Control of rabbit myxomatosis in Poland

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Summary: The authors present an epizootiological analysis of myxomatosis in Poland. The biological, physical and chemical properties of virus strains used for the production and control of 'Myxovac M' vaccine are discussed. The long-term stability, safety and efficacy of the vaccine are demonstrated. Laboratory experiments were confirmed in large-scale field observations.

KEYWORDS: Disease control – Epidemiology – Myxomatosis – Rabbits – Vaccination.

INTRODUCTION

The majority of economic losses in the rabbit population in Poland are caused by coccidiosis, haemorrhagic septicaemia, myxomatosis and viral haemorrhagic disease (VHD). Of these, myxomatosis and VHD are included in the Office International des Epizooties List B of contagious diseases. Control of VHD is examined in an accompanying paper in this issue of the Review (16).

The first outbreaks of myxomatosis in Poland occurred in the regions of Poznan and Silesia in 1955, near the western and southern borders of the country (17, 22, 24, 30, 31). In 1954, myxomatosis was also reported in neighbouring East Germany and Czechoslovakia (3, 12). Since 1965, as a result of a decision of the Ministry of Agriculture in Poland, myxomatosis has been listed as one of the notifiable diseases in the country. Until 1970, myxomatosis was controlled by slaughtering all rabbits involved in outbreaks. Since 1971, as the number of animals increased and the disease situation persisted, vaccination of rabbits in affected regions has been recommended, with the use of vaccines containing Shope fibroma virus. Vaccinations limited economic losses but failed to restrain the spread of myxomatosis. Significant progress in the control of the disease was achieved when an attenuated strain of myxomatosis virus was used (13).

MATERIALS AND METHODS

Sources of data for epizootiological analysis

Data on the number of outbreaks, numbers of dead or slaughtered rabbits, and the territorial range of myxomatosis were taken from monthly reports of infectious diseases issued by the Veterinary Service of the Ministry of Agriculture in Poland. Data concerning vaccine production were obtained from the producer.

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Virus

Virulent ZA and RP strains of myxoma virus were isolated at the National Veterinary Research Institute (NVRI) in Pulawy, from rabbits infected during outbreaks in Poland (15). Sanar (challenge standard strain isolated by Sanarelli) and MAV (an attenuated strain isolated by MacKercher and Saito [MSD]) (18), were kindly provided by the Institute for the Control of Biological Preparations and Drugs in Brno, Czech Republic. MAV was replicated in a rabbit kidney (RK) 13 cell line provided by the Central Laboratory of Sera and Vaccines in Warsaw, Poland. All virulent strains were stored at -18°C in tissues of dead or scarified rabbits or as 20% homogenates. MAV was stored as tissue-culture fluid or was freeze-dried with DSG-72 protective medium (11). The influence on virus survival of long-term storage at various temperatures was also studied.

Estimation of virus titre

Determination of the 50% infective dose (ID\textsubscript{50}) for all myxoma strains was conducted using intradermal injections in the shaved back of at least two experimental animals (13). The 50% lethal dose (LD\textsubscript{50}) for all virulent strains and the tissue culture infective dose (TCID\textsubscript{50}) for MAV were also calculated.

Serological tests

Presence of antibodies against myxoma virus was determined by complement fixation (CF) and gel precipitation (GP). CF and GP were also used to determine the presence of viral antigen in tissues of rabbits infected with myxoma virus (6).

Animals

Experiments were conducted on rabbits of New Zealand and California breeds, and cross-bred animals, of various ages and weights, mostly between 1.5 kg and 3.0 kg. Vaccination and challenge with myxoma viruses were generally performed subcutaneously (s.c.) or intradermally (i.d.), but sometimes by the intraconjunctival (i.c.) and intratestinal (i.t.) routes. In some experiments, dogs, cats, guinea-pigs, mice and hares were also used.

Post-mortem examination and sampling

All rabbits and other animals – both those which died and those which survived experimental infection – were subjected to necropsy. Skin and internal organs were sampled for histopathological and electron microscopic examinations.

Electron microscopy

Ultra-thin sections were prepared and stained according to the method described by Reynolds (27). These were examined and photographed at a magnification ranging from 8,000× to 62,000×.

Chemical properties

Resistance of the virus to ether (1) and chloroform (4) was examined. The inactivating properties of the following substances were also tested using different temperatures, concentrations of chemicals and times of inactivation: formalin, biethylenoimine (BEI), ethyl alcohol, sodium hydroxide (NaOH), iodine in Pollena JK and potassium persulfate in Virkon-Naturan.
Vaccine tests

Myxomatosis vaccine 'Myxovac M' was tested for sterility, safety, level of virus attenuation and efficacy (13).

Calculation of results

Titres of TCID$_{50}$, ID$_{50}$ and LD$_{50}$ were calculated using the method described by Reed and Münch (26). Means were presented as $\bar{x}$ (arithmetical) and $\bar{x}_g$ (geometric).

RESULTS

Occurrence and spread of myxomatosis in Poland

Epizootiological analysis of myxomatosis in Poland revealed that between 1955 and 1965 only a few outbreaks occurred each year, and these were confined to the Silesia region (22, 30, 31). Kinetics of prevalence, numbers of outbreaks and numbers of animals slaughtered after 1966 are presented in Figure 1. The critical year was 1983, when 5,663 outbreaks were reported and 53,401 rabbits were slaughtered. Territorial spread of the disease is presented in Figure 2. The present clinical picture of myxomatosis differs from that described by Fenner and Marshall in 1957 (9). Very large, protuberant skin lesions have modified to become relatively flat, and despite

![Graph showing the number of myxomatosis outbreaks and the number of dead or slaughtered rabbits between 1966 and 1992.](image-url)
epidemiological differences, moderately attenuated strains of the virus are now common in Poland. Over the last few years, the virulence of the virus strains has decreased and infected rabbits very often recover from the disease. In a small percentage of cases, respiratory tract symptoms caused by secondary infection have also been observed. Experimental infection of rabbits aged 2 and 4 weeks (born to vaccinated females) resulted in generalised myxomatosis symptoms, developed over 7 days. Approximately 90% of rabbits at different ages showed generalised myxomatosis between 6 and 12 days after infection with virulent ZA or RP strains, and between 7 and 8 days after infection with Sanar strain (23).
Seasonal variation of myxomatosis prevalence is observed. Between 1966 and 1992, 40.14% of myxomatosis outbreaks were detected in September (Fig. 3). This variation is linked to the biological cycle of mosquitoes. However, this did not explain the three outbreaks which were noted in February and March. Therefore, the persistence of the virus in tissues of infected rabbits was studied. Results of this experiment are shown in Table I. Myxoma virus persists in the skin of infected rabbits for 19 days (5, 6). Multiple observations revealed that myxomatosis did not spread through contact between infected and healthy rabbits kept in the same cages (23).

![Seasonal prevalence of rabbit myxomatosis in Poland between 1966 and 1992](image)

**Fig. 3**

**Seasonal prevalence of rabbit myxomatosis in Poland between 1966 and 1992**

**Characteristics of Polish vaccine against myxomatosis in rabbits**

The MAV strain was passaged 10 times in RK 13 cells at 35-36°C. The virus titre was $10^{6.0}$ TCID$_{50}$ as determined in RK 13 cells, and $10^{6.5}$ ID$_{50}$ when measured by i.d. inoculation. The virus replicated with characteristic and significant cytopathic effect forming 1-3 mm plaques under agar gel (13, 25). In intradermally inoculated rabbits, small tumors developed after 4-5 days at the injection site only. The plaques reached maximum size (15 mm) after 7 days and usually disappeared before the end of the three-week observation. In cytoplasm of cells inoculated with MAV, and in ultra-thin sections of the skin of rabbits infected with ZA strain, viral particles of the same size (290 x 200 nm) and morphology were detected by electron microscopy (23).

The MAV strain was susceptible to ether, chloroform, exposure to pH 3 and the other inactivators used. The virus was also inactivated after 10 min by heating at 100°C, and after 1 h at 56°C. Virus survived for more than 752 days when kept at 4°C. Long-term stability of the virus was achieved when freeze-dried with protective medium DSG-72.
TABLE I

Persistence of myxoma virus and antigen in different types of tissue of rabbits vaccinated with an attenuated strain (MAV strain) or infected with a virulent strain (ZA strain)

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>MAV strain</th>
<th>ZA strain</th>
<th>ZA strain 21 days after MAV strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4  7  19</td>
<td>7  19  35</td>
<td>63</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- site of injection</td>
<td>XO O  -</td>
<td>XO XO O  O</td>
<td>XO O</td>
</tr>
<tr>
<td>- eyelid</td>
<td>XO O  O</td>
<td>XO ND O  ND</td>
<td>XO O</td>
</tr>
<tr>
<td>- other site</td>
<td>XO O  ND</td>
<td>XO XO O  ND</td>
<td>X  -</td>
</tr>
<tr>
<td>Muscular tissue</td>
<td>O -  ND</td>
<td>XO XO - ND</td>
<td>X  -</td>
</tr>
<tr>
<td>Testicle or ovary</td>
<td>XO O  ND</td>
<td>XO XO O  O</td>
<td>XO O</td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- regional</td>
<td>XO O  -</td>
<td>XO XO O  O</td>
<td>XO -</td>
</tr>
<tr>
<td>- other</td>
<td>-  O  ND</td>
<td>XO XO O  O</td>
<td>X  -</td>
</tr>
<tr>
<td>Liver</td>
<td>O -  -</td>
<td>XO XO -  -</td>
<td>X  -</td>
</tr>
<tr>
<td>Spleen</td>
<td>O -  -</td>
<td>XO XO O  -</td>
<td>X  O</td>
</tr>
<tr>
<td>Kidney</td>
<td>O O  O</td>
<td>XO XO O  O</td>
<td>XO O</td>
</tr>
<tr>
<td>Lung</td>
<td>-  O  -</td>
<td>XO XO -  -</td>
<td>XO O</td>
</tr>
<tr>
<td>Blood **</td>
<td>-  X  -</td>
<td>X X -  -</td>
<td>X  -</td>
</tr>
</tbody>
</table>

X: presence of virus demonstrated by intradermal injection
O: presence of virus antigen demonstrated by complement fixation test
ND: no data
* figures represent no. of days
** virus antigen was not examined in blood

The annual decrease of virus titre after storage at 4°C was not higher than log_{10} 0.3, and was only log_{10} 0.4 at room temperature. Vitality of the virus after 10 years of storage was confirmed.

The MAV strain was free from microbiological contamination, and was safe for adult mice, guinea-pigs, dogs, cats and hares when up to 4 ml of the virus was administered by the s.c., i.m., i.p. or i.t. routes and when administered 0.03 ml i.c. to suckling mice. No reversion of virulence was observed after 10 serial passages of the virus by i.t. inoculation accompanied by simultaneous inoculation of hydrocortisone acetate (4 injections of 50 mg every 3 days). Safety of the virus for pregnant females and for suckling rabbits was also confirmed (23). The cross-reactivity between MAV and the virulent ZA, RP and Sanar strains was demonstrated by serological tests and challenge.

Between 1982 and 1983, nine experimental batches of the vaccine were prepared. These were free from microbiological contamination and safe for laboratory animals, including rabbits, when vaccinated i.d., s.c. or through puncture of the ear, with 1-50 doses recommended. Over 900 days of storage at 4°C, the titre of MAV decreased from $10^{5.5}$ to $10^{4.5}$ TCID_{50} (Table II).
### TABLE II

**Efficacy of myxomatosis vaccine Myxovac M after storage at +4°C**

<table>
<thead>
<tr>
<th>Period of storage</th>
<th>Log$_{10}$ of virus titre</th>
<th>No. of rabbits vaccinated</th>
<th>Period of observation</th>
<th>Results of challenge *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{TCID}_{50}$</td>
<td>$\text{ID}_{50}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7 days</td>
<td>5.5</td>
<td>6.3</td>
<td>8</td>
<td>18 days</td>
</tr>
<tr>
<td>18 months</td>
<td>5.3</td>
<td>6.1</td>
<td>6.3</td>
<td>8</td>
</tr>
<tr>
<td>21 months</td>
<td>5.0</td>
<td>4.5</td>
<td>5.9</td>
<td>6</td>
</tr>
<tr>
<td>22 months</td>
<td>5.0</td>
<td>4.5</td>
<td>5.9</td>
<td>6</td>
</tr>
<tr>
<td>38 months</td>
<td>5.0</td>
<td>5.9</td>
<td>6.3</td>
<td>8</td>
</tr>
</tbody>
</table>

* no. of resistant rabbits/no. of challenged rabbits

TCID$_{50}$: 50% tissue culture infective dose

ID$_{50}$: 50% infective dose

Examination by accelerated ageing revealed that heating at 100°C for 1 min, at 56°C for 1 h or at 37°C for 14 days caused a decrease in titre similar to that achieved after 18 months of storage at 4°C. Freeze-dried vaccine was completely inactivated by heating at 100°C for 30 min, at 56°C for 4 h and at 37°C for 48 days. Prevalence of post-vaccinal immunity and efficacy of the vaccine after storage at 4°C for 38 months are shown in Tables II and III. The minimal protective dose was estimated as 1/1,000 of the field dose. Experimental vaccines (batch III-IX/83) were administered to approximately 64,300 rabbits. No post-vaccinal side-effects were observed, except local tumors in some rabbits.

The use of Myxovac M in the 'endangered' zone provided protection to all vaccinated rabbits. The effect of administration of the vaccine during outbreaks varied, and efficacy was estimated at between 37.2% and 95.6% (14).

### TABLE III

**Prevalence of immunity against challenge with virulent ZA strain of myxoma virus in rabbits vaccinated with Myxovac M**

<table>
<thead>
<tr>
<th>Interval between vaccination and challenge</th>
<th>No. of rabbits vaccinated and challenged</th>
<th>No. of healthy animals</th>
<th>No. of animals with local lesions</th>
<th>No. of animals with generalised myxomatosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>4</td>
<td>–</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1 day</td>
<td>4</td>
<td>–</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2 days</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>4 days</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>11 days</td>
<td>4</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>18 days</td>
<td>4</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>36 days</td>
<td>15</td>
<td>15</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>36 days *</td>
<td>8</td>
<td>–</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

* control rabbits
Large-scale production of Myxovac M began at ‘Biowet’ in Pulawy in 1986. Since then, 32 batches containing a total of 2.255 million doses of the vaccine have been produced. All series were sterile, safe and efficacious. The batch control procedure revealed that, after challenge, only 9 of 166 vaccinated rabbits showed local lesions, while all control animals showed generalised myxomatosis. Since 1989, the vaccine based on technology developed by the authors at the NVRI has been also produced in Germany.

DIAGNOSIS AND CONTROL OF MYXOMATOSIS

Diagnosis of the disease is based on clinical and epidemiological observations, as well as pathological examinations. In the case of an atypical form of the disease, or when the disease is reported for the first time in a given area, reliable laboratory diagnosis is obligatory. Myxoma virus infection is commonly verified by gel precipitation or complement fixation, together with intradermal inoculation of live rabbits.

Under Polish law, the district veterinary officer must be notified by rabbit breeders or veterinary practitioners of each clinical case of myxomatosis. The district veterinary officer is obliged to ensure clinical and pathological examinations, as well as detailed anamnesis. The following measures should be undertaken immediately or on receiving a report from the diagnostic laboratory:

- designation of the focus of infection
- slaughter of all diseased or suspect animals and destruction of carcasses by incineration
- disinfection of rabbit cages, houses and the surrounding area
- establishment of ‘protective’ and ‘endangered’ zones around the contaminated area
- prohibition of any transport of live animals, skins, feed, etc.
- vaccination of all rabbits in the protective zone and prohibition of commerce involving rabbits, carcasses and skin.

In Poland, the vaccination programme is the most effective measure in protecting rabbits against myxomatosis. All rabbits over six weeks of age must be vaccinated against myxomatosis between April and October. Additionally, protection of cages against mosquitoes is required.

DISCUSSION

The Polish Ministry of Agriculture estimates the number of rabbits in the country at 6-10 million. Over 80% of these animals are bred in small colonies. There are also larger farms of 500-2,000 rabbits and only one with 15,000 animals. The reproduction cycle in small farms begins in March or April and the number of susceptible rabbits reaches a maximum between July and October. This information explains the relatively small number of dead or slaughtered animals in outbreaks of myxomatosis (Fig. 1). A distinct relation was observed between the quantity of rainfall and the number of outbreaks. In dry and hot summers, the number of outbreaks decreased. In Poland, the peak of morbidity appears in September, one month later than in France. This is also due to the
considerably smaller wild rabbit population. As in France, seasonal prevalence of myxomatosis and the number of outbreaks are connected with the presence of various biological vectors (fleas and mosquitoes) which, in turn, is determined by the climate of each province. The role of genetic resistance and less virulent strains should also be taken into account (2, 7, 8, 10, 21, 28).

The safety of the attenuated myxomatosis vaccine was demonstrated, and results of these experiments were in accordance with those reported by Jiran et al. (20) and Saurat et al. (29). Detailed experiments on the safety of the vaccine were performed to exclude the possibility of reversion of virulence (18, 19). The stability of myxoma virus in freeze-dried Myxovac M vaccine and the dynamics of post-vaccinal immunity were comparable to the results published by Jiran et al. (20) and Saurat et al. (29). Persistence of the virulent strain in tissues of vaccinated and infected rabbits was considerably limited (Table I).

CONCLUSION

Myxomatosis still causes problems for breeders of domestic rabbits in Poland, although the economic losses are not very high. The number of foci of disease depends on weather conditions, the reliability of diagnostic methods, the efficiency of stamping out of rabbits during outbreaks and the effectiveness of the vaccination programme.
así como también la inocuidad y la eficacia de esta vacuna. Las experiencias realizadas en laboratorio fueron posteriormente confirmadas en el terreno, a gran escala.


**REFERENCES**


