

## AN ACUTE OUTBREAK OF MYXOMATOSIS IN TWO GREEK RABBITRIES

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### ABSTRACT

A case of myxomatosis in vaccinated and non-vaccinated rabbits is described, and detection of *Myxoma virus* was performed in this study. In two commercial farms being 150 km distant each other, myxomatosis occurred after the import of animals from a common supplier. The disease was manifested firstly in the existing non-immune population of does and fatteners, and later in all vaccinated animals, being 2 to 3 months immune at the time of first symptoms. Morbidity was almost 100% with nasal discharge, listlessness, fever, eyelid swelling, eye and nasal purulent discharge, papules in the ears, facial oedema, and swelling of the anagenital region, and animals always died. Examination by PCR revealed the presence of a 492-bp specific product in all assays of the symptomatic animals tested from both farms, having 100% nucleotide sequence identity with the homologous major envelope gene region of the *Myxoma virus* Lausanne strain. The simultaneous occurrence of myxomatosis in the vaccinated and non-vaccinated rabbits of both farms suggests that the supplier was possibly the source of a viral isolate with increased virulence.

**Key words:** Myxomatosis, Rabbit, PCR.

### INTRODUCTION

Myxomatosis in rabbits is caused by a *Leporipoxvirus*, a virus of the *Poxviridae* family. There are two antigenic groups, the South American strains which natural host is the wild rabbit *Sylvilagus brasiliensis*, and the Californian strains which natural host is the wild rabbit *Sylvilagus brachmani*. Both are causing benign cutaneous fibroma in their respective hosts (Fenner and Ross, 1994). However, these strains are highly contagious and lethal for the European rabbits (*Oryctolagus cuniculus*). In 1950s, the South American virus was deliberately used in Australia, while later on in France, a second strain was used in order to reduce the harmful free-living rabbit populations that were incriminated for causing local ecological destruction. In recent years, the latter strain is spread in most of Europe (Marlier *et al.*, 2000). The disease was never reported in Greece for at least 30 years. Since vaccinations are not practiced in the Greek rabbitries, only the imported breeding replacement stock is vaccinated for myxomatosis once in the country of origin, but not later on in the farms. In this study, a case of a myxoma virus strain in vaccinated and non-vaccinated rabbits is described.

### MATERIALS AND METHODS

#### Animals and experimental design

The observations were performed in two commercial farms being 150 km apart. Housing was individual for the breeding does and bucks, while group housing (8 rabbits per cage) was applied for fattening animals from weaning (35 days of age) to slaughter at 90 days of age. Each building

contained does, bucks and fatteners in the same airspace. Farm A was operating already for five years, using a small grandparent stock (20 animals) to produce its own F1 breeder animals (around 400 does). Farm B was newly established, having its first batch of 2-month old F1-breeding rabbits imported at the end of December 2006 (400 breeding rabbit does). All animals were imported from Italy, and were once vaccinated for viral hemorrhagic disease and myxomatosis (homologous Borghi strain vaccine) at the farm of origin, 2 weeks prior to their export to Greece, at the age of 1.5 months. At the farm locations day/night temperatures were approximately 14°C/7°C during February and 25°C/15°C during May. No serious health problems, apart from occasional cases of pastereullosis and postweaning diarrhea, were evident in farm A during their operation. In the mid of February 2007, both farms had imported vaccinated F1-does from the same breeder (140 in farm A and 170 in farm B).

### PCR assay

For the diagnosis, eyelids were collected from 5 breeding and 5 fattening sacrificed sick animals of both farms, and DNA was isolated using a Nucleospin<sup>®</sup> Tissue kit (Macherey-Nagel GmbH & Co. KG). A PCR using a pair of primers (For: GGG GGA ATC TGA TAA AAA C; Rev: GCC AAA CGA TAC ATC CAC) amplifying part of the major envelope gene (Farsang *et al.*, 2003) was used to detect Myxoma virus. DNA extracts from tissues of a non-infected laboratory rabbit were used as negative controls. The PCR was performed using 0.5 units Dynazyme II DNA polymerase (Finnzymes, Finland) and the reaction mixture additionally contained 4% DMSO, 0,2 µM of each primer, and 1 µl of extracted DNA. The cycling profile consisted of 40 cycles applying a 47°C annealing temperature.

## RESULTS AND DISCUSSION

At the end of February 2007, several of the preceding older does in farm A, and few in farm B, have shown nasal discharge and eyelid swelling, not responding to antibiotic treatment. These signs became more severe next month in farm A, and had explosive character around the weaning period (mid-April). Clinical signs such as listlessness, fever, eyelid swelling, eye and nasal purulent discharge, papules in the ears, facial oedema, and swelling of the anagenital region have been observed in does and in fattening rabbits, leading to massive deaths. In farm B, severe clinical signs had occurred 15 to 30 days later. By the end of May, the existing population of young rabbits in farm A (6,500 animals), and all preexisting does and their offspring in both farms (around 4,000 in each farm) had died.

The does of the common import batch remained clinically healthy up to the parturition period, in both farms. At that time, these does were 4.5 months old in farm A and 5.5 month-old in farm B. Since then, a disease pattern similar to that seen in the older does has also been observed in them and their offspring.

A 492-bp specific product was obtained in assays of all the symptomatic animals tested. Direct sequencing of three PCR products (one from a fattener of farm A and two from a fattener and an old doe of farm B) confirmed the specific detection of Myxoma virus by revealing 100% nucleotide sequence identity with the homologous region of the Myxoma virus Lausanne strain (EMBL no. AF170726).

The simultaneous occurrence of myxomatosis in both farms, purchasing animals from the same supplier, suggests that the latter was possibly the source of infection in these two farms. This possibility is supported by the laboratory findings derived by the two farms. A question arises as to how it is possible that healthy vaccinated does can import a virulent virus in a naïve herd. It is likely that vaccinated animals could carry a virulent strain without showing yet or at all any signs of the disease. The possible presence of some “escaped” animals with defective immunity e.g. not properly immunized or being sick or stressed during vaccinations, definitely facilitates the spread of a virus. Even, in healthy immunocompetent animals, vaccinations can reduce but do not totally prevent the infection with a virulent strain (Marlier *et al.*, 2000). It could be that, a virulent strain was imported at

mid February and spread in both farms. The spread in farm A was faster, apparently due to the larger number of susceptible animals at the time of infection (non-immune does and their offspring, e.g. around 6,900 animals) compared to farm B (400 vaccinated does).

In farm B, the preexisting does were already immune for 2 months at the mid of February at the time of probable infection, and of course they remained so when the first massive deaths occurred in April. Considering that most homologous vaccines establish protective immunity lasting for 6 or even 9 months (Stanford *et al.*, 2007), the vaccinated does of that farm should have been protected. If it had been a drawback on vaccination of some individuals (stressed, sick, escaped, or delayed vaccinees), only a limited number of animals would have been affected. On the contrary, in our case, all does had been infected and died by 5.5 months after their vaccination, suggesting that the suspected present viral isolate has demonstrated an increased virulence. The fact that the natural infection did not act as a booster for the previously vaccinated does is also interesting and possibly indicates that the challenge dose was both high and virulent. The entire second batch of newcomer does in both farms, have similarly died 4 to 5 months after their vaccination, when apparently their immunity was fading away. Therefore, the efficacy of vaccinations seems to depend, not only on the vaccine, but also on the potency of the infective strain as well.

### CONCLUSIONS

The simultaneous occurrence of myxomatosis in the vaccinated and non-vaccinated rabbits of both farms, suggests that the supplier might possibly be the source of a viral isolate with increased virulence.

### REFERENCES

- Farsang A., Makranszki L., Dobos-Kovacs M., Virag G., Fabian K., Barna T., Kulcsar G., Kucsera L., Vetesi F. 2003. Occurrence of atypical myxomatosis in Central Europe: clinical and virological examinations. *Acta Vet. Hung.*, 51, 493-501.
- Fenner F., Ross J. 1994. Myxomatosis. In "The European Rabbit. The history and biology of a successful colonizer". Eds H.V. Thompson, C.M. King, Oxford University Press, Oxford, UK, 205-240.
- Marlier D., Mainil J., Boucraut-Baralon C., Linden A., Vindevogel H. 2000. The efficacy of two vaccination schemes against experimental infection with a virulent myxomatous or a virulent nodular myxoma virus strain. *J. Comp. Path.*, 122, 115-122.
- Stanford M.M., Werden S.J., McFadden G. 2007. Myxoma virus in the European rabbit: interactions between the virus and its susceptible host. *Vet. Res.*, 38, 299-318.

