

AN OUTBREAK OF MYXOMATOSIS IN GREEK RABBIT FARMS DURING 2007

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ABSTRACT

Myxomatosis is considered as one of the most frequent epizootic viral diseases of rabbit all over Europe. In Greek rabbitries, where all parent stock is exclusively imported from European countries with high myxomatosis incidence, surprisingly the presence of the disease was limited. In the present study, the diagnosis and isolation of a wild, highly pathogenic Myxoma virus is reported, causing a myxomatosis outbreak in Greece. The last myxomatosis outbreak in Greece was reported in 1973 and till February 2007 no further cases had ever been reported. No vaccination strategy against Myxoma virus was ever been applied and early in the spring 2007 the spread of the disease was inevitable, as no special preventive measures had ever been taken for the imported carrier animals. At that period, a myxomatosis outbreak was reported in two commercial rabbit farms, with a population of 500 and 800 does, situated within a 150 km distance that shared the same parent stock supplier. In both farms, morbidity and mortality reached 80-90% and myxomatosis diagnosis was based on clinical signs and laboratory confirmation that included virus isolation, histopathology profile and molecular detection by standard PCR. Samples from 15 fattening rabbits were processed for virus isolation according to OIE standard protocol using the established RK-13 cell line. Typical Myxoma virus cytopathogenic effect was always present after the second passage. Apart from the pathognomonic gross facial lesions, samples from eyelids and dermis presented histologically myxoid material mixed with collagenous tissue. Total DNA was extracted from tissues carrying lesions and from infected RK-13 cell culture supernatants and a 492bp segment of the major envelope gene was amplified. The specific PCR product was further analyzed by sequencing. Sequence alignment was performed against all available Myxoma virus genome sequences in Genbank. Compared to the major envelope gene of the Lausanne strain, the Greek isolate exhibited a 96% sequence homology and was submitted to Genbank and assigned accession number EU399816. Disease eradication was based on euthanasia that was performed to the total rabbit farm population, together with thoroughly applied hygienic measures. Preventive policy by vaccination program was not encouraged by the national veterinary authorities, in order to be maintained the myxomatosis free status of Greece. On the other hand, veterinary services were alerted and quarantine measures were strongly recommended to rabbit farmers every time that rabbit parent stock was imported. Preliminary genetic analysis of the Greek viral isolate indicates sequence identity with the Lausanne reference strain.

Key words: Myxomatosis, Rabbits, Virus isolation, Histopathology, Molecular analysis, Greece.

INTRODUCTION

Myxomatosis is a widely spread fatal disease of the European rabbit (*Oryctolagus cuniculus*) caused by Myxoma virus, a double stranded DNA virus belonging to the *Leporipoxvirus* genus of the *Poxviridae* family (Fenner, 1994).

Myxomatosis is considered as one of the most frequent enzootic viral diseases of wild and farmed rabbits, which is present in two forms: the nodular (classical) and the amyxomatous (respiratory) form (Marlier *et al.*, 2000; Stanford *et al.*, 2007). The nodular myxomatosis is characterised by pathognomonic skin lesions on the face mainly affecting the ears and oculo-respiratory areas. The mortality rate of the affected animals varies between 20-100%, according to the viral strain. Amyxomatous myxomatosis symptoms concern mainly the respiratory track, while skin nodules are usually number and size limited. Natural transmission of the nodular form is carried out by blood-sucking insects whereas the amyxomatous myxomatosis is transmitted mainly by direct contact.

In the present study, the isolation of a wild highly pathogenic Myxoma virus is reported, causing a myxomatosis outbreak in Northern Greece (about 500 km from Athens), in the spring of 2007, after almost 34 years from the last reported cases in 1973 (Stoforos *et al.*, 1974).

The outbreak started in February 2007 by affecting two commercial rabbit farms, with a population of 500 and 300 does, which were situated in a 150 km distance, having the same parent stock supplier that imported animals from a neighboring country. According to farmer's reports, morbidity approached 100% while mortality reached almost 80-90%. In Greece, vaccination against myxomatosis is not permitted and therefore the Greek animals had significantly limited immune protection against the virus. The parent stock, young females and males that were imported, had been vaccinated in the country of origin.

MATERIALS AND METHODS

About 15 euthanised fattening rabbits were sent to our laboratory in May 2007, from both affected commercial rabbit farms. Samples were taken from all submitted rabbits and pathologic material was properly processed for viral isolation in RK-13 cell line, histopathology and molecular detection by standard PCR.

Virus isolation

Samples were processed for virus isolation according to OIE standard protocol (OIE, 2007), using the established RK-13 cell line, in minimal essential medium (MEM) containing 5% foetal calf serum, 100 IU/ml penicillin; 100 µg/ml streptomycin; 100 µg/ml gentamycin; and 5 µg/ml amphotericin (fungizone). The inoculum was consisted of the supernatant fluid of pooled homogenised tissue segments and oculo-nasal secretions of euthanised rabbits, in MEM with 2% calf serum supplemented with antibiotics.

Tissue homogenates were freeze-thawed twice to ensure release of virus from cells and centrifuged at 600 g for 10 min. Six-well plates showing 90% confluent cell growth were inoculated with 0.5-ml volumes of the supernatant. Samples for the study of viral excretion were incubated for 1 h at 37°C and 0.25-ml volumes of the suspending viral transport medium were inoculated on to 90% confluent RK13 cells in 24-well plates. Inoculated cultures were observed daily for cytopathogenic effect (CPE).

Histopathology

Segments of eyelids and derma were fixed in 10% neutral buffered formalin and embedded in paraplast. The blocks were cut at a thickness of 5 µm and the sections were stained with haematoxylin and eosin (HE).

DNA extraction, PCR and molecular analysis

Total DNA was extracted from tissues carrying lesions and from RK-13 cell culture supernatants, following virus propagation using the DNeasy Blood and tissue kit (Qiagen, USA), according to

manufacturer's instructions. Primer selection and PCR amplification of the major envelope gene (492 bp) of the Lausanne strain was performed according to previously published protocol (Farsang *et al.*, 2004) in a iCycler (BIO-RAD, USA). PCR products were electrophoresed in 1.5% agarose gel (Promega, USA) and visualized in a UV transilluminator (BIO-RAD, USA).

Purification of PCR products was performed prior to sequencing with Nucleofast 96 PCR plates, (Macherey-Nagel, Germany). DNA sequencing with the above primer pair took place in a 3730 DNA analyzer (Applied Biosystems, USA). Analysis of sequence alignments was performed with ClustalW2 software (EMBL) and MEGA version 3.1 (Kumar *et al.*, 2004), against Myxoma virus genomes retrieved from Genbank.

RESULTS AND DISCUSSION

The facial lesions of the submitted animals were characteristic and pathognomonic to Myxomatosis infection. Ear nodules, facial edema, edema of the eyelids, blepharoconjunctivitis, ocular and nasal discharge, diffuse and exudative skin lesions and edema of the anogenital area as well as hemorrhages of the internal organs, were found. Histopathological lesions referred to myxoid material mixed with collagenous tissue in the dermis and general inflammatory reaction.

Typical Myxoma virus CPE was present in RK-13 cells, after the second passage of the pathologic material in the susceptible cell line. The 492 bp segment of the major envelope gene of Myxoma virus was detected by PCR in both the histological material and in culture supernatants following virus propagation in RK-13 cells. Compared to the major envelope gene of the Lausanne strain, the Greek isolate exhibited a 96% sequence homology which was submitted to Genbank and assigned accession number EU399816.

The last myxomatosis outbreak in Greece was reported in 1973 (Stoforos *et al.*, 1974) and till February 2007 no further cases had ever been reported. In Greek rabbitries, where all parent stock is exclusively imported from European countries with high myxomatosis incidence, surprisingly the presence of the disease was significantly limited. On the other hand, no vaccination strategy against Myxoma virus was ever been applied and early in the spring of 2007, the introduction and spread of the disease was inevitable, as no special preventive measures had ever been taken for the imported carrier animals, that were vaccinated in the country of origin.

In this recent outbreak, vaccinated imported parent stock was not efficiently protected against myxomatosis infection, while naive fattening population was primarily and heavily affected. Disease eradication was based on euthanizing the whole rabbit farm population of the affected farms, together with thoroughly applied hygienic measures. Preventive policy by vaccination program was not encouraged by the national veterinary authorities, in order to be maintained the Myxomatosis free status of Greece. On the other hand, veterinary services were alerted and quarantine measures were strongly recommended to rabbit farmers every time that rabbit parent stock was imported. Preliminary genetic analysis of the Greek viral isolate indicates sequence identity with the Lausanne reference strain. Further molecular and antigenic analysis of the above isolate is currently underway in our lab.

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