# Full diagnostic approach aimed to eradicate Myxomatosis from an industrial farm

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**ABSTRACT:** An integrated approach was used in a rabbit commercial farm during a case of Myxomatosis. The collaborative activity of various actors, was finalised to eradicate the disease accomplishing those rational sanitary measures provided by the law but without stopping the production. Myxomatosis was definitively eradicated, thanks to repeated sampling of sera and naso-conjunctival swabs to detect respectively antibodies by cELISA and viral antigen by using PCR. This work emphasizes that, in association with early diagnosis performed with sensitive and specific methods, cooperation, application of strict hygienic and biosecurity measures and vaccination programs are essential to recover from Myxomatosis. Indeed, there is the urgent need to define new and updated standard measures applicable for an effective control of this disease, endemic in Italy since 1955.

Key words: Myxoma virus, Serology, PCR, Vaccination.

**INTRODUCTION** – Myxomatosis is a lethal systemic disease of European rabbits (*Oryctolagus cuniculus*), caused by a rabbit poxvirus (Myxoma Virus - MV) (Fenner 1959). It was firstly introduced in Italy in the early '50 and it was soon included in the list of notifiable diseases (O.A.C.I.S 15/9/1955). Such control measures (complete halt of animals' movements, also for slaughtering, for 6 months after the last clinical case or for 15dd after stamping out and disinfections) have a negative economic impact on farmers and limited efficacy to eradicate the disease. In fact, myxomatosis became rapidly endemic in all the country mainly due to the presence of reservoirs among wild and domestic rabbits. New and more actual control measures are therefore needed considering: i) the improved zootechnical properties of rabbit production; ii) the updated data on epidemiology and characteristics of the disease; iii) the availability of efficacious vaccines. Following the notification of myxomatosis in a commercial farm in Vercelli province, we arranged with local veterinary authorities and in agreement with the farmer, to not interrupt animal production and to try to contemporary eradicate the disease. We traced the virus diffusion and circulation in the farm by combining direct diagnosis i.e. virus identification using advanced molecular methods, with indirect serological methods for the detection of specific antibodies in all the breeders.

**MATERIALS AND METHODS** – The farm has 400 does in a single unit on two floors and around 6000 fattening rabbits. There is an annexed slaughterhouse, which has a

working capacity of 1000/1200 rabbits/month. On November 2010, clinical myxomatosis was diagnosed among breeders. The diagnostic confirm was obtained by testing 20 naso-conjunctival swabs with PCR and by specific antibody titration of 12 sera with cELISA. The measures applied to reducing viral load in the farm included suppression of the clinically affected animals, accurate cleaning and disinfections of equipment and cages, vaccination with live attenuated vaccine. Nevertheless, on January 2011 the disease was still present. Therefore, in agreement with veterinary authorities, the clinically affected animals (breeders and growing rabbits) were immediately suppressed and further diagnostic analysis conducted. In particular, all the 282 remaining asymptomatic does were tested for specific anti-MV antibodies using the cELISA method (Botti *et al*., 2007; Lavazza *et al*., 2004). The semi-quantitative result of the test permitted to attribute a titre to each serum and to interpret the values obtained (Table 1).

**Table 1** – Expected antibody titres related to the possible condition of induction

| Condition of induction | Average titres detected |
|---|---|
| MV convalescent rabbits | 1/2560 -1/10240 |
| Vaccinated rabbits | 1/40-1/320 |
| Multi-vaccinated rabbits | 1/320 – 1/1280 |

The PCR for MV was done by using the protocols developed at the National Reference Centre for lagovirus, fully described by Cavadini *et al*. (2010). Thereafter, the following diagnostic investiga-tions were performed both on vaccinated animals and "sentinels", at variable intervals (20dd, 6, 9, 14 and 18 months), established according to the results obtained at each control.

**RESULTS AND CONCLUSIONS** – At the first check, all the tested rabbits were serologically positive with titres ranging from 1/20 to 1/10240 (Table 2). This result was expected, being all the does vaccinated or vaccinated and infected. It was likely that rabbits with titre ≤1/160 (16.0%) were vaccinated but not infected. Thus, these does were immediately moved to a clean and empty compartment to avoid any direct contact with the infected ones. Does with a titre between 1/320 and 1/640 (38.7%) were considered, with regard to MV infection, as "doubtful", being multi-vaccinated or alternatively previously infected and convalescing. Does with titres ≥1/1280 (45.2%) were considered as infected and their titres were inversely related to the time elapsed since infection. It was decided to immediately eliminate these "high titres" does aiming to reduce the virus load and its diffusion in the farm. The group of does with medium titres (>1/160 -<1280) were allocated in a separate area and set under observation. Three weeks after, 30 rabbits of this group were again tested both serologically and virologically (PCR from naso-conjunctival swabs). We did not detect any relevant difference (data not shown) in titres for any tested rabbit but a decreasing trend of titres with time was evident. Virologic PCR tests were all negative. During this visit, we also checked the 12 rabbit male breeders, all vaccinated, present in the farm, which resulted, similarly to the does of the second group, seropositive with titres ≤1/640. On the whole, these results were suggestive of the absence of viral circulation and of new

**Table 2** – Anti-MV antibody titres detected in does at the first time check

| Ab titre | N° rabbits | % positive |
|---|---|---|
| 10 | 0 | 0 |
| 20 | 3 | 1.1 |
| 40 | 5 | 1.8 |
| 80 | 19 | 6.7 |
| 160 | 18 | 6.4 |
| 320 | 56 | 19.9 |
| 640 | 53 | 18.8 |
| 1280 | 80 | 28.8 |
| 2560 | 31 | 11.0 |
| 5120 | 12 | 4.3 |
| 10240 | 3 | 1.1 |

infections, and were proving the effectiveness of the biosecurity measures applied. Even the choice of testing the same animals with both molecular and serological methods was fully justified, considering the necessity to achieve an early and reliable detection of infected animals. Further controls were then conducted at variable intervals. The visual inspection for early clinical signs was associated to serological testing vaccinated rabbits and "sentinels", that are essential to detect the possible circulation of even low virulent wild virus. On July 2011 we checked 90 does, of which 50 vaccinated at weaning and 40 non-vaccinated "sentinels". We detected medium-low titres (≤1/320) in around half of the vaccinated animals (the titres decrease quickly and almost disappear within few months) and very low titres, closed to the positive threshold (1/10-1/20) and presumably aspecific, in few sentinels (n.6). On October 2011, we tested 83 non-vaccinated, 80 days old, restocking does. Seventy-one of these were seronegative, 11 had a threshold titre (1/10) and just one had a 1/40 titre. Again, these results proved the absence of even previous infection and the full eradication of Myxomatosis from the farm. On March 2012, we examined 55 non-vaccinated, 120 days old, restocking does. They resulted all seronegative, demonstrating again the goodness of the technical approach adopted. The last control was done on July 2012 by testing 61 rabbits, of which 30 does vaccinated four month before and 31 growing, 80 days old, rabbits. All growing rabbits resulted seronegative and the vaccinated does have, on average, low titres. This may further support the need to perform vaccination quite often (every 4 months) and the strict adoption of a well-defined vaccination program. In conclusion, we succeeded to definitively eradicate Myxomatosis from the farm, thanks to repeated sampling of sera and swabs to detect respectively antibodies and antigen by using specific and sensitive methods. The cELISA was shown to be a useful technique to quantify the immune response after vaccination but also a reliable method for diagnosing Myxomatosis, when used in association with PCR. This integrated approach, operated by various people with different roles, was finalised to eradicate the disease accomplishing those rational sanitary measures provided by the law but without stopping the production. Such collaboration should become the rule, especially in the case of highly diffusive disease, which severity may jeopardize the farming activity due to the high direct and indirect expenses entailed. This experience emphasized a successful control and monitoring system based on the integrated use of different diagnostic techniques. Indeed, it also reiterated the operative difficulties that may originate from the full uncritical application of the in-force policy rules, which are obsolete, ineffective and too much hindering. Thus, there is the urgent need to define new and updated standard measures applicable for an effective control of Myxmatosis, a disease endemic in Italy since 1955.

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