

Isolation of Aujeszky's disease virus from two hunting dogs in Belgium after hunting wild boars

De isolatie van het Aujeszkyvirus bij twee jachthonden in België na het jagen op everzwijnen

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ABSTRACT

This article contains two separate case reports of the isolation of Aujeszky's disease virus (ADV) from a hunting dog. Each of the two dogs developed neurological symptoms and died of ADV within five days after hunting on wild boars in the Ardennes. Because ADV has been eradicated in the domesticated pig population, it was hypothesized that wild boars infected with ADV were the source, since one of the dogs was fed with the intestines of shot wild boars, and the other was fed with the intestines and other organs.

SAMENVATTING

In deze casuïstiek worden twee verschillende gevallen beschreven van de isolatie van het Aujeszkyvirus (AV) bij jachthonden. Twee honden vertoonden elk neurologische symptomen en stierven binnen de vijf dagen na het jagen op everzwijnen. Er wordt verondersteld dat met AV-geïnfecteerde everzwijnen de bron van besmetting waren omdat één hond de ingewanden van de geschoten everzwijnen als voer had gegeten en de andere de ingewanden en andere organen van de geschoten everzwijnen te eten kreeg.

INTRODUCTION

Aujeszky's disease virus (ADV), also called pseudorabies virus, is a worldwide distributed swine alphaherpesvirus that infects wild and domestic swine as natural hosts (Kluge *et al.* 1999).

The last decade a great effort has been made in many European countries to eliminate the virus from domestic pigs by the introduction of control and eradication programs. While the virus became progressively cleared from the domestic pig herds, it became clear that ADV was also circulating in the wild boar population in many European countries (Lari *et al.*, 2006, Capua *et al.*, 1997). In Belgium, ADV was isolated from wild boars on a private estate in the northern part of the country (Cay *et al.*, 2008). The results of a limited Belgian serological survey done on shot wild boars in the southern part of the country, showed a mean seroprevalence of 30% (Dr Czaplicky, oral communication). This demonstrates a circulation of ADV among wild boars throughout the whole country.

CASE

History

A first dog was presented to the veterinarian on Monday, the 17th of December 2007 with swollen cheeks and lips. According to the owner, the dog had entered a badger burrow the day before (Sunday) and his injuries were thought to be due to a battle with a

badger. The veterinarian noticed only lesions of the skin and a stripped right face at the level of the ear, but at that time no nervous disorders were observed. The dog was given antibiotics and anti-inflammatory drugs and was left with the veterinarian for further care. In the afternoon, the general condition of the dog was declining. He showed abnormal behavior and had a foamy salivation. That evening the condition of the dog was worse, he showed signs of severe pruritus, he was continuously itching his right face at the level of the ear, his foamy salivation was abundant, he became very aggressive towards the veterinarian and his movements became uncoordinated. Later on, convulsions appeared. At that time, ADV was forwarded as a possible cause. According to the owner, the dog had gone hunting in the Ardennes on Wednesday the 12th of December 2007 and had eaten offal from the shot wild boars. That night the dog died and the next morning the body was sent for necropsy to the Pasteur Institute. After rabies virus was eliminated as a possible cause of death, the brains were sent to our institute.

A second hound was presented to the veterinarian on Friday the 14th of December 2007, at which time his general condition was already very bad. He was nearly in shock, with abundant salivation and muscular tremor. He was no longer able to stand up. According to the owner, the hound had gone hunting wild boars the weekend before in the Ardennes and had been given organs of the shot wild boars to eat. On Saturday the 15th of December, the hound was dead and the body was sent to the Pasteur Institute for

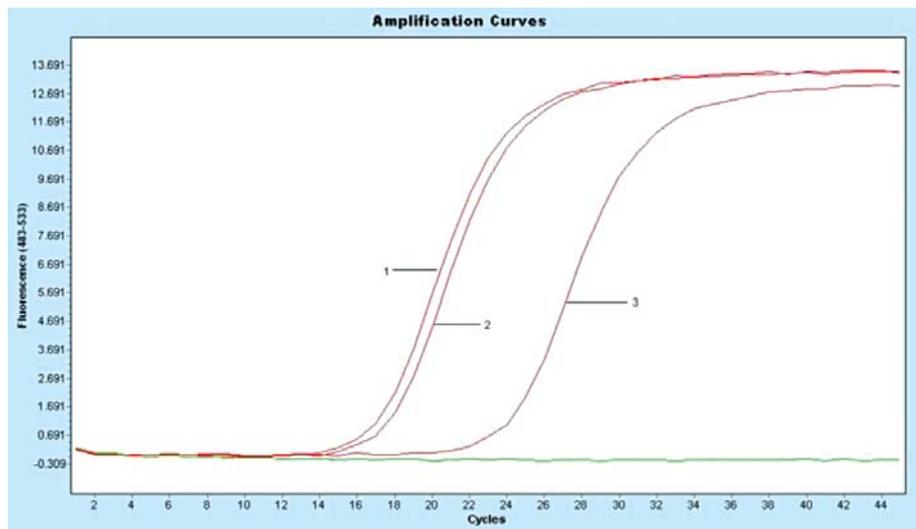


Figure 1. Realtime PRC analysis of the extracted DNA from the brain suspensions of the two dogs. Curves 1-2: dog 1 and dog 2, curve 3: positive control.

necropsy and analysis for rabies. The tests for rabies virus were negative and the brains of the hound were sent to our institute for further examination.

DIAGNOSTIC METHODS

Realtime PCR

A 10% (w/v) homogenate extract from the brains was prepared in PBS and 200 μ l of this homogenate was used in a gB specific Realtime PCR. DNA was first extracted with the Qiagen DNA kit and 1 μ l of extracted DNA was further used in a Realtime PCR with ADV gB specific primers and Taqman probe, as described previously (Cay *et al.*, 2008).

Virus isolation

The 10% (w/v) homogenate extract was centrifuged at 300 g and the supernatant was inoculated on a confluent monolayer of PK-15 cells and incubated in a CO₂ incubator at 37°C.

Cell cultures with a cytopathic effect were fixed with isopropanol and incubated with an ADV-specific fluorescein-conjugated antiserum.

RESULTS

Realtime PCR

The Realtime PCR procedure demonstrated that ADV was present in the brain suspensions of the two dogs (Figure 1).

Virus isolation

After four days of incubation at 37°C, a cytopathic effect with large syncytia, which are typical for ADV, was observed in the cell cultures. Staining with FITC-conjugated anti-ADV antibodies confirmed the presence of ADV in the cell culture.

CONCLUSION

Two hunting dogs, each originating from a different region in the Ardennes, showed neurological

symptoms and were brought to the veterinarian for examination. No treatment was effective and ADV was suspected as a possible cause of their death. After rabies virus was eliminated as a possible cause of death, the brains were further analyzed for ADV. The presence of ADV in the brains of both of the hunting dogs was demonstrated by Realtime PCR and virus isolation.

Together with the previous isolation of ADV from wild boars in the northern part of the country, this case report suggests that ADV is circulating among wild boars in Belgium. The strains isolated from the dogs were highly pathogenic for the dogs and, although so far there have been no reports of the transmission of ADV from wild boars to domestic pigs, constant vigilance is imperative. A full characterization of the new isolates is being carried out, and the possibility of their spreading from wild boars to domesticated pigs is currently under investigation.

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