



Research paper

Isolation and genetic characterization of *Neospora caninum* from naturally infected sheep

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ABSTRACT

Neospora caninum is considered one of the main causes of abortion in cattle but can also cause abortion in sheep. There is limited knowledge of the *N. caninum* population infecting sheep, and only one *N. caninum* isolate from a pregnant sheep from Japan has been reported. This study describes the *in vitro* isolation and genetic characterization of two new sheep isolates of *N. caninum* implicated in ovine reproductive failure. We used IFN- γ -knockout mice inoculated with PCR-positive brain homogenates from two clinically healthy but congenitally infected lambs at 4.5 months of age for parasite isolation. The lambs were born to dams from a sheep farm that had experienced pregnancy failure caused by *N. caninum* in successive generations. Tachyzoites were microscopically visualized in peritoneal flushes from all inoculated mice and were also observed in MARC-145 cell cultures within one week after inoculation with peritoneal flushes. Two *N. caninum* isolates, Nc-Spain11 and Nc-Spain12, were obtained from each lamb. The genotyping of the Nc-Spain11 and Nc-Spain12 isolates based on 9 microsatellite markers showed identical multilocus genotype (MLG). Comparison between a previous *N. caninum* genotype dataset including 80 MLGs from Argentinean, Spanish, Mexican, German and Scottish bovine isolates and the Japanese sheep isolate showed that the Nc-Spain11 and Nc-Spain12 MLG was unique and differed from the other MLGs. eBURST analyses showed that the Nc-Spain11 and Nc-Spain12 MLG was genetically clustered with other bovine MLGs and one ovine MLG, and the nearest genetic relationship was with an MLG from a bovine abortion collected in the same geographical area of Galicia.

1. Introduction

Neospora caninum and *Toxoplasma gondii* are phylogenetically closely related protozoan parasites that cause reproductive failure in ruminants worldwide. *N. caninum* is considered the main cause of abortion in cattle, whereas *T. gondii* is one of the main abortifacient agents in small ruminants (Dubey, 2009; Dubey and Schares, 2011). The relevance of neosporosis to abortion in small ruminants is considered limited, but recent studies have suggested that its importance in sheep flocks may be underestimated in terms of reproductive losses (Moreno et al., 2012; González-Warleta et al., 2014, 2018). Endogenous transplacental transmission occurs in infected ewes, and *N. caninum* reactivation can cause abortion in sheep in successive pregnancies (González-Warleta et al., 2014, 2018). There is limited knowledge of the *N. caninum* population infecting sheep. *N. caninum* has been isolated in culture only from one pregnant sheep in Japan (Koyama et al., 2001).

N. caninum was isolated in Brazil via a bioassay using dogs that shed oocysts after consuming the brains of seropositive sheep, but no *N. caninum* isolates were obtained in cell culture from mice, gerbils and large vesper mice infected with these oocysts (Pena et al., 2007). Here, we describe the first isolation and genetic characterization of *N. caninum* from two congenitally infected lambs from dams of a commercial sheep farm that had previously experienced reproductive failure caused by *N. caninum* (González-Warleta et al., 2014).

2. Materials and methods

2.1. Ethics statement

All protocols involving animals were in strict accordance with the proceedings described in Spanish and EU legislation (Law 32/2007, R.D. 53/2013, and Council Directive 2010/63/EU) and the established

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