

DETECTION OF MYXOMATOSIS VIRUS IN THE SEMEN OF BUCKS EXPERIMENTALLY INFECTED AFTER VACCINATION WITH A SHOPE'S FIBROMA VIRUS

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Abstract - The authors studied the possibility of transmitting Myxomatosis virus through semen in bucks vaccinated with a vaccine containing the Fibroma of Shope virus and infected 45 days later with a Myxomatosis wild virus. Semen samples were collected twice a week using an artificial vagina. The rabbits in the positive control group transmitted the virus through the semen for a long time and eventually all died. The vaccinated and successively infected rabbits overcame the infection unharmed and five of the six transmitted the virus through the semen. The presence of the virus in the seminal liquid of vaccinated rabbits and of the control was shown by biological tests and infection of cell cultures. The transmission of Myxomatosis virus through the semen, even in vaccinated rabbits, represents a serious risk factor in the practice of artificial insemination.

INTRODUCTION

In modern rabbitries, the practice of artificial insemination (a.i.) is widespread and for many raisers it has become indispensable (FACCHIN, 1995). A.i. offers practical, managerial and economic advantages and is believed to be an efficient method for controlling the diffusion of many infectious diseases. Yet, the seminal material may be a vehicle for transiting some pathological, diffusible diseases due to a manifested or latent disease condition in the bucks. The presence of the virus in seminal liquid is extremely dangerous in the practice of a.i. particularly since for some diseases it is very difficult to identify the viremic phase during which there is a massive presence of virus in the sperm (CICERI *et al.*, 1993).

Myxomatosis is still one of the most feared diseases by rabbit raiser; the disease may progress in an acute form, which is quick and lethal or in a subacute-chronic form characterized by the absence of myxomes, predominance of respiratory symptoms and the possibility of aerogenous transmission (BRUGÈRE, 1991 ; BRUN *et al.*, 1981 ; CASTRUCCI, 1978 ; JOUBERT *et al.*, 1982 ; SCATOZZA, 1977). These forms of "amyxomatosa" Myxomatosis are very difficult to diagnose quickly.

In a recent study (SCUOTA *et al.*, 1993), the authors showed the possibility of transmitting Myxomatosis virus in the seminal liquid of experimentally infected bucks. The results showed that the transmission of the virus through the semen can also occur in the pre-symptomatic phase of the disease. There is virus spreading also in concomitance with initial symptoms (conjunctivitis, blepharitis) which are not associated only with Myxomatosis, during the time which the infected animal continues to give semen. The authors also report the possibility of transmitting Myxomatosis by a.i., using semen at a low titer (CASTELLINI *et al.*, 1994).

Based on previous experiences, the aim of this study was to determine if vaccination of the bucks could prevent the spread of Myxomatosis by a.i. The evaluation was made by studying the virus in the semen of bucks experimentally infected after vaccination with a heterologous vaccine containing the Shope's Fibroma virus.

MATERIALS AND METHODS

Virus : A highly virulent wild strain of Myxomatosis virus provided by the IZS of Padova was used.

Vaccine : The vaccine "Myxoshope" produced by IZS of Umbria and Marche and distributed by Gellini S.p.a. of Aprilia containing Shope's Fibroma virus was used.

Animals : A total of 186 male New Zealand white bucks were used. All rabbits were unvaccinated, Myxomatosis free and seronegative.

Of these :

15 bucks, seven-month-old, were used for the vaccination, experimental infection and related controls. These were divided into four groups:

- 1) 3 rabbits were neither vaccinated nor infected (negative control).
- 2) 3 rabbits were vaccinated but not infected (negative control)
- 3) 3 rabbits were not vaccinated but infected (positive control)
- 4) 6 rabbits were both vaccinated and infected (animals in experiment).

171 rabbits, 4-month-old, were used for biological tests. All animals were kept in complete isolation.

Cell cultures : The cells used were rabbit kidney cells RK13 cultivated on EAGLE (Earle base) agar added with 10% bovine serum.

Vaccination : The vaccination was given by subcutaneous injection in the rib region inoculating 0.5 ml of vaccine following the suggestions of the producer. The formation of a nodule at the point of injection confirmed that the vaccine virus took.

Experimental Infection : six vaccinated and three unvaccinated rabbits were infected by inoculation with 25 infecting doses of the virus. The six vaccinated subjects were infected 45 days after vaccination.

Semen sampling: Twice a week for 50 days or until the death of the animal, semen was collected using a commercially available artificial vagina according to current practices. Each sampling was carried out using sterile test tubes and vaginas.

Infection of cell cultures : 0.2 ml of each ejaculate were seeded after dilution in EAGLE on RK 13 cells. After five days of incubation at 37 °C., the infection was evaluated by immunofluorescence techniques according to TITOLI *et al.* (1972).

Biological tests : Tests were carried out inoculating a male rabbit by subcutaneous injection 0.2 ml of undiluted seminal material. At the death of the animals, the virus was detected in the smears from organs by Direct Immunofluorescence. In addition, cell cultures were infected with homogenates of the target organs for the re-isolation of the virus. The surviving animals were kept under observation for 70 days.

RESULTS AND DISCUSSION

The results regarding mortality and the transmission of Myxomatosis virus with seminal liquid of the 15 rabbits used for the experiment are given in Table 1.

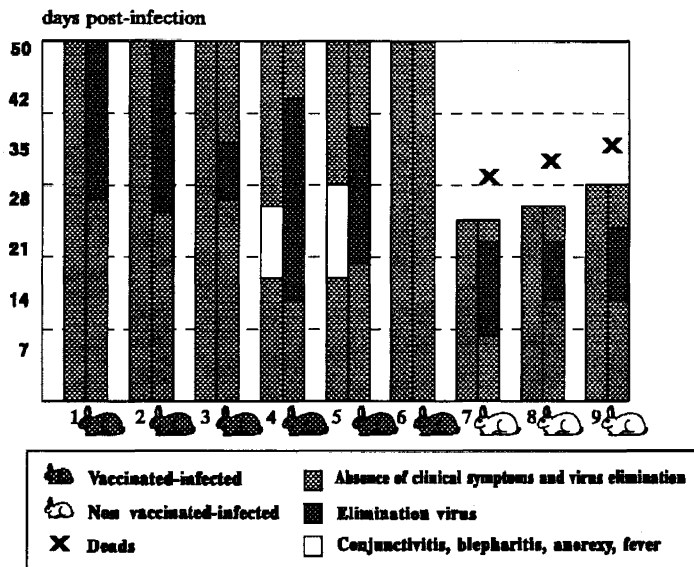
Table 1 : Protocol of research and results obtained.

		Non infected animals		Infected animals	
		non vaccinated (group 1)	vaccinated (group 2)	non vaccinated (group 3)	vaccinated (group 4)
Animals	n.	3	3	3	6
Elimination of virus	(%)	0	0	100	83.33
Deads	(%)	0	0	100	0

The rabbits in groups 1 and 2 (negative controls) never transmitted the virus through seminal liquid. The rabbits in group 3 (positive control) started transmitt the virus from the third sampling after infection and continued as long as it gave semen. All of the rabbits of this group died of Myxomatosis at different times.

None of the vaccinated-infected rabbits (group 4) died. Three were not affected by the disease but they transmitted the virus through seminal liquid many times. Two animals showed light symptoms: fever and anorexia for a day, conjunctivitis and blepharitis and did not give semen at the fifth sampling. They transmitted the virus through semen from the fourth sampling onward until they were clinically healed. One rabbit did not show any symptoms, always gave semen and never transmitted the virus. These results concerning the infected animals are given in Figure 1.

Figure 1 - Bucks response to Mixomatosis contamination.



We can conclude that vaccination with a heterologous virus, while allowing the vaccinated rabbits to overcome the infection, does not lessen the risk related to the diffusion of Myxomatosis virus through a.i. Therefore, while it is necessary to scrupulously observe all the norms of direct and indirect prophylaxis for this disease, it is likewise indispensable to accurately and systematically verify the health condition of bucks.

The possibility of transmitting the wild virus by means of seminal liquid of experimentally infected rabbits previously vaccinated with homologous virus will be the object of the next study.

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Ricerca del virus della Mixomatosi nel liquido seminale di conigli riproduttori infettati sperimentalmente dopo la vaccinazione effettuata con il virus del Fibroma di Shope - Gli AA hanno indagato sulla possibilità di eliminazione del virus della Mixomatosi attraverso il seme di conigli riproduttori, vaccinati con un vaccino contenente il virus del Fibroma di Shope e infettati dopo 45 giorni con un virus selvaggio della Mixomatosi. I prelievi del seme sono stati effettuati 2 volte a settimana utilizzando una vagina artificiale. I conigli del gruppo di controllo positivo hanno eliminato il virus col seme per un lungo periodo e sono tutti venuti a morte. I conigli vaccinati e successivamente infettati, hanno superato indenni l'infezione e cinque su sei hanno eliminato il virus col seme. La presenza del virus nel liquido seminale dei conigli vaccinati e di quelli di controllo è stata dimostrata mediante prove biologiche ed infezione di colture cellulari. L'eliminazione del virus della Mixomatosi con il seme, anche in conigli vaccinati, rappresenta un grave fattore di rischio nella pratica della inseminazione artificiale.