

Microsatellite markers for the molecular characterization of the polish isolate (NC-PolB1) of *Neospora caninum*

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Neospora caninum is a protozoan parasite with a complex life cycle and wide range of intermediate and definitive hosts. It has been recognized as a cause of neuromuscular disease and reproductive disorders in infected animals worldwide. Neosporosis, the disease caused by this parasite, is now considered to be the main cause of infectious bovine abortion around the world. Due to reproductive failure *Neospora* is a cause of major problems in livestock operations and serious economic losses.

In Poland, the presence of the parasite was confirmed in cattle, bison, red deer, fallow deer as well as dogs, foxes, badgers, raccoon dogs and wolves.

The isolation of the parasite from the tissues of infected animals and *in vitro* culture are extremely difficult. Therefore, in Poland, isolation of viable *N. caninum* has been achieved in only a few cases, so far.

The first polish isolate of *N. caninum* (NC-PolB1) was obtained from the brain tissue of a transplacentally infected calf. *Neospora*-like tachyzoites were detected in Vero cell culture 66 days after inoculation. The identity of the parasite was later confirmed by polymerase chain reaction.

Despite of extensive data about the biology and epidemiology of the parasite, analysis of the genetic diversity as well as differences in pathogenicity between *N. caninum* isolates is limited.

The aim of this study was to demonstrate phylogenetic differences between the polish *Neospora* isolate NC-PolB1 and other isolates.

DNA was extracted from tachyzoites of the reference strain NC1 and the polish isolate NCPolB1 maintained in *in vitro* culture.

Molecular and phylogenetic analyses were conducted using selected specific microsatellite sequences. Genotyping was performed using the following nine microsatellite markers: MS4, MS5, MS6A, MS6B, MS7, MS8, MS10, MS12 and MS21.

The obtained PCR products were purified and sequenced. Sequences were aligned and assembled with the bioinformatic software,VectorNTI. The Basic Local Alignment Search Tool (BLAST) was used to search for similarities between the newly obtained sequences.

The generated sequences showed from 89% up to 96% similarity to the *N. caninum* isolates deposited in GenBank including NC Spain6, Nc SweB1, NC Spain2H, KBA 2, NC GER1.

The newly obtained sequences were submitted to GenBank and deposited there with the following accession numbers: (GenBank: MK248845, MK248846).