germany, and entered the zoo in the Federal State of Brandenburg in 2012.

2.2. Necropsy and sampling

Foetal membranes, the fresh and the mummified foetuses were submitted for necropsy to the Berlin-Brandenburg State Laboratory, Frankfurt (Oder), Germany. The mummified foetus was preserved in total in 4% neutral buffered formalin and brain tissue was used for histological analysis only.

The fresh foetus underwent a complete necropsy including histological analysis of brain, heart, lung, liver, kidney and foetal membranes. Heart blood was collected, stored frozen at − 20 °C and centrifuged (1800 × *g*; 10 min; 4 °C) prior to serological examination. Samples from lung, spleen, kidney, liver, foetal membranes and cerebrum were sampled for PCR.

A serum sample of the moose cow could not be collected at the time of abortion and to avoid unnecessary stress or sedation the zoo gave no permission to sample the animal later.

2.3. Histological and immunohistochemical analysis

Samples of brain, heart, lung, liver, kidney and foetal membranes were fixed in 4% neutral buffered formaldehyde for histology. All formalin fixed tissue samples were routinely processed and embedded in paraffin wax. Sections were cut to 5 µm thickness and stained with haematoxylin and eosin (H & E). Immunohistochemical (IHC) staining of deparaffinised tissue sections was performed with the VECTASTAIN Elite ABC Kit as recommended by the manufacturer (Vector Laboratories, Burlingame, California, USA) after heat-mediated antigen retrieval in 10 mM citrate-buffer, pH 6.0 (Uzeda et al. 2013), blocking with normal goat serum and by using a 1:200 diluted polyclonal rabbit *N. caninum*-specific antiserum (Peters et al. 2000). The slides were finally treated with an AEC (3-amino-9-ethylcarbazole) substrate-chromogen (Agilent, Santa Clara, California, USA), and counterstained with haematoxylin.

2.4. DNA and RNA extraction

Tissue samples were homogenized with Hank's Salt Solution (Biochrom AG, Germany) using FastPrep-24™ (MP Biomedicals, USA). DNA and RNA were extracted with the innuPREP AniPath DNA/RNA Kit-KFFLX (Analytik Jena, Germany) according to the manufacturer's instructions. Negative extraction controls and internal controls (housekeeping gene or external synthetic nucleic acid depending on the subsequent PCR) were used to control nucleic acid extraction.

2.5. Detection of infection with potential abortion associated bacterial and viral pathogens

Routine diagnostic workup included bacteriology of placenta, lung, spleen, and abomasal content using standard culture media and protocols. In addition, infections with different bacterial and viral pathogens were excluded by real-time PCRs for *Brucella* spp. (Probert et al. 2004), *Chlamydia* spp. (Ehricht et al. 2006), *Coxiella burnetii* (Klee et al. 2006), BHV1 (Wernike et al. 2011), BTV (Adiavet BTV Realtime PCR Kit, BioX Diagnostics, Belgium), and BVDV (LSI VetMAX™ BVDV 4ALL, Life technologies, USA).

2.6. Conventional end-point PCRs to detect Toxoplasma (T.) gondii and N. caninum infection

To detect *T. gondii* and *N. caninum* DNA, conventional end-point PCRs were performed as described (Legnani et al. 2016; Schares et al. 2011). PCR primers (Toxoplasma: TOX4/TOX5 (Homan et al. 2000) and TOX8/TOX5 (Homan et al. 2000; Reischl et al. 2003); Neospora: Np6 + /Np21 + (Müller et al. 1996)) were used at a final concentration of 0.5 µM and dNTPs at 250 µM each (Amersham Biosciences, Piscataway, USA). DyNAzyme II DNA polymerase (Finnzymes, Espoo, Finland) was added at 1 U/25 µl with the provided buffer. The reaction mix was supplemented with bovine serum albumin at a concentration of 20 µg/ml. Water PCR Reagent (Sigma-Aldrich, Taufkirchen, Germany) served as negative control. The reactions were performed in a thermal cycler (Eppendorf Mastercycler, Personal Thermal Cycler, Hannover, Germany). Amplification products were visualized after electrophoresis in 1.5% agarose gels stained with ethidium bromide. A 100 bp DNA ladder (Invitrogen GmbH, Karlsruhe, Germany) served as size standard.