



Genetic characterization of *Neospora caninum* from aborted bovine fetuses in Hokkaido, Japan

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Introduction

- Neospora caninum* has been implicated in bovine abortions in Japan, with nationwide distribution and high economic losses.
- However, genetic analysis has not been conducted on the isolates from cattle abortions in Japan, and to date, only one isolate has been analyzed from a pregnant sheep without clinical disease.
- Evidence suggests that *Neospora caninum* isolates differ in their virulence, which may explain variations in disease outcomes and epidemiology
- Nevertheless, multilocus microsatellite genotyping revealed extensive genetic diversity among *N. caninum* isolates, indicating predominant clonal propagation in cattle and geographically related population substructures
- This study, for the first time, aimed to determine the genetic relationship between *N. caninum* isolates causing cattle abortions in Japan and other worldwide isolates.

Materials and Methods

Sample information

- Four brain samples were obtained from aborted bovine fetuses upon outbreak of an abortion epidemic in a dairy cattle farm in Shihoro, Hokkaido, Japan (2010).
- Additionally, one sample was obtained from an aborted fetus in a farm located in the same area (2018).

Genetic characterization of *N. caninum*

- was performed by microsatellite analysis employing 12 MS markers: MS1A, MS1B, MS2, MS4, MS5, MS6A, MS6B, MS7, MS8, MS10, MS12, and MS21.
- The sizes of the 6-FAM-labeled PCR products for all of the MSs were determined using an 8-capillary 3500 DNA analyzer (Applied Biosystems, Foster City, CA, USA) with Gene Scan-500 (LIZ) Size Standards (Applied Biosystems) and the GeneMapper™ Software 5 (Applied Biosystems).
- Amplicons from the non-labeled MS7 and MS10 primers were cloned using pCR™4-TOPO® TA Cloning Kits for Sequencing.

Data analysis

- A previous *N. caninum* MLGs dataset was used to identify genetic relationships; all analyses were conducted using R software with the adegenete and poppr packages.

Results and Discussion

- Assigned genotypes showed high frequencies of mixed alleles in the sequenced markers MS7 and MS10, raising concerns about the subpopulation structures of *N. caninum* infecting animals in Japan.
- Seven Japanese genotypes of *N. caninum* are clustered into two subpopulations, one of which was unique and distinct from European and American subpopulations. Meanwhile, another cluster was genetically related to the Mexican and Spanish genotypes.
- These findings may be attributed to clonal spreading by vertical and horizontal transmission as well as geographically related population substructuring.

Legend

(A) Scatter plot of discriminant analysis of principal components based on the first two linear discriminant functions in k = 12. The 12 posterior clusters are shown in different colors.

(B) Bar plot of posterior cluster membership probability for the 114 isolates. The bar plots show the probability of the isolate belonging to each posterior cluster. The colors are the same as those of the posterior clusters in (A). The priori population of the isolates is shown at the top of the bar plot, and the name of the isolate is indicated under the bar plot.

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