

A comparison between PCR and CIEP for early detection of Aleutian mink disease virus

Testing of mink by counter-immunoelectrophoresis (CIEP) and elimination of seropositive individuals has not been effective in permanently eradicating the Aleutian mink disease virus (AD virus) from many ranches in Nova Scotia and elsewhere. Some ranchers have established excellent biosecurity systems and have followed strict biosecurity measures and disinfection of their cages and equipment, yet the virus showed up again and again. Why?

Obviously there are many routes for re-entry of the virus on a ranch: wildlife, contaminated feed, trucks, personnel boots and clothing, etc. An entirely different possibility is that the virus was not really eradicated from some ranches even if all replacement animals were negative on CIEP.

Some CIEP-negative mink may carry the virus, and lots of it, i.e., they are false negatives on CIEP. There are a couple of explanations for infected mink with negative CIEP test results. One reason is that some animals may have become infected just a few days prior to blood sampling for the test. The amount of AD virus in the body reaches its peak around 10 days after infection, while antibody levels increase slowly and take time to reach a detectable level by CIEP. How soon after exposure to the virus can the infection be picked up by CIEP or polymerase chain reaction (PCR)? This was the question which we looked at in this study. Remember, the CIEP tests for antibodies to the virus which are produced by the mink, not the virus itself, whereas PCR directly tests for the virus.

In five experiments, we gave 44 black and 12 sapphire mink a shot of a local strain of the AD virus. We killed the animals 10 days after inoculation and collected blood and seven organs from each mink and tested these for the presence of the virus by PCR, and for antibodies against the virus by CIEP. The CIEP tests were performed in duplicate on each sample.

We observed that all mink were clearly infected on day 10 after inoculation, because viral DNA was detected by PCR in the plasma, spleen, lymph nodes, bone marrow, and lung samples of all inoculated mink, and in most of the small intestine, kidney and liver samples. These results were consistent with the belief that peak virus replication is around day 10 after infection.

In contrast, not one of the 44 inoculated black mink was CIEP positive, but 19 (43.2%) showed an inconclusive CIEP result. Two of the 12 sapphire mink were CIEP positive and one showed inconclusive results (25.0%). The results imply that low levels of antibodies during the early stages after virus exposure resulted in failure of CIEP to detect infection.

The duplicate CIEP test agreed in 94.1% of the cases, and no positive and negative results were observed in duplicate samples of the same animal, showing the high reproducibility of the CIEP test. We concluded that the CIEP test, although accurate, is not reliable for early detection of infection by the AD virus. The results also suggested that the inconclusive CIEP tests are most likely an indication of infected animals with low levels of antibodies, and must be considered as positive for eradication purposes.

We also observed considerable differences among individual mink for production of detectable levels of antibodies, indicating that the genotype of the animal plays an important role in the earliest time that infection can be detected by CIEP. In previous experiments, infection was detected by CIEP between 5 to 28 days after exposure to the virus. Because of this animal variation, the false negative CIEP rate is going to be different on different ranches.

Saliva swabs, fecal samples, and rectal swabs were PCR positive or inconclusive in 49%, 41.0%, and 85.1% of the inoculated mink, suggesting that these sampling sites, although noninvasive, were not reliable. Collecting rectal swabs was the easiest and the results were more promising, but collecting saliva swabs was tough on the mink.

Obtaining uncontaminated urine samples for the PCR test was difficult and the virus was not detected in urine of two infected mink which were tested.

Conclusion and practical relevance

- Although the CIEP is a reliable test for detecting antibodies against the AD virus, it failed to detect infection 10 days after mink were exposed to the virus. PCR accurately detected the virus in blood and lymphoid organs (spleen, lymph nodes, bone marrow) at this time.
- The genotype of individual mink plays an important role in the pattern of antibody production and CIEP results.

What options do ranches have?

- PCR testing is much more expensive to do than CIEP, and many ranchers cannot afford to use it regularly.
- Two CIEP tests on each animal, performed about 20 days apart, could help in detecting more infected animals.
- Methods for detecting antibodies with higher sensitivity than CIEP have been developed, which could increase the chance of picking up infected animals early on in their infection. Such tests are more complex and more costly than CIEP, and are therefore not used for routine herd screening. Given the various limitations of what is available, new, economical methods of testing must be investigated.

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For more information, please refer to the following published paper
A.H. Farid, I. Hussain and I. Arju, 2015. Detection of Aleutian mink disease virus DNA
and antiviral antibodies in American mink (*Neovison vison*) 10 days post-inoculation.
Journal of Veterinary Diagnostic Investigation,
<http://www.ncbi.nlm.nih.gov/pubmed/25862712>