Network for wildlife health surveillance in Europe

Diagnosis Card

Myxomatosis

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Etiology

Myxomatosis is an infectious disease of lagomorphs caused by the *Myxoma* virus (MYXV), a poxvirus (family *Poxviridae*; subfamily *Chordopoxvirinae*; genus *Leporipoxvirus*) that was first isolated from laboratory rabbits (*Oryctolagus cuniculus*) in Uruguay in 1898. The MYXV genome encodes about 170 genes, among which approximately 70 encode immunomodulatory and host-interactive factors (Kerr, 2021) that are involved in subverting the host immune system and other anti-viral responses. Prototype strains of virus deriving from the Australian and European outbreaks have been designated to characterise the various virulence grades (from grade I to grade V). The ha-MYXV/MYXV Toledo isolate from Iberian hares (*Lepus granatensis*) in 2018 in Spain is a recombinant strain, characterised by an insertion of four novel poxviral genes towards the 3' end of the negative strand of its genome (Agueda-Pinto *et al.,* 2019; Dalton *et al.,* 2019).

Affected species (wildlife, domestic animals, humans)

The natural hosts are two wild leporid species: *Sylvilagus brasiliensis* in South America (South American strains) and *S. bachmani* in California, USA (Californian strains), in which MYXV causes subclinical infection or localized cutaneous fibromas. Following deliberate introductions into Australia (1950) and Europe (1952) as a biological control measure for wild European rabbits (*Oryctolagus cuniculus)*, MYXV has become endemic and widely distributed in wild rabbit populations, and can spill over into farmed, laboratory and pet rabbits. European brown hares (*Lepus europeus*) and Italian hares (*Lepus corsicanus*) (Rossini *et al*, 2024) may rarely develop generalised disease. However, a cross-species jump of MYXV has been documented in the Iberian Peninsula, where high mortalities in Iberian hare populations have been reported (Garcia-Bocanegra *et al*., 2019; Abade Dos Santos et al., 2020; Agulló-Ros et al., 2023). Additionally, cases of this emerging MYXV variant have been reported in European brown hares in Spain and Great Britain. There is no known risk of non-leporid species (including human) infection with MYXV.

Epidemiological characteristics and disease course

Wild European rabbits (*Oryctolagus cuniculus*) act as reservoirs, and insects (mainly mosquitoes and fleas but also midges, ticks and lice) can transmit the virus. In areas where wild and domestic rabbits are in close proximity (i.e. in farmed settings), virus can also be transmitted by direct contact. MYXV is shed in ocular and nasal secretions or from skin lesions and it may also be present in semen and genital secretions, indicating infection of the testicles, where the virus can persist for months after recovery and be re-excreted when immunosuppression occurs (Marlier *et al*, 2000). There is no age or sex predilection. Two forms of the disease are observed: the nodular (classical) form and the amyxomatous (respiratory) form. Nodular myxomatosis is naturally transmitted by biting insects and mainly observed in wild and domestic rabbits. Although described since the 1980s in wild rabbit populations (Joubert *et al*, 1982), the amyxomatous form is considered as more significant for farmed rabbits and it is considered an adaptation to direct transmission in the absence of competent vectors, presumably via respiratory and conjunctival secretions. So far, this form is commonly reported in countries with substantial rabbit meat production.

Clinical signs and gross lesions

The classical form of myxomatosis is characterised by florid skin lesions and severe immune dysfunction, accompanied by supervening bacterial infections of the respiratory tract. After infection with a highly virulent strain, the first clinical sign is a lump at the site of infection, which increases in size, becomes protuberant and ulcerates. An acute blepharoconjunctivitis and an oedematous swelling of the genital area gradually develop. The secondary skin lesions appear on about the sixth or the seventh day. Death usually occurs between the 8th and 15th day post-infection. After infection with milder or low virulent strains, the same clinical signs evolve more slowly and are less severe, and in surviving animals, the lesions progressively heal. The mortality rate fluctuates between 20 and 100% according to the grade of virulence of the viral strain. Secondary bacterial infections (in particular *Pasteurella* sp. and *Bordetella* sp.) are typical in rabbits that survive longer than 10–14 days after infection and may be the major cause of death in rabbits infected with subacute strains of MYXV.

The clinical signs of amyxomatous myxomatosis are mainly respiratory and ocular (swollen eyelids, blepharoconjunctivitis and rhinitis) with fewer and smaller cutaneous lesions. Perineal oedema is also present. The clinical expression by amyxomatous viruses appears to depend on the presence of bacterial pathogens such as *Pasteurella multocida* (Marlier et al, 2000).

The main macroscopic lesions caused by ha-MYXV infection in hares include bilateral blepharoconjunctivitis, epistaxis, rectal bleeding, oedema of the nasal, oral, genital and anal orifices, and intense congestion and internal haemorrhages in several internal organs, while visible myxomas are usually not found.

Histological lesions

Histopathology of cutaneous lesions shows that the large lumps found in the skin are mainly due to an accumulation of mucinous material with destruction of the connective tissue architecture in the dermis rather than to an intense cellular proliferation (Marcato & Rosmini, 1986). The derma and epidermis are invaded by granulocytes and enlarged, stellate, reticulo-endothelial cells with a large nucleus and abundant cytoplasm, called “myxoma cells”. These cells destroy the endothelium of small vessels causing extravasation of red cells; they also replicate in the spleen and lymph nodes causing complete loss of lymphocytes from both B-cell and T-cell zones. After the viraemic phase, the virus spreads throughout the body and causes genital and visceral lesions, mainly congestive with vascular damage. In the lung the lesions are of variable intensity and the characteristic epidermal lesions are also observed in the bronchial epithelium.

In Iberian hares infected with ha-MYXV, the most common histopathological findings include hyperplastic epidermis with predominant hyperkeratosis and myxoid matrix in the dermis. ha-MYXV-positive keratinocytes exhibit hydropic degeneration and cytoplasmic inclusion bodies. Alveolar oedema, interstitial pneumonia, marked lymphoid depletion in the spleen and necrosis in the liver and testes have also been observed.

Differential diagnosis

Clinical signs of classic myxomatosis are fairly clear-cut, although bacterial upper respiratory tract infections and bacterial conjunctivitis/keratoconjunctivitis can cause confusion and misdiagnosis. Ha-MYXV infection in Iberian hare casuses vascular lesions compatible with Pasteurellosis, EBHS and RHD. In cases of amyxomatous forms, particularly after infection with strains of moderate to low pathogenicity, the clinical signs are difficult to distinguish from bacterial respiratory disease (particularly with *Pasteurella*, which is also often associated). Rabbit fibroma virus (RFV, formerly Shope fibroma virus) produces a simple fibromatous local lesion that should be distinguished from MYXV. Genital lesions should be distinguished from those induced by *Treponema paraluisleporidarum* (Hare and Rabbit Syphilis).

Criteria for diagnosis

The different techniques available vary in their ability to detect MYXV in typical myxomatous lesions, oedema of the eyelids or genital oedema. Nevertheless, the diagnosis of attenuated typical myxomatosis or of atypical (amyxomatous) forms usually requires viral identification. In addition to classical direct methods i.e. the isolation of the virus by inoculation of sensitive cell lines and identification of the virus by electron microscopy and immunological methods, nowadays, the MYXV is easily and quickly identified by demonstration of its nucleic acid. Indeed, molecular techniques can reveal subclinical infection (e.g. by testing conjunctival swabs).

Infection of rabbits with MYXV strains induces a strong adaptive immunological response with the production of specific antibodies of the IgM and IgG classes, which, associated with clinical signs and/or clinicopathological abnormalities compatible with myxomatosis, usually confirm the diagnosis (Kerr, 1997).

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

In the case of classical form, the identification of the virus in skin lesions (myxomas), eyelid, genital mucosa, conjunctival/nasal swabs and internal organs (lungs, liver, spleen, kidney etc) can be

done using electron microscopy (EM), direct immunofluorescence (IF), isolation on cell-culture and eggs embryos, ELISA and PCR. For the amyxomatous respiratory form of the disease, nasal and conjunctival swabs and respiratory tissues (e.g. lungs) may be collected for viral identification.
Tissue cryosections fixed in methanol can be directly immunostained with fluorescein-conjugated anti-MYXV serum or MAbs. An *in-vivo* direct immunofluorescence (DIF) test on impressions of cornea, eyelid and conjunctival cells has also been described (DIF-ET).
Histopathology and immunohistochemistry (with hyperimmune serum or specific MAbs) can be conducted in formalin fixed lungs and skin lesions.
Isolation of the virus in cell culture can be accomplished using primary cultures of rabbit kidney (RK) cells, or with established cell lines, such as RK-13, SIRC, Vero and BGMK. The specificity of the cytopathic effect is usually confirmed by EM, Indirect fluorescent antibody tests (IFAT) or Immunoperoxidase monolayer assay (IPMA). MYXV can be also cultured on the chorioallantoic membrane of embryonated chicken eggs.
Polymerase chain reaction (PCR) (Cavadini et al., 2010) or real-time PCR (Albini et al., 2012; Duarte et al., 2014), can be used to amplify genome fragments of MYXV in diagnostic material including eyelid, ear and nasal myxomas, crusts and/or lung lesions, nasal and conjunctival swabs or semen. All these methods are also able to detect the recombinant ha-MYXV strain (Garcia-Bocanegra et al., 2019; Dalton et al., 2019; Abade Do Santos et al., 2022; Rossini et al., 2024).
To detect serum anti-MYXV antibodies in rabbits, enzyme-linked immunosorbent assays (ELISAs) are preferred, for their simplicity, speed, low cost and high sensitivity and specificity. Two very similar indirect ELISAs (I-ELISA), with the antigen directly coated to the solid phase, show similar performances (Gelfi *et al*, 1999; WOAH, 2021). At the WOAH Reference Laboratory for Myxomatosis (WOAH, 2021), serological tests are routinely performed by using a competitive ELISA (C-ELISA), centred on the use of a MAb (1E5) that specifically recognises the MYXV immunodominant envelope protein (IMV – open reading frame M071L). As this protein is also expressed in the ha-MYXV hare strain and as the method does not depend on secondary specific antispecies antibodies, it could also be used to detect antibodies in hare species.

EWDA proposed harmonized protocol (for harmonization at large scale)

Direct diagnosis: PCRs (one step PCR and real-time PCR) are the universal method able to ascertain viral positivity in a short time with high sensitivity from skin lesions, internal organs or mucosal swabs. Isolation in eggs embryo and cell cultures, and direct viral demonstration with IFAT and EM are alternative and/or confirmatory methods.
Indirect diagnosis: the detection of specific antibodies against MYXV is achieved by ELISAs that should be considered the elective method for serosurveys.
All samples (including sera) must be kept refrigerated (4ºC) until transport to the laboratory (within 24-48 hours) stored at -20ºC until serological and molecular processing.

Laboratories that can be contacted for diagnostic support

- Reference laboratory of the WOAH for *Myxomatosis* (see: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/). Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER), National Reference Centre for Viral Diseases of Rabbits, Brescia (Italy) (https://www.izsler.it/chi-siamo/per-chi-e-con-chi-lavoriamo/centri-di-referenza/internazionali/oie-reference-laboratory-for-myxomatosis-of-rabbits/).
- École Nationale Vétérinaire de Toulouse Laboratoire Interactions Hôtes-Agents Pathogènes, Toulouse (France) (stephane.bertagnoli@envt.fr).
- Instituto Universitario de Biotecnología de Asturias, Departamento de Bioquímica y Biología Molecular, Edificio Santiago Gascón, Universidad de Oviedo, Oviedo, Spain (https://iuba.uniovi.es).
- Animal Health and Zoonosis Research Group (GISAZ), University of Córdoba, Spain, (nacho.garcia@uco.es).

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