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MARLIER D., BERTAGNOLI S.

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VIRUS INFECTIONS OF RABBITS

MARLIER D*. and BERTAGNOLI S**.

*Department of Bird and Rabbit Medicine, Faculty of Veterinary Medicine, University of Liège, Bld de Colonster 20, Bat B42, Sart-Tilman, B4000, Liège, Belgium.
** UMR de Microbiologie Moléculaire, Institut National de la Recherche Agronomique and Ecole Nationale Vétérinaire, 23 Chemin des Capelles, F31076 Toulouse, France.

Introduction

The domestic rabbit, derived from the European wild rabbit (*Oryctolagus cuniculus*), can be naturally infected by several viruses. Nevertheless, nowadays two important viral diseases of European rabbits still exist: myxomatosis and rabbit viral haemorrhagic disease (RVHD).

The etiological agent of myxomatosis, myxoma virus (MV), is a large double strand DNA virus belonging to the *Leporipoxvirus* genus of the *Poxviridae* family (Francki *et al*, 1991). It induces a mild disease in cottontail rabbits (*Sylvilagus* spp) but a systemic and usually fatal one in the European rabbits (Fenner and Ratcliffe, 1965).

Rabbit viral haemorrhagic disease (RVHD), a highly contagious disease in wild and domestic rabbits, was first described in People's Republic of China in 1984 (Liu *et al*, 1984). RVHD spread throughout Europe during the years 1987 to 1989 (Morisse *et al*, 1991). Infected rabbits -adults or young animals older than two months- usually die within 48 to 72 hours of necrotizing hepatitis and haemorrhagic syndrome (Marcato *et al*, 1991). The causative agent was characterized as a member of the *Caliciviridae* family (Ohlinger *et al*, 1990; Parra and Prieto, 1990).

Because of the importance of myxomatosis and RVHD in intensive rabbit production, this review will be mostly devoted to these two diseases.

Myxomatosis

History

Myxomatosis is a major viral disease of wild and domestic European rabbits (Oryctolagus cuniculus). The aetiological agent was first isolated from a colony of laboratory rabbits in Uruguay in 1898 and identified as a poxvirus in 1927. The natural hosts are two species of leporid: *Sylvilagus brasiliensis* in South America (South American strains) and *Sylvilagus bachmani* (Californian strains) in California (Fenner, 1994). In its natural hosts, the viral strains produce only a benign fibroma, generalized disease occurring only in juvenile animals. In the European rabbits (*Oryctolagus cuniculus*), the South American and Californian strains of myxoma virus are highly lethal but produce different kinds of disease mainly less prominent clinical signs with the Californian strains (Fenner, 1994).

Myxomatosis was first recognised in North America in 1930, where

outbreaks of the disease occurred in rabbitries in southern California. Myxomatosis remains enzootic in the western United States, some cases sporadically occurring in domestic rabbits (Patton and Holmes, 1977).

Since 1950, myxomatosis has been used for the biological control of the rabbit pest in Australia. In the initial epidemic in Australia, myxomatosis was estimated to have killed as many as 99.8% of infected wild rabbits, and many populations were reduced by more than 90% (Kerr and Best, 1998). Myxomatosis was deliberately introduced into France in 1952 and soon became enzootic throughout Europe (Arthur and Louzis, 1988).

Scientifically, the most interesting aspect of these introductions of myxoma virus in the field was to determine the evolutionary changes that might happen to the virus and to its host. This problem was mainly investigated both in Australia and in England. A complete discussion is beyond the scope of this review so only general outline will be given. In both countries, the trend was the same. On one hand, the viral strains of very high virulence were replaced by strains of intermediate virulence even though more attenuated strains were sometimes recovered from the field. On the other hand, selection of resistant rabbits was stimulated by the emergence of attenuated viral strains that allowed the survival of moderately resistant animals (Kerr and Best, 1998).

Studies on the co-evolution between myxoma virus and rabbits are still in progress and recent results seem to indicate that, in turn, the selection of more virulent viral strains is now in progress because resistant rabbits are less effective transmitters of the virus (Kerr and Best, 1998).

Interestingly, apart from these changes of virulence, a new form of the disease (amyxomatous myxomatosis) has also emerged. The clinical signs of amyxomatous myxomatosis are mainly respiratory, skin nodules being few and small. It might be thought that amyxomatous myxomatosis would not spread via vectors but through direct contact, and would arise predominantly in intensive enclosed rabbitries. However, this last notion must be viewed with caution since the disease has also been observed in wild rabbits. So far, these forms of myxomatosis have been reported only in France (Brun *et al.*, 1981; Joubert *et al.*, 1982), Spain (Rosell *et al.*, 1994) and more recently in Belgium (Marlier and Vindevogel, 1996).

Aetiology

Myxoma virus (MV), the agent responsible for myxomatosis, is a member of the *Poxviridae* family, which are amongst the largest animal viruses. It has been designated by the International Commitee on Taxonomy of Viruses as the type species of the *Leporipoxvirus* genus (Francki *et al*, 1991). As many other poxviruses, myxoma virus has a very narrow host range, and is preferentially transmitted mechanically by biting arthropods. By electron microscopy, the virions of myxoma virus are indistinguishable from those of the prototype poxvirus, vaccinia virus. Myxoma virus has a double-stranded DNA of 161 kbp, with a central part containing highly conserved enzymatic and structural genes required for maintenance of normal viral life cycle. Peripheral regions of the DNA, within and near the inverted terminal repeats (ITR) at both sides of the genome, encode non-essential factors that contribute to the modulation of the host response to infection (Drillien *et al*, 1987; Upton *et al*, 1987; Upton and Mc Fadden, 1986). Several MV proteins have been described and shown to be associated with virulence: myxoma growth factor (Upton *et al*, 1987) is related

to cellular EGF; M11L acts in an unknown pathway as an anti-inflammatory factor (Graham *et al*, 1992) , MT-2 (Upton *et al*, 1990) and M-T7 (Upton *et al*, 1992) are homologues of cellular receptors of TNF and IFN-g, respectively. MT-1 is a secreted protein which binds to chemokines (Graham *et al*, 1997; Lalani *et al*, 1999). Functions of virulence factors MT-5 (Mossman *et al*, 1996) and MT-4 (Barry *et al*, 1997) are much more unclear. Moreover, to date, two genes coding for serpins (**ser**ine **p**roteinase **in**hibitors; (Carrell and Travis, 1985)) have been identified so far. *SERP-1* (Upton *et al*, 1990) is located in two copies in the ITR and is a secreted factor implicated in the modulation of the inflammatory response (Macen *et al*, 1993; Nash *et al*, 1997; Nash *et al*, 1998). Serp2 has been identified near the right ITR (Petit *et al*, 1996) and is closely related to cowpoxvirus-encoded Crm A. It has been shown to be an intracellular inhibitor of caspase-1 and Granzyme B, and to interfere with both inflammation and apoptosis (Messud-Petit *et al*, 1998; Turner *et al*, 1999).

Clinical signs and pathology

The clinical signs of myxomatosis differ according to the strain of virus, its passage history and its virulence (Fenner, 1994). Two forms of the disease have been identified to date: the nodular (classical) form and the amyxomatous (respiratory) form.

Florid skin lesions and severe immunodysfunction, accompanied by supervening Gram-negative bacterial infections of the respiratory tract, characterize the nodular myxomatosis syndrome caused by a virulent myxoma virus strain. Prototype strains of virus deriving from the Australian and European outbreaks have been designed which characterise the various virulence grades (from grade I to grade V) as determined in laboratory rabbits (Fenner and Ratcliffe, 1965).

After infection with a grade I (the most virulent) strain, the first sign of infection is a lump at the site of infection, which increases in size and usually becomes protuberant and ulcerates. An acute blepharo-conjunctivitis and an oedematous swelling of the perineum and scrotum gradually develop. The secondary skin lesions appear on about the sixth or the seventh day (Fenner, 1994). Death usually occurs between the eighth and fifteenth day after infection.

After infection with grade II to V strains the clinical signs are usually the same at the exception that they evolve more slowly and are less intense. When animals survive, the lesions progressively heal. The mortality rate fluctuates between 100 and 20%, according to the viral strain. The natural mode of transmission of the nodular form is by biting insects. This form is mainly observed in small-scale rabbitries (Arthur and Louzis, 1988).

The clinical signs of amyxomatous myxomatosis are mainly respiratory, skin nodules being few and small. After inoculation of SPF rabbits with five amyxomatous myxoma virus strains (Marlier *et al.*, 1999a), the main clinical observations were the abnormal appearance at the inoculation site and the small number or absence of secondary skin lesions. An acute serous blepharo-conjunctivitis, the intensity of which varied with time and virus strain, was a consistent observation. It began with conjunctival oedema and redness associated with photophobia, and progressed to a thickening of the eyelids that sometimes led to closure of the eyes. Acute respiratory distress was only observed in some animals. When the same five strains were inoculated to conventional rabbits (Marlier *et al.*, 2000b), a much more acute myxomatosis

syndrome was reproduced. Both the respiratory and cutaneous expression of the disease was more pronounced in conventional rabbits than in SPF animals. However, the main clinical signs were of the respiratory type. Some animals showed secondary skin lesions, but the skin nodules were of reduced size and never became prominent or exudative. From the comparison of three studies on amyxomatous myxomatosis (Marlier *et al.* 1999a; 2000b, 2000c), it cannot be concluded that the pneumotropism of amyxomatous myxoma virus strains is greater than this of nodular strains; only the expression of the ectodermotropism is clearly reduced. The development of a more or less severe respiratory distress is due to bacterial superinfections, probably complemented by immunosupression. Therefore, the clinical diagnosis of the amyxomatous form of myxomatosis is clearly more difficult than for the classical one.

In nodular forms, the symptomatology is so characteristic that diagnosis can be made on the basis of the clinico-pathological syndrome. At the opposite, the diagnosis of atypical (amyxomatous) forms most frequently implies the isolation of the virus by inoculation of sensitive cell lines such as the RK-13 cell line (ATCC CCL37) and identification of the virus as myxoma virus by indirect immunofluorescence or indirect immunoperoxidase test (Marlier *et al.*, 1999b).

Epidemiology

There are few studies on myxomatosis in intensive rabbit production unit either about the nodular or about the amyxomatous forms.

Deutrich and Hausburg (1986) reported that the mean annual proportion of rabbit farms affected with clinical forms of myxomatosis was 13.5 % during 1978 - 1980.

Rosell *et al.* (1992) found that the proportion of farms with clinical cases of myxomatosis varied from 13.0 to 22.8 % from January 1986 to December 1990.

Ghram *et al.* (1996) detected antibody to MV in 54.9% of the rabbitries; no clinical signs being observed in 72% of these infected farms.

In a study of 66 rabbits with no history of vaccination against myxomatosis, which died of pulmonary lesions, myxoma virus was isolated from 10.6% of rabbits and serological evidence of MV infection was demonstrated in 44% of rabbits. No relationship could be established between presence of specific antibodies to MV and the observed pulmonary lesions or the results of bacteriological examinations of lungs (Marlier *et al*., 2000a).

Marlier *et al.* (2000d) have studied the seroprevalence of myxoma virus specific antibodies in 16 farms considered free of myxomatosis on the basis of the absence of typical clinical signs. MV antibodies were detected by ELISA (sensitivity 100%, specificity 90%) in all 16 farms, the corrected seroprevalences (95% confidence interval) being 55.2 ± 7.73% and 37.0 ± 6.13% for does and broilers respectively. The association between some conditions (herd sizes, types of rabbitries, presence of recurrent respiratory, digestive or reproductive troubles) and seroprevalence of MV antibodies was prospected by means of univariate Tarone's chi-square test and by means of logistic regression analyses. In all models, the seroprevalence of MV antibodies was significantly higher in herds (does and broilers) with recurrent respiratory or digestive troubles than in herds without these problems. In broilers, the

seroprevalence was higher in herds where animals were housed totally or partially in outdoors rabbitries than in totally enclosed rabbitries. The effect of herd sizes on the presence of MV antibodies was the same in does and broilers, the intermediate sizes being at lower risk than the smaller and larger ones. In does, the univariate analysis shows that the seroprevalence is higher in herds with reproductive problems than in herds without these troubles. The logistic regression model because of confounding effect with herd sizes could not confirm this finding.

Rabbit Viral Haemorrhagic Disease (RVHD)

History

The Rabbit Viral Haemorrhagic Disease (RVHD) is a new acute disease of domestic and wild rabbits present in Europe since 1987. This disease was first reported in 1984 by Liu *et al* in the People's Republic of China, where it killed some 470,000 rabbits in the first 6 months. By 1988 it had spread throughout eastern and western Europe and had reached North Africa (Morisse *et al*, 1991). In 1988, cases occurred in Mexico, but rigorous control practices were successfully employed on a large scale in the eradication of RVHD from this country (Gregg *et al*, 1991). Recently it was accidentally released in Australia (Mutze *et al*, 1998) and New Zealand.

Aetiology

The causative agent was characterized as a member of the *Caliciviridae* family (Ohlinger *et al*, 1990; Parra and Prieto, 1990). It has been designated by the International Commitee on Taxonomy of Viruses as the type species of the new genus *Lagovirus* (Pringle, 1998). The virions are nonenveloped, 40 mm in diameter, and have icosahedral symmetry. The genome of the Rabbit Haemorrhagic Disease Virus (RHDV) is a 7.4 kb single-stranded positive-sense RNA with only two open reading frames (ORFs). ORF1 extends from nucleotide 10 to nucleotide 7042, and encodes for a polyprotein that is cleaved into non-structural proteins and the major capsid protein VP60 (Meyers *et al*, 1991; Rasschaert *et al*, 1995). ORF2 overlaps the 3' end of ORF1 by 17 nucleotides and encodes for a 12 kDa polypeptide (Para and Prieto, 1990; Meyers *et al*, 1991), recently described as a minor capsid protein VP10 (Wirblich *et al*, 1996). Molecular epidemiology studies have revealed a low genomic variation within isolates collected from different geographic areas and over a period of several years (Le Gall *et al*, 1998), suggesting that, to date, a single RHDV serotype exists. Nevertheless, serological studies indicated that RHDV was present before the disease was first notice, and, as it was speculated, an avirulent variant of RHDV, named RCV, has been characterized (Capucci *et al*, 1996).

Clinical signs and pathology

The disease is responsible for high economic losses in rabbitries, as well as for high mortality rate in wild rabbits. RVHD only affects domestic, farmed and wild rabbits over two months of age of the species *Oryctolagus cuniculus* (Marcato *et al*, 1991). The incubation period is between 16 to 48 h, and infection usually leads to peracute or acute clinical disease. In peracute cases, rabbits usually died suddenly with very few clinical signs. In acute cases several clinical signs can be observed, although they are not all present in all cases: elevated

temperatures, rapid respiration and cyanosis, anorexia. Sometimes, affected rabbits develop signs of central nervous system disease, have foamy bloody discharge from the nostrils, diarrhoea or constipation. Death usually occurs between two and three days post-infection. Morbidity and mortality rates in a population can be as high as 90-100%. In late stages of an epidemic, subacute and chronic forms are sometimes seen. The clinical signs observed with these forms are the same as those seen in acute forms but the evolution of the disease is slower and the mortality rates are lower, and some animals survive to the infection (Kroneman and Horzinek, 1994). A major characteristic of RVHD is to be age-linked. Rabbits less than four weeks of age, even in the absence of maternal antibodies, do not develop clinical signs or pathological lesions, although they are infected and develop lifelong immunity (Morisse *et al*., 1991, Rodak *et al*., 1991).

RHDV replicates especially in the cytoplasm of hepatocytes, resulting in a severe necrotizing hepatitis, particularly affecting hepatocytes in the peripheral areas of the lobules of the liver (Morisse *et al*, 1991). So, at postmortem, constant pathological features include an enlarged, pale, yellow, grey friable or congested liver with a distinct lobular pattern, an enlarge and often dark spleen, and multifocal petechial haemorrhages in the liver and also in other organs such as lung, kidney, heart or trachea. Systemic endothelial damage probably caused by viraemia would lead to disseminated intravascular coagulation followed by consumption coagulopathy. The hypothesis is supported by the presence of numerous fibrin thrombi in the microvasculature of many organs together with the demonstration in the reduction in platelets numbers (Marcato et al., 1991; Ueda et al., 1992). Usually, the clinical diagnosis can be made by the history of an acute disease with high mortality in adult rabbits associated with the obvious gross lesions observed at necropsy.

Epidemiology

RVHD can spread rapidly by various routes and vectors. Under field conditions, infection commonly occurs through direct animal to animal contact, faecal-oral route being probably the most important method of spread (the virus is present in all excretion and secretion products) (Marcato *et al*, 1991). The high stability of the virus leads to local contamination of the environment, and RVHD can be spread via contaminated material such as feedstuffs, water, and bedding. It was shown in Australia that some insects are a major potential source of the virus for oral or conjunctival transmission to wild rabbits (Asgari *et al*, 1998). The passive transmission of the virus to new areas via movement of people, equipment and wild or domestic animals (including, of course, rabbits) may also occur. Finally, rabbit's products have also been implicated in the spread of the disease, and rabbit meat seems to be an important potential source of infectivity, especially via trade exchanges (Gregg *et al*, 1991). In commercial rabbitries, the occurrence of unapparent infection with the non-pathogenic RCV virus might explain the patchy nature of outbreaks of RVHD.

In Belgium, RVHD was diagnosed for the first time in 1990. Since, it has become a major problem. RVHD was found to be the cause of death in 23% of the rabbits necropsied in the Bird and Rabbit clinic of the University of Liège between January 1994 and June 1998. The distribution of the percentages of RVHD cases relates to the type of rabbitries, RVHD being far more common in craft rabbitries than in commercial ones (Marlier and Vindevogel, unpublished

data). To date, classical RVHD cases are still found but atypical cases with reduced mortality rates and slow development of the disease in the rabbitries seem to occur more frequently than in the previous years (Marlier and Vindevogel, unpublished data).

Prophylaxis and control of myxomatosis and RVHD

Control of these diseases is difficult because of their epidemiological characteristics. Indeed, nowadays, it is not feasible to control the diseases in populations of wild rabbits. Myxomatosis and RVHD can only be controlled in domestic and commercial rabbit colonies by the combination of physical measures and vaccination.

Physical measures

Producers of domestic rabbits who live in countries where myxomatosis occurs in wild rabbit may need to protect their stocks from infection. First, mosquito proofing of animal quaters is desirable, and the health status of new animals should be known (otherwise, rabbits should be quarantined). Colonies with the disease should be sacrificed, the premises controlled for insects and disinfected.

Measures to prevent introduction of the RHDV include restricted access and disinfections of all equipment entering or leaving a breeding facility. It is important to prevent any contact with wild rabbits or other rabbits whose disease status is unknown (any new rabbit should be quarantined for at least 1 month). The level of flies, mosquitoes and other insects needs to be kept as low as possible. Cages and equipment can be disinfected with solutions of either formalin (1-2%) or sodium hypochlorite (0,5%), or sodium hydroxide (10%). When RVHD has occurred, colonies with the disease should be slaughtered, all infectious material (included suspect feed) should be removed and the premises disinfected, before restocking.

Vaccination

Vaccination is very efficient in reducing the spread of myxomatosis, especially for breeding animals. This is currently achieved by using heterologous vaccines based on Shope fibroma virus, another Leporipoxvirus, or homologous vaccines based on cell culture attenuated strains of myxoma virus. In France, primary vaccination is done with Shope fibroma virus, and a cell culture attenuated strain of myxoma virus, named SG33, is used for booster injections (Saurat *et al*, 1978). Nevertheless, it was observed that some attenuated strains of myxoma virus might show, sometimes, residual pathogenicity for young rabbits (Brun *et al*, 1981).

Vaccines have been developed in Europe to protect rabbits from RVHD. They are prepared as an inactivate homogenate (with formalin or β propiolactone) of experimentally infected rabbit tissue mixed with adjuvant (oil or aluminium hydroxide) (Argüello Villares, 1991). Parenterally administrated, they induce a good immune response that protects older animals from fatal infection. Booster vaccinations are commonly given (at yearly or half yearly intervals) even though it is thought that vaccination can provide life-long immunity (Argüello Villares, 1991). Since *in vitro* systems are not available for efficient virus propagation, virus antigens still has to be produce in rabbits. This

is not ideal because of ethics and moreover, large amounts of highly infectious material need to be handle. In recent years, VP60 protein has been produced in several heterologous systems (Bertagnoli *et al*, 1996; Boga *et al*, 1994; Laurent *et al*, 1994). The recombinant proteins obtained in all these systems have been shown to induce protective immunity. In some cases, the recombinant capsid protein VP60 self-assembled into virus like particles (VLPs). These VLPs turned out to be highly immunogenic and to induce a good protection against the disease (Laurent *et al*, 1994). All these systems of recombinant VP60 production may lead to improved methods of vaccine manufacture.

Nevertheless, in industrial and traditional breeding conditions, RHDV specific maternal antibodies may block the effect of inactivated or subunit vaccines used at weaning. The use of an attenuated recombinant virus may resolve this kind of problem. To protect rabbits against myxomatosis and RVHD simultaneously, we have constructed recombinant myxoma viruses based on the attenuated SG33 strain expressing the RHDV VP60 protein (Bertagnoli *et al*, 1996b). We also tried to improve safety of SG33 virus by deletion of thymidine kinase (TK) gene (Jackson and Bults, 1992) or ORFs coding for previously identified pathogenesis factors like MGF (Myxoma Growth Factor) (Opgenorth *et al*, 1992; Upton *et al*, 1987) and M11L (Graham *et al*, 1992; Opgenorth *et al*, 1992). One intradermal injection of both recombinant myxoma-RHDV viruses was able to protect young rabbits against a virulent RHDV challenge, as efficiently as the commercial vaccines (Argüello Villares, 1991), under the conditions used for vaccine controls in France. As it was previously reported (Argüello Villares, 1991; Laurent *et al*, 1994; Boga *et al*, 1994; Bertagnoli *et al*, 1996a), the RHDV protective immunity was rapidly established since all the rabbits vaccinated 5 or 15 days before challenge were protected. One intradermal injection of classical dose of either recombinant myxoma-RHDV viruses gave the same level of protection against myxomatosis as the SG33 virus strain. Therefore the immunogenicity of the myxoma virus as a live vaccine was not significantly impaired by the insertion of a foreign gene into the TK or M11L-MGF genes of the SG33 virus strain. Moreover, in our experiments, we have not observed any signs of residual myxomatosis pathogenicity for young rabbits after administration of both recombinant viruses. This result has to be confirmed under breeding conditions. Thus, in young domestic rabbits, an early and efficient protection against RVHD and myxomatosis may be obtained by intradermal myxoma-RHDV recombinant viruses administration. Furthermore, we have also shown that the protection induced by the oral route with higher doses of recombinants viruses was as complete as the one induced by the intradermal vaccination (Bertagnoli *et al*, 1998). Recently, new recombinant myxoma-RHDV viruses based on a naturally attenuated field strain of myxoma virus have been shown to confer horizontal transmissible protection either by direct contact or in flea-mediated process (Bàrcena *et al*, 2000). These results give rise to the opportunity of wild rabbit vaccination.

Other virus infections

Contrary to most other animal species, natural virus infections are rarely described in rabbits either those belonging to the genus *Oryctolagus* or to the genus *Sylvilagus*.

1/ *Miscellaneous DNA virus infections*

Two herpesviruses of *Lagomorpha* have been described to date: *leporid herpesvirus 1* specific of *Sylvilagus floridanus* and *leporid herpesvirus 2* specific of *Oryctolagus cuniculus* (Hudson, 1994a, 1994b). These two viruses were initially isolated from spontaneously degenerating primary kidney cultures of their assumed respective hosts. Obviously, only *leporid herpesvirus 1* is a pathogenic organism, no clinical symptoms accompanying experimental inoculation of rabbits with *leporid herpesvirus 2*. In 1992, Onderka *et al.* have isolated a third herpesvirus from New Zealand White rabbits coming from two rabbitries with high mortality rates. The primary lesions were multifocal haemorrhagic dermatitis, localized pneumonia and severe splenic necrosis. These observations had never been confirmed to date.

There are two pathogenic papovaviruses of rabbits. The first is the cottontail rabbit papillomavirus that is often called "Shope papilloma virus" (Moreno-Lopez, 1994a). The natural host of this virus is *Sylvilagus floridanus*. The lesions found in natural cases of papillomatosis are localized keratinised tumours mostly present on the skin of the head, neck, shoulder, abdomen and inner part of the thighs. Eventually, these tumours can metastasise to other organs causing the death of the animal. Experimentally, the European rabbit (*Oryctolagus cuniculus*) can be infected with this virus. The clinical syndrome is similar to the one observed in *Sylvilagus floridanus* but a threefold higher incidence of malignant papillomas are found in domestic rabbit. In domestic rabbits, the lesions contained little or no demonstrable virus and can only rarely be passaged serially (Moreno-Lopez, 1994a). No natural infection of domestic rabbit has ever been demonstrated. The second papovavirus is the "oral papillomatosis virus of rabbits" which infects domestic rabbits causing papillomas of the tongue. Usually, these papillomas are of small sizes and can be found on the underside of the tongue. In rabbits inoculated with the oral papillomatosis virus, the lesions takes 9 to 38 days to appear. In natural cases, the virus might be transmitted from mother to offspring during the suckling period. This infection is not highly contagious and spread from infected animals to noninfected rabbits does not seem to occur naturally.

A parvovirus has been described in rabbit. The strain is called lapine parvovirus (LPV) F-7-9 and was isolated in 1977 (Matsugana *et al.*, 1977). Little is known about this infection. Experimentally infected rabbits show very mild clinical signs of listlessness and inappetence lasting for a few days. Histologically, mild to moderate catarrhal enteritis was observed in the small intestine (Matsugana and Chino, 1981). According to Metcalf *et al.* (1989), natural parvovirus infection would be very common in laboratory rabbit as 75% of the animals have high antibody titres to LPV.

2/ *Miscellaneous RNA virus infections*

As in many animal species, rotaviruses were observed (Percy and Barthold, 1993; Morisse *et al*., 1982) or isolated (Sato *et al.*, 1982) from faeces of rabbit with diarrhoea. Rotaviruses are endemic in many rabbitries and they usually induce only mild clinical signs in absence of secondary bacterial infections. Maternal antibodies protect rabbit less than 1 to 2 month of age. After this age, according to the level of infection in the farms, rabbits undergo only a subclinical infection that reinforce their immunity, or suffer from diarrhoea (Peeters, 1989). At histopathology, there is a diffuse infiltration of lymphocytes in the *lamina propria* of the small intestine associated with villus shortening and

fusion. The presence of virus in the faeces can be confirmed by electronic microscopy or by ELISA. Experimentally, a synergistic effect between rotavirus and *Escherichia coli* seem to occur, causing more severe diarrhoeal disease in weaning rabbits than that resulting from either pathogen alone (Thouless *et al.*, 1996).

In rabbits, coronavirus infections have been associated both with cases of pleural effusion disease (Fennestad *et al.*, 1975) and with diarrhoea in young rabbits (Lapierre *et al.*, 1980). Pleural effusion disease (PED) was observed for the first time in 1966 in laboratory rabbits inoculated with the Nichols strain of *Treponema pallidum* (Jorgensen, 1968). Later, it was shown that these PED cases were caused by a coronavirus infection (Fennestad *et al.*, 1975). To date all known coronavirus strains come from rabbit previously inoculated with *Treponema pallidum* (Fennestad *et al.*, 1986). At necropsy, there is effusive pleuritis with clear straw-colored fluid in the thorax (Fennestad *et al.*, 1975). Right ventricular dilatation and pulmonary oedema are also commonly found (Fujiwara, 1994). Acute rabbit coronavirus infection results in virus-induced myocarditis and congestive heart failure (Alexander *et al.*, 1992). Pulmonary alveolar epithelial cells are swollen with increase in the number of macrophages (Fujiwara, 1994). No cases of PED have been reported to date in commercial rabbitries. Specific pathology of enteritis in rabbits due to coronavirus is still unclear as are the real pathogenic characteristics of the virus strains. No data are available on the links between coronavirus strains causing PED and diarrhoea.

3/ Unclassified putative viral infections

The presence of viruses in the lungs of rabbits that died of pneumoenteritis has been suspected on the basis of a positive acridine-orange staining and the presence of inclusion bodies in histological sections (Rai et al., 1985). Virus-like particles similar in morphology and morphogenesis to paramyxoviruses have been observed by transmission electron microscopy in lungs of a rabbit that died of pneumonia (Ducatelle et al., 1994). Attempts to isolate these putative agents have not been undertaken.

Bibliography

Alexander, L.K., Small, J.D., Edwards, S., Baric, R.S. 1992. An experimental model for dilated cardiomyopathy after rabbit coronavirus infection. Journal of Infectious Diseases, 166, 5, 978-985.

Argüello Villares, J.L. 1991. Viral haemorrhagic disease of rabbits: vaccination and immune response. Revue Scientifique et Technique de l'Office International des Epizooties10, 2:471-480.

Arthur, C.P. and Louzis, C. 1988. A review of myxomatosis among rabbits in France. Revue Scientifique et Technique de l'Office International des Epizooties, 7, 937-976.

Asgari, S., J. R. E. Hardy, R. G. Sinclair and B. D. Cooke. 1998. Field evidence for mechanical transmission of Rabbit Haemorrhagic Disease Virus (RHDV) by flies (Diptera: Calliphoridae) among wild rabbits in Australia. Virus Research, 54: 123-132.

Bàrcena, J., M. Morales, B. Vasquez, J. A. Boga, F. Parra, J. Lucientes, A. Pagès-Mante, J. M. Sanchez-Vizcaino, R. Blasco and J. M. Torres. 2000. Horizontal transmissible protection against myxomatosis and Rabbit Hemorrhagic Disease by using a recombinant myxoma virus. Journal of Virology, 74, 3: 1114-1123.

Barry, M., S. Hnatiuk, K. Mossman, S. F. Lee, L. Boshkov, and G. McFadden. 1997. The myxoma virus M-T4 gene encodes a novel RDEL-containing protein that is retained within the endoplasmic reticulum and is important for the productive infection of lymphocytes. Virology. 239**:**360-77.

Bertagnoli S., J. Gelfi, F. Petit, J.F.Vautherot, D. Rasschaert, S. Laurent, G. Le Gall, E. Boilletot, J. Chantal, and C. Boucraut-Baralon. 1996a. Protection of rabbits against rabbit viral haemorrhagic disease with vaccinia-RHDV recombinant. Vaccine, 14, 506-510.

Bertagnoli S., J. Gelfi, F. Petit., C. Boucraut-Baralon and Milon A. 1998. Vaccination contre la myxomatose et la maladie hémorragique virale des lapins : construction et utilisation d'un virus myxomateux recombinant. C.R. VIIèmes Journées de la Recherche Cunicole Fr, Lyon, Mai 1998. INRA-ITAVI Edit., pp 57-60.

Bertagnoli S., J. Gelfi, J.F.Vautherot, D. Rasschaert, S. Laurent, G. Le Gall, E. Boilletot, F. Petit, C. Boucraut-Baralon and Milon A. 1996b. Protection against Myxomatosis and Rabbit Viral Haemorrhagic Disease with recombinant Myxoma viruses expressing Rabbit Hemorragic Disease Virus capsid protein. Journal of Virology., 70, 5061-5066.

Boga, J.A., R. Casais, M.S. Marin, J.M. Martin-Alonso, R.S. Carmenes, M. Prieto, and F. Para. 1994. Molecular cloning, sequencing and expression in *Escherichia coli* of the capsid protein gene from rabbit haemorrhagic disease virus (Spanish isolate AST/89). Journal of General Virology, 75:2409-2413.

Brun, A., A. Godard, and Y. Moreau. 1981. La vaccination contre la myxomatose, vaccins hétérologues et homologues. Bull. Soc. Sci. Vét. et Méd. comparée 83, 5:251-254.

Brun, A., Saurat, P., Gilbert, Y., Godart, A., Bouquet, J.F. 1981. Données actuelles sur l'épidémiologie, la pathogénie et la symptomatologie de la myxomatose. Revue de Médecine Vétérinaire, 132, 585-590.

Capucci, L., P. Fusi, A. Lavazza, M. L. Pacciarini and C. Rossi. 1996. Detection and preliminary characterization of a new rabbit calicivirus related to Rabbit Hemorrhagic Disease Virus but nonpathogenic. Journal of Virology, 70, 12: 8614-8623.

Carrell, R. W., and J. Travis. 1985. Alpha-1-antitrypsin, and the serpins: Variation and countervariation. Trends Biochemical Sciences. 10:20-24.

Deutrich, V., Hausburg, G. 1986. Control of myxomatosis by area vaccination. Monatsch. Veterinarmed, 41, 615-621.

Drillien, R., D. Spehner, D. Villeval, and J. P. Lecocq. 1987. Similar genetic organization between a region of fowlpox virus DNA and the vaccinia virus HindIII J fragment despite divergent location of the thymidine kinase gene. Virology. 160:203-9.

Ducatelle, R., Vanrompay, D., Charlier, G. 1994. Paramyxovirus associated with pneumonia in a dwarf rabbit. World Rabbit Science, 2, 47-52.

Fenner, F. 1994. Myxoma virus. In: Virus Infections of Vertebrates, Vol. 5. Virus Infections of Rodents and Lagomorphs, A.D.M.E. Osterhaus, Ed., Elsevier Science B.V., Amsterdam, pp. 59-71

Fenner, F., and F.N. Ratcliffe. 1965. Myxomatosis. Cambridge University Press, London.

Fennestad, K.L., Jensen, H.J.S., Moller, S., Bentzon, M.W. 1975. Pleural effusion disease in rabbits. Acta Pathologica Microbiologica Scandinavia, 83, 541-548.

Fennestad, K.L., Mansa, B., Christensen, N., Larsen, S., Svehag, S.E. 1986. Pathogenicity and persistence of pleural effusion disease virus isolates in rabbits. Journal of General Virology, 67, 993-1000.

Francki, R.I.B., C.M. Fauquet, D.L. Knudson and F. Brown (eds.). 1991. Classification and nomenclature of viruses. Fifth Report of the International Committee on Taxonomy of Viruses. Archive of Virology (supp.2), Springer-Verlag, 450 p.

Fujiwara, K. 1994. Rabbit coronavirus. In: *Virus infections of vertebrates. Volume 5. Virus infections of rodents and lagomorphs*. Osterhaus, A.D.M.E. (Ed.) Elsevier Science B.V., pp. 265-266.

Ghram, A., Benzarti, M., Amira, A., Amara, A. 1996. La myxomatose en Tunisie: étude séro-épidémiologique dans la région de Monastir (Tunisie). Archives de l'Institut Pasteur de Tunis, 73, 167-172.

Graham, K. A., A. Opgenorth, C. Upton, and G. McFadden. 1992. Myxoma virus M11L ORF encodes a protein for which cell surface localization is critical in manifestation of viral virulence. Virology. 191:112-24.

Graham, K. A., A. S. Lalani, J. L. Macen, T. L. Ness, M. Barry, L. Y. Liu, A. Lucas, I. Clark-Lewis, R. W. Moyer, and G. McFadden. 1997. The T1/35kDa family of poxvirus-secreted proteins bind chemokines and modulate leukocyte

influx into virus-infected tissues. Virology. 229**:**12-24.

Graham, K.A., A. Opgenorth, C. Upton, and G. McFadden. 1992. Myxoma Virus M11L ORF encodes a protein for which cell surface localization is critical in manifestation of viral virulence. Virology, 191:112-124.

Gregg, D. A., C. House, R. Meyer and M. Berninger. 1991. Viral Hemorrhagic Disease of rabbits in Mexico: epidemiology and viral Characterization. Revue Scientifique et Technique de l'Office International des Epizooties, 10: 435-451.

Hudson, J.B. 1994a. Herpesvirus cuniculi (leporid herpesvirus 2). In: *Virus infections of vertebrates. Volume 5. Virus infections of rodents and lagomorphs*. Osterhaus, A.D.M.E. (Ed.) Elsevier Science B.V., p. 141.

Hudson, J.B. 1994b. Herpesvirus sylvilagus (leporid herpesvirus 1). In: *Virus infections of vertebrates. Volume 5. Virus infections of rodents and lagomorphs*. Osterhaus, A.D.M.E. (Ed.) Elsevier Science B.V., p. 142-145.

Jackson R.J., and H.G. Bults. 1992. The Myxoma Virus thymidine kinase gene: sequence and transcriptional mapping. Journal of General Virology, 73:323-328.

Jorgensen, B.B. 1968. Spontaneous deaths among rabbits inoculated with *Treponema pallidum* less then 2 weeks before. Zentralblad Versuchstierk, 10, 46-54.

Joubert, L., Duclos, P.H., Tuaillon, P. 1982. La myxomatose des garennes dans le Sud-Est: la myxomatose amyxomateuse. Revue de Médecine Vétérinaire, 133, 739-753.

Kerr, P.J., Best, S.M. 1998. Myxoma virus in rabbits. Revue Scientifique et Technique de l'Office International des Epizooties, 17, 256-268.

Kroneman A., Horzinek M.C. Rabbit haemorrhagic disease. In: Virus infections of vertebrates. Volume 5. Virus infections of rodents and lagomorphs. Ed. Osterhaus A.D.M.E. Elsevier Science B.V. 1994, 391-402.

Lalani, A. S., J. Masters, K. Graham, L. Liu, A. Lucas, and G. McFadden. 1999. Role of the myxoma virus soluble CC-chemokine inhibitor glycoprotein, M- T1, during myxoma virus pathogenesis. Virology, 256**:**233-45.

Lapierre, J., Marsolais, G., Pilon, P., Descôteaux, J.P. 1980. Preliminary report on the observation of a coronavirus in the intestine of the laboratory rabbit. Canadian Journal of Microbiology, 26, 1204-1208.

Laurent, S., J.-F. Vautherot, M.-F. Madelaine, G. Le Gall, and D. Rasschaert. 1994. Recombinant rabbit hemorrhagic disease virus capsid protein expressed in baculovirus self-assembles into virus-like particles and induces protection. Journal of Virology, 68, 10:6794-6798.

Le Gall, G., C. Arnauld, E. Boilletot, J. P. Morisse and D. Rasschaert. 1998. Molecular epidemiology of rabbit haemorrhagic disease virus outbreaks in France during 1988-1995. Journal of General Virology, 79: 11-16.

Liu, S.J., H.P. Xue, B.Q. Pu, and S.H. Qian. 1984. A new viral disease in rabbits. Anim. Husb. Vet. Med. 16, 6:253-255.

Macen, J. L., C. Upton, N. Nation, and G. McFadden. 1993. SERP1, a serine proteinase inhibitor encoded by myxoma virus, is a secreted glycoprotein that interferes with inflammation. Virology, 195:348-63.

Marcato, P.S., Benazzi, C., Vecchi, G., Galeotti, M., Della Salda, L., Sarli, G., Lucidi, P. 1991. Clinical and pathological features of viral haemorrhagic disease of rabbits and the European brown hare syndrome. Revue Scientifique et Technique de l'Office International des Epizooties, 10, 2, 371-392.

Marlier D., Herbots, J., Detilleux, J., Lemaire, M., Thiry, E., Vindevogel, H. 2000d. Cross-sectional study of the association between pathological conditions and existence of myxoma virus antibodies in intensive rabbit farms. Preventive Veterinary Medicine. Submitted for publication.

Marlier, D., Boucraut-Baralon, C., Vindevogel, H. 1999b. Diagnosis of myxomatosis by an indirect immunoperoxidase test. Vlaams Diergeneeskundig Tijdschrift, 68, 142-144.

Marlier, D., Cassart, D., Boucraut-Baralon, C., Coignoul, F., Vindevogel, H. 1999a. Experimental infection of specific pathogen-free New Zealand White rabbits with five strains of amyxomatous myxoma virus. Journal of Comparative Pathology, 121, 369-384.

Marlier, D., Mainil, J., Boucraut-Baralon, C., Linden, A., Vindevogel, H. 2000c. The efficacy of two vaccination schemes against experimental infection with a virulent amyxomatous or a virulent nodular myxoma virus strain. Journal of Comparative Pathology, 122, 115-122.

Marlier, D., Mainil, J., Linden, A., Vindevogel, H. 2000a. Infectious agents associated with rabbit pneumonia: isolation of amyxomatous Myxoma virus strains. The Veterinary Journal, 159, 171-178.

Marlier, D., Mainil, J., Sulon, J., Beckers, J.F., Linden, A., Vindevogel, H. 2000b. Study of the virulence of five strains of amyxomatous myxoma virus in crossbred New Zealand White / Californian conventional rabbits, with evidence of long-term testicular infection in recovered animals. Journal of Comparative Pathology, 122, 101-113

Marlier, D., Vindevogel, H. 1996. La myxomatose amyxomateuse: isolement de trois souches en Belgique. Annales de Médecine Vétérinaire, 140, 343-346

Matsugana, Y., Chino, F. 1981. Experimental infection of young rabbits with rabbit parvovirus. Archives of Virology, 68, 257-264.

Matsugana, Y., Matsuno, S., Mukoyama, J. 1977. Isolation and characterization of a parvovirus of rabbits. Infection and Immunity, 18, 2, 495-500.

Messud-Petit, F., J. Gelfi, M. Delverdier, M. F. Amardeilh, R. Py, G. Sutter, and S. Bertagnoli. 1998. Serp2, an inhibitor of the interleukin-1beta-converting enzyme, is critical in the pathobiology of myxoma virus. Journal of Virology, 72:7830-9.

Metcalf, J.B., Lederman, M., Stout, E.R., Bates, R.C. 1989. Natural parvovirus infection in laboratory rabbits. American Journal of Veterinary Research, 50, 7, 1048-1051.

Meyers, G., C. Wirblich, and H.J. Thiel. 1991. Rabbit hemorrhagic disease virus- molecular cloning and nucleotide sequencing of a calicivirus genome. Virology, 184:664-676.

Moreno-Lopez, J. 1994a. Cottontail rabbit papillomavirus. In: *Virus infections of vertebrates. Volume 5. Virus infections of rodents and lagomorphs*. Osterhaus, A.D.M.E. (Ed.) Elsevier Science B.V., p. 161-164.

Moreno-Lopez, J. 1994b. Oral papillomatosis virus of rabbits. In: *Virus infections of vertebrates. Volume 5. Virus infections of rodents and lagomorphs*. Osterhaus, A.D.M.E. (Ed.) Elsevier Science B.V., p. 165-166.

Morisse, J.P. 1982. Isolement d'un Rotavirus sur lapins à diarrhée. Recherche du pouvoir pathogène. Recueil de Médecine Vétérinaire, 158, 12, 805-808.

Morisse, J.-P., G. Le Gall, and E. Boilletot**.** 1991. Hepatitis of viral origin in Leporidae: introduction and aetiological hypotheses. Revue Scientifique et Technique de l'Office International des Epizooties, 10, 2:283-295.

Mossman, K., S. Lee, M. Barry, L. Boshkov, and G. McFadden. 1996. Disruption of M-T5, a novel myxoma virus gene member of the poxvirus host range superfamily, results in dramatic attenuation of myxomatosis in infected European rabbits. Journal of Virology, 70:4394-4410.

Mutze, G., B. Cooke and P. Alexander. 1998. The initial impact of rabbit hemorrhagic disease on European rabbit populations in South Australia. Journal of Wildlife Diseases, 34: 221-227.

Nash, P., A. Lucas, and G. McFadden. 1997. SERP-1, a poxvirus-encoded serpin, is expressed as a secreted glycoprotein that inhibits the inflammatory response to myxoma virus infection. Adv Exp Med Biol. 425:195-205.

Nash, P., A. Whitty, J. Handwerker, J. Macen, and G. McFadden. 1998. Inhibitory specificity of the anti-inflammatory myxoma virus serpin, SERP-1. Journal of Biological Chemistry, 273:20982-91.

Ohlinger, V.F., B. Haas, G. Meyers, F. Weiland, and H.J. Thiel. 1990.

Identification and characterization of the virus causing rabbit haemorrhagic disease Journal of Virology, 64, 7:3331-3336.

Onderka, D.K., Papp-Vid G., Perry A.W. 1992. Fatal herpesvirus infection in commercial rabbits. Canadian Veterinary Journal, 148, 2, 93-95.

Opgenorth A., K.A. Graham, N. Nation, D. Strayer. and G. McFadden. 1992. Deletion analysis of two tandemly arranged virulence genes in Myxoma virus, M11L and Myxoma Growth Factor. Journal of Virology, 66:4720-4731.

Parra, F. and M. Prieto. 1990. Purification and characterization of a calicivirus as the causative agent of a lethal hemorrhagic disease in rabbits. Journal of Virology 64, 7:4013-4015.

Patton, N.M., Holmes, H.T. 1977. Myxomatosis in domestic rabbits in Oregon. Journal of the American Veterinary Association, 171, 560-562.

Peeters, J.E. 1989. Ademhaling en spijsverteringsstoornissen in de industriële slachtkonijnenhouderij. Diergeneeskundig Memorandum, 3, 90-135.

Percy, D.H., Barthold, S.W. 1993. Pathology of laboratory rodents and rabbits. Ed. Iowa State University Press, Ames, Iowa. p. 179-224.

Petit, F., S. Bertagnoli, J. Gelfi, F. Fassy, C. Boucraut-Baralon, and A. Milon. 1996. Characterization of a myxoma virus-encoded serpin-like protein with activity against interleukin-1 beta-converting enzyme. Journal of Virology, 70:5860-6.

Pringle, C. R. 1998. Virus taxonomy – San Diego 1998. Archive of Virology, 143: 1449-1459.

Rai, R.B., Singh, D., Singh, R.N. 1985. Aetiological agents associated with pneumoenteritis in rabbits. Indian Journal of Animal Sciences, 55, 163-168.

Rasschaert, D., S. Huguet, M.F. Madelaine, and J.F. Vautherot. 1995. Sequence and genomic organization of a rabbit haemorrhagic disease virus isolated from a wild rabbit. Virus Genes, 9, 2:121-132.

Rosell, J.M., Badiola-Saiz, J.I. , De La Fuente, L.F. 1992. Viral infection of rabbits in Spain. Study of field results during 1986 - 1990. Rivista di Coniglicoltura, 29, 45-50. (in Italian).

Rosell, J.M., Gonzales, J.L., Rueda, A., Gallego, E., Flores, J.M. 1984. Mixomatosis atipica en Espana. Medicina Veterinaria, 1, 401-412.

Sato, K., Inaba, Y., Miura, Y., Tokuhisa, S., Matumoto, M. 1982. Isolation of lapine rotavirus in cell cultures. Archives of Virology, 71, 267-271.

Saurat, P., Y. Gilbert, and J.-P. Ganière. 1978. Etude d'une souche de virus myxomateux modifié. Revue de Médecine vétérinaire, 129, 3:415-451.

Thouless, M.E., DiGiacomo, R.F., Deeb, B.J. 1996. The effect of combined rotavirus and Escherichia coli infections in rabbits. Laboratory Animal Science, 46, 4, 381-385.

Turner, P. C., M. C. Sancho, S. R. Thoennes, A. Caputo, R. C. Bleackley, and R. W. Moyer. 1999. Myxoma virus Serp2 is a weak inhibitor of granzyme B and interleukin- 1beta-converting enzyme in vitro and unlike CrmA cannot block apoptosis in cowpox virus-infected cells. Journal of Virology, 73:6394-404.

Ueda, K., Park, J.H., Ochiai, K., Itakura, C. 1992. Disseminated intravascular coagulation (DIC) in rabbit haemorrhagic disease. Japanese Journal of Veterinary Research, 40, 133-141.

Upton C., J.L. Macen, and G. McFadden. 1987. Mapping and sequencing of a gene from Myxoma virus that is related to those encoding Epidermal Growth Factor and Transforming Growth Factor Alpha. Journal of Virology, 61:1271-1275.

Upton, C., and G. McFadden. 1986. Tumorigenic poxviruses: analysis of viral DNA sequences implicated in the tumorigenicity of Shope fibroma virus and malignant rabbit virus. Virology, 152:308-21.

Upton, C., J. L. Macen, D. S. Wishart, and G. McFadden. 1990a. Myxoma virus and malignant rabbit fibroma virus encode a serpin-like protein important for virus virulence. Virology, 179:618-31.

Upton, C., K. Mossman, and G. McFadden. 1992. Encoding of a homolog of the IFN-gamma receptor by myxoma virus. Science, 258:1369-72.

Upton, C., A. Opgenorth, P. Traktman, and G. McFadden. 1990b. Identification and DNA sequence of the Shope fibroma virus DNA topoisomerase gene. Virology, 176:439-47.

Wirblich, C., H. J. Thiel and G. Meyers. 1996. Genetic map of the calicivirus rabbit hemorrhagic disease virus as deduced from *in vitro* translation studies. Journal of Virology, 70: 7974-7983.