

Isolation, identification and maintenance in cell culture of the first Polish isolate of *Neospora caninum*

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Abstract

The protozoan parasite *Neospora caninum* was isolated from the brain of a 12-h-old calf, born to seropositive cow and suspected of having *Neospora* infection. The calf brain was homogenized and injected into *Vero* cell lines that were grown in RPMI medium supplemented with 1% horse serum and 50U/ml antibiotics (streptomycin and penicillin). The cell cultures were examined daily for the presence of tachyzoites. *Neospora*-like tachyzoites in *Vero* cells were detected after 9 and 1/2 weeks post injection of brain homogenates.

The identity of the parasites in the brain homogenate and in cell lines was confirmed by polymerase chain reaction amplification of a *N. caninum* portion of the gene5 region using specific oligonucleotide primers Np6 and Np21 (Yamaga et al. 1996).

The isolated tachyzoites of the Polish strain as well as the tachyzoites of reference strain NC-1 were used to prepare antigen for electrophoresis and

Western blot analysis. Western blot analysis revealed no major antigenic differences between reference strain NC-1 and the Polish isolate.

The Polish isolate of *N. caninum* has been forwarded to the European *N. caninum* reference centre at the Moredun Research Institute, where it will be analyzed using molecular tools and compared to the different isolates already typed at their centre. Preliminary results support that there are no major differences among the Polish and other strains of *Neospora*. This is the first successful isolation of a viable *N. caninum* tachyzoites from the brain of a naturally infected calf in Poland, and the isolate has been designed as NC-PolB1.

References

- Yamaga M., Flechtner O., Gottstein B. 1996. *Neospora caninum*: specific oligonucleotide primers for the detection of brain "cyst" DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). *Journal of Parasitology* 82: 272-279.