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Review

Myxomatosis in Australia and Europe: A model for emerging infectious diseases

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ABSTRACT

Myxoma virus is a poxvirus naturally found in two American leporid (rabbit) species (*Sylvilagus brasiliensis* and *Sylvilagus bachmani*) in which it causes an innocuous localised cutaneous fibroma. However, in European rabbits (*Oryctolagus cuniculus*) the same virus causes the lethal disseminated disease myxomatosis. The introduction of myxoma virus into the European rabbit population in Australia in 1950 initiated the best known example of what happens when a novel pathogen jumps into a completely naïve new mammalian host species. The short generation time of the rabbit and their vast numbers in Australia meant evolution could be studied in real time. The carefully documented emergence of attenuated strains of virus that were more effectively transmitted by the mosquito vector and the subsequent selection of rabbits with genetic resistance to myxomatosis is the paradigm for pathogen virulence and host–pathogen coevolution. This natural experiment was repeated with the release of a separate strain of myxoma virus in France in 1952. The subsequent spread of the virus throughout Europe and its coevolution with the rabbit essentially paralleled what occurred in Australia. Detailed molecular studies on myxoma virus have dissected the role of virulence genes in the pathogenesis of myxomatosis and when combined with genomic data and reverse genetics should in future enable the understanding of the molecular evolution of the virus as it adapted to its new host. This review describes the natural history and evolution of myxoma virus together with the molecular biology and experimental pathogenesis studies that are informing our understanding of evolution of emerging diseases.

Contents

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1. Introduction

The modern world has seen an ever expanding number of novel diseases of humans and animals (Jones et al., 2008; Keesing et al., 2010). Understanding and predicting the outcome of emerging pathogens is not easy and is likely to differ in each case. Infectious diseases such as malaria, smallpox, tuberculosis and bubonic plague have had a major impact on human populations and at least some infectious diseases appear to have influenced human gene frequencies (Dobson and Carper, 1996; Hill, 2006; Vannberg et al., 2011); the outcome of the current AIDS pandemic on human genetics can only be speculated. Good mammalian models for natural selection by pathogens are uncommon but there is one example of a massive biological experiment that was replicated on two continents, this is the release of myxoma virus (MYXV), the cause of myxomatosis, as a biological control for the European rabbit (*Oryctolagus cuniculus*) in Australia and subsequently in Europe (Fenner and Fantini, 1999). The shift in species, initial extreme virulence and subsequent host–pathogen coevolution, in a species with prolific reproduction and short generation time, plus the ability to undertake experimental studies in the same host species, made myxomatosis the classic paradigm for what happens as an emerging pathogen adapts to a new host. In particular, the nexus between virulence and transmission overturned the cosy idea that pathogens should adapt to cause minimal harm to their hosts (Anderson and May, 1982; Massad, 1987; Dwyer et al., 1990). More recently, molecular and genomic studies offer the prospect of understanding the molecular basis of this evolution.

This review firstly describes the natural history of MYXV, its use as a biological control and subsequent host–pathogen coevolution in Australia and Europe. It then turns to the pathogenesis of MYXV and the current knowledge of MYXV genes and the experimentally defined or inferred functions of the encoded proteins for pathogenesis. Finally, the current and future evolution of myxoma virus is briefly examined.

2. Natural history of myxoma virus

2.1. Myxomatosis

Myxomatosis was originally described following an outbreak of a novel lethal disease in laboratory rabbits at the Institute of Hygiene in Montevideo, Uruguay in 1896 (Sanarelli, 1898; Fenner and Ratcliffe, 1965), although the disease was apparently known to rabbit breeders (Aragão, 1927), and subsequently investigated following outbreaks at the Institute Oswaldo Cruz in Brazil (Moses, 1911; Aragão, 1927; Fenner and Ratcliffe, 1965). These were European rabbits (*O. cuniculus*), the common domestic and laboratory rabbit, which are not native to the Americas. Sanarelli (1898) suggested that the disease was due to a virus, making myxomatosis one of the earliest diseases of animals to be associated with a virus. Sanarelli was unsuccessful at infecting other species, including humans, by inoculation of infectious material from the diseased rabbits although he believed that an inoculated dog developed mammary tumours because of the virus (Hobbs, 1928).

Clinical signs of myxomatosis in rabbits can be observed from about 4 days after infection with virulent virus, initially as conjunctival inflammation accompanied by an elevated rectal temperature; a raised cutaneous lesion at the inoculation site may be visible but in natural infections this may not be noticed. By 6 days, anogenital swelling is present and cutaneous papular secondary lesions can be seen on the face and ears; serous and later mucopurulent discharge from the nostrils and conjunctivae becomes increasingly prominent (Fenner and Ratcliffe, 1965; Best and Kerr, 2000). Rabbits with typical acute late stage myxomatosis 8–10 days after infection have a swollen head and face, swollen drooping ears, mucopurulent blepharoconjunctivitis with swollen, closed eyelids and mucopurulent rhinitis with occlusion of the nasal passages (Fig. 1A). There are multiple discrete cutaneous swellings (sometimes termed tumours or myxomas) ranging from a few millimetres to several centimetres in diameter over the body (Fig. 1B and D). The anogenital region is grossly swollen and

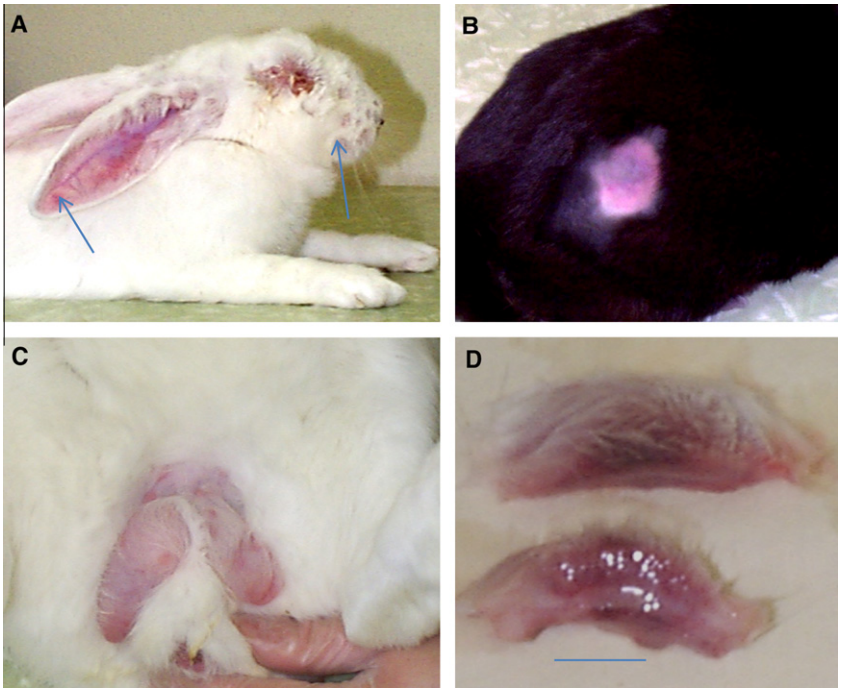

Fig. 1. Myxomatosis in the European rabbit. (A) Acute myxomatosis in laboratory rabbit. Note swollen head and eyelids, mucopurulent conjunctivitis and rhinitis, swollen base of ears and secondary lesion on ear (arrow) and head. (B) Primary lesion at inoculation site about 10 days after infection (Image Dr. S. M. Best). (C) Oedema of scrotum and secondary skin lesions 10 days after infection. (D) Cut section of primary lesion 10 days after infection; upper image is looking down on the sectioned lesion and lower image is in cross-section (scale bar = 1 cm).

oedematous. In males, the scrotum and testes are extremely swollen (Fig. 1C). In late stages of the disease, the head and neck are extended and respiration is slow, laboured and stertorous. Death typically occurs between 8 and 12 days after infection. Virus is present in the secretions and skin lesions and is readily transmitted by direct contact and biting arthropods.

2.2. Virus properties

Myxoma virus (MYXV) is a poxvirus (family *Poxviridae*; subfamily *Chordopoxvirinae*; genus: *Leporipoxvirus*) (Fig. 2), like other poxviruses it is a large brick shaped virion: $286 \times 230 \times 75$ nm (Farrant and Fenner, 1953) possessing a double stranded DNA genome with closed single strand hairpin loops at the termini and terminal inverted repeat regions (TIR) at each end of the genome. The Lausanne (Lu) strain of MYXV encodes 158 unique open reading frames in its 161.8 kb genome, 12 of which are duplicated in the 11,577 bp TIRs (Cameron et al., 1999). Morphologically, based on negative staining and thin section electron microscopy (Farrant and Fenner, 1953; Duteyrat et al., 2007), MYXV is indistinguishable from the orthopoxvirus Vaccinia virus (VACV), which is the best studied of the poxviruses and serves as the model for poxvirus genomics, replication and structure–function studies (Moss, 2007; Condit et al., 2006).

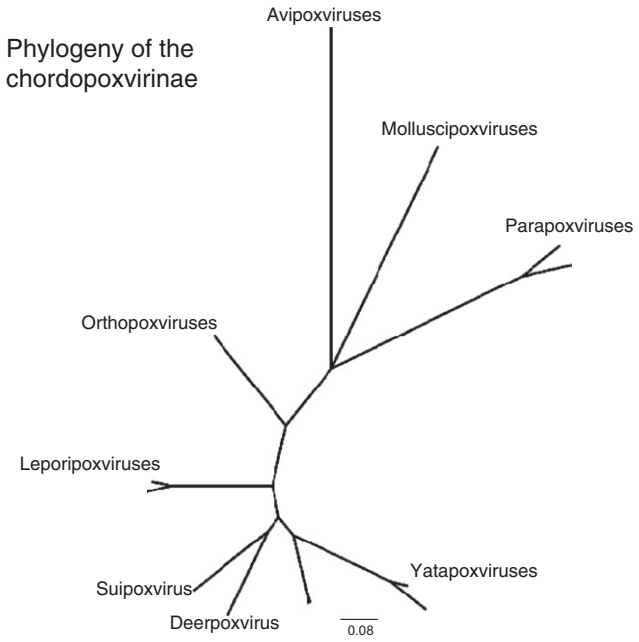

Fig. 2. Poxvirus phylogeny. Unrooted maximum likelihood tree based on the amino acid sequence of the RNA helicase/nucleophosphohydrolase II protein (VACV I8; MYXV M044).

2.3. Natural hosts, geographic range and related leporipoxviruses

The natural host of MYXV is the South American leporid *Sylvilagus brasiliensis*, also called the tapeti or jungle rabbit. In the tapeti, MYXV causes a localised cutaneous fibroma, which persists for some weeks without apparently inconveniencing the animal, although in very young animals more serious disease is reported to occur (Aragão, 1943; Fenner and Ratcliffe, 1965). The virus adheres to the mouthparts of mosquitoes or other biting arthropods probing through the virus-rich epidermis overlying the fibroma and can then be passively inoculated into a new host at a subsequent feed (Fenner and Ratcliffe, 1965). The skin lesions are the source of virus any virus in the blood meal is excreted in the faeces (Fenner et al., 1952); MYXV does not replicate in the vector (Aragão, 1943; Day et al., 1956). The virus naturally occurs in *S. brasiliensis* across a wide area of South America ranging from Panama to Argentina and mosquitoes or fleas can transmit the virus from the reservoir host to introduced European rabbits (Fenner and Ratcliffe, 1965).

Two leporipoxviruses closely related to MYXV occur in North American leporids. Rabbit fibroma virus (RFV; also called Shope fibroma virus) is found in *Sylvilagus floridanus* (eastern cottontail) in the East and central USA (Fenner and Ratcliffe, 1965), while the Californian strain of myxoma virus is found in *Sylvilagus bachmani* (brush rabbit) on the western coast of the USA and Baja peninsula of Mexico (Marshall and Regnery, 1960; Marshall et al., 1963; Luna, 2000). In their natural hosts these viruses cause a fibroma which is similar to that caused by MYXV in *S. brasiliensis* (Fenner and Ratcliffe, 1965). However, they have quite different properties in European rabbits. RFV causes a cutaneous fibroma in immunocompetent European rabbits and elicits immunological cross-protection to MYXV, which has led to its widespread use as a vaccine against MYXV. In contrast, the Californian strains of MYXV are lethal for European rabbits; some strains, such as MSW, causing a peracute syndrome with few signs of typical myxomatosis (Fenner and Marshall, 1957; Silvers et al., 2006). Although, clinical reports indicate more typical myxomatosis is also seen in domestic rabbits and the occasional rabbit survives infection (Kessel et al., 1934; Patton and Holmes, 1977).

There appears to have been specific adaptation of each of these viruses to their natural host; mosquitoes fed on *S. bachmani* infected with a South American strain of MYXV did not transmit the virus and mosquitoes could not transmit Californian virus to *S. brasiliensis*; similarly *S. floridanus* did not transmit the North American MYXV although limited mosquito transmission of a South American virus was achieved (Regnery and Marshall, 1971; Regnery, 1971). Experimentally, replication of South American and Californian virus can occur in other Sylvilagus species from North America although only the South American virus reaches transmissible titres (Regnery and Marshall, 1971; Regnery, 1971; Silvers et al., 2010). Interestingly, although European rabbits inoculated with RFV develop high titres of virus in the epidermis over the fibroma, this is a poor source of virus for mosquito transmission (Day et al., 1956).

A natural laboratory recombinant between RFV and MYXV termed malignant rabbit virus has also been described. In this virus, 7978 bp of RFV DNA has replaced an equivalent region of MYXV by homologous recombination (Upton et al., 1988, 1990). The virus causes a disease indistinguishable from myxomatosis (Strayer, 1992). It is likely that recombination can occur quite readily given the high degree of sequence conservation in the leporipoxviruses and it has also been reported between a South American derived French virus attenuated as a vaccine (SG33) and a Californian virus (Camus-Bouclainville et al., 2011).

3. Myxoma virus and biological control of the European rabbit

3.1. Natural history of the European rabbit

Paleontological and historical evidence indicate that the European rabbit is native to the Iberian Peninsula and possibly southern France. It was spread by humans outside of its natural range and subsequently established in France, northern and central Europe with most wild stock originating from domestic or captive animals (Flux, 1994; Rogers et al., 1994). Rabbits were introduced to Britain in the middle ages and domestic rabbits were introduced to Australia with British settlement in 1788. However, it was wild rabbits imported from Britain in 1859 that initiated the rapid spread of European rabbits across Australia (Rolls, 1969; Fenner and Fantini, 1999). By 1910, rabbits had spread from their initial focus, near Winchelsea in south-eastern Australia, over 3000 km west to reach the Indian Ocean and more than 2000 km north to the tropic of Capricorn. Rabbits were described as a "grey blanket" covering Australia.

European rabbits are ecological generalists that can exploit many different environments but are particularly well adapted to the Mediterranean environment of southern Australia (Thompson, 1994; Myers et al., 1994). Their grazing and browsing substantially modify the environment by preventing regeneration of trees and shrubs and encouraging weed invasions and soil erosion. The excavation of warrens aids survival particularly in the often extreme conditions of the Australian outback and its high fecundity enables the rabbit to rapidly expand populations under favourable conditions. Rabbits were and are the most destructive vertebrate pest in Australia causing massive agricultural losses and unquantified ecological damage (Williams et al., 1995). Feral European rabbits have also caused serious damage in Chile, Argentina, New Zealand and many islands (Flux, 1994).

3.2. History of introduction of myxoma virus into Australia

The use of myxomatosis as a biological control for rabbits in Australia was suggested as early as 1919 (Aragão, 1927; Fenner and Ratcliffe, 1965) and some early laboratory trials were conducted in the 1920s but no field releases were permitted. In the 1930s, systematic investigations were undertaken to determine the potential of myxomatosis to control rabbits in Australia. Trials of MYXV in rabbits were initially undertaken in Britain (Martin, 1936) followed by species-specificity testing and further rabbit trials in Australia (Bull and Dickinson, 1937). The virus was highly species-specific and lethal with case fatality rates >99%. However, although MYXV successfully reduced populations in enclosure experiments, it did not spread well in field trials. When native stickfast fleas (*Echidnophaga* spp.) were present and in the absence of European foxes (*Vulpes vulpes*), which removed sick rabbits, local spread did occur (Bull and Mules, 1944). Mosquitoes do not appear to have been present in sufficient numbers to act as significant vectors in these trials, which were predominantly conducted in very dry country, although laboratory trials had demonstrated the potential of both mosquitoes and fleas to transmit the infection (Aragão, 1943; Bull and Mules, 1944). The conclusion from these very extensive trials was that myxomatosis was unlikely to be useful to control rabbits in Australia except perhaps in local areas where vectors were present and predators were absent (Bull and Mules, 1944). This much quoted conclusion made on the basis of carefully conducted trials demonstrates just how difficult it is to predict the emergence of novel diseases.

During the same period, releases of MYXV were also undertaken in rabbit populations on Skokholm island off the coast of Britain in 1936, 1937 and 1938 without success, presumably because there were no effective vectors (Lockley, 1940, 1955) and in Denmark on Vejrø Island in 1936, 1937 and 1938, and Sweden in 1938 (Fenner and Ratcliffe, 1965). In Sweden, the virus did cause a local epizootic but then appears to have died out.

3.3. The first epizootic of myxomatosis in Australia

Rabbit numbers had built up considerably in Australia in the 1940s, which led to pressure for further trials of myxomatosis. In 1950, experimental trials with MYXV were undertaken around the Murray Valley in northern Victoria and southern NSW (Fig. 3) where the role of mosquito vectors could be assessed (Ratcliffe et al., 1952; Myers, 1954). The virus spread locally on the trial sites but with low efficiency and then apparently died out even in high density rabbit populations. This appears due to the fact that mosquitoes were present either in low numbers or the species present were subsequently shown not to feed on rabbits. However, MYXV obviously managed to trickle on at some sites and in December (summer in Australia) the virus re-emerged in epizootic form. Its transmission was driven by large irruptions of *Culex annulirostris*

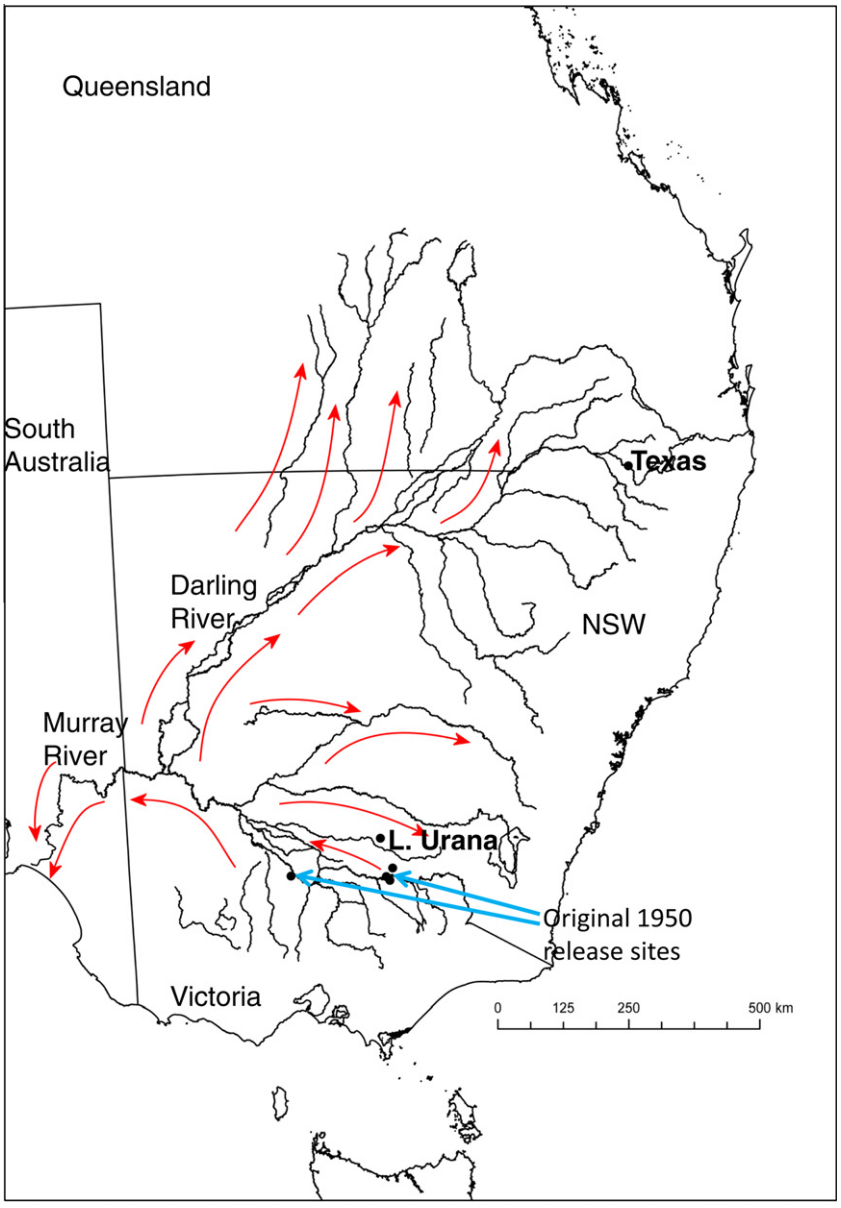

Fig. 3. Spread of myxoma virus in the first epizootic, summer 1950/51. South eastern Australia showing the rivers of the Murray–Darling Basin. The release sites at Gunbower, Corowa, Balldale and Rutherglen in the Murray River Valley are indicated as are the study sites at Lake Urana in NSW and Texas in Qld. Approximate spread of the virus is indicated by red arrows (based on Fenner and Ratcliffe, 1965; Ratcliffe et al., 1952; Brereton, 1953).

and *Anopheles annulipes* mosquitoes along the water courses and flood plains of the Murray–Darling Basin and the massive numbers of rabbits completely susceptible to the new virus (Ratcliffe et al., 1952). This was undoubtedly aided by flooding in the northern parts of the river system sending water down normally dry ramifying inland watercourses and enabling mosquito-borne spread over a vast area of inland northern New South Wales and southern Queensland west of the Great Dividing Range, which separates the east coast from the interior (Ratcliffe et al., 1952). "In places it was possible to drive for a day or more through country that had previously been swarming with rabbits and see only isolated survivors" (Ratcliffe et al., 1952) (Fig. 3). The speed and distribution of the epizootic along the watercourses comprehensively demonstrated the essential role of mosquitoes in transmission of MYXV, which spread over an estimated 1600 km from south to north and 1800 km east to west in the first 3 months. However, spread was very uneven with little spread in dry country. To the south, in Victoria, spread was mainly localised to the Murray River (Fig. 3) and to isolated point releases (Ratcliffe et al., 1952; Marshall et al., 1955).

Following this summer/autumn epizootic, the virus trickled on in local foci during the winter and then re-emerged in epizootic form the following spring and summer, particularly in areas not reached by the first epizootic. During the next 3 years MYXV spread throughout the rabbit infested areas of Australia aided by intensive inoculation campaigns. Estimated case fatality rates in these early epizootics ranged from 40% to 99.8% with estimates of reductions in populations ranging from >90% to no effect (Fenner et al., 1953).

4. Host pathogen coevolution of myxoma virus and the European rabbit

4.1. Attenuation of the virus

The virus released in Australia was derived from a strain isolated from a European rabbit in Brazil (Moses, 1911) and

Table 1
Virulence of field isolates of MYXV 1952/53.[a]

Date	Location	AST (days)	Range of ST
SLS		10.7	8–15
SLS		10.6	10–13
Nov 1952	Lake Urana	18.3	15–23
		20	18–21
		17.6	13–25
		26.8	13–28 (S)[b]
		15.2	10–23
		19.5	12–45
Dec 1952		20.4	15–28
		15.4	13–20
Dec 1952	Corowa NSW	17.4	11–30 (S)
		22.5	18–37
		12.5	10–19
		21.9	15–29 (S)
May 1952	Texas QLD	15.1	12–21
		12.9	11–17
Aug 1952		25.2	12–31 (S)
Nov 1952		17.7	16–19
April 1953		27.7	19–54
		18.9	15–27
May 1952	Dunroy NSW	21	13–41 (S)
Dec 1952		16.9	12–28

[a] Data are for laboratory rabbits from Myers et al. (1954) and Marshall et al. (1955) calculated using the transformation log10(ST-8) of survival times (ST) to calculate average survival times (AST) (Fenner and Marshall (1957)). Independent assays of SLS in laboratory rabbits are shown from each paper.
[b] Rabbit survived infection – AST for these viruses are from Fenner and Marshall (1957).

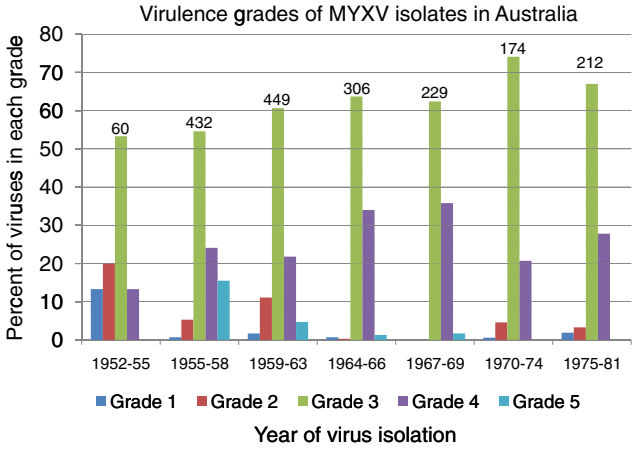

Fig. 4. Virulence of MYXV isolates from Australia 1952–81. Virus isolates were tested by the inoculation of a low dose (approximately 5–10 rabbit ID_{50}) into groups of 4–6 laboratory rabbits. Allocation into grades 1–5 was based on average survival times (AST), case fatality rate and clinical signs (see Tables 2 and 3). Numbers above the bar indicate the total number of viruses tested for that period. Data are from Fenner (1983).

subsequently supplied to researchers in the USA (Martin, 1936; Fenner and Fantini, 1999). It had undergone many passages in rabbits. In Australia, this virus was later termed the standard laboratory strain (SLS). Based on pre-release testing (Bull and Dickinson, 1937; Bull and Mules, 1944; Martin, 1936) it was estimated that SLS killed 99.8% of infected rabbits and this case fatality rate was borne out in the early epizootics. However, at some locations the kill rate was much lower either because rabbits avoided infection or because rabbits survived infection (Fenner et al., 1953).

In a large sampling of rabbits across sites in south eastern Australia with different observed epizootic patterns, 370 of 824 rabbits sampled between February and July 1952, were seropositive (45%) indicating recovery from myxomatosis. This ranged from 6% on one site to 95% seropositive on a second site sampled at the same time and only 10 km apart (Fenner et al., 1953). In one carefully studied site, at Lake Urana in Southern NSW (Fig. 3), it was estimated that the rabbit population was reduced from 5000 to 50 after the first epizootic at the site in the summer of 1951/52, which was initiated by rabbit inoculation in September 1951. This represented a percentage kill of 99% and a case fatality rate estimated at 99.8% (Myers et al., 1954). Clinical cases were observed to trickle on through the winter but the population grew to about 550 due to immigration and breeding. Virus was again released in the area in October 1952 but appears to have actually spread from the overwintering foci. In this second season, the estimated case fatality rate had dropped to 90% and more chronically affected rabbits were observed, suggesting that the overwintering had selected for an attenuated strain or strains of virus.

Testing of viruses isolated from mosquitoes at Lake Urana in the second epizootic revealed prolonged survival times compared to SLS. Average survival times (AST) for 8 virus isolates tested in groups of 5–8 laboratory rabbits ranged from 15.2 to 27 days; similar results were obtained from viruses isolated at Corowa about 70 km south on the Murray River (Myers et al., 1954) (Table 1). These viruses were still very lethal: 1 of 48 laboratory rabbits and 1/16 experimentally infected wild rabbits survived challenge with the viruses from Lake Urana. SLS by comparison had an AST of 10.7 days with 100% case fatality rate. Similar testing on a group of eight viruses isolated over 800 km north at Texas in Qld (Fig. 3) and Dunroy in northern NSW between May 1952 and April 1953 showed AST ranging from 12.9 to 27.7 days with 2/47 laboratory rabbits and 0/27 wild rabbits surviving challenge (Marshall et al., 1955) (Table 1). As at Lake Urana, this occurred in the face of repeated releases of virulent SLS. Thus a similar selection of slightly attenuated field strains was occurring in very different geographic areas of the rabbit's range and these field viruses outcompeted the more virulent SLS that was being released. Overall, with the 20 field strains tested in Table 1, only 5/119 rabbits survived infection, a case fatality rate of 95.8%, but this was 20 times the estimated survival rate for SLS.

Virulence testing was standardised to allow detection of very small differences in phenotype based on a combination of average

Table 2
Virulence grades of myxoma virus.[a]

Virulence grade	1	2	3[b]	4	5
Case fatality rate	99.5%	95–99%	70–95%	50–70%	<50%
Average survival time (days)[c]	⩽13	14–16	17–28	29–50	Not defined

[a] Modified from Fenner and Marshall (1957) and Fenner and Ratcliffe (1965).
[b] In some studies, grade 3 viruses were divided into 3A (case fatality rate of 90–95%; AST of 17–22 days) and 3B (case fatality rate of 70–90%; AST of 23–29 days) (Fenner and Ratcliffe, 1965).
[c] As survival times are not normally distributed they were transformed [$\log_{10}$ (ST-8)] for calculation of average survival times and fiducial limits and then backtransformed as shown above (8 days was chosen as the correction factor because infected rabbits did not die of myxomatosis before this time). Rabbits that survived infection were assigned a survival time of 60 days and mean survival times and standard deviations estimated according to Sampford (1954). If more than two rabbits survived infection virulence grades were assigned based on number of surviving rabbits and clinical signs (see Table 3).

Table 3
Symptomatology of virulence prototypes of MYXV.[a]

Virulence grade	Prototype virus (origin)	AST days	ST range	CFR%	Clinical signs
1	SLS (Brazil 1910)	10.8	8–15	100	Primary swelling detectable at inoculation site by d3; this primary lesion is not clearly demarcated from surrounding skin; secondary skin lesions and eyelid swelling present by d6–7; eyes closed by d9; discharge from eyes is mucopurulent late in infection; pronounced swelling of head, ears and perineum late in course together with slow laboured respiration and mucopurulent nasal discharge.
3	KM13 (Aust /Dec 1952)	21.5	13-S	88	Lump at inoculation site by d3–4 becomes large, flat, soft and clearly demarcated from surrounding skin. Similar timing of initial signs to SLS but development of disease is slower. Eyes do not usually close completely until d14–16. Eyelids often distorted rather than uniformly swollen. Swelling of head, ears and perineum is not as severe as SLS but may be very pronounced in late stages. Occasional animals have a mild course. Secondary bacterial infections of conjunctivae and upper respiratory tract.
4	Uriarra (Aust/Feb 1953)	26.2	15-S	58	Lump at inoculation site by d3–4 becomes hard, red and later purple/black. It is clearly demarcated by d12; the centre may become necrotic and scab. Numerous secondary lesions occur; may be discrete nodules on eyelids rather than generalised swelling. Perineal swelling is often extensive. Discharge from eyes and nose is less than with more virulent viruses.
5	Neuromyxoma (IC passage of SLS)	N/A	N/A	0	Hard red demarcated lesion at inoculation site regresses and scabs quite quickly. Secondary skin lesions are scanty nodules < 5 mm diameter. Swelling of head and perineum does not occur and the rabbit appears healthy during the infection.
5	Nottingham (UK/ April 1955)	N/A	14-S	23	Primary lesion seen at d5, may regress around d12 or proliferate to a large hard protuberant tumour, which persists for some weeks. Nodular, demarcated secondary lesions appear around d9. Swelling of the perineum is severe but head and ears are largely unaffected and eyelids only have localised nodules rather than generalised swelling.

[a] Descriptions are from Fenner and Marshall (1957). All viruses are derived from SLS except Nottingham, which is derived from Lu but is more typical of grade 5 viruses from the field than Neuromyxoma, which was derived by intracerebral passage in rabbits. Grade 2 viruses have essentially the same clinical pattern as grade 1 and no prototype strain was defined. AST – average survival time; ST – survival time; CFR – case fatality rate.

survival times, survival rates and clinical signs in groups of 4–6 adult laboratory rabbits infected with a low dose (5–10 rabbit infectious dose 50%; ID_{50}) of each virus. The challenge was to differentiate between viruses with a case fatality rate of >99% and perhaps 95% using manageable numbers of animals. Viruses were graded from 1 (high virulence) to 5 (low virulence) (Tables 2 and 3) (Fenner and Marshall, 1957). These assays relied on estimating mean survival times using low numbers of rabbits and hence were readily skewed by single rabbits. However, despite subsequent criticisms of the methods and statistical approaches (Ross and Sanders, 1987; Parer et al., 1994; Parer, 1995), a clear distinction could be seen between the highly lethal and reproducible results obtained over many trials with SLS and the longer survival times obtained with the more attenuated grade 2 and 3 viruses (see Table 1). The virulence divisions are relatively arbitrary and precise distinctions between the more attenuated viruses should probably not be made. For example, the prototype grade 4 virus Uriarra has an AST of 26.2 days i.e. it would be classified as grade 3 on AST alone but nearly 50% of infected animals survived infection (Table 3) (Fenner and Marshall, 1957). Importantly, this simple methodology allowed the standardised classification of hundreds of field samples and demonstrated very clearly the evolution of the virus that was occurring.

It rapidly became apparent that the predominant viruses in the field were of grade 3 virulence (Fig. 4). This was despite the regular release of SLS or an SLS-derived grade 1 virus known as the Glenfield strain (Gv). In fact, grade 1 or 2 viruses were usually only isolated within 6 months of deliberate releases in an area (Fenner and Marshall, 1957). Grade 3 viruses were still very lethal with case fatality rates of 70–95%. In laboratory testing, very few rabbits survived infection but infected rabbits survived for considerably longer on average than rabbits infected with a grade 1 virus, which generally died within 5 days of becoming infectious for mosquitoes. This provided a selection advantage as the infected rabbit was a potential source of virus for mosquitoes for longer.

The critical level of virus in the epidermis of skin lesions was around 10^7 ID_{50}/g for efficient mosquito transmission (Fenner et al., 1956) (Figs. 5 and 6). Skin lesions, particularly swollen eyelids and ears, are the source of virus; rabbits do not become infectious until these lesions appear (Fenner et al., 1952, 1956). Skin

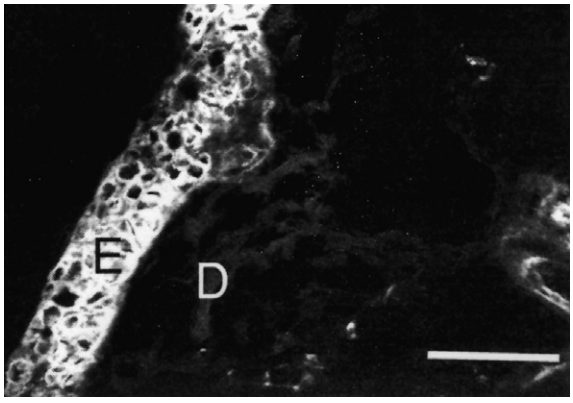

Fig. 5. MYXV in the epidermis of the primary skin lesion. Immunofluorescence of MYXV antigen in the epidermal cells of a primary skin lesion 10 days after inoculation. Mosquitoes or fleas probing through the epidermis will pick up virus on their mouthparts; E – epidermis; D – dermis; scale bar 100 μm (image from Best et al. (2000) Elsevier with permission).

lesions with titres below 10^7 ID_{50}/g tended to have low transmission potential (Fig. 7). For example, at 18 days after infection only 1/47 mosquitoes allowed to feed on a skin lesion with a titre of $10^{5.1}$ ID_{50}/g transmitted infection whereas 34/44 mosquitoes fed on a lesion with a titre of $10^{7.5}$ ID_{50}/g transmitted infection (Fenner et al., 1956).

Infections with highly attenuated viruses such as neuromyxoma (Figs. 6 and 7; Table 3), which was artificially attenuated by intracerebral passage (Hurst, 1937b), or grade 5 field isolates such as Nottingham (Fenner and Marshall, 1957), could be quickly resolved by the rabbit. This meant that the skin lesions had titres in the transmissible range for only a short period. Even if absolute titres were favourable for transmission, lesions which scabbed over rapidly could not be probed by mosquitoes. Furthermore, it was later shown that rabbits which died quickly following infection and rabbits which survived infection were poor sources of virus for insect transmission; most successful transmission occurred from rabbits that had prolonged survival times but ultimately died (Mead-Briggs and Vaughan, 1975).

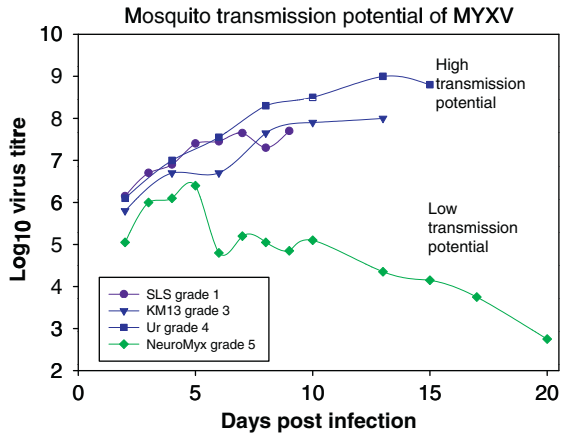

Fig. 6. Relationship between virulence, skin titre and transmission. Titres of virus in the primary skin lesions from rabbits inoculated at 10 sites with a high dose of virus (20,000 rabbit ID_{50}). Titres are log_{10} rabbit ID_{50} g^{-1} and are the geometric mean for two rabbits where both rabbits were alive; rabbits inoculated with SLS both died before d10; KM13 on d10 and d13 and Uriarra on d10 and d15; rabbits inoculated with neuromyxoma virus recovered. Data are from Fenner et al. (1956). The grade 5 neuromyxoma virus was generated by intracerebral passage in rabbits and is not typical of field strains with grade 5 virulence.

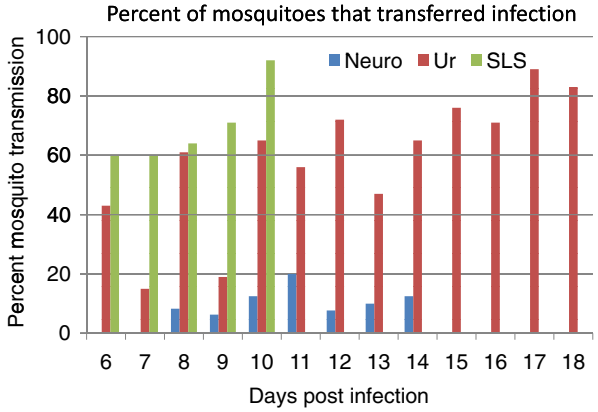

Fig. 7. Mosquito transmission of different virulence grades of MYXV. The percentage of mosquitoes that successfully transmitted infection following feeding on lesions of grade 1 (SLS), grade 4 (Uriarra) or grade 5 (neuromyxoma) viruses. Data are from Fenner et al. (1956).

4.2. Genetic resistance in wild rabbits

Even in the first epizootic, some rabbits survived infection. The emergence of more attenuated strains of MYXV increased the likelihood that rabbits with a degree of innate resistance would survive infection. Non genetic factors may also have contributed to increased survival rates: maternal antibody can modify the course of disease in young kittens infected with attenuated viruses (Fenner and Marshall, 1954) and high environmental temperatures can enhance survival of rabbits infected with moderately attenuated virus strains (Marshall, 1959; Marshall and Douglas, 1961; Sobey et al., 1968). In regions experiencing annual epizootics, the kittens born each breeding season will mostly have been the progeny of females born in the previous year that had survived myxomatosis. This imposed a very high selection pressure (Marshall and Fenner, 1958).

The best documented development of resistance was at Lake Urana in southern NSW. Within 7 years – representing seven breeding seasons or seven generations, the case fatality rate on challenge with a grade 3 virus (KM13) had fallen from 90% to 26% (Fig. 8) (Marshall and Fenner, 1958; Marshall and Douglas, 1961). Interestingly, over 50% of the progeny bred from challenged rabbits that had shown only mild clinical signs survived challenge with the grade 1 SLS (Marshall and Fenner, 1958). Resistance appeared to develop more rapidly in the hotter dry areas of Australia such as the Mallee in Victoria and the NSW Riverina (Lake Urana) than in cooler wetter areas such as Gippsland in south eastern Victoria (Marshall and Fenner, 1958; Marshall and Douglas, 1961; Edmonds et al., 1975). It has been proposed that this may relate to increased selection pressure as the Mallee and Riverina experienced annual epizootics whereas epizootics in Gippsland were less frequent (Fenner and Ross, 1994).

Simple inspection of Fig. 6 and more detailed modelling indicated that transmissibility should have been maximised by viruses of grade 4 virulence (Anderson and May, 1982; Dwyer et al., 1990); however, grade 3 viruses became the predominant strain in the field and persisted as such for at least the first 30 years of myxomatosis in Australia (see Fig. 4); there is no systematic analysis available after this period. Viruses with grade 3 virulence in laboratory rabbits would appear as lower virulence if tested in wild rabbits with genetic resistance. In fact, the grade 3 KM13 strain had a grade 5 phenotype in the wild rabbits from Lake Urana after 7 years of selection. While this suggests that more virulent viruses (as measured in laboratory rabbits) should re-emerge in the field, it is likely that reality is more complicated. For a start, the clinical severity of an epizootic as measured by case fatality rate, clinical disease picture and distribution of survival times will be influenced not just by the intrinsic virulence of the virus and the resistance of the rabbits but by biotic and abiotic factors such as weather, with cold increasing effective virulence and high temperatures ameliorating virulence (Marshall, 1959), nutritional status, age of the rabbit and intercurrent disease or parasitism (Joubert et al., 1982; Cattadori et al., 2007; Marlier et al., 2000c). In addition, predators will tend to remove sick rabbits that would survive in cage or pen trials.

Selection pressures may have been quite different for virus that could continue to trickle through a population during the winter, with few mosquitoes and low numbers of susceptible rabbits, where

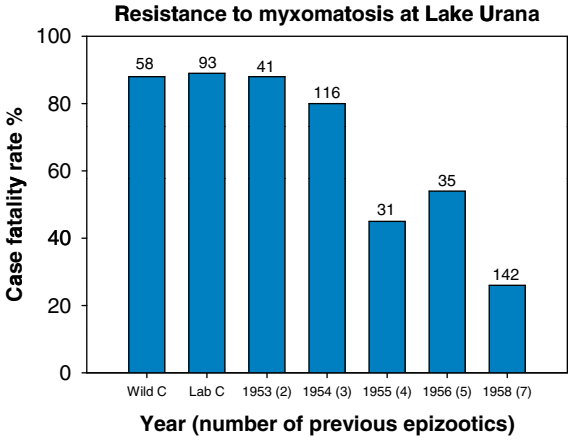

Fig. 8. Innate resistance to MYXV in Australian rabbits. Rabbit kittens were captured at Lake Urana, NSW, each year prior to the annual epizootic of myxomatosis and transferred to an animal house. At 4 months of age, seronegative rabbits were challenged with 5–20 rabbit ID_{50} of the grade 3 KM13 strain of MYXV. The percentage of rabbits that died is shown for each year. Wild C and Lab C are the results of challenge for wild rabbits from a population that had never been exposed to MYXV and laboratory rabbits, respectively. Figures above the bars indicate the number of rabbits in each experiment. In 1955, the kittens were most likely the progeny of rabbits that had survived the experimental release of the highly virulent Lu strain at Lake Urana the previous year. Data are from Marshall and Fenner (1958) and Marshall and Douglas (1961).

long persistence of infectious lesions may have been critical compared to a virus in summer/autumn following the breeding season with high numbers of susceptible rabbits and plentiful vectors where a highly virulent virus may have had an advantage (Fenner and Ratcliffe, 1965). This suggests that at a landscape level it might be possible for viruses of quite different virulence grades to be successful, which is supported by the presence of grade 1, 3, 4 and 5 viruses in field samples in the 30 years following the first epizootics (Fig. 4). Genetic plasticity for virulence could be an important feature of the success of MYXV. The release and spread of the European rabbit flea in the early 1970s as a year-round vector for MYXV may also have altered the dynamics of selection (see Section 4.2).

Although no large scale collections of viruses have been analysed for virulence using the standardised tests in the last 25 years, some limited testing showed the presence of both highly virulent grade 1 viruses and attenuated grade 4 and 5 viruses in the field during the 1990s (Saint et al., 2001; Kerr et al., 2010). The grade 1 viruses were of relatively low virulence (equivalent to grade 4 or 5) when tested in resistant wild rabbits under pen or cage conditions although two of the grade 1 viruses were considerably more virulent than the progenitor SLS in wild rabbits (Kerr et al., 2003, 2004). Interestingly, some recent viruses had a hypervirulent phenotype in laboratory rabbits causing rapid death with few clinical signs of myxomatosis (Kerr et al., 2004). This resembles the clinical disease caused by the virulent MSW Californian strain of MYXV (Silvers et al., 2006; Fenner and Marshall, 1957). However, in wild rabbits these viruses produced clinical myxomatosis although interestingly the primary lesion at the inoculation site was often very small. This suggests that ongoing coevolution continues between Australian rabbits and MYXV and it is interesting to note that Fenner and Ratcliffe (1965) predicted that one possible evolutionary outcome of ongoing selection for resistant rabbits was the emergence of virus strains that would be hypervirulent when tested in laboratory rabbits.

5. Enhancing biological control by myxomatosis in Australia

5.1. Release of virulent strains of myxoma virus

Unlike Brazil and California, there was no reservoir host in Australia to maintain virulent virus. However, there have been widespread releases of virulent SLS and the SLS-derived Gv strain, and from the 1970s to the end of the 20th century, the Lausanne strain (Lu). This was the virus used to initiate the epizootics in Europe. Testing in genetically resistant wild rabbits revealed that Lu and Gv were more virulent than SLS, although the virulence of the three viruses could not be distinguished in laboratory rabbits (Fenner, 1983). Fenner et al. (1957) released the Lu strain experimentally at Lake Urana and other sites because its florid clinical signs meant it could be readily differentiated from the Australian SLS-derived field strains. Although it initially established and spread in these experiments, the virulent Lu strain was quickly replaced by Australian field strains. Genetic studies have shown that despite the widespread release of Lu since 1970, all field strains examined have been derived from SLS (Saint et al., 2001; Kerr et al., 2010) and further field trials confirmed that Lu did not compete in the field (Berman et al., 2006). More generally, it appears that releases of virulent MYXV had little effect on the evolution of MYXV in Australia and may at best have had some local impact on rabbit populations (Fenner and Ratcliffe, 1965).

5.2. Novel vectors: Introduction of rabbit fleas to Australia

MYXV in Europe was transmitted by the European rabbit flea (*Spilopsyllus cuniculi*), which had not been introduced to Australia with imported rabbits. This flea was deliberately introduced into Australia and widely spread in the early 1970s in part because of the belief that flea transmission was associated with selection for higher virulence (Sobey and Menzies, 1969; Cooke, 1983; King et al., 1985). It provided a year-round vector for MYXV in the absence of mosquitoes and is credited with substantially increasing the effectiveness of myxomatosis at a time when the impact of the disease was perceived to be in decline. It was also believed that *S. cuniculi* was a more effective vector for the Lu strain of MYXV being released at the same time (Shepherd and Edmonds, 1977). Unfortunately *S. cuniculi* did not persist in the hot arid regions of Australia, where mosquitoes were also often absent, and so the Spanish rabbit flea (*Xenopsylla cunicularis*) was introduced to provide a vector more adapted to hot dry regions (Cooke, 1984, 1990a,b). Whether *X. cunicularis* has had an impact on rabbit control is unclear because the release of the flea in 1994–95 coincided with the introduction of the calicivirus, Rabbit Haemorrhagic Disease Virus (RHDV) as a second and very successful biological control for rabbits (Fenner and Fantini, 1999; Cooke, 2002).

5.3. Success or failure of biological control?

The attenuation of MYXV and the emergence of resistant rabbits in the decade following the release was a dramatic example of host–pathogen coevolution occurring in real time. It is also often seen as a failure of biological control. However, this is an oversimplification. The enormous drop in rabbit numbers in the early 1950s provided ecological breathing space and allowed a rapid regeneration of plant species together with increased agricultural productivity (Fenner and Ratcliffe, 1965). Although rabbit numbers recovered and in some areas, particularly the sparsely inhabited rangelands of inland Australia, rabbits re-emerged as a significant environmental problem, myxomatosis continued to act as a control on the population expansion and enhanced the value of conventional controls such as habitat destruction and poisoning. In addition, predators such as cats and foxes could continue to suppress rabbit numbers once the populations had been substantially reduced (Williams et al., 1995). As an example of the unnoticed effect of myxomatosis, the repeated introduction of an attenuated immunizing strain of MYXV experimentally into wild rabbit populations at two sites at Lake Urana saw one population increase by 8-fold and the other 12-fold over 2 years (Parer et al., 1985). This was in an area where 10 years earlier it had been estimated that between 50% and 60% of rabbits observed with myxomatosis recovered from infection (Williams and Parer, 1972). In the early 1990s, it was suggested that rabbit numbers were 5–25% of the population pre 1950, with the higher numbers in the ecologically more fragile rangelands (Williams et al., 1995; Fenner and Ross, 1994). Since the release of RHDV in 1995 it has been very difficult to determine the impact of myxomatosis on rabbit populations.

6. Repeating the experiment: Myxomatosis in Europe

6.1. Introduction in France and subsequent spread

On 14 June 1952, Dr. PFA Delille inoculated two wild rabbits on his estate at Maillebois, about 70 km west of Paris, with a strain of MYXV he had obtained from the Laboratorie de Bacteriologie in Lausanne, Switzerland (Fenner and Fantini, 1999). The virus, (Brazil Campinas/1949), now known as the Lausanne strain (Lu), was isolated in Brazil in 1949 and had undergone fewer than five rabbit passages. Lu has a similar case fatality rate to SLS in laboratory rabbits i.e. essentially 100% of infected rabbits die with an average survival time of 10–12 days, however, the clinical appearance is more florid with larger purple/red cutaneous lesions (see Fig. 14F).

Despite attempts to limit its spread, MYXV moved across France and over the next 10 years into all areas of Europe where rabbits were present in the wild (Arthur and Louzis, 1988; Fenner and Ross, 1994). It also spread into the commercial and backyard rabbit industries, where it has subsequently been partially controlled by vaccination and quarantine (Joubert et al., 1982; Marlier et al., 2000b, 2001) but continues to spread into new countries (Kritas et al., 2008). From continental Europe, MYXV was introduced into Britain in 1953 where again, although attempts at control were made, the virus spread and became enzootic. Interestingly, deliberate releases of SLS had been made in the Scottish Hebrides in 1952 and 1953 where it caused an epizootic on each occasion but then burnt out (Fenner and Ratcliffe, 1965).

Unlike in Australia, where mosquitoes were the main vector, in Europe and Britain the European rabbit flea *S. cuniculi* was a major vector and in Spain and Portugal other rabbit fleas such as *X. cunicularis* were present as vectors in addition to the more seasonal mosquitoes and Culicoides midges. This meant that the epidemiology was potentially different to the initial Australian situation as the fleas were present as vectors all year (Ross and Tittensor, 1986).

6.2. Impact of myxomatosis in Europe and Britain

In France, rabbit populations were estimated to have been reduced by 90–98% (Arthur and Louzis, 1988; Fenner and Ross, 1994) and in Britain it was estimated that the wild rabbit populations were reduced by greater than 99% (Fenner and Ross, 1994). In Europe, the wild rabbit occupied multiple sociological and ecological niches. In forestry and farming it was a serious pest animal but it was also a major resource for recreational hunters (Fenner and Ratcliffe, 1965; Joubert et al., 1982). In the Iberian penninsula, rabbits are a keystone species and the loss of rabbits due to myxomatosis, hunting and subsequently rabbit haemorrhagic disease has threatened the survival of top predators such as the imperial eagle and the lynx (Rogers et al., 1994). Rabbit grazing is crucial to maintaining particular environments such as chalk downs in what was regarded as a traditional state (Thompson, 1994). In addition, there is a substantial commercial and backyard production of domestic rabbits for meat and hair, (140 million rabbits were produced annually in France prior to myxomatosis), which has incurred ongoing production losses from myxomatosis and costs due to vaccination and quarantine (Fenner and Ratcliffe, 1965; Arthur and Louzis, 1988).

6.3. Host–pathogen coevolution: Changes in virulence of MYXV in Europe

Despite the differences in climate, environment, vectors and progenitor strain of MYXV, the overall evolution of MYXV and its new host, the European rabbit, was broadly similar in Europe and Britain to what occurred in Australia. Attenuated strains of virus were first observed nearly three years after the initial release in France. A virus isolated in April 1955 in Loiret, France was shown to be a typical grade 4 virus characterised by prolonged survival of infected laboratory rabbits (Fenner and Marshall, 1955). In Britain, grade 3 viruses were isolated about 12 months after the initial outbreak (Fenner and Marshall, 1957); a virus isolated from a mixed infection in April 1955 at Nottingham was shown to be markedly attenuated grade 5 (Fenner and Marshall, 1955). Whether there was a slower development of attenuated viruses in France compared to Australia and Britain or simply a lower rate of observation is not clear.

In a large scale survey from Britain in 1962 the majority of isolates were of Grade 3 virulence with virtually no grade 1 isolates (Fenner and Chapple, 1965) (Fig. 9). The proportion of more virulent viruses subsequently gradually increased (Ross and Sanders, 1987) with a high proportion of grade 2 strains of virus and relatively few grade 4 or 5 virus strains. This prevalence of high virulence viruses was quite distinct from the situation in Australia and possibly relates to the difference in the principal insect vector (Fenner and Ross, 1994) but may also reflect the very strong resistance present in rabbits by the mid-70s (see Fig. 11). The virulence of viruses in the rest of Europe has not been studied as systematically, however, in France between 1962 and 1968 and 1979–82 the majority of isolates were of grade 3 or 4 virulence (Joubert et al., 1972; Arthur and Louzis, 1988; Fig. 10). In Spain, 20 isolates made from 1992 to 1995 were tested for virulence. The tests cannot be directly compared with those done in previous studies because of the age of the rabbits (30 days rather than >4 months) and a large (10,000 pfu) dose of virus. However, it is obvious that 14 of the viruses were highly virulent with average survival times ranging from 9.8 to 14 days and mortalities of 100% in the inoculated and in-contact rabbits, 3 of the viruses were of intermediate virulence based on AST of 15–24.8 days but still killed 100% of challenged rabbits and in-contact rabbits; one virus was of lower virulence, 30–80% case fatality rate and AST of 29.7 days, while two isolates

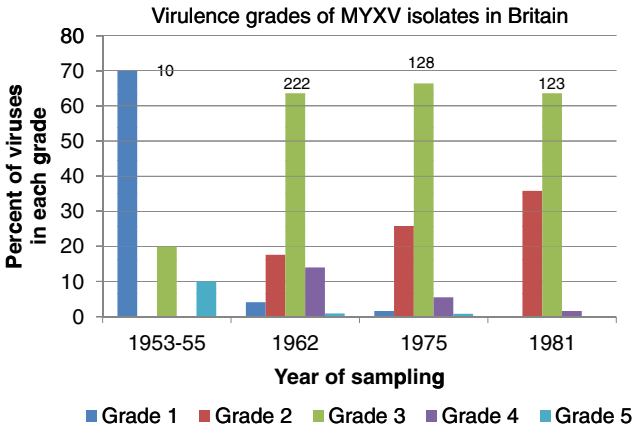

Fig. 9. Virus virulence in Britain. Virus isolates were tested by the inoculation of approximately 10–50 rabbit ID_{50} into groups of 5 or 6 laboratory rabbits. Allocation into grades 1–5 was based on AST, case fatality rate and clinical signs (see Tables 2 and 3). Numbers above the bar indicate the number of viruses tested for that period. Data are from Fenner and Marshall (1957), Fenner and Chapple (1965), Ross and Sanders (1987).

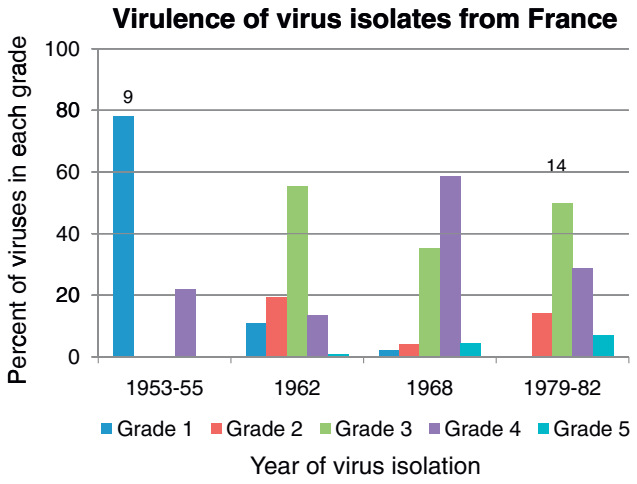

Fig. 10. Virus virulence in France. Data are from Fenner and Marshall (1957), Joubert et al. (1972), Arthur and Louzis (1988). Numbers of viruses tested were not given in Joubert et al. (1972).

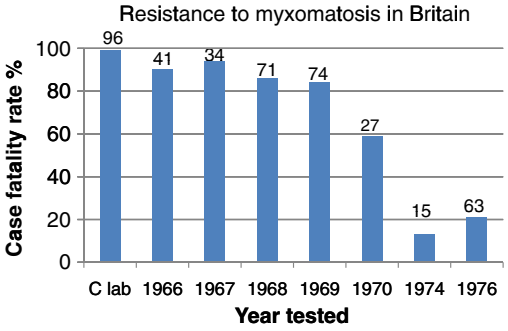

Fig. 11. Innate resistance to MYXV in rabbits from Britain. Kittens were captured at a study site in Norfolk or bred in captivity from adults captured at the site. Seronegative rabbits at 4 months of age were challenged with 50 rabbit ID_{50} of the grade 3 Brecon strain of MYXV. C Lab – laboratory rabbit controls. Numbers above the bars indicate the number of rabbits tested. Data are from Ross and Sanders (1977).

did not cause any mortalities although only one transmitted to in-contact rabbits (Bárcena et al., 2000b). Thus a range of virulence grades was present but with a tendency towards higher virulence.

6.4. Genetic resistance in rabbits in Europe

Rabbit numbers in Britain and France gradually recovered through the 1960s and 1970s. Genetic resistance to myxomatosis was identified in Britain using a method similar to that of Marshall and Fenner at Lake Urana in Australia (Ross and Sanders, 1977). As early as the first testing in 1966, wild rabbits from a population in Norfolk challenged with a grade 3 virus had a much longer average survival time than control laboratory rabbits (29.8 days vs. 20.2). The case fatality rate was 90% in wild rabbits and 99% in laboratory rabbits. The proportion of rabbits surviving the challenge increased each year from 1970 with only 21% succumbing in 1976 (Fig. 11). Case fatality rates in wild rabbits were still 100% when challenged with a grade 1 virus but average survival times were prolonged to 18 days compared to 12 days for laboratory rabbits. Further testing from 1978 to 1980 showed that resistance was widespread with rabbits from 3 widely separated locations having mortality rates of around 45% when challenged with the same grade 3 virus compared to 100% for laboratory rabbits (Ross and Sanders, 1984). More extreme resistance was also present, 21/44 rabbits from a population in Wiltshire survived challenge with the Grade 1 Cornwall strain (Ross and Sanders, 1984). Resistance in Britain was slow to emerge compared to Australia; possible explanations include: lower survival rates due to higher virulence virus strains, the cooler climate with no summer sparing effect, the flea as the main vector and hence ongoing transmission especially through the winter when the virus is effectively more virulent. However, once resistance appeared, it quickly became significant and widespread.

In France, resistance also emerged but there have been few systematic studies of any scale; Arthur and Louzis (1988) describe resistance as extremely variable across regions. Rogers et al. (1994) note that resistance seemed very intense in the Vaucluse (south of France around the Mediterranean) with no deaths in 11 rabbits compared to 5/7 from the Paris district but does not say with what strain the rabbits were challenged. In a small trial of rabbits from 3 sites: 1/7, 1/4 and 7/7 rabbits survived challenge with the grade 1 Lu strain (Galaup, 1988; cited by Fenner and Ross, 1994).

6.5. Amyxomatous myxoma virus strains

Two clinical forms of myxomatosis have been described in France and Belgium, the nodular type with typical clinical myxomatosis and widespread cutaneous lesions, which was seen from the time of the initial release and, since the 1970s, a type described as amyxomatous or respiratory. This form has fewer, smaller, cutaneous lesions than the nodular type, although many of the clinical signs are typical of myxomatosis such as the cutaneous lesion at the inoculation site, perineal oedema, swollen eyelids, serous or purulent blepharoconjunctivitis and serous/purulent rhinitis (Arthur and Louzis, 1988; Duclos et al., 1983). Both types have been observed in wild rabbits but the amyxomatous form is regarded as more significant for farmed rabbits and may be widespread but under-diagnosed (Marlier et al., 1999, 2000b, 2001). It has been suggested that the amyxomatous form represents an adaptation to contact transmission in the absence of vectors, presumably via respiratory and conjunctival secretions as direct contact is needed for transmission; rabbits housed in adjacent cages did not become infected (Joubert et al., 1982). However, such a specific adaptation remains to be demonstrated and may be unnecessary since myxoma virus has always transmitted efficiently to in-contact animals in pen trials (Martin, 1936; Bull and Dickinson, 1937; Mykytowycz, 1953, 1956; Bárcena et al., 2000b). While the absence of visible cutaneous lesions might limit vector transmission there are no data on virus titres in eyelids or base of the ears, which are key sites for vector transmission (Fenner and Woodroofe, 1953), or on the ability of vectors such as fleas to transmit the amyxomatous form.

It is possible that selection pressure on MYXV in rabbit farms is quite different to wild rabbits due to the use of vaccines that provide only partial protection and do not necessarily eliminate shedding following challenge (Marlier et al., 2000a; Pšikal et al., 2003; Kritas et al., 2008) and the presence of bacterial pathogens, which could serve to enhance the apparent virulence of MYXV strains thus leading to selection for more attenuated viruses. The virulence of amyxomatous viruses appears to depend on the presence of bacterial pathogens such as *Pasturella multocida*. Specific-pathogen-free rabbits infected with a series of five amyxomatous strains developed relatively mild disease but the same viruses inoculated into conventional rabbits were much more pathogenic (Marlier et al., 1999, 2000c).

The genetic basis for the amyxomatous phenotype has not been defined but it may be a common mutation since the Californian myxoma virus MSW and some Australian field strains also cause an apparent amyxomatous phenotype in laboratory rabbits and wild rabbits with small or no visible lesions at the inoculation site and few secondary lesions (Fenner and Marshall, 1957; Kerr et al., 2003; Silvers et al., 2006).

7. Similarities and differences between Australia and Europe

The viruses introduced into Australia and France differed in their passage history, virulence and disease signs. While the general clinical picture in infected rabbits is similar for Lu and SLS, Lu has a distinctive morphology of the cutaneous lesions, these are raised and swollen, sometimes described as "golf ball-like", with a red/purple colour whereas the SLS lesions are flatter and tend to be red/pink in colour, although flattened lesion morphology with slight attenuation readily emerges from Lu derived viruses (Woodroofe and Fenner, 1965; Fenner and Chapple, 1965). The Lu phenotype closely resembles the disease caused by other MYXV strains isolated from South America that have not been passaged in European rabbits (Fenner and Marshall, 1957). Emergence of attenuated viruses may have occurred somewhat later in France than in Australia but was similar in Britain to Australia and genetic resistance was quite slow to emerge in Britain compared to Australia.

In Australia ongoing widespread introductions of virulent virus were made for nearly 50 years following the initial release whereas

in continental Europe and Britain there was essentially a single point release although some deliberate spread occurred (Fenner and Ross, 1994). In France and other countries, active efforts were made to disrupt transmission of MYXV including vaccination of wild rabbits, initially with rabbit fibroma virus and later with attenuated strains of MYXV (Joubert et al., 1982; Arthur and Louzis, 1988; Fenner and Ross, 1994; Ferreira et al., 2009; Guitton et al., 2008). These vaccines may have altered selection pressures although it is unlikely that the scale was sufficient for any real impact. However, in commercial rabbitries, widespread use of vaccines that are imperfect and allow infection and shedding may select for virus that can spread in this environment. In addition, inadvertent selection for genetic resistance could be occurring in farmed rabbits. Virus can potentially spread from rabbit farms to wild rabbits and back thus creating a very different selection process to that in Australia where only limited commercial rabbit production occurs and vaccination has not been permitted since 1962. Attempts to restore rabbit populations were made by the introduction of *S. floridanus* from the USA to France for hunting and the importation of resistant wild rabbits from Australia (Arthur and Louzis, 1988; Fenner and Ross, 1994).

Despite these differences the overall evolutionary picture in wild rabbits was very similar in Europe and Australia with initial highly lethal epizootics that devastated the completely naïve rabbit populations followed by the emergence of attenuated viruses that had a transmission advantage and then selection for genetic resistance in the rabbit population. This coevolution of host and pathogen gradually reduced the impact of the disease and allowed some recovery of the rabbit population but at much lower numbers than previously.

MYXV has also been released and become established in parts of Chile and southern Argentina for control of feral European rabbits and on a number of islands. It was also trialled in New Zealand but failed to establish because of the lack of vectors.

8. Pathogenesis of myxomatosis

8.1. Pathogenesis in laboratory rabbits

Clinical signs depend on the strain of virus and the route of inoculation (Table 3) (Fenner and Marshall, 1957; Chapple and Muirhead-Thompson, 1964). With SLS, the first sign is a red swelling at the inoculation site 2–4 days after inoculation. This increases to 4–6 cm in diameter and 2 cm in height by 10 days after infection. Swelling of the eyelids and anogenital region appears from day 6 and the face and base of ears also become swollen. Serous conjunctival and nasal discharge occurs between days 6–8 and this becomes mucoid or purulent and forms crusts over the eyelids and nostrils. By day 10 the eyelids and surrounding tissue can be so swollen that the eyes are completely closed. Virus is present in all discharges and readily transmitted by contact. In males the scrotum becomes oedematous and grossly swollen. Small secondary lesions appear over the body from day 6. These are often first visible as red pinpoints on the eyelid margins. These secondaries

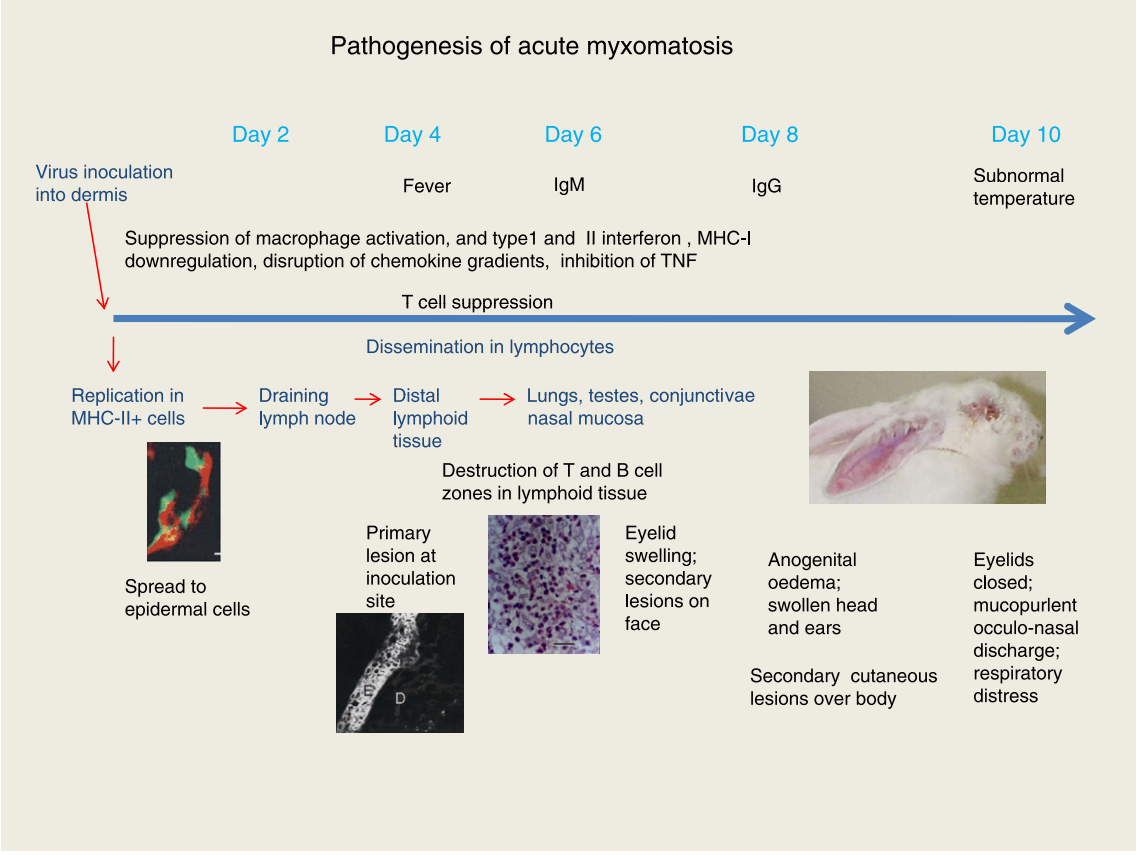

Fig. 12. Pathogenesis of virulent MYXV in susceptible European rabbits. Virus inoculated intradermally initially replicates in MHC-II positive cells at the dermal/epidermal junction and spreads to the overlying epidermal cells and to the draining lymph node. Replication in the epidermis induces cell proliferation and destruction with the formation of a raised primary lesion or myxoma. In the draining lymph node, MYXV replicates within T and B cell zones inducing massive loss of lymphocytes. It disseminates in infected lymphocytes and possibly monocytes to distal tissues including lymphoid tissue, lungs, testes, conjunctivae, nasal mucosa and distal skin. Generalised clinical signs of eyelid and facial swelling become apparent from days 5–6 with respiratory distress occurring from days 7 or 8. The virus suppresses host antiviral activity and both macrophage and T cell activation. However, IgM antibody can be detected by days 6–10 and IgG by day 10. Fever occurs from around day 4 but at late stages the rectal temperature may be subnormal. Death normally occurs between days 9–12.

gradually increase in size but death usually intervenes around day 10–12. Elevated rectal temperature occurs from about day 4 but becomes subnormal from day 8 or 9. At autopsy the main findings are the external features just described, lymph nodes are swollen up to 6× normal size and may be haemorrhagic, the spleen is usually grossly enlarged, the liver may have a mottled appearance and there may be petechial or larger haemorrhages on the surfaces of the viscera or serous membranes. In the acutely infected rabbit there may be very few other consistent findings (Hurst, 1937a; Best and Kerr, 2000).

The pathogenesis of myxomatosis is summarised in Fig. 12. Following intradermal inoculation of laboratory rabbits with SLS, viral replication can be visualised by immunofluorescence staining in MHC-II positive, dendritic-like cells at the epidermal/dermal junction and deeper in the dermis at 24 h but not at 12 h after infection (Best et al., 2000) (Fig. 13A). From these cells, virus spreads to epidermal cells and macrophages and other cells of the dermis. By 10 days after infection the virus is mostly found in the epidermal cells (see Fig. 5). A particular feature of the infection is the presence of large stellate or polygonal "myxoma cells" which appear to bud through or from the endothelium of the small blood vessels (Fig. 13B and C) (Hurst, 1937a; Ahlstrom, 1940; Best et al., 2000). These cells and the endothelial cells are positive for viral antigen. Within 24 h, SLS is present in the draining lymph node in cells of the paracortex directly under the subcapsular sinus and subsequently more generalised infection of lymphocytes of the cortex and paracortex occurs. Virus replication in the lymphoid tissue results in massive loss of lymphocytes from both the cortex and paracortex. This appears to be due at least in part to bystander apoptosis (Best et al., 2000). From the lymph node the virus spreads, probably in lymphocytes or monocytes, to distal tissues such as lung, testis, spleen and other lymphoid tissues and mucocutaneous sites such as the eyelids, anogenital region and upper nasal passages. Virus is not found free in the blood but is present in the white cell fraction (Fenner and Woodroofe, 1953). Secondary cutaneous lesions develop especially on the head and ears but can be all over the body. The virus-rich epidermis of the swollen eyelids and base of the ears provide key sites for mosquito transmission (Fenner and Woodroofe, 1953).

Skin and lymphoid tissue show the most dramatic histopathology; a combination of cell proliferation and destruction, particularly destruction of lymphoid tissues (Hurst, 1937a). The epithelial cells of the epidermis enlarge, proliferate and degenerate (Fig. 13D and E). These cells are packed with viral antigen. The underlying dermis is oedematous and disorganised, there may be an influx of polymorphs particularly lower in the dermis but few lymphocytes are visible (Fig. 13 B and C). "Myxoma cells" characterised by large swollen nuclei with fragmented chromatin (Rivers, 1930), appear to proliferate and migrate from the endothelium of the small blood vessels, which may be disrupted. These cells are frequently surrounded by polymorphs (Fig. 13 B and C).

In the lymphoid tissue there is often complete loss of the lymphocytes from both the T cell zones and the follicles (Fig. 13F). The reticular elements proliferate obliterating the sinuses, polymorphs

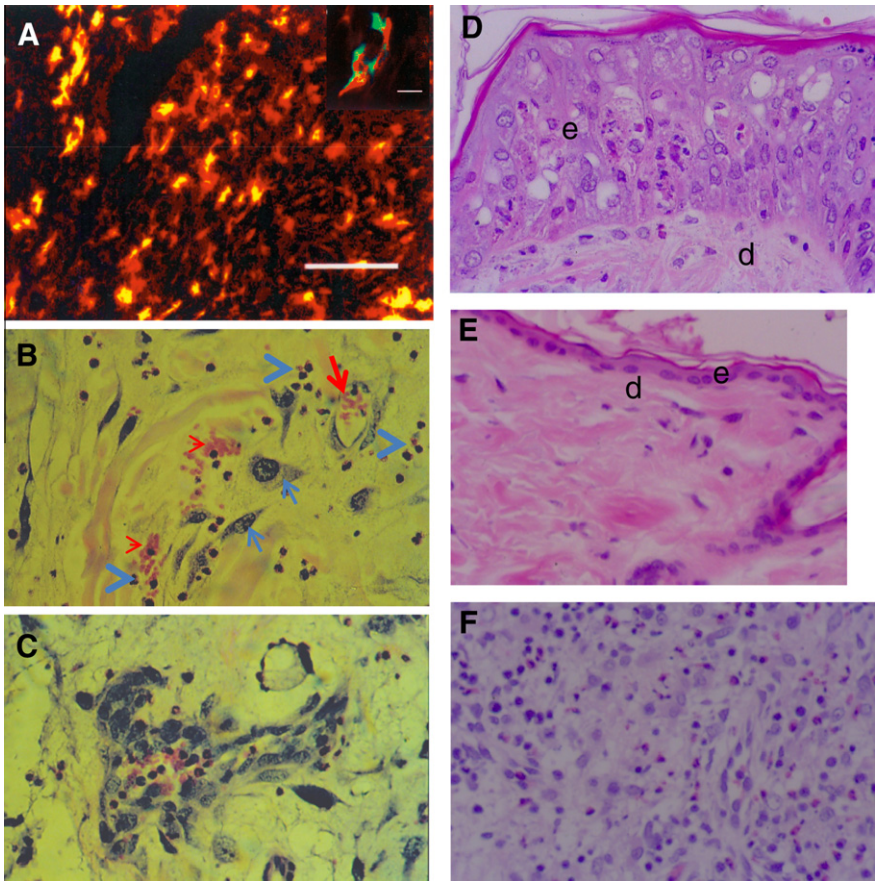

Fig. 13. Histology of myxomatosis. (A) Two colour immunofluorescence of skin at inoculation site at day 4. Red staining is MHC-II, virus antigen is stained green – overlapping colours are stained yellow. Inset shows artificial colour separation on a single cell. Scale bar = 80 μm. (B) Primary lesion: dermis showing typical "myxoma" cells (blue arrows) and "myxoma" cells budding from blood vessels (red arrows), blue arrowheads indicate neutrophils (heterophils in rabbits) and red arrowheads indicate erythrocytes. (C) Primary lesion: dermis showing blood vessel disruption by budding myxoma cells. (D) Skin section from primary lesion at inoculation site 10 days after infection (e indicates epidermis, d indicates dermis). (E) Normal skin section. Note the very thin epidermis in the rabbit. (F) Draining lymph node 10 days after infection showing complete loss of lymphocytes. Image A is from Best et al. (2000); Elsevier with permission; Images D, E and F provided by Dr. S. M. Best.

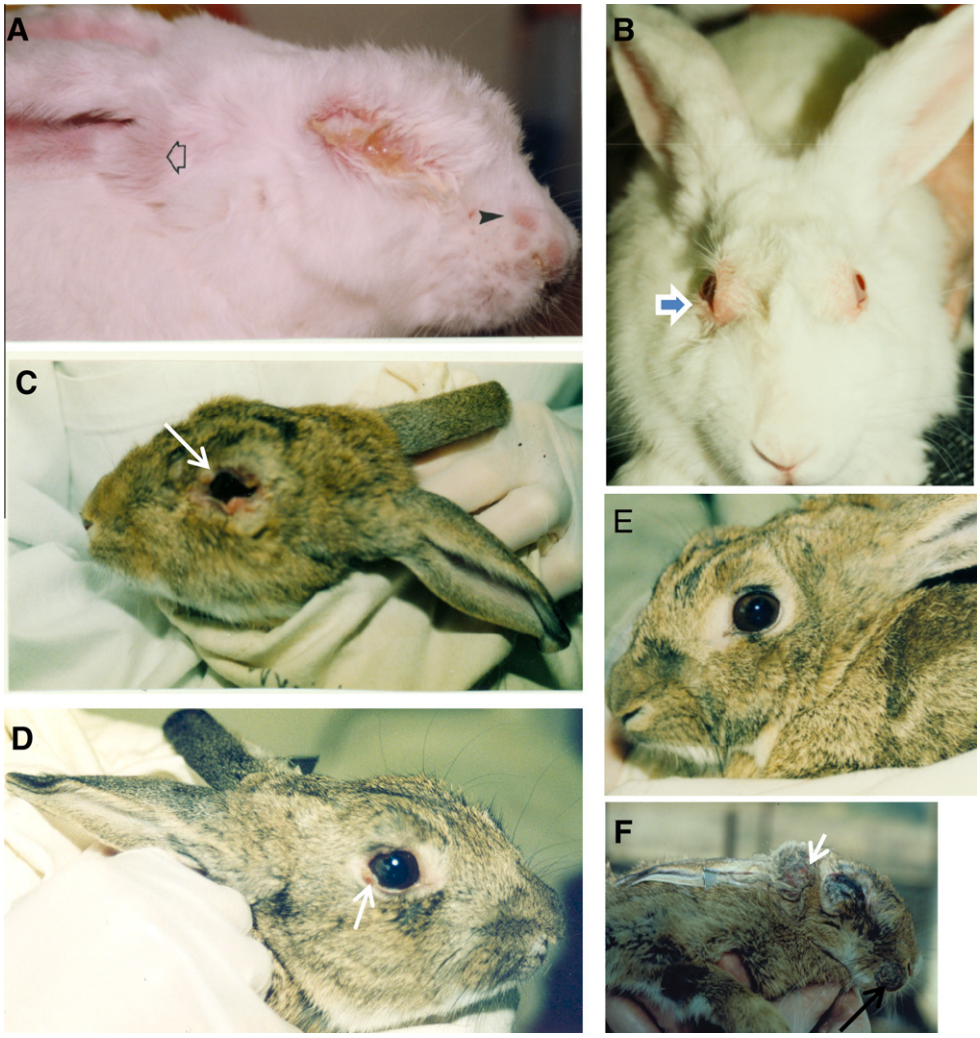

Fig. 14. Susceptible and resistant rabbits infected with virulent and attenuated viruses. (A) Susceptible laboratory rabbit 10 days after infection with the grade 1 virulent SLS. Note the completely closed eye, mucopurulent discharge, swollen head and ears. Arrow indicates swollen base of ear, arrowhead shows secondary lesions around the nose. (B) Susceptible laboratory rabbit 10 days after infection with the grade 5 Ur. Arrow indicates mild inflammation and swelling of eyelid. (C) Resistant wild rabbit 10 days after infection with SLS. Note the swollen head and base of ears but the eyes are open and the mucopurulent conjunctivitis present in the susceptible rabbit (Fig. A) is absent. Arrow indicates secondary lesion on eyelid, note the distortion of the lower lid. (D) Resistant wild rabbit 20 days after infection with SLS. Arrow indicates small secondary lesion on lower eyelid. Note the residual distortion of the upper eyelid but otherwise almost complete clinical recovery. (E) Resistant wild rabbit 10 days after infection with Ur. No clinical signs are visible apart from a primary lesion at the inoculation site (not shown). (F) Wild rabbit infected with the Lu strain of MYXV. Note completely closed eye and large secondary lesions at the base of the ear and on the muzzle (arrows). Lu is more virulent than SLS when tested in resistant rabbits. Figs. A, C, D, E are from Best and Kerr (2000); Elsevier with permission; Fig. B is from Dr. S. M. Best.

are often prominent and there may be haemorrhages from disrupted blood vessels. Myxoma cell proliferation from the small blood vessels is similar to the dermis (Hurst, 1937a; Best et al., 2000).

The same general processes occur in the lungs particularly in the peribronchial lymphoid tissue. The alveolar walls may show the typical proliferation and enlargement of the endothelial cells described for the dermis and lymphoid tissue leading to thickening of the alveolar walls sometimes causing loss of the alveolar lumen or alveolar haemorrhage. Hyperplasia and necrosis of the bronchial epithelium has also been described (Rivers, 1930; Hurst, 1937a; Ahlstrom, 1940).

The liver is not a major target for the virus, but infection of scattered hepatocytes occurs late in the course of infection with SLS (Mims, 1964). Three types of histological changes have been described in the liver: central lobular necrosis; dilated sinusoids with hyperplasia and degeneration of Kupfer cells and, occasionally, myxomatous changes in bile ducts and periportal connective tissue (Hurst, 1937a; Ahlstrom, 1940).

In the male, the testes and epididymis have high titres of virus accompanied by inflammation and degeneration (Hurst, 1937a; Fenner and Woodroofe, 1953; Fountain et al., 1997).

Local, and more generalised, immunosuppression occurs caused by specific viral proteins. This is seen as suppression of macrophage activity and loss of in vitro T cell proliferation in response to mitogens (Strayer, 1992; Jeklova et al., 2008; Cameron et al., 2005a,b) and the absence of pro-inflammatory cytokines such as IL-1β, IL-6 and MCP (macrophage chemoattractant protein) (Johnston et al., 2005a).

The cause of death in acute myxomatosis is unclear. Mims (1964) could not attribute death to virus replication causing pathology in vital organs. Secondary bacterial infection due to immunosuppression is often claimed to be the cause of death. However, in acutely infected rabbits signs of secondary bacterial infection are limited to the conjunctivae and upper respiratory tract; there is little gross pathology in the lungs. Asphyxiation due to nasal obstruction has also been proposed (Hobbs, 1928) but this is not always a feature. Moreover, rabbits infected with

hypervirulent viruses such as the Californian MSW strain or some Australian field strains die without overt signs of secondary infection suggesting that bacterial infection is not the primary cause of death in acute and peracute infections. Nor did antibiotic treatment have any influence on the outcome of acute infection (McKercher, 1952). Secondary bacterial infection is much more likely to be a complicating factor and a potential cause of death in rabbits infected with the more attenuated viruses; rabbits infected with grade 3 or 4 viruses may survive for some weeks in a miserable state with chronic respiratory disease and other complications of secondary bacterial infections. These infections may be particularly important in the pathogenesis of amyxomatous strains of MYXV (Marlier et al., 1999, 2000c).

Fenner and Marshall (1957) reported neurological signs following infections with Californian strains of MYXV and virus titres in the brain (Fenner and Ratcliffe, 1965). However, Silvers et al. (2006) did not find significant titres of MSW in brains from infected rabbits. Although some rabbits infected with field strains have relatively high titres of virus in the brain, this tends to be inconsistent (unpublished data). Resistant wild rabbits infected with a recombinant SLS expressing rabbit IL-4 had titres of 10^2–10^6 pfu/g of brain 8 days after infection (Kerr et al., 2004) while Swan (1941) reported virus in brains of rabbits 3–5 days after infection with MYXV and Hurst (1937c) also noted "appreciable amounts" of virus in the CNS. In domestic rabbits infected with Australian hypervirulent strains of MYXV, pulmonary oedema appears to be a significant cause of death, however, the pathogenesis of this has not been determined. It is possible that the massive destruction of lymphoid cells and the widespread tissue damage in the skin is triggering a septic shock type of condition in acute and peracute infections (Silvers et al., 2006; Stanford et al., 2007c). This is an area that would benefit from further investigation given the increasing recognition of the importance of cytokine storms in the pathogenesis of acute viral infections (Stanford et al., 2007b).

8.2. Infections with attenuated virus

The general course of disease in rabbits infected with attenuated viruses is similar to that already described. However, the time course may be delayed and prolonged depending on the virulence of the virus. There is also more variability in clinical signs and virus titres in skin lesions between rabbits (Fenner and Marshall, 1957; Fenner and Ratcliffe, 1965; see Table 3). Detailed pathogenesis studies with the attenuated grade 5 Ur strain, from which most rabbits recover, showed that virus titres were similar to SLS in the primary lesion, tended to be lower in the draining lymph node, but were 10–100-fold lower in the distal lymph node, spleen and lung, suggesting that the rabbit was better able to control replication at distal sites (Best and Kerr, 2000). Lymphocyte depletion was not a consistent feature of infection although many apoptotic lymphocytes were present in the draining node. Inflammation in the dermis at the inoculation site with a prominent mononuclear cell infiltrate was a major difference to SLS. Myxoma cells and endothelial cell proliferation were not features of Ur infection in the skin or lymph nodes (Best et al., 2000). In rabbits that survive, the cutaneous lesions gradually scab and resolve leaving prominent scarring. Chronic respiratory disease (snuffles) frequently occurs. Numerous studies have been done with gene knock-out viruses to define the role of individual genes in virulence (see Section 9).

8.3. Immune responses

Infected rabbits develop IgM and IgG antibodies to MYXV which can be detected in the serum by ELISA and neutralization tests as early as days 6–10 after infection with SLS or Lu. This is despite the immunosuppression induced by the virus and the profound destruction of lymphoid tissue including follicles and the fact that these rabbits were going to die over the next few days (Best and Kerr, 2000; Jeklova et al., 2008). Serum antibodies can be detected for at least 2 years in naturally infected wild rabbits and presumably persist for the life of the rabbit (Fenner et al., 1953; Kerr, 1997). Maternal IgG crosses the placenta and disappears from kittens by 6–9 weeks after birth (Fenner and Marshall, 1954; Kerr, 1997). Fenner and Marshall (1954) demonstrated that passively administered antibody could modify the course of virulent virus infection in young kittens from seronegative mothers; 3/18 infected kittens survived challenge with a very low dose (10 ID_{50}) of SLS and four further kittens did not become infected. In kittens with maternal antibody, 37/68 challenged with SLS by mosquito bite did not become infected but the infected kittens died albeit with prolonged survival times. Survival rates were higher when challenge was with a more attenuated virus (Fenner and Marshall, 1954). This indicates that antibody alone can provide some protection from natural infection and that maternal antibody could alter the epidemiology of myxomatosis particularly with attenuated viruses. However, in a separate study no advantage of maternal antibody was found in kittens exposed to MYXV by fleas or contact compared to animals with no antibody (Sobey and Conolly, 1975).

As with other poxvirus infections, cell mediated immunity and neutralising antibody are probably essential for clearing the virus and providing long term protection from reinfection and disease (Seet et al., 2003; Panchanathan et al., 2008). This will require activated dendritic cells and appropriate cytokine environments to produce an effective Th1 skewed CD4 + T cell response to MYXX. MYXV suppresses lymphocyte and macrophage activation (Cameron et al., 2005a,b) and inflammatory responses (Kerr and McFadden, 2002) and destroys or suppresses lymphocytes. Lymphocytes from rabbits infected with virulent virus do not respond to mitogen stimulation in vitro (Cameron et al., 2005a; Jeklova et al., 2008). Recovered rabbits challenged experimentally with MYXV develop a reaction at the inoculation site which is much more rapid than seen with primary infection suggesting that a cell mediated immune response is occurring in response to virus antigen (Fenner et al., 1953; Joubert et al., 1972; Kerr, 1997; Kerr et al., 2004).

8.4. Clearance of virus – Does persistent infection play any role in epidemiology?

Experimentally, rabbits recovered from myxomatosis do not normally develop generalised myxomatosis following challenge even with high doses of virulent virus (Fenner et al., 1953; Kerr, 1997; Kerr et al., 2004), although localised skin lesions and a rise in serum antibodies have been described in recovered rabbits continually exposed to virus and fleas (Sobey and Conolly, 1975). One rabbit that had survived clinical myxomatosis as a kitten due to passive immunization later developed generalised myxomatosis on challenge as an adult (Fenner and Marshall, 1954). However, vaccinated rabbits can have quite limited protection particularly if challenged with high doses of virus. In one study, 50–95% of rabbits developed clinical myxomatosis and shed virus only 7 weeks after vaccination when challenged with 1500 pfu of MYXV (Marlier et al., 2000a). In addition, there have been reports of previously recovered wild rabbits observed with clinical myxomatosis (Williams et al., 1972; Fullagar, 1977; Fenner and Ross, 1994). Such observations need to be interpreted carefully since bacterial upper respiratory tract infections mimic many of the clinical signs of myxomatosis and can confuse diagnosis even by experienced field workers.

The idea that rabbits can remain persistently infected with MYXV with no clinical signs of disease and subsequent recrudescence of "latent virus" has been proposed to explain the appearance of myxomatosis at isolated field sites and the introduction

of myxomatosis into rabbit farms (Dunsmore et al., 1971; Williams et al., 1972; Arthur and Louzis, 1988; Kritas et al., 2008). Two experiments have been done to test this hypothesis. In one trial, 20 wild rabbits recovered from myxomatosis were housed in an isolated room and subjected to heat/cold stress and adrenocorticotrophic hormone (ACTH) treatment over a 7 month period. One rabbit developed signs of myxomatosis; conjunctival material from this rabbit inoculated into two susceptible rabbits led to the death of both inoculated rabbits with "typical myxomatosis". However, conjunctival discharges from other rabbits in the group with swollen eyelids and conjunctivitis did not transmit any disease (Williams et al., 1972). Virus isolation was not reported. Fenner and Ross (1994) note that other workers were unable to repeat these results. In the second study, 11 domestic rabbits recovered from infection with MYXV 98 days earlier were stressed by daily injections of ACTH for 10 days. The rabbits developed swollen eyelids and serous blepharoconjunctivitis as well as other signs associated with mild clinical myxomatosis; 7 rabbits died of bacterial bronchopneumonia. However, despite the similarity of clinical signs to myxomatosis, no virus could be detected in eyelids, conjunctival or respiratory secretions or in the mononuclear cell fraction of the blood (Marlier et al., 2000c). It seems likely that the clinical signs were due to bacterial disease in the face of immunosuppression. In 3 of the rabbits that died, MYXV was detected in the testis (an immune privileged site) suggesting that virus can persist in the testis for at least 125 days after infection (Marlier et al., 2000c). Fountain et al. (1997) demonstrated that MYXV DNA could be detected by PCR for at least 120 days in the testes of laboratory rabbits following infection with a highly attenuated grade 5 virus. However, infectious virus was not detected after day 30. Although MYXV has been transmitted in semen (Marlier et al., 2000c), the epidemiological significance of this for wild rabbits is unclear; it could provide a means of MYXV entry into commercial rabbit farms.

Williams et al. (1972, 1973) reported that more than 50% of wild rabbits previously recovered from myxomatosis became seronegative in winter/spring. They proposed that this allowed recrudescence of latent virus that boosted antibody titres and initiated epizootics of myxomatosis. This was based on measurements of antibody from blood samples absorbed onto filter paper and analysed using gel immunodiffusion with an antigen extract from MYXV (Sobey et al., 1966, 1970b). No comparison was made with any alternative assay, such as virus neutralization, to test these observations. The immunodiffusion assay has low sensitivity; serum neutralization tests were 100× more sensitive and detected antibody in rabbits that were negative by gel immunodiffusion (Vaughan and Vaughan, 1968). In contrast, antibody measured by complement fixation persisted for years at a reasonable titre after an initial drop and neutralization antibodies could be detected for similar periods (Fenner et al., 1953). In Britain, at multiple sites, the majority of rabbits were seropositive during the winter (Vaughan and Vaughan, 1968). Other studies using ELISA and neutralization assays did not show fluctuation of antibody titres from month to month in Australian wild rabbits over a 12 month period (Kerr, 1997).

There is no evidence of genuine latency for any poxvirus although viruses such as *Molluscum contagiosum* may persist for prolonged periods (Damon, 2007). MYXV is adapted to persist in skin lesions of its natural host by suppressing and evading the immune response. In European rabbits, Fenner et al. (1956) isolated MYXV from secondary tumours 60 days after infection and demonstrated mosquito transmission 40 days after infection. In wild European rabbits experimentally infected with a field strain of MYXV it was possible to transmit virus by needlestick from eyelids and secondary lesions for at least 48 days after inoculation (Kerr et al., 2003) and transmission by contact for at least 47 days has been reported (Mykytowycz, 1953). The related leporipoxvirus, RFV, can be transmitted by mosquitoes for as long as 10 months from the lesions of eastern cottontails infected as young kittens thus providing a mechanism for overwintering in the absence of vectors (Kilham and Dalmat, 1955). Even though European rabbits normally quickly clear RFV, it was recoverable from skin lesions of some European rabbits with more disseminated disease for 108 days after infection (Hurst, 1937c). However, in all these cases there was visible evidence of ongoing lesions. Other studies have noted contact transmission of very attenuated MYXV based on seroconversion in the absence of clinical signs of myxomatosis (Bárcena et al., 2000b). Obviously, MYXV can persist for prolonged periods in some rabbits, whether genuine recrudescence of disease and virus shedding can occur and have epidemiological significance remains to be clearly demonstrated.

8.5. Vaccination against myxomatosis

Early attempts at vaccination using virus inactivated by formaldehyde, phenol, chloroform, crystal violet, nitrogen mustard or heat or by using soluble viral antigens were largely unsuccessful with both South American and Californian MYXV (Hobbs, 1928; Kessel et al., 1934; Rivers et al., 1939; McKercher, 1952). In only one study was significant resistance to challenge, measured as prolonged survival time and recovery of some rabbits, obtained. This protocol used 5–7 injections, 3–5 days apart, of heat inactivated South American MYXV and the protection was enhanced by the addition of live pneumococci as an adjuvant (Hyde, 1939a; McKee, 1939).

However, in the 1930s it was shown that previous infection with RFV protected rabbits from challenge with South American MYXV (Shope, 1932, 1938; Hurst, 1937c; Hyde, 1939b). RFV induces a cutaneous fibroma 1.5–4 cm in diameter at the inoculation site depending on the virus strain. This resolves over 2–5 weeks without overt disease except in very young kittens or immunosuppressed rabbits where generalised disease may result (Fenner and Woodroofe, 1954; Hyde and Gardner, 1939; Allison, 1966; Allison and Friedman, 1966; Joiner et al., 1971; Smith et al., 1973; Pathak and Tompkins, 1974). The protection delivered by RFV varies with the strain of virus; Boerlage is more effective than OA but causes a larger fibroma (Fenner and Woodroofe, 1954). Rabbits immunized with OA succumbed to challenge with MYXV 2–5 months after immunization while some rabbits immunized with Boerlage still resisted challenge with very low doses of MYXV (10 ID_{50}) up to 12 months later (Fenner and Woodroofe, 1954). Higher challenge doses were associated with much less protection (Fenner and Woodroofe, 1954; Marlier et al., 2000a). Importantly, vaccinated rabbits can become infected on challenge and shed MYXV (Fenner and Woodroofe, 1954; Marlier et al., 2000a).

RFV was used to protect laboratory and subsequently farmed rabbits from myxomatosis in Australia (Fenner and Woodroofe, 1954) and was used in Europe after the release of MYXV. RFV is still used as a heterologous vaccine to protect domestic, pet and farmed rabbits against myxomatosis in the UK and other European countries and was also used in attempts to protect wild rabbits in Europe (Arthur and Louzis, 1988). Vaccination needs to be repeated at least every 6 months and possibly more frequently depending on the challenge conditions. Vaccination of domestic rabbits against MYXV has not been permitted in Australia since 1962 for fear that the live virus vaccine could establish in the wild population and inhibit the effects of myxomatosis.

Attenuated homologous MYXV vaccines have also been widely used. The first of these was produced from the Californian MSD strain of MYXV by serial passage in rabbit kidney cells (McKercher and Saito, 1964; Saito et al., 1964).This virus is used in Europe to protect rabbits (Gorski et al., 1994). Interestingly, a virus which

appears to be genetically derived from this vaccine has been isolated from rabbits with mild myxomatosis in the Czech Republic (Pšikal et al., 2003).

The best characterised attenuated live MYXV vaccine is the French strain SG33 (Saurat et al., 1978; Camus-Bouclainville et al., 2011), derived from a naturally attenuated field isolate, which was further attenuated by serial passage at 33 °C in rabbit kidney cells followed by serial passage in chick embryo fibroblasts. SG33 has a 13.5 kb deletion at the right-hand end of the genome encompassing a number of virulence genes and truncating the terminal inverted repeats. It also appears to have recombined with a Californian virus strain at some point (Camus-Bouclainville et al., 2011), possibly the vaccine strain. SG33 has been widely used in both domestic and wild rabbits but like RFV does not necessarily prevent infection and shedding. The duration of protection can be quite short with case fatality rates of 20–30% following challenge with 1000 ID_{50} 6–8 weeks after vaccination. Lower challenge doses were associated with better protection and are probably a more realistic test (Picavet et al., 1992). Similar results with high challenge doses were obtained by Marlier et al. (2000a). Because the SG33 strain was associated with immunosuppression in some rabbit facilities, it has been recommended to vaccinate with RFV and then with SG33 3 weeks to 2 months later followed by boosters with SG33 every 4–6 months (Brun et al., 1981; Vautherot et al., 1997). Other attenuated homologous live vaccines include the Italian Borghi strain (Cavadini et al., 2010) and the Spanish Poxlap (Marlier, 2010).

For vaccines, there is a clear trade-off between protection against myxomatosis and attenuation, and hence safety. Experimentally, less attenuated viruses stimulated a stronger immune response (Adams et al., 2008b). A completely safe vaccine with no side effects or potential for transmission can be produced by deletion of the rabbit host-range M063 gene (Barrett et al., 2007a). However, immunization with M063 deletion mutants provides only short term protection against challenge (Adams et al., 2008a).

The SG33 strain has been engineered to express the RHDV capsid protein and shown to protect against both myxomatosis and RHD (Bertagnoli et al., 1996). An attenuated field strain of MYXV from Spain (strain 6918; Morales et al., 2009) has been similarly engineered and tested in laboratory and field studies as a vaccine against both myxomatosis and RHD for wild rabbits (Bárcena et al., 2000a; Torres et al., 2000, 2001; Angulo and Bárcena, 2007).

8.6. Drug therapy for myxomatosis

Several drugs are in development for human diseases caused by orthopoxviruses such as VACV, monkeypox virus (MPXV) and variola virus (VARV) (De Clercq, 2010). The two most advanced candidates are ST-246, an antiviral developed against the F13 protein of VACV, and CMX001, a derivative of a nucleoside analogue called cidofovir. Cidofovir has broad activity against poxviruses including orthopoxviruses, molluscipoxvirus and parapoxvirus in humans (Andrei and Snoeck, 2010). ST-246 is ineffective against MYXV because the F13 orthologue in MYXV is too diverged to be recognised by the drug, but CMX001 inhibits MYXV replication in cultured cells as effectively as VACV, and has the potential to be used as an antiviral against MYXV in rabbits or humans (G. McFadden, Personal Communication). CMX001 administered orally has been demonstrated to protect rabbits from lethal disease following infection with the orthopoxvirus rabbitpox (RPXV) either administered for 5 days starting 24 h prior to infection or administered from the onset of clinical disease 3 days after infection; delaying treatment to day 4 was less effective (Adams et al., 2007; Rice et al., 2011). Cidofovir administered either as an aerosol or intravenously has also been shown to be effective against RPXV in rabbits (Verreault et al., 2012).

8.7. Pathogenesis studies in resistant rabbits

Despite studies on resistance dating back into the 1950s there has been remarkably little analysis of the mechanism of innate resistance. Resistant rabbits are not resistant to infection, but are more likely to control the infection and recover (Fig. 14). For example, wild rabbits infected with SLS developed severe clinical myxomatosis during the first 10 days after infection but from about day 12 were visibly controlling the disease and 4/5 made a rapid recovery (Kerr et al., 2004). By contrast laboratory rabbits infected with the same virus all died between days 10 and 12 (Robinson et al., 1999). As occurs with attenuated viruses in laboratory rabbits, there is a wide range of clinical signs and survival times in resistant rabbits infected with MYXV. Some rabbits have severe, including lethal, generalised myxomatosis, others have a milder but generalised form of the disease, while a few show nothing but a primary lesion at the inoculation site followed by seroconversion (Ross and Sanders, 1977; Kerr et al., 2003).

There are some indications from experimental pen trials with wild rabbits that low doses of virus, particularly attenuated virus, may not infect a small proportion of resistant rabbits (Vaughan and Vaughan, 1968; Kerr et al., 2003), however, virtually all rabbits exposed to epizootics of myxomatosis in the wild seroconvert indicating that they do become infected (Kerr et al., 2010).

In vitro testing of primary fibroblasts or lymphocytes prepared from resistant or laboratory rabbits showed no difference in productive infection with virulent MYXV suggesting that resistance was not due to any lack of receptor or cell competence for virus replication (Best and Kerr, 2000). In comparisons of pathogenesis of laboratory and wild rabbits infected with SLS, viral replication was particularly constrained in draining and distal lymph nodes, spleen and lung with titres 10–100-fold lower in wild rabbits than in laboratory rabbits (Fig. 15) (Best and Kerr, 2000). The depletion of lymphocytes from the lymphoid tissue was still present but varied between rabbits and by 15 days after infection lymph nodes had recovered (Best et al., 2000). Similarly, at the inoculation site, although high titres of virus were present, there was also a pronounced inflammatory response and mononuclear cell infiltrate. The general picture was similar to laboratory rabbits infected with attenuated virus, although with some differences in detail, suggesting that resistance effectively reduces the virulence of the infecting virus. When infected with a grade 5 virus, resistant rabbits showed very few clinical signs of myxomatosis apart from a primary lesion at the inoculation site. Fig. 14E shows a typical resistant wild rabbit infected with a grade 5 virus. At the molecular level, resistant rabbits had strong expression of nitric oxide synthase at the infection site, which was not present in laboratory rabbits (Labudovic, van Leeuwen and Kerr unpublished) suggesting that activation of macrophages was occurring, which in laboratory rabbits is suppressed by MYXV (Cameron et al., 2005a,b).

These studies suggested that the innate immune response is probably critical for resistance. Skewing of the response towards an effective antiviral innate response would lead to an effective T and B cell response that controlled infection and allowed recovery similarly to the resistance model for mousepox caused by ectromelia virus (ECTV) (Kerr and McFadden, 2002; Chaudhri et al., 2004). This hypothesis was tested by challenging resistant wild rabbits with recombinant MYXV expressing rabbit IL-4 with the aim of skewing the immune response towards a Th2 pattern. SLS-IL-4 completely overcame genetic resistance. A very attenuated recombinant Ur virus was also engineered to express IL-4. This increased its case fatality rate for laboratory rabbits to essentially 100% from effectively zero but resistant wild rabbits all survived infection with this virus (Kerr et al., 2004).

Genetic resistance can also be overcome by infection with more virulent viruses, thus Lu was demonstrated to be more virulent

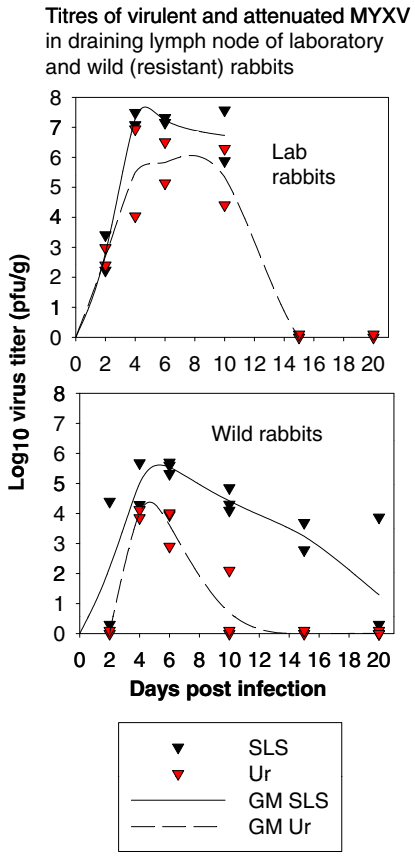

Fig. 15. Replication of MYXV in resistant and susceptible rabbits. (A) Titres of virulent SLS and attenuated Ur in the lymph node draining the inoculation site of fully susceptible laboratory rabbits. (B) Titres of SLS and Ur in the lymph node draining the inoculation site of wild resistant rabbits. Lines show geometric mean titres for each virus. Data are replotted from Best and Kerr (2000).

than SLS when tested in resistant rabbits (Fenner and Ross, 1994) (Fig. 14F) and the Californian MSW strain of MYXV was able to completely overcome resistance in Australian wild rabbits (Silvers et al., 2006). Understanding the physiological and genetic basis for resistance is an obvious area of research which should be advanced by the current sequencing of the rabbit genome and the use of high throughput sequencing for transcriptional studies.

9. Genetics of resistance

9.1. Selection studies

The genetic basis of resistance is not understood. Whether there is a single gene responsible for resistance with multiple alleles encoding various levels of resistance or multiple genes has never been defined. Similarly, it is not clear whether there is a gene dosage effect with homozygotes having stronger resistance than heterozygotes. An interesting question is whether there is any evolutionary cost to strong resistance, for instance by increasing susceptibility to intestinal or respiratory pathogens and parasites due to a skewing of the immune response. Attempts to understand the genetics of resistance to MYXV were made by selecting laboratory rabbits for resistance (Sobey, 1969). After six generations of selection an average of 21% survived challenge with SLS and the distribution of survival times was markedly extended. However, this survival was strongly age skewed with rabbits 10–18 weeks of age having a much higher survival rate than older rabbits (Sobey et al., 1970a). Beyond this period there was little increase in resistance (Sobey and Conolly, 1986; Fenner and Ross, 1994). This was considered a similar level of resistance to that seen in the field after a similar period of selection i.e. 6–7 years of annual epizootics (Fenner and Ross, 1994). Heritability of resistance was estimated at 0.35–0.40 (Sobey, 1969).

9.2. Sire effect

A reanalysis of the data from the selection studies led to the concept that resistance was due, in part, to some temporary "non-genetic" factor transmitted to his offspring by a male that had survived infection. This was termed the "sire effect" (Sobey and Conolly, 1986). Rather oddly, this factor was also considered to be passed on, for at least 6 months, by females that had previously mated with a recovered male when mated with a non-exposed male. It was also considered that the resistance was at least partially specific for the virus strain to which the male had been exposed, although the genetic differences between Australian strains are very limited. In a reanalysis of a separate set of experimental data, rabbits whose sires had been challenged less than 10 months prior to their birth had a 20% survival advantage compared to rabbits whose sires were challenged >10 months earlier (Parer et al., 1995). This advantage was not observed in rabbits whose sires had been immunized with RFV rather than challenged with MYXV. A similar effect based on reanalysis of infections of wild rabbits from immunized parents was reported by Williams and Moore (1991).

If real, the sire effect would exaggerate the impact of genetic resistance in the wild rabbit population. No prospective studies have been undertaken to reproduce this effect and no mechanism has been postulated to explain it. However, studies using wild rabbits bred in an animal house from parents that had never been exposed to myxomatosis, and so could not exhibit the postulated sire effect, confirmed that Australian wild rabbits have very high resistance to myxomatosis that is best explained by natural selection for genetic variants (Kerr et al., 2004, 2010; Best and Kerr, 2000).

10. Molecular basis of pathogenesis

10.1. Virulence and host-range genes

The complete genome sequence of the Lu strain of MYXV was published in 1999 (Cameron et al., 1999).[1] Comparison with other poxvirus genomes such as VACV allows the division of the genes into two basic classes: first, those associated with virus structure and assembly, gene transcription, DNA replication, cell entry and exit – with some minor differences, these genes tend to be conserved both in sequence and gene order between poxviruses (Gubser et al., 2004). The second group of genes encode proteins important for suppression and evasion of host cell and tissue, intrinsic, innate and adaptive antiviral responses and are often referred to as immunemodulatory and host-range genes. These genes tend to occur towards the termini of the genome, are less conserved between poxviruses and generally not necessary for replication in cultured cell lines. Disruption of these genes frequently attenuates the virus. Genes encoding proteins with known roles in pathogenesis and host-range are described in Table 4. In addition, there are at least 15 proteins which may play important roles in pathogenesis based

[1] Genes are numbered based on their location in the MYXV genome from the left hand end with the direction of transcription indicated by L or R eg *M010L*. Genes in the TIR are indentified by L/R eg *M007L/R*. Following recent usage (Condit et al., 2006; Moss, 2007) proteins are identified by the same number as the gene with the transcription direction omitted ie M010. If the protein has a well known functional name, that has been added in brackets eg M010 (Myxoma growth factor; MGF). Where genes from rabbit fibroma virus are referred to they are prefaced with S rather than M (Willer et al., 1999).

Table 4
The impact on virulence of proteins that modulate the host response to infection.

Gene	Protein function (No. of amino acids; transcription time: E, I, L)	Effect on virulence of knock out	Reference
M001L/R	Chemokine binding (260; E)	Generalized myxomatosis; 1/6 survived	Lalani et al. (1999)
M002L/R	TNF binding; antiapoptosis (326; E)	Moderate to severe myxomatosis; 5/8 animals survived	Upton et al. (1991), Macen et al. (1996), Schreiber et al. (1997), Sedger et al. (2006)
M004L/R	RDEL motif; antiapoptosis (237; E)	Small rapidly resolved primary lesions; 1/8 rabbits had a secondary; all animals recovered	Hnatiuk et al. (1999), Barry et al. (1997)
M005L/R	Host-range; antiapoptosis; E3 Ub ligase(483; E)	Primary lesion only; rapid resolution; no signs of clinical myxomatosis	Mossman et al. (1996a)
M007L/R	Secreted IFN- γ binding protein; chemokine binding (263; E)	12/13 rabbits mild to moderate disease; lymphocyte infiltration	Mossman et al. (1996b), Lalani et al. (1997)
M008.1L/R	Serp 1; secreted serine proteinase inhibitor (369; L)	Moderate to severe generalised myxomatosis; 5/8 rabbits recovered from infection; enhanced inflammatory response	Macen et al. (1993)
M010L	Myxoma growth factor; epidermal growth factor homologue (85; E)	Moderately severe generalised myxomatosis; 25% of animals became moribund; 75% recovered	Opgenorth et al. (1992)
M011L	Antiapoptotic factor (166; E)	All rabbits survived; large protuberant demarcated primary; large secondaries; mild conjunctivitis/rhinitis	Opgenorth et al. (1992), Graham et al. (1992)
M013L	Pyrin domain inflammasome; NFκB inhibition (126; E)	Mild clinical signs rapidly resolved; small secondaries; no mortality; rapid inflammatory response	Johnston et al. (2005a), Dorfleutner et al. (2007), Rahman et al. (2009), Rahman and McFadden (2011)
M029L	Type I interferon resistance/PKR inhibition (115; E)	Not tested	Cameron et al. (1999), Myskiw et al. (2011)
M062R	Host range (158;E/L)	Abortive infection in rabbits and rabbit cells	Liu et al. (2011)
M063R	Host range (215; E)	No virus replication in rabbits and rabbit cells	Barrett et al. (2007a)
M128L	CD47 homologue; macrophage inhibition (281; L)	Mild generalised disease; rapid resolution; no mortality	Cameron et al. (2005b)
M130R	Unknown function; localised to ER/Golgi, (122; L)	Clinical generalised myxomatosis but no deaths	Barrett et al. (2009)
M131R	Superoxide dismutase inhibition (163; L)	All animals euthanised days 10–11; RFV is attenuated	Cao et al. (2002), Teoh et al. (2005). (2003)
M135R	Immunomodulatory (178; E)	All animals survived; mild disease with little generalization	Barrett et al. (2007b)
M138L	Sialyltransferase (290; E)	Severe fatal myxomatosis; survival time prolonged	Jackson et al. (1999)
M141R	OX-2 homologue (218; E)	Mild generalised disease, rapid resolution, all survived; increased macrophage and T cell activation	Cameron et al. (2005a)
M148R	Ankyrin repeat; putative E3 Ub ligase (675; L)	Moderate generalised myxomatosis; 2/5 rabbits euthanised at 21 days; mononuclear inflammatory response	Blanié et al. (2009), Zhang et al. (2009)
M149R	Ankyrin repeat; putative E3 Ub ligase (490; E/L?)	Moderate generalised myxomatosis with delayed secondaries; 5/5 rabbits survived	Blanié et al. (2009), Zhang et al. (2009)
M150R	NFκB inhibition; E3 Ub ligase (494; E)	Rapid inflammatory response at primary site; few small secondaries; 12/12 recovered by d21	Camus-Bouclainville et al. (2004), Zhang et al. (2009), Blanié et al. (2010)
M151R	Serp 2 (333; E)	Local primary but few or no secondary lesions; 7/10 infected rabbits recovered; 3/10 euthanised –respiratory disease	Messud-Petit et al. (1998)
M152R	Serp 3 (266; L)	4/10 infected rabbits recovered; 6/10 euthanised because of respiratory disease; no 2° lesions	Guérin et al. (2001)
M153R	MHC downreg; E3 Ub ligase (206; E)	Generalized myxomatosis; 4/12 rabbits euthanised day 14, the remainder recovered	Mansouri et al. (2003), Guérin et al. (2002), Collin et al. (2005)
M156R	Interferon resistance; eIF2α homologue (102; L)	Not tested	Ramelot et al. (2002)

Table 5
MYXV genes encoding potential modulators of the host response to infection.

Gene	Protein function	Reference
M003.1L/R	VACV B15 orthologue; Bcl-2-fold	González and Esteban (2010)
M006L/R	Gene family; putative E3 Ub ligase	Zhang et al. (2009)
M008L/R	Gene family; putative E3 Ub ligase	Zhang et al. (2009)
M009L	Gene family; putative E3 Ub ligase	Zhang et al. (2009)
M014L	Putative E3 Ub ligase	Zhang et al. (2009)
M064R	Potential host range	Cameron et al. (1999)
M104L	Potential immunomodulatory	Cameron et al. (1999)
M121R	NK cell receptor homologue	Cameron et al. (1999)
M122R	NK cell receptor homologue	Cameron et al. (1999)
M136R	Homology to VACV A52; Bcl-2-fold	Moss (2007), González and Esteban (2010)
M139R	Homology to VACV A52; Bcl-2-fold	González and Esteban (2010)
M140R	Putative E3 Ub ligase	Zhang et al. (2009)
M143R	RING-E3 Ub ligase; possible apoptosis regulator	Hovey Nerenberg et al. (2005), Brick et al. (1998)
M146R	VACV N1 orthologue;TLR signal inhibition; Bcl-2-fold antiapoptosis	Cameron et al. (1999), Moss (2007), González and Esteban (2010)
M154L	Vac M2 orthologue; downreg of NFκB?	Cameron et al. (1999), Gedey et al. (2006)

on their orthology with other poxvirus proteins or predicted functions (Table 5). The immunomodulatory and host-range genes of MYXV evolved in *Sylvilagus* species of leporid not in *Oryctolagus*. However, all of the functional analysis of these genes and their encoded proteins has been done in the European rabbit where the pathogenesis of MYXV is quite different. These differences and the overall pathogenesis must be due to the actions of some of these gene products.

10.2. Replication, assembly and exit from infected cells

Much of the understanding of poxvirus replication comes from research on VACV. Poxviruses replicate exclusively in the cytoplasm of infected cells. Infection is associated with activation of multiple cell signalling cascades which can in part determine cell permissivity for MYXV (Masters et al., 2001; Johnston et al., 2003; Villa et al., 2010). Following cell entry, the virion core is transported to the perinuclear region where partial uncoating occurs and early gene transcription takes place using enzymes and transcription factors packaged in the virion (a poxvirus class of immediate early genes has also been suggested (Assarsson et al., 2008). This is followed by transcription of intermediate and late gene classes (Broyles, 2003; Moss, 2007). Viral assembly occurs in virus factories termed virosomes. Two forms of infectious virus are produced: intracellular mature virions (MV; previously termed IMV), which are released upon cell lysis and extracellular enveloped virions (EV; previously termed EEV), which are produced from an intermediate form termed wrapped virion (WV; previously IEV), which has acquired a double membrane from the Golgi or endoplasmic reticulum. EV express an extra set of viral encoded proteins on the envelope. WV are transported to the cell membrane by microtubules and bud through the plasma membrane losing one layer of the double membrane to become EV. Some EV remain associated with the cell membrane and have been called cell associated enveloped virus (CEV). Both MV and EV are infectious; in the VACV model for poxviruses, EV are critical for cell to cell spread and for dissemination in the infected host while MV are thought to be more important for transmission (reviewed by Roberts and Smith (2008)). Electron microscopy of infected cells has demonstrated that MYXV produces both MV and EV particles (Duteyrat et al., 2007). MYXV does not form intracellular A type inclusion bodies typical of some poxviruses as it lacks the necessary genes. In addition, MYXV lacks an orthologue of the VACV A36 protein, one of the 6 proteins found in VACV EV (Barrett et al., 2001), although the other MV and EV proteins are encoded by MYXV.

10.3. Poxvirus entry into cells

No specific cell receptor has been defined for any poxvirus (Bengali et al., 2009; Roberts and Smith, 2008). VACV MV attaches to the cell surface and can either fuse its membrane with the cell membrane, mediated by a complex of viral fusion proteins, inserting the viral core directly into the cytoplasm, or enter cells by a process of endocytosis (Townsley et al., 2006) or macropinocytosis (Mercer and Helenius, 2008; Mercer et al., 2010), subsequent fusion of the MV membrane and endosomal/pinosome membrane inserts the viral core into the cytoplasm (Townsley et al., 2006; Bengali et al., 2009). EV particles rupture their envelope on binding to cell glycosaminoglycans exposing the MV membrane in apposition to the plasma membrane and allowing either fusion of the MV membrane at the cell surface or uptake by macropinocytosis and subsequent fusion within the endosome (Roberts et al., 2009; Roberts and Smith, 2008). Differences in entry requirements for MYXV compared to VACV have been demonstrated in human cancer cells (Villa et al., 2010). MYXV was not able to enter by fusion at the cell surface but entry was inhibited by prevention of endosome acidification suggesting that the endosomal/macropinocytosis pathway was used.

10.4. Subversion of the host-antiviral response by MYXV

Like other viruses, MYXV hijacks, subverts and manipulates key elements of the intracellular and extracellular environment to facilitate its replication, dissemination and transmission (Masters et al., 2001; Johnston et al., 2003). Within the cell, MYXV proteins inhibit interferon (IFN) and inflammatory cytokine production, cell death due to apoptosis and the recognition of infected cells by innate and adaptive immune responses. MYXV regulates the external environment by secreting proteins that bind tumour necrosis factor (TNF) (M002), chemokines (M001; M007) and Type II IFN (M007); inhibit inflammatory cascades (M008.1/Serp 1), and stimulate cell proliferation through epidermal growth factor receptors (M010/MGF) and ErbB signalling (Seet et al., 2003; Tzahar et al., 1998). In addition, virus proteins expressed on the cell surface inhibit activation of macrophages and T cells (M128; M141) (Cameron et al., 2005a,b). These mechanisms are considered in more detail below; some are relatively rabbit-specific while others operate more broadly across species and cell types.

10.4.1. Subversion of pattern recognition receptor signalling

Initial recognition of virus infection in cells occurs through pattern recognition receptors (PRRs) (Wilkins and Gale, 2010; Bowie and Unterholzner, 2008) which recognise pathogen associated patterns (PAMPs) on the cell surface, in endosomes or the cytoplasm. Signalling via PRRs stimulates gene transcription, expression of interferons and activation of proinflammatory cytokines such as IL-1β and IL-18 through cleavage by activated caspase 1 in the inflammasome. Type 1 IFN and IL-1β/IL-18 stimulate antiviral responses by paracrine and autocrine binding to specific receptors leading to transcription of the interferon response genes and other proinflammatory molecules such as TNF that attract inflammatory cells, and mediators such as complement to the site of infection. This proinflammatory antiviral response is critical for the development of an adaptive immune response with specific CD8+ and CD4 + T cells and neutralising antibody which can clear the virus and provide protection from subsequent infection.

NFκB has a central position in transcriptional control of responses to viral infection from PRRs and signalling by proinflammatory cytokines such as TNF and IL-1β. Pathways leading to NFκB activation are major targets for poxvirus manipulation (Mohamed and McFadden, 2009). Two MYXV proteins (M013 and M150) have been implicated in inhibiting activation of NFκB and a further five may have a role in disruption of signalling leading to activation of NFκB. In addition, disruption of TNF signalling by the M002 protein will also potentially disrupt NFκB activation (Mohamed and McFadden, 2009).

M013 is an early protein of 126 amino acids with an N-terminal pyrin domain (PYD) implicated in inhibition of caspase 1 activation in the inflammasome (Johnston et al., 2005a). In human monocytic cells, M013 also binds to NFκB1/p105 inhibiting its degradation and preventing the translocation to the nucleus of the NFκB family member RelA (p65) in response to MYXV infection (Rahman et al., 2009). This prevented the expression of proinflammatory cytokines controlled by NFκB independently of M013 binding to ASC-1 (apoptosis associated speck like protein containing CARD) in the inflammasome (Rahman et al., 2009; Johnston et al., 2005a; Rahman and McFadden, 2011). However, in HEK 293 cells Dorfleutner et al. (2007) reported that expression of M013 activated NFκB and enhanced TNFα activation of NFκB. Deletion of *M013L* profoundly attenuated MYXV (Johnston et al., 2005a).

M150 is a 494 amino acid early protein with 9 ankyrin (ANK) repeats and a C-terminal F-box (Camus-Bouclainville et al., 2004). In MYXV infected cells, M150 is colocalised with NFκB in the nucleus although direct binding has not been demonstrated. M150 binds cellular Skp and Cullin-1 through the F-box to form an E3 Ub ligase complex which would be expected to target bound proteins to the 26S proteosome for degradation (Camus-Bouclainville et al., 2004; Blanié et al., 2010). Deletion of *M150R* significantly attenuated the virus (Camus-Bouclainville et al., 2004).

M154, an early protein of 196 amino acids, following cleavage of a predicted N-terminal signal sequence, is an orthologue of VACV M2 (Cameron et al., 1999). VACV M2 localises to the endoplasmic reticulum (ER) and inhibits NFκB activation via an ERK2 pathway (Gedey et al., 2006; Hinthong et al., 2008). The function of M154 has not been determined although it does have an EDEL ER retention motif at the C-terminus in a similar position to VACV M2. Interestingly, *M154L* has been duplicated in some Australian isolates of MYXV and in the Californian MSW and MSD strains (Kerr et al., 2010; Labudovic et al., 2004).

Four MYXV proteins (M003.1; M136; M139; M146; see Table 5) have been proposed as orthologues of poxvirus proteins that act as PRR signal inhibitors. This is based on homology with poxvirus A46, N1, N2 and C1 protein families (González and Esteban, 2010). These proteins are predicted to form a Bcl-2 like fold, but only the N1 orthologues are predicted to bind proapoptotic proteins such as Bax and Bak and inhibit cell death (González and Esteban, 2010; Bahar et al., 2011). The roles of the MYXV proteins have not been determined and it should not be assumed that they have identical functions to their orthologues.

M131 is a late expressed protein of 163 amino acids, which is packaged into the virion. It is an inactive homologue of cellular superoxide dismutase (SOD), which is believed to interfere in the activation of cellular SOD leading to an increase in reactive oxygen species (ROS) in the infected cell (Teoh et al., 2003, 2005; Cao et al., 2002). ROS are important positive and negative regulators of signalling induced by PRRs (West et al., 2011) but deletion of M131 had no effect on virulence of MYXV although deletion of S131 attenuated RFV.

10.4.2. Inhibition of IFN activity

Interferons (types I (IFN α/B); II (IFN-γ) and III (IFN-λ) are key mediators of antiviral responses and most viruses including poxviruses have evolved mechanisms to disrupt or evade the activity of IFNs (Malmgaard, 2004; Sadler and Williams, 2008; Perdiguero and Esteban, 2009; McFadden et al., 2009; Versteeg and García-Sastre, 2010). MYXV expresses three proteins which directly modulate or are predicted to modulate IFN responses. M007 is a rabbit-specific IFN-γ receptor homologue (Upton et al., 1992) secreted from infected cells at $>10^7$ molecules per hour (Mossman et al., 1995b). M007 competes with the IFN-γ receptor to bind IFN-γ with high affinity (Kd 1.2 nM). M007 also binds CC, CXC and C chemokines through a heparin binding domain thus potentially disrupting chemokine gradients by inhibition of chemokine-glycosaminoglycan binding (Lalani et al., 1997; Liu et al., 2000; Dai et al., 2010). Deletion of the *M007L/R* genes significantly attenuated the virus; infection was characterised by mild to moderate clinical disease with only 1/13 rabbits severely affected. Histologically, a prominent feature was the influx of lymphocytes to the sites of virus replication but whether inhibition of this by wild-type virus was due to IFN-γ inhibition or disruption of chemokine gradients has not been defined (Mossman et al., 1996b).

MYXV also encodes orthologues of the VACV *K3L* gene (*M156R*; Ramelot et al., 2002) and the VACV *E3L* gene (*M029L*; Cameron et al., 1999; Barrett et al., 2001), which inhibit the action of type 1 IFN. K3 and M156 mimic eIF2α and act as competitive substrates for phosphorylation by protein kinase R (PKR) (Ramelot et al., 2002). VACV E3 binds dsRNA in the cytoplasm and thus prevents upregulation of PKR and 2′–5′-oligoadenylate synthase (Perdiguero and Esteban, 2009). In addition, the C terminal domain of VACV E3 inhibits the antiviral activity of ISG15 (IFN stimulated gene; ubiquitin like modifier) (Guerra et al., 2009). M029 binds dsRNA and prevents activation of PKR and induction of IFN-β in HeLa cells (Myskiw et al., 2011). M029 lacks the N-terminal Z DNA/RNA binding region of E3, which is important in VACV pathogenicity and suppression of signalling responses (Dai et al., 2011; Barrett et al., 2001; Myskiw et al., 2011). Rabbit type 1 IFN had no effect on MYXV replication in primary rabbit embryo fibroblasts indicating that MYXV is able to disrupt rabbit IFN signalling (Barrett et al., 2007b).

Two further orthologues of VACV IFN inhibitors are encoded by MYXV but do not inhibit IFN. VACV B18 is a secreted (and cell surface) receptor for type I IFN, however, the MYXV orthologue (M135) lacks the C-terminal half of B18, is localised to the cell surface, and does not bind IFN (Barrett et al., 2007b). VACV VH1 phosphatase, an enzyme packaged in the virion, dephosphorylates STAT1 and STAT2 following type 1 IFN signalling and STAT1 following type II IFN treatment to prevent IFN responsive gene transcription. The MYXV orthologue of VACV VH1, M069, while essential for virus replication (Mossman et al., 1995a), does not appear to dephosphorylate STAT1 or STAT2 (Wang et al., 2009b).

10.4.3. Inhibition of caspase 1 activation and IL-1β and IL-18

ASC-1 and caspase 1 are downstream of multiple PRR activation pathways. M013 interacts with ASC-1 in the inflammasome through a PYD-PYD interaction (Johnston et al., 2005a; Dorfleutner et al., 2007). This binding inhibits the cleavage of procaspase 1 to caspase 1 and therefore the activation of pro-IL-β and pro-IL-18. Deletion of *M013L* significantly attenuated MYXV (Johnston et al., 2005a).

M151 (Serp 2), a serine proteinase inhibitor (serpin) packaged in the virion, has been demonstrated to inhibit caspase 1 cleavage of human IL-1β in vitro (Petit et al., 1996; MacNeill et al., 2006; Zachertowska et al., 2006). Whether this is the role of M151 in vivo has not been determined. In vitro, Serp 2 also inhibited human caspase 8, granzyme B and possibly caspase 10 and could protect cells from apoptosis (MacNeill et al., 2006). Deletion of *M151R* attenuated MYXV (Messud-Petit et al., 1998). MYXV does not encode an orthologue of the orthopoxvirus IL-1/IL-18 binding protein.

10.5. Regulation of cell death

Induction of cell death following virus infection is a major host defence mechanism and many viruses have evolved methods of regulating cell death in infected cells (Lamkanfi and Dixit, 2010). Five MYXV proteins have been identified as inhibitors of cell death in rabbit lymphocytes infected with MYXV: M002, M004, M005, M11, M013; all have major roles in virulence.

M002 was originally identified as a secreted protein that specifically binds rabbit TNFα, competitively inhibiting receptor ligation and signalling (Schreiber and McFadden, 1994, 1996; Schreiber et al., 1996, 1997). In addition, M002 has a highly conserved viral preligand assembly domain (vPLAD) at its N-terminus which acts as a dominant negative inhibitor of TNFR signalling in the infected cell and inhibits apoptosis in RL-5 T lymphocytes and primary peripheral blood mononuclear cells infected with MYXV (Macen et al., 1996; Sedger et al., 2006). Deletion of *M002L/R* significantly attenuated MYXV (Upton et al., 1991).

M004 consists of 221 amino acids, following cleavage of an N-terminal signal sequence, with a C-terminal ER retention signal. It is retained within the ER of the infected cell and deletion of the *M004L/R* genes attenuated MYXV (Barry et al., 1997; Hnatiuk et al., 1999). The function of the protein and the mechanism by which it inhibits cell death are unknown.

M005 is a major host range protein of MYXV (Werden and McFadden, 2008). An early protein of 483 amino acids with 7 ANK repeats and a C- terminal F box, M005 prevents growth arrest of infected cells at the G0/G1 cell cycle checkpoint by degradation of p27/Kip1 (a cyclin dependent kinase inhibitor) (Johnston et al., 2005c). M005 functions as an E3 Ub ligase, binding Skp-1, Cullin-1 through the F-box and the substrate p27/Kip1, which is ubiquitinated and degraded in the proteosome. M005 also binds and

activates the Ser/Thr kinase, Akt. Akt has critical roles in regulation of cell cycle progression and cell survival (Wang et al., 2006; Werden and McFadden, 2008). Deletion of *M005L/R* significantly attenuated MYXV infection of rabbits (Mossman et al., 1996a).

M011 is a structural homologue of Bcl-2, which localises to the mitochondrial membrane in infected cells where it blocks cells death by binding cell death factors Bak and Bax through their BH3 domains (Everett et al., 2000; Douglas et al., 2007; Kvansakul et al., 2007; Su et al., 2006; Wang et al., 2004b). RL-5 cells and primary rabbit monocytes infected with *M11L* knock-out virus undergo apoptosis (Macen et al., 1996; Everett et al., 2000), although Opgenorth et al. (1992) demonstrated replication of *M11L* knock-out virus in primary lymphocytes stimulated with the mitogen ConA but not in unstimulated cells. M11 is a critical virulence factor (Opgenorth et al., 1992).

M013, in addition to its role in inhibition of caspase 1 cleavage of IL-1β and IL-18 in the inflammasome and inhibition of NκB activation, is needed for productive infection of rabbit lymphocytes and monocytes (Johnston et al., 2005a). Inhibition of ASC activation may be important for avoidance of pyroptosis (Duprez et al., 2009).

It has been suggested that replication in lymphocytes and monocytes is critical for MYXV dissemination within the infected rabbit and hence for development of generalised disease (Johnston et al., 2005a; Mossman et al., 1996a; Barry et al., 1997). However, *M002L/R*, *M004L/R*, *M005L/R* and *M011L* knock out viruses do reach distal tissues (Best and Kerr, unpublished) but do not replicate to high titres at distal sites. Ur, a grade 5 virus with a natural disruption to *M005L/R*, disseminates and causes generalised myxomatosis (Best and Kerr, 2000; Collins, van Leeuwen and Kerr, unpublished). In addition *M002L/R* knock-outs caused some generalised disease (Upton et al., 1991) and *M11L* knock-out causes secondary lesions distal to the inoculation site (Opgenorth et al., 1992), while small secondary lesions were noted for *M013L* knock-outs (Johnston et al., 2005a). It seems that the barrier to dissemination is not absolute but rather that these proteins are essential for suppression of antiviral responses and efficient replication at distal sites.

10.6. Inhibition of effector cells of the innate and adaptive immune response

CD8 + T cell recognition of infected cells by MHC-1 presentation of viral peptides is critical for clearance of virus by the adaptive immune system. MYXV downregulates MHC-1 expression on the surface of infected cells (Boshkov et al., 1992; Zuniga et al., 1999).This is caused by M153; one of a family of herpes and poxvirus encoded E3 ubiquitin ligases with an N-terminal RING-CH domain and two transmembrane domains (Guérin et al., 2002; Mansouri et al., 2003; Collin et al., 2005; Zhang et al., 2009). M153 also downregulates Fas/CD95 from the cell surface, which could inhibit cell killing through activation of caspase 8 by FasL expressing T cells (Guérin et al., 2002; Seet et al., 2003). In infected CD4 + T cells, M153 downregulates CD4 potentially interfering with CD4 + T cell help in the adaptive immune response (Mansouri et al., 2003). M153 has also been demonstrated to downregulate ALCAM 1 (Activated Leukocyte Cell Adhesion Molecule; CD166) potentially also disrupting T cell recognition of infected cells (Bartee et al., 2006). Deletion of *M153R* significantly attenuates MYXV (Guérin et al., 2002). No functional analyses of the consequence of MHC downregulation on activation of rabbit T cells have been published but downregulation of MHC-1 by ER retention and TAP transport inhibition in cowpox virus (CPXV)-infected mice prevented activation of CD8 + T cells and was a critical virulence determinant (Byun et al., 2007; Alzhanova et al., 2009).

At least four other proteins are involved in modulating T cell responses and inflammation. M141 is a viral homologue of CD200 expressed on the cell surface and is postulated to act as a negative regulator of macrophage activation by engaging CD200R on macrophage/APCs leading to decreased T cell activation (Cameron et al., 2005a). Similarly expression of the viral CD47-like protein M128 on the infected cell surface appears to prevent macrophage activation (Cameron et al., 2005b). Deletion of either gene made the virus non-lethal. The secreted serpin M008.1 (Serp 1) is important in inhibiting inflammation at the site of virus replication and is a key virulence factor (Macen et al., 1993). In addition, the secreted M001 protein binds CC chemokines and inhibits the influx of inflammatory cells particularly monocytes and macrophages to sites of MYXV replication although deletion of *M001L/R* had relatively minor effects on virulence (Lalani et al., 1999; Graham et al., 1997).

Downregulation of MHC-1 might be expected to mark the infected cell for NK cell-mediated killing (Lanier, 2008; Vivier et al., 2011). Two MYXV proteins, M122 and M121, have some homology to NK cell receptors (Cameron et al., 1999). These proteins are orthologous to VACV A34 and A33, respectively, which have homology to C-type lectin domains. A33 is expressed on the viral envelope and the surface of VACV infected cells. Its crystal structure was recently determined and shown to form a dimer that had a similar structure to NK cell receptor C-type lectin domains (Su et al., 2010). Nothing is known about the interaction of NK cells and MYXV proteins, indeed Laybourn et al. (1990) and Piriou et al. (2000) were unable to detect functional NK cells in rabbits using cell killing assays although other workers have reported apparent NK cell activity (Takahashi et al., 1991; See et al., 1999). CPXV and MPXV encode a soluble inhibitor of the NKG2D NK cell activation receptor which blocks NK cell killing in CPXV infected cells (Campbell et al., 2007).

10.7. Cell and host permissivity for MYXV replication

MYXV productively infects only a very small group of leporid species although it can replicate on the chorioallantoic membrane of hen eggs, in suckling mouse brain and infect mouse periportal hepatic cells if inoculated via the bile duct – although in the latter case it did not spread to adjacent cells (Fenner and Ratcliffe, 1965; Andrewes and Harisijades, 1955; Mims, 1964; Regnery and Marshall, 1971; Regnery, 1971). Two MYXV host-range genes, *M062R* and *M063R*, which are orthologous to the VACV *C7* host-range gene, are essential for productive replication in rabbit cells and rabbits (Barrett et al., 2007a; Liu et al., 2011). *M062R* is also required for replication in some otherwise permissive human and primate cell lines. M062 and M063 proteins co-immunoprecipitate in infected cells and bind to human SAMD9 (sterile alpha motif containing protein 9), which may be involved in innate immune responses and preventing permissivity to poxvirus infections (Liu et al., 2011).

Host-range restriction probably results from the species-specificity of the immune and inflammatory suppressive factors encoded by MYXV (McFadden, 2005). This was elegantly demonstrated using STAT-1 knock-out mice, which died following intracerebral inoculation of high doses of MYXV, implying that induction of and signalling by type 1 IFNs is a critical host barrier to productive infection (Wang et al., 2004a).

There is no evidence that MYXV can successfully replicate in humans. As already noted, Sanarelli inoculated serum from rabbits infected with MYXV into two people without any evidence of successful infection although both developed conjunctivitis (Hobbs, 1928). In early 1951, following the initial spread of MYXV in Australia, three senior Australian scientists, Frank Fenner, MacFarlane Burnett and Ian Clunies–Ross, inoculated themselves with MYXV to demonstrate that it was harmless for humans (Fenner and Ratcliffe, 1965; Burnet, 1968). Similarly, a serological study of humans exposed to mosquitoes and rabbits infected with MYXV in

California found no evidence of seroconversion (Jackson et al., 1966). This inability to replicate in humans may be an important safety feature for the potential clinical use of MYXV for virolytic oncotherapy in humans (Spiesschaert et al., 2011).

Despite its narrow host range, MYXV replicates in a variety of cell types including chicken, mouse, non-human primate, human primary cells and human cell lines. Cell permissivity for productive replication is determined by whether the cell supplies the necessary host factors for virus replication (Masters et al., 2001; Johnston et al., 2003; McFadden, 2005; Werden et al., 2007; Stanford et al., 2007a) and whether MYXV can suppress the innate and intrinsic antiviral defences of the infected cell. Different cell lines and primary cells from human and mouse have been exploited to understand some of the factors involved in permissivity and to shed light on factors restricting cross-species jumps for viruses. Suppression of the type I IFN response triggered by PRRs is particularly critical but TNF is also important for restriction in human primary cells (Wang et al., 2004a, 2008, 2009a, 2009b; Johnston et al., 2005b; Bartee et al., 2009; Dai et al., 2011).

11. Pathways of host–pathogen evolution

Myxomatosis is the classical case of a species jump which occurred when a novel pathogen was introduced to a new species. Where the ecological conditions for transmission were present: high numbers of susceptible rabbits plus efficient vectors, introduction of MYXV into European rabbit populations in Australia, Europe, Britain, Chile and Argentina was successful. Where introductions failed either vectors were not present or the numbers of rabbits were limited so the disease burnt out. While there may have been some adaptation to European rabbits in the laboratory prior to field introduction, the different passage histories of SLS and Lu suggest that this was unimportant.

Two other points may be made; firstly, MYXV is a DNA virus that replicates its genome without the high error rate of RNA viruses so it is unlikely that there was significant genetic variation in the initial virus populations, and secondly, the dose of virus delivered by a mosquito is generally very low – perhaps 1–10 ID_{50} (Fenner et al., 1956) so that an almost limit dilution occurred at each transmission suggesting that rare variants in an infected rabbit had little chance of transmission. The point introduction of a single genotype meant that random mutations were the only way to generate genetic variation on which natural selection could act. Even though repeated reintroductions were made in Australia they were essentially of the same genotype until the introduction of Lu.

The key to MYXV evolution is transmissibility – virus persisting at sufficient titres in epidermal lesions for long enough to be transmitted by insects or being shed in sufficient concentrations for contact transmission. Whether the host survives the infection is largely immaterial as it is subsequently immune to reinfection (Anderson and May, 1982). Paradoxically, to be more effectively transmitted the virus needed to become slightly less efficient in its new host. In the natural Sylvilagus host, vector transmission occurs from a virus-rich fibroma without overt disease. Susceptible kittens are born every year and there is some evidence that immunity to the virus is not long-lasting (Aragão, 1943; Fenner and Ratcliffe, 1965). The virus proteins that disrupt the local immune response to enable virus persistence in this fibroma facilitate systemic spread and cause fulminant disease in European rabbits. Disruption of major virulence genes can prevent generalised disease but usually this means that the rabbit controls virus replication and recovers quickly. Quantitative studies on virus titres and transmission have not been done with knock-out viruses but it seems reasonable to conclude that this would mean limited opportunity for transmission particularly in resistant rabbits. For MYXV, there is a strong nexus between virulence and transmissibility in European rabbits. In a very elegant experiment, Mead-Briggs and Vaughan (1975) demonstrated that the most effective rabbits for flea transmission of MYXV strains of different virulence grades were those that survived for a prolonged period but eventually succumbed to the disease. Only an average of 12% of fleas from rabbits that died by 16 days and 8.3% of fleas from rabbits that survived, transmitted infection. This implies that genetically resistant rabbits that are able to readily control infection will tend to be poor sources of virus for transmission.

This may mean that there is no simple evolutionary pathway for the virus involving a single gene mutation or a stepwise series of mutations that would lead to a phenotype in the European rabbit that mimics that of MYXV in its natural host. However, if the rabbit population has sufficient genetic variability and myxomatosis exerts sufficient pressure for natural selection to continue to increase the level of resistance to MYXV, then it is possible that disseminated, generalised disease might become uncommon. This could drive the virus towards evolution of a localised fibroma type disease assuming that the virus had sufficient genetic flexibility to evolve further in this direction. As Fenner and Ratcliffe (1965) pointed out, rabbits infected with such viruses that did not have strong genetic resistance would die of acute myxomatosis as happens in the original American range of the virus. The more likely scenario envisaged by Fenner and Ratcliffe (1965) was that there would not be a continued selection for genetic resistance beyond a certain point because the virus would be unable to evolve any further to drive selection. In this case, MYXV would continue to cause epizootics with potentially significant mortality rates but relatively little impact on the rabbit population because of the rabbit's high reproductive rate.

Alternatively, the virus could evolve further towards contact transmission but this is likely to only be important in local populations or rabbit farms. The amyxomatous phenotype may represent a loss of selection pressure for cutaneous lesions rather than a genuine selection for enhanced contact transmission. However, like the amyxomatous types, some Australian field strains also do not induce a significant primary lesion at the inoculation site in wild rabbits, and this has also been described in British wild rabbits (Vaughan and Vaughan, 1968), which means that viral dissemination and replication to high titres at distal sites, such as eyelids and base of the ears, is critical for mosquito or flea transmission. This suggests that the maintenance of virulence genes that enable dissemination will be critical for virus success in the wild rabbit population (Kerr et al., 2003).

Field viruses have multiple mutations (Morales et al., 2009; Dalton et al., 2010; Belsham et al., 2010; Muller et al., 2010; Saint et al., 2001; Kerr et al., 2010). For example, the completely sequenced highly attenuated Spanish 6918 strain isolated in 1995 has differences in 73 positions compared to the Lu precursor virus (Morales et al., 2009).While disruptions to known virulence genes such as *M135R* and *M148R* in 6918 provide obvious explanations for attenuation (Morales et al., 2009), such viruses are not representative of the predominant grade 3 viruses from the early radiations of MYXV or of the grade 2 viruses that seem more prevalent in Britain and possibly in Spain (Bárcena et al., 2000b). In Australian field isolates there has been duplication of two potential virulence genes *M154L* and *M156R* by expansion of the TIR regions, a similar duplication involving more genes has occurred in the Californian MSW and MSD strains of MYXV suggesting that this may be a common mechanism to create genetic variation (Kerr et al., 2010; Labudovic et al., 2004). In both cases, this duplication has deleted the 3′ 923 nucleotides of the M009 gene indicating that this gene is probably not important for virulence/transmission in European rabbits or in brush rabbits. Similarly, a highly virulent Australian isolate has a disruption in the M036 gene suggesting that this gene is not required for virulence (Saint et al., 2001). Interestingly

rabbit fibroma virus (strain Kazza) has multiple genes fragmented that are involved in virulence in MYXV (Cameron et al., 1999; Willer et al., 1999). This suggests that MYXV more closely resembles the ancestral leporipoxvirus and that adaptation of rabbit fibroma virus to eastern cottontails may have made these genes redundant. In CPXV, a gene fragment has evolved a new role as a protein that downregulates MHC-1 showing that gene fragments resulting from gene disruptions can evolve new and important roles (Alzhanova et al., 2009). Mapping and understanding the roles of mutations in adaptation and evolutionary pathways will require genomic sequence analysis, pathogenesis studies and reverse genetics. There are likely multiple pathways to success in a virus with such a large genome.

The genetic basis for resistance in wild rabbits is not understood. If, as seems likely, genes involved in the innate immune response are responsible for resistance it is possible that MYXV is driven to adapt to this resistance by mutations in particular genes involved in suppressing innate pathways. These mutations could potentially be quite subtle, at the level of single amino acid substitutions rather than gene disruptions. It raises the question of how far resistance could become specific to particular virus strains and how this would influence further host–pathogen evolution.

12. Conclusions and further work

Myxomatosis was first studied in 1896 when the very concept of a virus was novel. The introduction of MYXV into European rabbits in 1950 predates the solving of the structure of DNA. A lack of immunological reagents, the costs of housing and animals and lack of inbred rabbit lines have limited studies on MYXV pathogenesis in rabbits. However, in the 21st century, high throughput sequencing, sequencing of the rabbit genome, transcriptomics and viral reverse genetics potentially provide new tools to understand the genetic basis of resistance and the molecular drivers of the coevolution of the rabbit and MYXV. The intensive studies currently being done on MYXV in human cancer cells, while aimed at developing new tools for treating cancer, are also increasing our understanding of how MYXV interacts with the cell at the molecular level (Spiesschaert et al., 2011). The pioneering studies done in Australia and subsequently other countries on the evolution of MYXV still stand out as one of the best data sets for what happens as a novel pathogen adapts to a new host. Now there is an opportunity to bring together molecular, ecological and genetic studies to understand the past, present and future of this pathogen in its new host.

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