Detection of the Aleutian mink disease virus in soil and water

Message: We identified the best method for detecting the AD virus in water and soil samples, but it is a lengthy process

Virology 101

Everyone knows that bacteria can grow in water, on food, in your mouth, in a manure pile, and so on. They are everywhere and they can grow on and in almost everything. One bacterium becomes millions in a matter of hours if its environment is favorable. Did you know that viruses cannot grow outside of their host? The AD virus only grows in the mink body (and in a few other species), but one virus remains a lonely virus outside the mink body. Not only do they not grow in the environment, they also become continuously degraded and diluted. This is the first problem to recognize when we want to detect the AD virus in water or soil samples.

You may ask how it is that viruses cause infection if they become diluted and degraded? First, some viruses are more resistant to harsh environmental conditions and spread over longer distances than bacteria. Second, fewer viruses, compared with bacteria, are needed to cause infection. The AD virus is particularly stable in the environment, remains in water and soil for a long time and still can cause infection. No wonder some old timers call the AD 'a new shed disease'. Dig up the soil for a new building and AD viruses that were trapped in the ground for who knows how long, will pop up.

Finding a needle in a hay stack or finding viruses in water; which one is easier?

Finding a needle in a hay stack is a statement commonly used to say that it is difficult to find something small in a big pile. Let me tell you that finding the AD virus in soil and water is much more difficult than finding a needle in a hay stack! To find a needle in a hay stack, first spread the hay stack and then use a magnetic metal detector (one with a long handle is easier to use!) and go over the layer of hay. The needle will stick to the magnet.

Unfortunately viruses do not have a 'magnetic personality', but they have something similar to that. They have negative electric charge under some conditions, which will help us to capture them. We pass water or washed soil through a special, positively charged filter. The negatively charged virus will attach to the filter and then we change the electric charge of the filter, releasing the virus from the filter and washing it into a small tube. This is called adsorption-elution filtration. We can also make the virus positively charged and capture it with negatively charged filters.

We need a large volume of water to make sure that enough viruses are in the sample, but we must make this large volume very small so that the virus is concentrated enough

to allow for detection by PCR (polymerase chain reaction). The trick is to use at least 0.5 litre of water, capture the viruses and bring the volume down to 0.00002 of a litre (25000 fold) before testing it, without losing many viruses. It is easy to say, but many steps have to be optimized to make sure that as many AD viruses as possible are captured and detected. Keep in mind that no one has tried to detect the AD virus in soil or water before.

A summary of our research

We conducted four experiments to optimize all the steps required to capture the AD virus from soil and water samples.

Experiment 1: Eight different sources of water were used. These were water samples from the Atlantic Ocean, a natural river, washed compost, washed manure, and washed soil as well as laboratory-made salt water and river water, with ultra-pure (Millipore) water as a control. A known amount of the AD virus was added to each, serially diluted (up to 100,000 times), viral DNA was extracted by five commercially available DNA extraction kits and viral recovery was measured by PCR. The results showed that the sources of water and the DNA extraction kits had large effects on viral detection by PCR. Big differences were observed for detection limit of the kits for various types of water, and no kit was superior for all types of water. The minimum number of viruses that was detected by a specific kit from some water sources was up to 100,000 times greater than others. The best DNA extraction kit for each of the eight water sources was identified in this experiment.

Experiment 2: Three commonly used filtration methods, with both positively and negatively charged filters, were tested to determine viral recovery limit from 0.5 liter of the eight water sources, each mixed with a known amount of the virus, and each was 10-fold serially diluted (up to 100,000 times). The best filter (1MDS) and the most efficient filtration methods for virus recovery were identified. The 1MDS filter detected the AD virus in various water sources 10 to 1000 times better than the other two filters. It was also observed that virus recovery from samples with high mineral contents (laboratory made salt water, Atlantic Ocean water and natural river water) was at least 10 times lower than virus recovery from the other water samples for all filter systems.

Experiment 3: Ultra-clean water, tap water and washed-soil water were mixed with a known amount of the AD virus, 10-fold serially diluted (up to 100,000 times) and four volumes (0.5, 1.0, 2.0 and 3.0 litres) were tested by the three filter methods to measure viral recovery limit. Volume of water did not have any effect on viral recovery, although 2 and 3 litres of washed-soil water clogged the filters. It was concluded that between 0.5 and 1.0 litre of water should be sufficient for AD virus detection. Although larger volumes

of water contain more viruses, the efficiency of virus capturing will decrease because of the blockage of filters with suspended materials and dissolved organic compounds.

Experiment 4: Soil samples (50 grams) were mixed with a known amount of the AD virus, washed with 0.45, 0.9 or 1.8 litres of ultra-pure water, and the virus was captured by the three filter methods. The same filter that worked the best for water samples (1MDS) was also superior to other filter systems for virus recovery from washed soil. The results also showed that washing 50 g of soil with 0.9 litre of water produced a better result than 0.45 or 1.8 litres of water.

Conclusions

A method for the detection of the AD virus, or even similar viruses, in the environment has not been previously developed. The techniques that were evaluated in this study for capturing AD virus, concentrating the virus and DNA extraction are useful for monitoring the movement of this virus in the environment and can be used by ranchers and environmental agencies. It was concluded that type of water has a great effect on the success of the AD virus detection in environmental samples, and the 1MDS filter method was generally superior over the other methods.

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