Myxomatosis

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Summary
Myxomatosis, a major disease of European rabbits (Oryctolagus cuniculus), is enzootic on several continents. The disease is infectious, virulent and contagious. The pathogen is a virus of the family Poxviridae, genus Leporipoxvirus. In its classic form the disease is often fatal, characterised by severe immunosuppression and the appearance of skin pseudotumours (myxomas); it is conducive to effective mechanical transmission by many biting arthropods. Atypical clinical forms, referred to as amyxomatous, of variable severity and with an apparent preference for direct transmission, have recently emerged in Europe. Virus–host interactions have been particularly well studied since the voluntary introduction of the myxoma virus into Australia and Europe, revealing a remarkable process of co-evolution. Molecular analysis has recently demonstrated the extraordinary evolutionary capacity of the myxoma virus.

Keywords
European rabbit – Host–virus co-evolution – Mechanical vector transmission – Myxoma virus.

Background
Myxomatosis is an infectious, virulent and contagious disease affecting the European rabbit Oryctolagus cuniculus. It is primarily transmitted by vectors and is caused by the myxoma virus, a poxvirus of the genus Leporipoxvirus, which is antigenically related to the Shope fibroma virus. It was described for the first time in 1896 in Uruguay by Giuseppe Sanarelli, following the emergence of a deadly new disease affecting laboratory rabbits. It was characterised by the appearance of pseudotumours called ‘myxomas’ on the head and the anogenital and dorso-lumbar regions, usually associated with respiratory disorders and blepharo-conjunctivitis, leading to the death of the individual within ten days in the severest cases. The disease was named myxomatosis after the Greek muxa, meaning mucus, and oma, meaning tumour.

The close resemblance between the myxoma virus and other members of the family Poxviridae, such as the smallpox or fowlpox vaccinia virus, was demonstrated for the first time in 1927 (1). A remarkable feature of the myxoma virus is that, while it causes only mild disease (localised cutaneous fibroma) in its natural hosts, American rabbits of the genus Sylvilagus, in European rabbits of the genus Oryctolagus it causes serious disease that can result in 100% mortality. As it is so highly pathogenic to European rabbits, the myxoma virus was deliberately introduced to control rabbit populations in Australia in 1950, then in France in 1952, from where it spread across the whole of Europe, including Great Britain. It was introduced into Australia as a biological agent as part of an overall policy to control rabbit populations, while in France it was introduced illegally by a landowner wishing to eradicate the species from his property (2, 3). More than 60 years after its introduction into Europe and Australia, myxomatosis has now become enzootic, with regional and seasonal micro-epizootics (Fig. 1). In addition to the acute forms, which kill rapidly, there are now subacute and attenuated forms that are not always fatal and confer immunity (4). Nevertheless, myxomatosis remains one of the leading causes of death in wild rabbits, with declining populations recorded in Europe over the past 30 years.

The myxoma virus
Like all poxviruses, the myxoma virus is large (286 × 230 × 75 nm) with a double-stranded DNA genome 161.8-kb long, with inverted terminal repeats of 11,557 pb, encoding 158 single open reading frames and 12 that are duplicated in the inverted terminal repeats. The myxoma virus infects...
only lagomorphs and is divided into two major phylogenic groups:

- Californian strains, for which the natural host is *Sylvilagus bachmani*

- South American strains, for which the natural host is *Sylvilagus brasiliensis*.

The viruses from these two subpopulations cause myxomatosis in European rabbits and sometimes in brown hares (*Lepus europaeus*) (5). The strains introduced into Europe and Australia are South American in origin.

The evolution of pathogenicity observed in populations of European rabbits in enzootic zones is partly a result of genetic modifications that alter the functions of some virulence genes. The complete genome sequence of a naturally attenuated strain isolated in Spain in 1995, originating from the Lausanne strain introduced into France in 1952, revealed 73 genetic modifications, resulting in the inactivation of four genes, three of which appear to play a role in the expression of pathogenicity (6). More recently, the complete sequencing of many strains of various grades, collected between 1950 and 1999, has shown the remarkable ability of the myxoma virus to evolve, with an estimated average substitution rate of $10^{-3}$ substitutions per site per year, one of the highest ever observed for a double-stranded DNA virus, approaching that of the smallpox virus (7, 8). These studies also showed that multiple, complex mechanisms underlie the expression of myxoma virus pathogenicity or attenuation, as manifested by phenotypic convergence between strains with no obvious genetic matches. The evolutionary process of the myxoma virus is characterised by an unusually high nucleotide substitution rate for a double-stranded DNA virus, frequent changes in virulence and a marked ability to spread.

**Clinical study**

Myxomatosis may take a very striking form when infection is by South American strains, but remains more discreet when infection is by Californian strains, although in both cases mortality can reach 100%. We often see secondary bacterial infections with Gram-negative bacteria, especially *Pasteurella multocida* and *Bordetella bronchiseptica*, in the respiratory tract and conjunctiva, which contribute to the lethality of the disease. The epidemiology and clinical course of myxomatosis caused by South American strains has been studied in depth in Australia, where attenuated forms of the disease were identified shortly after the virus was introduced. Fenner and Marshall (1957) proposed a system for describing the virulence of viral strains and grading them according to the case fatality rate and the average survival time of experimental animals under laboratory conditions (Table I) (9).
Under this system, grade I strains are hypervirulent (Lausanne and standard laboratory strains), while grade V strains are highly attenuated, with the other grades between the two extremes.

**Clinical signs**

In the classic nodular form of the disease, the myxomas do not regress and the associated immunosuppression contributes to its virulence (10). In 1979, amyxomatous forms of the disease emerged in Europe. They exhibit a noticeable decrease in skin tropism, with the myxomas replaced by diffuse swelling of the eyelids (oedematous blepharitis) and sometimes of the scrotum and ears (11, 12). In amyxomatous forms, respiratory clinical signs appear to predominate and the (false) semblance of increased pneumotropism (in reality unchanged) led to their initial classification as ‘respiratory’ forms (13).

There are several types of clinical course for infection by European and Australian strains (Fig. 2):

– Grades I and II produce acute forms of the disease and are usually fatal. The incubation period is five days. In 6–7 days, in three successive locations, different types of secondary myxomas appear: cephalic, with blepharo-conjunctivitis, oedematous swelling, distortion of the face (leontiasis), purulent nasal discharge and weeping; anogenital, accompanied by regional oedema; and dorsolumbar and tarsometatarsal, distorting the shape of the back and hindquarters. Death occurs 10–15 days after inoculation (14). The hemispherical, hazelnut-sized, weeping myxomas usually become necrotic and confluent but remain painless. The general immune system dysfunction exacerbates frequent bacterial secondary infections.

– Strains with attenuated virulence (grade V) cause mild, localised, only slightly exudative and self-healing forms of the disease. Rabbits present small numbers of pea-shaped, very firm, non-exudative, mainly auricular and metatarsal nodules, rapidly covered by a crust (14).

<table>
<thead>
<tr>
<th>Virulence grade</th>
<th>Case fatality rate (%)</th>
<th>Average survival time (days)</th>
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<tbody>
<tr>
<td>I</td>
<td>&gt;99</td>
<td>&lt;13</td>
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<tr>
<td>II</td>
<td>95–99</td>
<td>14–16</td>
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<tr>
<td>III</td>
<td>70–95</td>
<td>17–28</td>
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<td>IIIa</td>
<td>90–95</td>
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<td>IIIb</td>
<td>70–90</td>
<td>23–28</td>
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<td>IV</td>
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<td>29–50</td>
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<tr>
<td>V</td>
<td>&lt;50</td>
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**Fig. 2**

Domestic rabbits with myxomatosis: clinical signs caused by different strains of myxoma virus

Source: National Polytechnic Institute, National Veterinary School of Toulouse (INP-ENVT)
Intermediate pathogenicity strains (grades III and IV) result in forms of the disease between these two extremes. The essential difference between the European and Australian strains is the protuberant cutaneous myxomas in the former (14).

The hyperacute form of the disease caused by Californian strains of the myxoma virus kills rabbits within approximately one week, often exhibiting only minor external clinical signs, such as inflammation and oedema of the eyelids. Cutaneous haemorrhage may be seen in the final phase and convulsions often precede death, revealing acute neurotropism rather than ectodermotropism (10).

Epidemiology, mode of transmission and the role of arthropod vectors

The chief mode of transmission is biting arthropods, which are passive vectors, and the primary route of inoculation is intradermal (15). Culicidae, Siphonaptera and Simuliidae are the main vectors, with lice, ticks and mites playing only a minor role. The effectiveness of such transmission varies, depending largely on the virus titre of cutaneous lesions (a threshold of $10^7$ ID$_{50}$/g of damaged skin was defined in a study of transmission by mosquitoes), and on the size of vector populations. Transmission by direct contact was also found in domestic rabbits (13, 16). Although this could explain certain winter epizootics (17), its relative importance is difficult to evaluate in the presence of vectors. Nevertheless, it was direct contact that enabled the spread of myxomatosis in the Kerguelen Islands, between its introduction in 1955–1956 and the introduction of rabbit fleas in 1987 (18, 19).

Diagnosis

Diagnosis is based on the observation of clinical signs and the epidemiological context. Clinical diagnosis can be difficult because the clinical signs induced by attenuated viral strains are barely discernible and the cutaneous tropism associated with amyxomatous strains is reduced. It is therefore useful to confirm clinical suspicions with a laboratory diagnosis.

As a final note, myxomatosis is included in the Terrestrial Animal Health Code (Terrestrial Code) of the World Organisation for Animal Health (OIE), meaning that Member Countries and territories are required to report outbreaks of the disease in accordance with the provisions of the OIE Terrestrial Code (24).

Prophylaxis

For myxomatosis, as with many other contagious viral animal diseases, only preventive, not curative, control is possible because no truly effective in vivo virostatic agents are available. Myxomatosis control, which is used only for livestock, generally consists of sanitary measures and vaccination.

Sanitary prophylaxis

Mechanical and chemical protection against vectors is the foremost sanitary requirement for rabbit breeding.

Medical prophylaxis

Heterologous vaccination

The Shope fibroma virus vaccine was the first to be used and it continued to be used for decades. This heterologous live vaccine can be administered from 28 days of age, either subcutaneously or via intradermal injection. Intradermal injection appears to induce a better immune response and therefore confers better clinical protection than subcutaneous injection. Booster injections are administered every two to six months, depending on the risk.

The advantage of this vaccine is its safety, because it causes only very mild immunosuppression and can be used in animals with respiratory diseases. However, it should not be used in very young animals (which are hyper-receptive to the fibroma virus and may suffer generalised fibromatosis and die) or in pregnant females. High corticoid levels during pregnancy may stimulate the fibromatous lesion that it causes (14). However, this vaccine provides only partial short-term protection, always less than a homologous vaccine. Therefore, although this vaccine is used frequently, it gives only relative protection – not enough to cope with highly virulent strains, although the wild strains likely to be encountered in the field are of intermediate virulence.

Homologous vaccination

A number of studies have yielded attenuated viral strains by passages in cell culture, either from the Californian MSD strain (Saito strains: Borghi, MAV, etc.), or from South American isolates of the Lausanne strains (SG33, Pisa, Leon 162, etc.) (25). In all cases, the protection conferred
by intradermal or subcutaneous administration, while incomplete, is much better than that conferred by the Shope fibroma virus (good clinical protection in the early stage and for around six months). However, unlike the Shope fibroma virus, these vaccines are not perfectly safe, especially for very young rabbits.

In addition, in intensive breeding systems with poor hygiene the immunosuppressive effect of vaccination using the modified virus with significant residual pathogenicity has been known to upset the delicate balance between the host and the bacterial respiratory diseases for which it serves as an asymptomatic carrier and those whose clinical signs develop insidiously (26).

In conclusion, the myxoma virus, like many poxviruses, is an effective viral vector because different attenuated strains have been used to create recombinant vaccines to allow simultaneous vaccination against myxomatosis and rabbit haemorrhagic disease (27, 28, 29).

Impact in the wild

In both Australia and France it has been estimated that rabbit populations were reduced by more than 90% during the initial spread of the virus (30, 31). Thereafter they recovered gradually as a result of host–pathogen co-evolution (25, 30, 32). The least virulent strains were rapidly isolated (9, 33). In parallel, studies have shown that rabbits have developed resistance to the disease (34, 35, 36, 37, 38, 39), although the genetic origin of such resistance has not been clearly established (40, 41). Nonetheless, this resistance has had a limited effect because it reduces the mortality rate and the severity of the clinical signs only for a particular viral strain (3, 36). The third factor to have reduced the impact of the disease is the development of immunity within populations (3, 42).

In Australia, less virulent viral strains were detected in 1952–1953 (33), and in 1955 grade III strains started to dominate in the wild. They have a selective advantage in terms of persistence and spread, stemming from a trade-off between the survival of infected rabbits and the level of viral shedding. Highly virulent strains are hampered by the short survival time of infected rabbits, giving them just a few days in which to spread the virus. Conversely, the highly attenuated strains are hampered by the small number of viral particles shed by infected rabbits, although their survival time is longer. Two strategies have been adopted to boost the effectiveness of biological control by the disease. The first was to introduce rabbit fleas (Spilopsyllus cuniculi and Xenopsylla cunicularis), the main vectors of the disease in Europe, to encourage virus spread and increase the impact of the disease (43, 44). This approach was used in the sub-Antarctic islands of the Kerguelen archipelago (18). The second strategy was to regularly introduce virulent strains of myxomatosis (Lausanne strain, grade I). However, none of these strains has ever been more virulent than strains circulating in the wild, so introducing them has had little effect on the impact of myxomatosis on a wider scale, although local effects on the disease have been reported (45, 46). In Europe, while a decrease in the virulence of viral strains has been observed, there is a higher proportion of grade II strains than in Australia (3). Two non-mutually-exclusive hypotheses could explain this difference. First, the Lausanne strain introduced into France in 1952 was more virulent than the standard laboratory strain introduced into Australia in 1950. Second, in Europe, the spread of the deadliest strains is helped by fleas because, when a rabbit dies of myxomatosis, the fleas attempt to infest a new host, thereby transmitting the virus (3).

Recent studies have described the mechanisms that determine the impact of myxomatosis on populations. Maternal antibodies play a key role in these mechanisms by reducing the severity of the infection while allowing activation of the immune system (47). When the virus circulates effectively within populations, it helps to maintain strong herd immunity, thereby limiting the disease's impact (48). A large population and long breeding season favour the persistence of the virus in populations and limit the impact of the disease (49). On a larger scale, population fragmentation disrupts circulation of the virus between populations and hence the maintenance of immunity, promoting the emergence of severe forms of the disease (50).

The impact of myxomatosis is therefore determined not only by host–pathogen co-evolution but also by the broader dynamics and spatial structure of rabbit populations (47).

Conclusion

Myxomatosis is a model disease in many respects. It provides a powerful example of a pathogen that acquires virulence by switching hosts. Its spread among European rabbit populations in the wild has made it one of the most intensively studied models of host–pathogen co-evolution (3, 51). On rabbit farms, the development of vaccines has brought the disease under control and it is no longer a priority animal health issue. In the wild, myxomatosis remains a major biological conservation issue that can be viewed from two diametrically opposed standpoints. In Europe, where rabbits are a heritage species, the introduction of myxomatosis has had dire consequences for ecosystems. It has triggered a sharp decline in rabbit populations, exacerbated by changing habitats and the emergence of rabbit haemorrhagic disease. Declining populations altered
the balance of ecosystems, endangering specialist rabbit predators such as the Iberian lynx (*Lynx pardinus*), Spanish imperial eagle (*Aquila adalberti*) and Bonelli’s eagle (*Aquila fasciata*), as well as species dependent on rabbit burrows, such as the ocellated lizard (*Timon lepidus*). Conversely, in Australia, the decline in rabbit populations has allowed ecosystems to recover from the damage caused by their pressure on vegetation. Although it may not be enough to resolve rabbit-related problems, the use of myxomatosis as a biological control agent has been deemed a success because estimated population levels in the early 1990s were a mere 5–25% of pre-1950 levels (25).

References


