

**PREVALENCE OF RABBIT HEMORRHAGIC DISEASE (RHD)
IN WILD RABBITS (*ORYCTOLAGUS CUNICULUS*)
IN FLANDERS, BELGIUM, 1999 – 2002**

*Prevalentie van Viraal Hemorragisch Syndroom (VHS) bij wilde konijnen
(*Oryctolagus cuniculus*) in Vlaanderen, België, 1999-2002*

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ABSTRACT

During the period of July 1999 through June 2002, carcasses of wild rabbits that had been shot or found dead and livers originating from wild rabbits that had been shot for consumption were collected in Flanders. One hundred and twelve carcasses were suitable for necropsy and histological and bacteriological analysis; histological analysis was possible in 41 livers. Considering the 112 rabbit carcasses only, Rabbit Hemorrhagic Disease (RHD) was found to be present in 33.9% of the cases. RHD was the most prevalent wild rabbit pathology detected in this study, before staphylococcosis (12.5%), and myxomatosis (10.7%). None of the liver samples from rabbits shot for consumption were positive for RHD. Of the 38 histologically RHD positive samples, 24 were analyzed with the hemagglutination (HA) technique, yielding 58.3% positive results. Seven samples that were histologically positive for RHD but HA negative were examined by transmission electron microscopy and were found positive for calicivirus. This proves that HA-negative RHD strains are circulating in the Flemish wild rabbit population.

SAMENVATTING

Van juli 1999 tot juni 2002 werden wilde konijnenkadavers (geschoten of dood aangetroffen) en levers van wilde konijnen die geschoten werden voor consumptie, verzameld in het Vlaamse Gewest. Honderdentwaalf kadavers waren geschikt voor lijkschouwing en histologische en bacteriologische analyse. Eenenveertig levers waren voldoende vers voor histologische analyse. Het Viraal Hemorragisch Syndroom (VHS) werd vastgesteld in 33,9% van de kadavers. VHS was daarmee de belangrijkste ziekte, vóór stafylokokkose (12,5%) en myxomatose (10,7%). Geen enkel van de leverstalen afkomstig van de konijnen geschoten voor consumptie, was VHS-positief. Van de 38 histologisch VHS-positieve stalen werden er 24 onderzocht met de hemagglutinatie-techniek (HA). 58,3% van die stalen werd positief bevonden. Zeven stalen, die histologisch positief waren voor VHS maar HA-negatief waren, werden elektronenmicroscopisch bekeken en waren positief voor het calicivirus. Dit toont aan dat er HA-negatieve VHS-stammen circuleren in de Vlaamse wilde konijnenpopulatie.

INTRODUCTION

Rabbit Hemorrhagic Disease (RHD) was first reported in China in 1984 (Liu *et al.*, 1984). It subsequently spread worldwide and was first reported in Belgium in 1990 (Peeters, 1990). RHD is an acute viral disease of domestic and wild rabbits that is caused by a calicivirus (Parra and Prieto, 1990) and transmit-

ted through fecal material and direct contact. The virus can survive in the environment for several days (Peeters, 1990).

The virus can replicate in young animals of three weeks and older, but symptoms are only seen in rabbits older than 2 months (Peeters, 1990). Two to three days after infection, there is a mortality of 80 to 100%, usually peracute without noticeable signs of illness.

Some animals are lethargic, have respiratory problems and show nervous symptoms such as spasms or opisthotonus (Peeters, 1990; Ohlinger *et al.*, 1993). The disease also causes fever (Xu and Chen, 1989). In partially immune animals, the disease proceeds more slowly.

Pathognomonic lesions at necropsy include congestion and hemorrhages of the thymus, hemorrhagic tracheitis with frothy contents, hemorrhages and edema of the lungs, and congestion of the kidneys. The liver can be pale and fragile with accentuation of the lobular markings, or congested (Peeters, 1990; Ohlinger *et al.*, 1993). These lesions may also be absent, however. Histologically, a diffuse necrosis of the liver is seen, especially in the portal areas of the lobuli (Peeters, 1990; Ohlinger *et al.*, 1993). Diagnosis is possible by means of histological analysis of the liver, immunofluorescence or ELISA. Hemagglutination (HA) is of limited value because there have been reports of HA negative RHD strains (Kesy *et al.*, 1996).

The wild rabbit population acts as a virus reservoir. Several authors report the presence of the virus in wild rabbits in the vicinity of affected rabbitries (Galassi *et al.*, 1989; Morisse, 1990; Rosell *et al.*, 1989), and rabbits fed with fresh greens run a greater risk of being affected by RHD (Peeters *et al.*, 1990). Moreover, RHD outbreaks in free-living populations of wild rabbits have been reported in a number of countries, such as Spain (Villafuerte *et al.*, 1994), the U.K. (Anonymous, 1994), France (Marchandeu *et al.*, 1998) and Australia (Mutze *et al.*, 1998; Saunders *et al.*, 1998). Hunting associations have been observing alarming drops in the numbers of wild rabbits (Simón *et al.*, 1995). Flemish hunters have also repeatedly reported seeing high numbers of wild rabbits in the spring, with significant decreases during the summer and autumn. This reduction is illustrated in Table 1, which presents the hunters' catch in the Weimeersjacht at Uitbergen (a territory of about 120 Ha in East Flanders) for the period of 1995–1999. Especially in 1999, RHD was believed to be largely responsible for the decline in the wild rabbit population (personal communication of G. Frulleux).

No data were available concerning the prevalence of RHD among the wild rabbits in Flanders, however. A study was therefore conducted at the request of and financed by the Institute for Forestry and Game Management, Forest and Green Areas Division (Department of Environment and Infrastructure, Ministry of the Flemish Community). This study commenced on July 1, 1999, and was terminated on June 30, 2002.

Table 1. Number of wild rabbits shot in the Weimeersjacht at Uitbergen, East Flanders (120 Ha) in 1995–1999.

Year	Number	Decrease compared to 1995
1995	178	/
1996	138	22%
1997	59	67%
1998	41	77%
1999	9	95%

MATERIAL AND METHODS

One hundred and twenty-two rabbits, shot or found dead, and 42 livers from rabbits shot for consumption, were collected. Ten carcasses could not be analyzed due to postmortem decay, absence of internal organs (disemboweled by hunter) or destruction of the liver by the shot. Furthermore, one liver was too putrid to allow further analysis. Therefore, of the 164 samples, 153 were analyzed completely, and one putrescent rabbit was examined only for myxomatosis.

The samples were kept frozen at -20°C until necropsy and analysis. The freezing procedure was used because the collaborators in the field did not have easy access to 10% formalin for immediate fixation. The livers were examined for histopathological lesions after fixation in 10% formalin and hematoxylin-eosin (HE) staining. Seven samples were additionally analyzed by transmission electron microscopy (TEM). Hemagglutination (HA) was performed as described by Pu *et al.* (1985). Skin samples of rabbits showing myxomata or other lesions compatible with myxomatosis at necropsy were histologically examined after fixation in 10% formalin and hematoxylin-eosin (HE) staining. Lung samples were bacteriologically examined to allow differential diagnosis with pasteurellosis and other pulmonary infections.

For 115 of the 153 samples, the (approximate) date of the sample collection was available. Table 4 shows how they were distributed per year. For 140 of the 153 samples, the region in which the samplings were made was known. Figure 1 shows where the samples were collected and where positive cases were detected. There was a more dense sample collection in the provinces of East Flanders, Flemish Brabant and

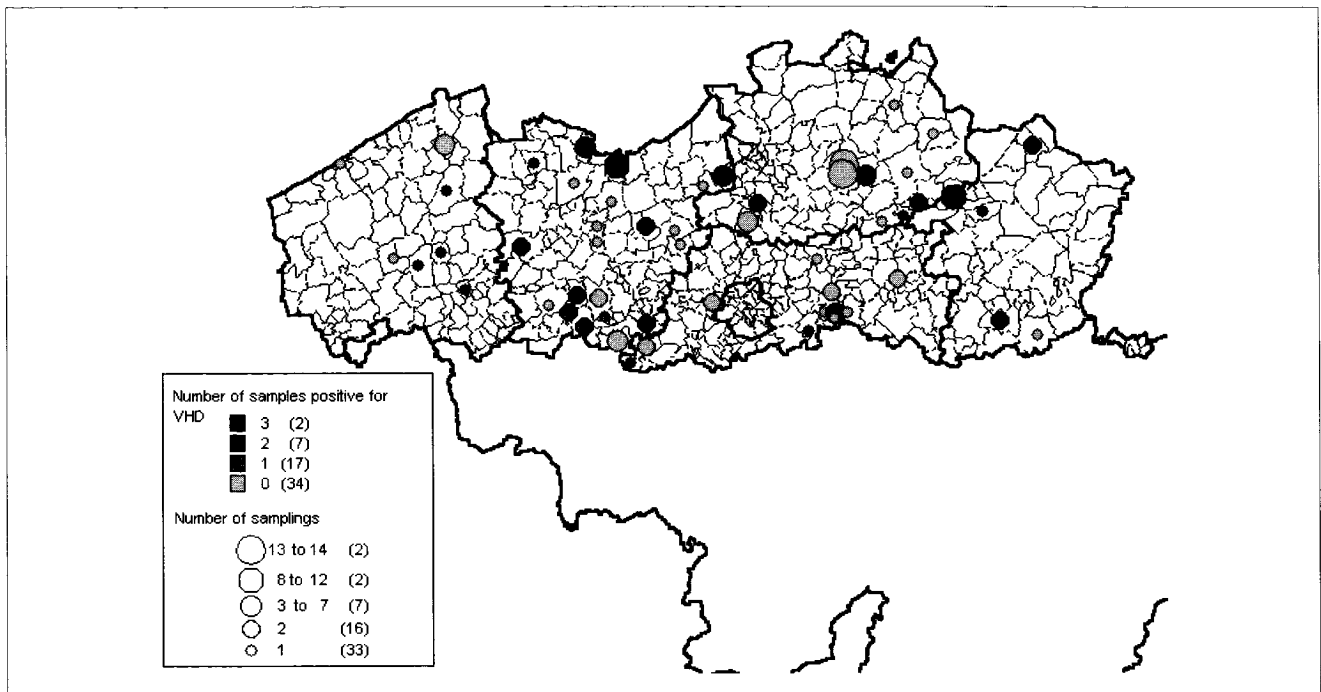


Figure 1. Geographical situation of the samplings and of the RHD positive samples. Sufficient data were available for 140 of the 153 samples, allowing their inclusion in this map.

Antwerp, but samples from all five Flemish provinces were available. The site data were introduced into the geographical software MapInfo to evaluate the spread of the samplings and the RHD positive samples.

RESULTS

Macroscopically, lesions compatible with RHD, trauma, septicemia, myxomatosis, lung pathology and liver pathology were found in the complete carcasses. Table 2 shows the results for the rabbit carcasses re-

ceived complete and in a sufficiently fresh condition to allow further examination. Myxomatosis was found in 13 out of 122 rabbits, or 10.7%. Lung staphylococcosis and liver pathology (i.e. hepatic coccidiosis or bacterial hepatitis) were found in 14 and 10 out of 112 cases, or 12.5% and 8.9%, respectively. No pasteurellosis was detected. In a number of cases, two simultaneous pathologies were found. These are summarized in Table 3.

Thirty-eight (24.8%) of the 153 livers histologically examined were positive for RHD. Only one of the

Table 2. Pathologies diagnosed in the wild rabbits.

Pathology	N ¹	n ²	%
RHD	112	38	33.9%
Trauma³	122	38	31.1%
Lung staphylococcosis	112	14	12.5%
Lesions compatible with septicemia	112	14	12.5%
Myxomatosis	122	13	10.7%
Liver pathology⁴	112	10	8.9%

¹: Number of samples examined

²: Number of cases detected

³: Shot wounds or traffic casualties

⁴: Mainly hepatic coccidiosis and histological lesions compatible with bacterial hepatitis

Table 3. Combined pathologies as detected in the wild rabbits.

	Lung staphylococcosis	Liver pathology ¹	Trauma ²
RHD	2	1	1
Liver pathology¹	1	/	2
Trauma²	2	/	/
Myxomatosis	2	1	2

¹: Mainly hepatic coccidiosis and histological lesions compatible with bacterial hepatitis.

²: Shot wounds or traffic casualties.

Table 4. Distribution per year of the samples for which the date of collection was specified.

Year	Carcasses	Livers	Subtotal
1998	1	0	1
1999	32	7	39
2000	42	1	43
2001	14	0	14
2002	2	16	18
No data	31	18	49
Total	122	42	164

RHD positive rabbits was shot, and the hunter who brought it in reported that he had shot the animal because it was ill. Of the 38 livers histologically positive, 24 were additionally tested by HA. Of these 24 samples, only 14 (58.3%) were HA positive. Seven samples, histologically RHD positive but HA negative, were also analyzed by TEM. All seven were positive for calicivirus.

Thirty-eight (33.9%) carcasses were positive for RHD. Excluding the rabbits without macroscopical signs of disease that had been shot or had been run over in traffic, 38 out of 83 animals or 45.8% were found positive. Except for two samples collected in the spring, all RHD-positive samples were recorded in the period June to January. Most RHD-positive samples were collected from June 2000 to January 2001. RHD was detected in all five provinces investigated. There seemed to be a concentration of HA-ne-

gative cases in the northern part of Limburg and on its border with Antwerp, but such cases were found in East Flanders and Flemish Brabant as well. Only in West Flanders were no HA-negative cases detected.

DISCUSSION

One hundred and sixty-four samples were collected, 122 of which were complete carcasses. Collecting dead wild rabbits is no easy task, since diseased animals will most often retreat into their burrows and die there, out of sight and out of reach. This has also been reported in other studies (Cooke, 1996). The limited number of samples does not allow thorough conclusions, but does give an idea of the RHD situation in the Flemish wild rabbit population.

33.9% of the carcasses were positive for RHD. After exclusion of the rabbits without macroscopic signs of disease that had been shot or run over in traffic, this figure increases to 45.8%. Therefore, RHD is clearly the most prevalent pathology found in this study. These percentages are comparable with those found by Spanish researchers (Simón *et al.*, 1995): 36.7 to 60.8% positive samples, with an average of 51.1%. Lung staphylococcosis was diagnosed in 12.5% and myxomatosis in 10.7% of the cases. However, staphylococcosis and other pathologies such as pasteurellosis may have been underestimated because the bodies were kept frozen until analysis.

All RHD positive samples originated from rabbit carcasses. None of the livers of the animals shot for consumption was RHD positive. This indicates that normal looking rabbits that have been shot are not suited for RHD detection. However, Simón *et al.* (1995) reported that apparently healthy animals that had been shot also gave HA positive results. The authors

gave two possible explanations: first, that there could be subacute and chronic cases of RHD in endemic areas and, secondly, that some results could have been false positive. Capucci *et al.* (1991) demonstrated the occurrence of 8% false positive results due to cross-reactions with other pathogens such as *Pasteurella* and parvovirus. Simón *et al.* (1995) calculated a specificity of the HA technique of 95.74%, with a predictive value of 97.01% for positive results.

Except for two samples, which were collected in the spring, all RHD positive cases were collected during the period of June to January. In June, many young rabbits have reached the age of susceptibility to RHD (Lange *et al.*, 1994). The period of August 15th to November 15th is known to be a critical period for RHD outbreaks (Anonymous, 1992). However, the limited number of samples and time data in this study do not allow any conclusions regarding a possible annual RHD cycle in our wild rabbit population. Simón *et al.* (1995) could not draw any conclusions regarding the relationship between the time of year at which the samples were collected and the number of RHD cases, although most of the cases coincided with the time of greatest supply of samples, which was October to April. Some authors have found that the disease can occur at any time of the year under natural conditions, while others consider that the incidence may be lower during the summer, with a higher risk in the spring and autumn (Cancelotti *et al.*, 1989; Pagès Manté, 1989; Capucci *et al.*, 1990; Marcato *et al.*, 1991). This may be due to the presence of a greater number of susceptible animals, greater climatic variability, and better virus survival. Calvete *et al.* (2002) reported an annual cycle in the occurrence of RHD outbreaks, which were detected predominantly in winter and spring. In Calvete's study, the peaks of maximum mortality from RHD in adult rabbits were detected at the beginning of the second half of the breeding season, when the highest proportion of pregnant females was found, and when most young rabbits were still limited to the burrows. RHD epidemiology may be largely defined by the persistence of the virus inside the burrows, which researchers associate with the large number of rabbit deaths from RHD inside them (Cooke, 1996). During the breeding season, adult rabbits increase their use of the burrows considerably, which facilitates the simultaneous infection of a high proportion of the adults, causing the annual winter outbreak, which would continue while there were sufficient susceptible young rabbits in the population (Cooke *et al.*, 2000).

RHD was detected in samples from all five Flemish provinces. HA negative strains were detected in all these provinces except West Flanders. The low number of samples received from West Flanders is probably the reason for this lack of detection. Nonetheless, a concentration of HA negative strains was seen in the northern part of Limburg and its border with Antwerp.

Histology is a more sensitive method for RHD detection than HA, since HA negative RHD strains have been described (Kesy *et al.*, 1996). Seven liver samples, histologically RHD positive but HA negative, were also examined by EM and found to be positive for calicivirus. The histopathological lesions found in these cases were pathognomonic for RHD. No indications could be found linking the noted lesions to freezing or other causes of liver necrosis. These samples can therefore be considered to be positive for RHD variant strains that are HA negative. In the Spanish study of Simón *et al.* (1995) such strains were also found, but the researchers interpreted these results as being caused by the presence of too little virus in the organs or the occurrence of degraded forms of the virus. A more recent study (Calvete *et al.*, 2002) proposes the possibility of a new pathogenic non-hemagglutinating variant of the RHD virus circulating in the wild rabbit populations. Our findings – using TEM for virus detection in HA negative but histologically positive liver samples – now confirm that HA negative RHD strains are circulating in the Flemish wild rabbit population.

The question as to whether the burrows are the main reservoir of the RHD virus is an important point for future research, because the density of burrows in an area might determine how the disease manifests itself and its impact on rabbit populations. Also, little is known about wild rabbit ecology, and more research is needed both on the epidemiology of RHD in wild rabbit populations and on possible measures to help them to recover (Calvete *et al.*, 2002).

Unfortunately, the expectations of Löliger and Eskens (1991) that the slowly increasing antigen spread would lead to the buildup of an immune balance have not been fulfilled. At this point, there are no means of protecting the wild rabbit population against RHD. However, in Spain experiments are taking place with a myxomatosis and RHD recombinant vaccine to test the possibilities of vaccination *per os* or by using fleas (Barcena *et al.*, 2000; Torres *et al.*, 2001). When testing of the vaccine, administered by means of subcutaneous injection, was carried out on a small island, 56% of the non-vaccinated contact wild rabbits were found to have significant serum titers of

anti-myxomatosis and anti-RHD antibodies one month after the vaccination had been performed. Gortázar *et al.* (2002), however, advise against vaccination. They claim that vaccination is too expensive and, when using recombinant vaccines, a certain risk is involved for wild rabbit populations. Further experiments are needed and it will take some time before a practical vaccination strategy is available to protect our wild rabbit population.

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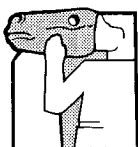
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Uit de faculteit



Emeritus professor Georges Peeters steeds alert

Vele lezers van dit tijdschrift hebben nog les fysiologie gekregen van Prof. Georges Peeters, de eerste decaan van onze nieuwe faculteit in 1968. Hij is met pensioen sedert 1985, maar hij houdt niet op zich uitgebreid te informeren door lectuur, kranten en TV. Hij is fysisch wel gehavend, maar zijn geest is onaangetast. Men ziet hem hier zitten in zijn rolstoel aan zijn dagelijkse massieve tafel, aandachtig lezend. De granieten kop van Georges Peeters is goed bewaard en vrijwel zonder rimpels. Een zeer recent boek over humane fysiologie is hij zopas ten derde male aan het lezen: "dit heb ik ook gegeven, dit meer, dat minder". Hij heeft nog altijd zijn wetenschappelijke gedrevenheid en zucht naar kennis om de kennis. In het tijdschrift *Natuur en Techniek* werden enkele jaren geleden de Grote Namen in de Belgische Wetenschap opgesomd, 36 namen en de man aan de leestafel is er één van, de enige veearts: de Wetenschappelijke Prijs Joseph Maisin 1971-1974 voor geneeskunde. Men mag hem dus de witte raaf van het Merelhof (zijn woonplaats) noemen!

F. Verschooten

