Association d'Epidémiologie et de Santé Animale – September 7th to 9th 2016, Crown Plaza Hotel, Liege (Belgium)

Réseau de Surveillance Sanitaire de la Faune Sauvage en RW

SPW Service public de Wallonie

Université de Liège

Aesa Association d'Epidémiologie et de Santé Animale

7th-9th sep 2016 Liège (Belgium) Congress 25 years

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

Oral presentation by Volpe Rosario

Friday, the 9th of September 2016

INTRODUCTION

- 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 (60 to 90% mortality rate occurring 24-48h after infection).
- 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.**

!! This strain a) is a very virulent one

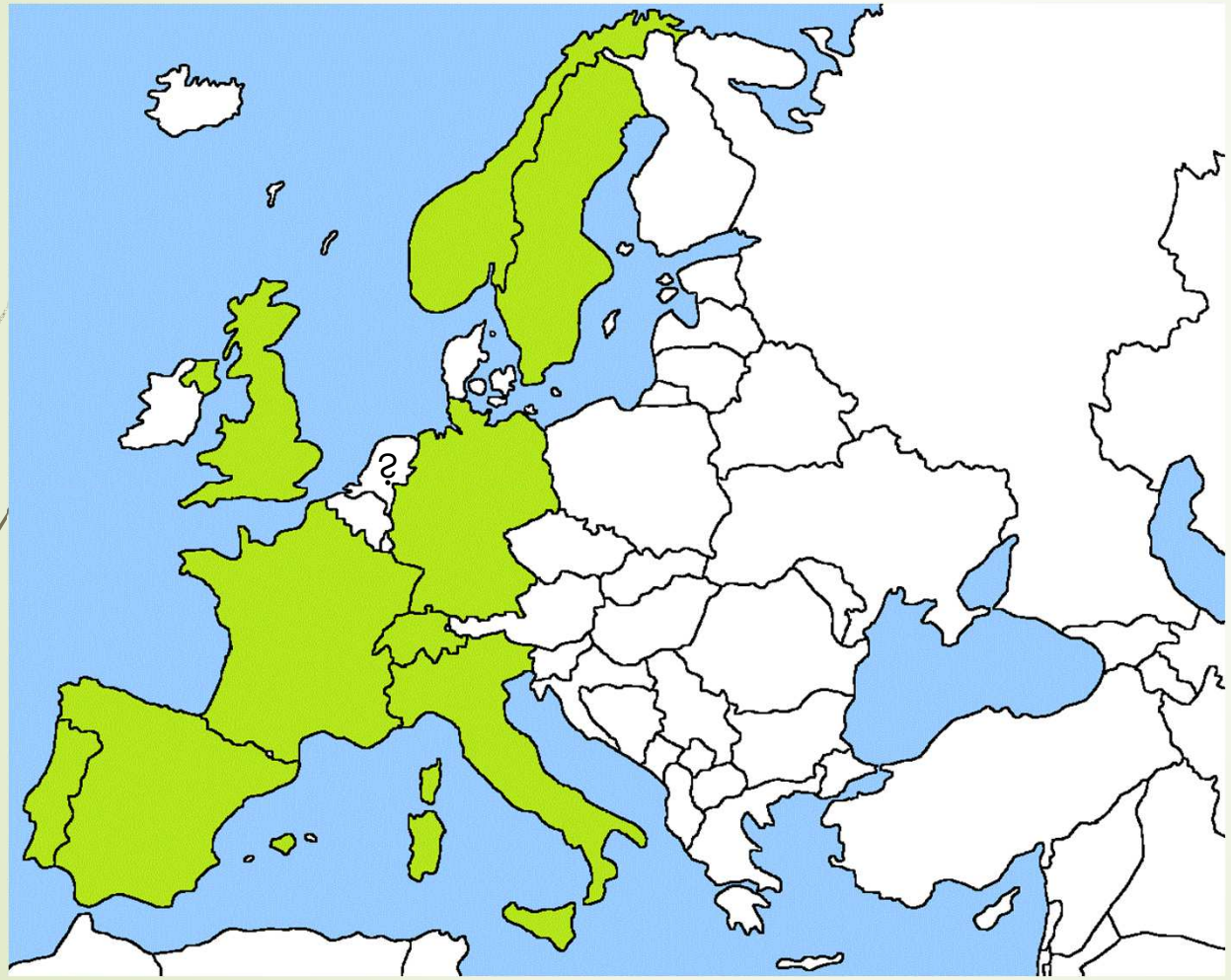
b) has a very distinct antigenic profile and high mortality rate (up to 70%)

→ but take more time → virus excreted longer

c) is able to overcome immunity to classical RHDV strains, including vaccine strains !

→ to cause the disease also in vaccinated and new-born rabbits

RHDV2 spread in Europe from 2010 till August 2016 …



In Europe

- 2010: France
- 2011: Italy, Spain
- 2012: Portugal, Sweden, Malta
- 2013: Germany, United Kingdom
- 2014: Norway, Switzerland
- 2015: Belgium
- (communication talk – AESA/ paper in progress)
- 2016: Canary Islands (Spain)

Out of Europe

- Recently, in Canada and Australia (2015)(communication talk – EWDA)

OBJECTIVES

- The objectives of this study are

(1) To describe the anatomopathologic patterns and the results of the 7 RHDV2 confirmed wild rabbits in 2015 (South Belgium)

(2) To reports all RHD-suspected wild rabbits received and analysed by the Surveillance Network of Wildlife Diseases (Prof. A. Linden) since 2013

Now Belgium member of contaminated country

What about these RHDV2 belgian cases ?

→ Until today: already described in rabbitries in Belgium (Marlier D. *et al.*, 2014) but **NOTHING in the WILD**

No official control program in Belgium ! This disease is not for compulsory declaration(AR 3 février 2014). Only volontary prevention assessment ! (AFSCA, Press release, August 2016)

MATERIAL and METHODS

- In november 2015, the Surveillance Network of Wildlife Diseases (Faculty of Veterinary Medicine of the University of Liege – FMV) 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 following a systematic protocol: each rabbit

(1) was weighted

(2) age was determined by the presence/absence of the distal ulna protuberance,

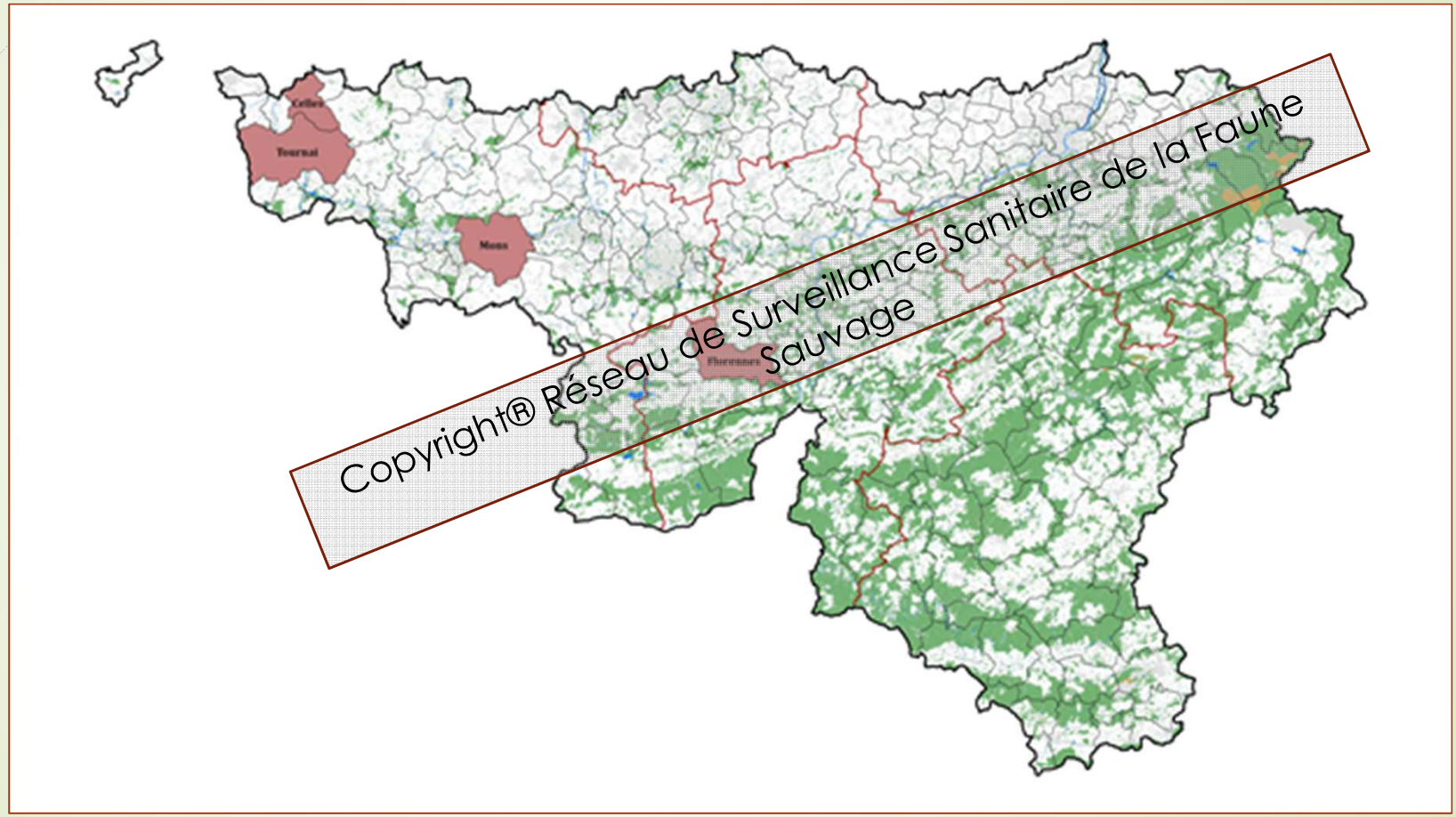
(3) stomach was investigated to exclude poison,

(4) spleen was systematically driven into Yersinia CIN culture media for detection of *Yersinia pseudotuberculosis*

(5) 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)

(6) feces were gathered for parasitology (Service of Parasitology, FVM).

Found dead spots (Southern Belgium):



Results (1)

- **At necropsy**, animals (5 adults: 3 males/2 females and 2 juveniles: 1 male/1 female) were in good condition. Examinations of the carcasses showed congestion of lungs/kidneys BUT livers were macroscopically normal.

- For **bacteriology**, all spleens were negative for *Yersinia pseudotuberculosis*.

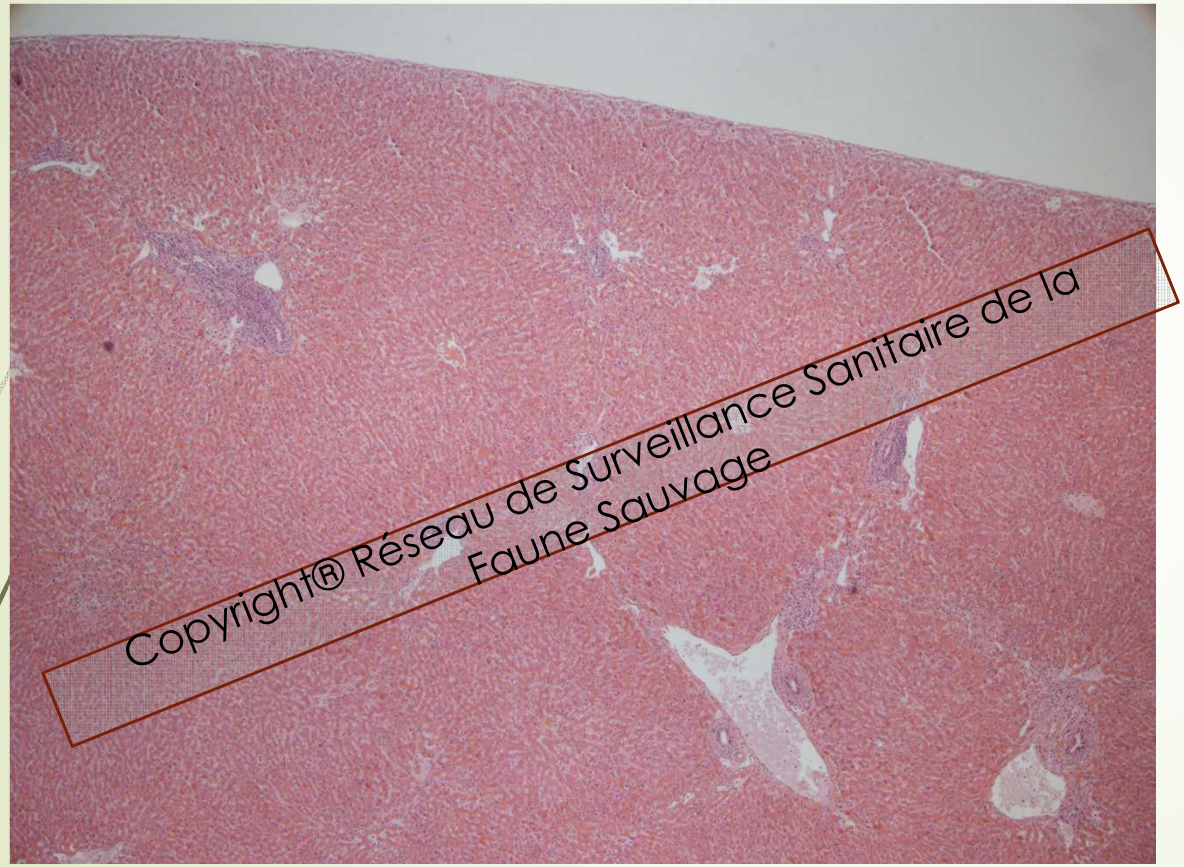
- For **parasitology**, only one animal presented an abnormal high rate of coccidiosis in feces. The others parasites found are those commonly seen in rabbits like some coccidia, *Trichuris* spp., *Strongyles*, *Nematodirus* spp., *Cittotaenia* spp., …

Results (2)

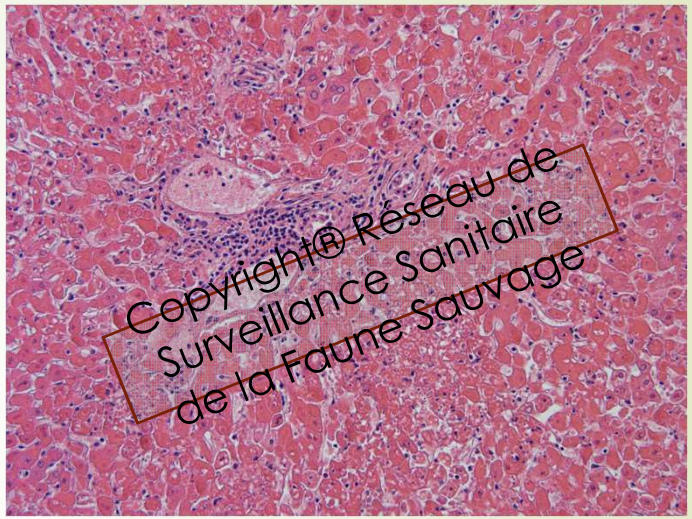
- **Histopathological** examination revealed haemorrhagic lung lesions in one animal while 5 of them presented severe necrotic hepatitis, sometimes associated with peri-angiocholitis.
- Samples of livers were sent to Scanelis Laboratory **for RHDV RT-qPCR diagnostic**. The results were positive for the new variant RHDV2 in 5 out of the 7 rabbit livers. All the samples were negative for the classic RHDV2.

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.

Histopathological lesions (liver):

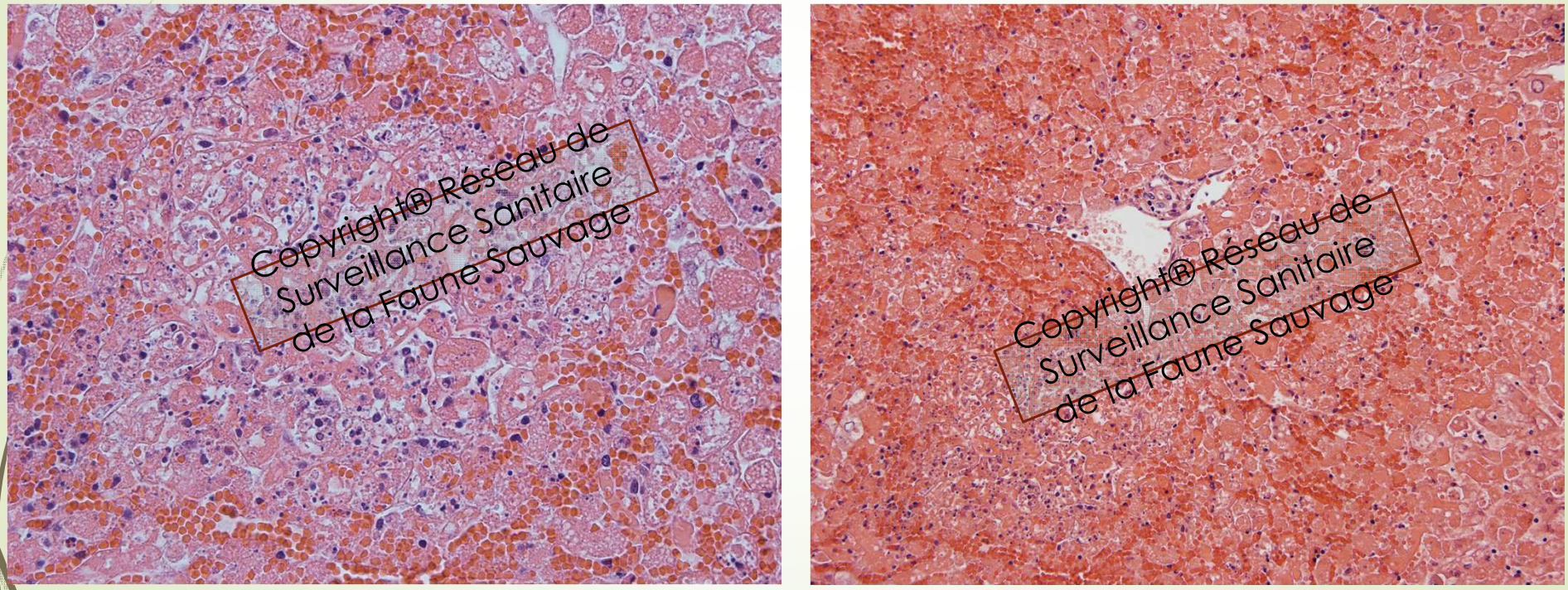


Liver, x10, HE: inflammation cells in portal spaces



Liver, x40, HE: inflammatory cells

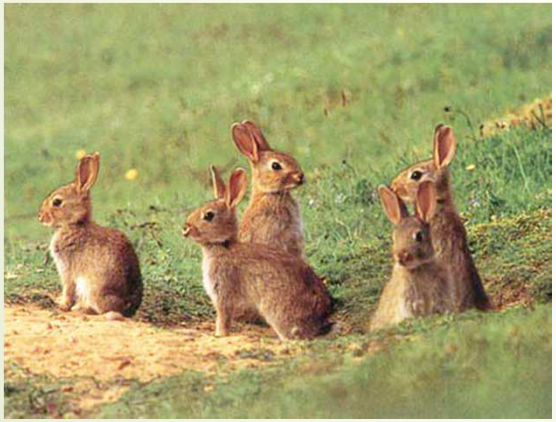
Hepatocytes necrosis +++



Liver, x40, HE: hepatocytes necrosis

News from the 12th European Wildlife Disease Association (EWDA – August 27th-31st, 2016, Berlin, Germany)

- Detection of the new emerging rabbit haemorrhagic disease type 2 virus (RHDV2) in European brown hares (*Lepus europaeus*) from Spain and Italy (Velarde Rosera *and al.,* oral communication at EWDA).
- *These specific features suggest that RHDV2 could represent a new viral emergence from an unknown source, including a possible species jump of lagoviruses between species of lagomorph genus.*



Oryctolagus cuniculi

Jump

Lepus europaeus
L. capensis
L. corsicus

Just for information: European Brown Hare Syndrom Virus (EBHSV)

- Since first description in hare (Sweden 80's): EBHSV is restricted to Europe.

Sensitive species to EBHS	
YES	**NO**
European Brown hare (*Lepus europaeus*)	Iberian hare (*Lepus granatensis*)
Mountain hare (*Lepus timidus*)	Broom hare (*Lepus castroviejoi)*
Italian hare (*Lepus corsicanus*)	Cape hare (*Lepus capensis*)
Eastern cottontail (*Sylvilagus floridanus*)	European rabbit (*Oryctolagus cuniculus*)

Sensitive species to RHDV2	
YES	**NO**
European rabbit (*Oryctolagus cuniculus*)	Eastern cottontail (*Sylvilagus floridanus*)
European Brown hare (*Lepus europaeus*)	Broom hare (*Lepus castroviejoi)*
Mountain hare (*Lepus timidus*)	Iberian hare (*Lepus granatensis*)
Cape hare (*Lepus capensis*)	

Lavazza A et al., oral communication EWDA 2016

CONCLUSIONS

- This is the first report which confirms the presence of RHDV2 **in wild populations of rabbits** in Southern Belgium
- According to our samples bank, the virus was already present **in 2013** 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
- Next step: RHDV2 in wild hares !
- Furthermore, the epidemiological meaning of this «new» lagovirus is still to be clarified

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- Forest **Rangers**

Thank you paying attention

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