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A RE-EMERGING DISEASE OF HARES (*Lepus europaeus*): HARE FIBROMATOSIS

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Key words: hare, poxvirus, hare fibromatosis, re-emerging disease

Abstract

Since 1961 (Leinati *et al*.) fibromatosis in hares has not been reported in the Northern part of Italy. After 40 years, in the same geographic area an outbreak of fibromatosis in game hares occurred. Two hundred and fifty (25%) hares showed typical skin lesions, with no difference in prevalence related to the age. Skin tumours of 1 to 3 cm in diameter were present on legs and ears. After 4-6 weeks skin lesions disappeared and the animals spontaneously recovered. Hystologically many large cells with large nuclei and numerous periodic acid-Schiff inclusion bodies in their abundant cytoplasm were observed. E.M. confirmed the presence of poxviruses. Cross-infection trials and serological examinations on challenged rabbits, hares and cottontails are in progress to confirm origin and nature of the virus.

Zusammenfassung (??)

Résumé (??)

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Introduction

Fibromatosis is a disease, different from mixomatosis, typical of hares. It was for the first time described in Italy (4) and France (3), even if at that time there had already been several reports of its occurrence, some of them long time before mixomatosis had been introduced in Europe (6, 7).
It is caused by a leporipoxvirus antigenically related more closely to the agent of rabbit (Shope) fibroma than to myxoma virus (1, 5).
The clinical disease is characterised by single or multiple protruding solid tumours, occurring mostly on ears and legs. The virus is probably mechanically transmitted by arthropods, but animals could also become infected by direct contact. The presence of skin microlesions or trauma is considered a predisposing factor. Morbidity is usually high but mortality is low and most of adult hares spontaneously recover within 1-3 months. Since 1961 in Northern Italy and 1964 in the rest of Europe fibromatosis in hares has not being reported anymore (2).

Clinical cases

At the end of January 2001, in the same geographic area where the disease was first detected in 1959 (Pavia province, Lombardia region; lat. 44,916, long. 9,0146), we observed an outbreak of fibromatosis in a farm of game hares. Two hundred and fifty (25% of the effective) hares showed typical skin lesions, with no difference in prevalence related to the age. Skin tumours of 1 to 3 cm in diameter were present on ears and legs (**Figures 1-2**). After 4-6 weeks skin lesions changed: tumours reduced in size and sometime spontaneously detached. Bleeding scars or dry crusts were then observed and at last most of the animals spontaneously recovered (**Figure 3**). Four hares died for concurrent infections (pasteurellosis and staphylococcosis) and then submitted to necropsy and laboratory examinations. More recently two affected hares showing typical lesions were hospitalised in the animal facilities of IZSLER, kept in isolated rooms and checked daily. At present they are being used for serological controls and experimental trials in order to reproduce the disease.
At the end of 2001 similar lesions were detected in a single hare (**Figure 4**) imported from Hungary and released in an area of Novara province, close to Pavia province, for restocking of hunting areas.

Material and methods

Samples of typical tumours taken during necropsy were submitted to routine examinations i.e. bacteriological, histological (EE staining and Alcian PAS) and virological (identification by electron microscopy using the negative staining drop method and isolation on chicken embryo and RK13 cell culture).
An attempt of reproducing experimentally the disease is still in course. Rabbits, hares and cottontails (*Sylvilagus floridanus)* have been infected using a 1:10 (v/v) PBS homogenate of a nodule (1.5 cm in diameter). The animals of each species were infected through eye inoculation, intradermal injection or direct contact with affected hares. Blood samples were taken from all animals before the challenge and at 1 and 2 months post infection. Two other time-point bleeding are scheduled at 3 and 5 months p.i.; the whole set of sera will be then examined using serological methods (competition ELISA) set up to detect anti-rabbit mixomatosis antibodies.

Results

Bacteriological examination resulted negative on solid nodules, whilst from ulcerated ones *Staphylococcus* sp. and other environmental bacteria were isolated.
Histologically we observed areas of consistent proliferation of new connective tissue in which large spindle or star-shaped fibroblasts with large nuclei and numerous periodic acid-Schiff (PAS) inclusion bodies in their abundant cytoplasm were evident. The vascular hyperplasia, characterised by new blood vessels showing dilated walls and containing large amounts of erythrocytes, was evidence of a recent fibroblastic proliferation. A particular amount of large PAS-positive fibroblasts was sometime visible around the wall of the wider new blood vessels.
Electron microscopic examination was systematically carried out on the homogenates of the nodular tissues taken from all the infected hares showing typical lesions. It permitted to observe several viral particles morphologically resembling poxvirus (**Figure 5**). Similar particles, but in a lower amount, were found when we examined by EM crust material scraped from the scar of an infected hare 4 weeks after the housing in the isolation room.
The attempts of *in vitro* isolation were positive with regard to embryo egg inoculation, i.e. typical pocks were observed on chorion-allantoic membrane (CAM) after three passages and poxvirus particles were observed examining CAM extracts. On the contrary we did not observe any cytopathic effect (CPE) on RK13 cell culture nor positivity for poxvirus by EM, even after 7 passages.
The results of serological investigations will be available at the end of the experimental trial.

Discussion

This outbreak of hare fibromatosis occurred in a “close” farm of game hares, with a good hygienic standard. The morbidity in the farm was quite high and this could be due to passive transfer of virions through equipment and cages, and favoured by microlesions on the skin of captive hares.
It is difficult to determine the origin of the disease because no new animals had been recently introduced, neither the disease had been diagnosed in free living hares of the same area. Nevertheless the detection of similar lesions in a hare imported from abroad for restocking of hunting areas suggests the hypothesis of a re-appearance of the virus in Italy by this way.
Pavia province was among those at the beginning considered as endemic for hare fibromatosis by Leinati et al. (1959). Thus, the possibility that other species could act as reservoirs cannot be excluded. It has to be noted that in this province as well as in other neighbouring province of Piedmont region the population of *Sylvilagus floridanus* is sharply increasing and it is well known that cottontails are naturally resistant to leporipoxviruses such as myxomatosis virus.
The re-appearance of hare fibromatosis after 40 years should draw our attention to the risk that it could become widespread on the territory, causing serious problems and taking an epidemiological relevance. In fact, even if it usually causes low mortality rates, the hares become weaker and could become affected by secondary infections more easily. In addition, they remain carrier for a long time (>1 month) and so could spread large amount of viral particles, increasing the rate of diffusion of the disease in the field.
In conclusion, specific prophylaxis and control measures must be implemented for all the three categories of hares involved in hunting management and mainly used for restocking hunting areas. These hares, i.e. captive hares from close farms, imported hares and free-living hares from the so called “restocking areas” are normally moved to areas even far away, and thus a strict control of their health status and a permanent monitoring of the “receiving areas” are needed to avoid the dissemination of fibromatosis as well as any other infectious disease.

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