

Isolation of *Neospora caninum* from a calf in Malaysia

ABSTRACT

In order to attempt isolate the protozoan parasite *Neospora caninum*, an *N. caninum* seropositive pregnant Sahiwal Friesian cross heifer from a large-scale dairy farm in Malaysia was kept for observation until parturition at the Veterinary Research Institute, Ipoh. The heifer gave birth to a female calf that was weak, underweight and unable to rise. Precolostral serum from the calf had an *N. caninum* indirect fluorescent antibody test titre of 1:3200. It died 12 h after birth and necropsy was performed. Brain homogenate from the calf was inoculated into 10 BALB/c mice that were kept for 3 months after which brain tissue from the mice was inoculated onto 24 h fresh monolayer Vero cell lines. The cell cultures were examined daily until growth of intracellular protozoa was observed. DNA of the organisms from the cell cultures was analyzed by PCR and DNA sequencing. DNA fragments of the expected size were amplified from the isolate using *N. caninum*-specific primers, and sequence analysis of ITS1 clearly identified the isolate as *N. caninum*. This is the first successful isolation of *N. caninum* from a bovine in Malaysia, and the isolate is designated Nc-MalB1.

Keyword: *Neospora caninum*, Cattle-protozoa, Isolation, Malaysia