

## Occurrence of the new pathogenic variant of rabbit haemorrhagic disease virus (RHDV2) in wild populations of rabbits in Southern Belgium.

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Rabbit haemorrhagic disease (RHD) is a highly infectious and fatal disease of the European rabbit (*Oryctolagus cuniculus*), responsible for important economic losses in the rabbit industry. The aetiological agent of the disease is a RNA virus (RHDV, *Lagovirus*, *Caliciviridae*) first detected in China in 1984. Currently RHDV is endemic in most parts of Europe, Asia and North Africa. Phylogenetic analyses of RHDV strains have identified 3 distinct groups : the classic RHDV, the variant RHDVa and RHDV2. This latter has been detected in France for the first time in 2010 in domestic and wild rabbits (Le Gall-Reculé G *et al.*, 2013) and since then has spread throughout Europe, replacing the circulating RHDV/RHDVa strains in most european countries. RHDV2 has already been detected in Belgium in rabbitries (Marlier D *et al.*, 2014). Here, we report for the first time the presence of RHDV2 in wild rabbits in Southern Belgium.

In november 2015, the Surveillance Network of Wildlife Diseases received seven dead wild rabbits for necropsy. The discovery of 7 fresh carcasses found at the same time in a same area (Hainaut province) emphasised the infectious or intoxication hypothesis as cause of death. *Postmortem* examinations were performed at the Faculty of Veterinary Medicine (FVM) of the University of Liege according to a systematic protocol based on gross lesions, histopathological and targeted microbiological analysis. For necropsy, each rabbit (1) was weighted and age was determined by the presence/absence of the distal ulna protuberance, (2) stomach was investigated to exclude poison, (3) spleen was systematically driven into *Yersinia* CIN culture media for detection of *Yersinia pseudotuberculosis*, (4) lungs and livers were systematically (a) packaged into 10% formaldehyde solution for histopathology analysis (Service of Pathology, FVM) and (b) frozen at -20°C for RHDV analysis (Scanelis Laboratory, Toulouse, France) and finally (6) feces were gathered for parasitology (Service of Parasitology, FVM).

At necropsy, animals (5 adults: 3 males/2 females and 2 juveniles: 1 male/1 female) were in good condition. No hematomas or broken bones were detected, only one displayed clues of diarrhoea. Examinations of the carcasses showed congestion of lungs/kidneys and livers were macroscopically normal. No foreign body or suspicious particles was seen in the stomachs, only one rabbit was hardly infested by tapeworms in the gut. Histopathological examination revealed haemorrhagic lung lesions in one animal while 5 of them presented severe necrotic hepatitis, sometimes associated with peri-angiocholitis. Only one animal presented an abnormal high rate of coccidia in feces. Samples of livers were sent to Scanelis Laboratory for RHDV RT-qPCR diagnostic. The results were positive for the new variant RHDV2 in 5 out of the 7 rabbit livers. All the samples were negative for the classic RHDV.

To determine if RHDV2 was already present before 2015 in wild rabbits in the region, we tested a series of livers that had been collected in 2013 and 2014 for a retrospective study. Among the 25 rabbit livers checked, 12 presented necrotic hepatitis and were sent for analysis. Ten were confirmed positive by RT-qPCR for RHDV2.

In conclusion, this is the first report which confirms the presence of RHDV2 in wild populations of rabbits in Southern Belgium. Additional data are needed to strengthen the epidemiological picture and to determine how RHDV2 is spread in other provinces in Southern Belgium.

#### References

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