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Orf virus infection in sheep or goats

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ABSTRACT

Orf virus, a member of the genus *Parapoxvirus*, is the causative agent of contagious ecthyma ('Orf'). It is a pathogen with worldwide distribution, causing significant financial losses in livestock production. The disease mainly affects sheep and goats, but various other ruminants and mammals have been reported to be infected as well. It is also a zoonotic disease, affecting mainly people who come in direct or indirect contact with infected animals (e.g. farmers, veterinarians). The disease is usually benign and self-limiting, although in many cases, especially in young animals, it can be persistent and even fatal. Production losses caused by *Orf virus* are believed to be underestimated, as it is not a notifiable disease. This review of literature presents all latest information regarding the virus; considerations regarding treatment and prevention will be also discussed.

1. Introduction

Contagious ecthyma ('Orf') is a contagious disease, caused by the epitheliotropic *Orf virus*, a member of the genus *Parapoxvirus*. The disease has a worldwide distribution and a significant financial importance. The disease affects primarily sheep and goats; camels, South American camelids, Cervidae (deer, reindeer), other ruminants (bighorn sheep, chamois, dall sheep, mountain goats, musk oxen, serows, steenboks, tahr), dogs, cats and squirrels. The disease also has a zoonotic potential, although it is more of an occupational hazard to people working with animals (e.g. farmers, animal carers, veterinarians).

2. Classification, structure and genome

Orf virus is the prototype species of the genus *Parapoxvirus*, belonging to the family Poxviridae, sub-family Chordopoxvirinae. Parapoxviruses include four species currently recognised by the International Committee on Taxonomy of Viruses: *Orf virus*, *Bovine popular stomatitis virus*, *Pseudocowpox virus* and *Parapoxvirus* of red deer. These can be differentiated from other poxviruses from their ovoid shape, the relatively small size (220–300 × 140–170 nm, ~260 × 160 nm for *Orf virus*) and the high G + C content of 64% on average compared to 30–40% of other poxviruses (Wittek et al., 1979). However, their most characteristic feature is a unique spiral (criss–cross) tubule-like pattern of their coat.

These viruses consist of linear double-stranded DNA (130–160 kbp in size), with a large number of homologues to most structural genes of poxvirus vaccinia, suggesting a similar structure and morphogenesis (Delhonet al., 2004; Mercer et al., 2006). Like poxvirus vaccinia, *Orf virus* has also at least two types of infectious particles: the mature virion, in which the outer membrane is derived from the endoplasmic reticulum, and the extracellular virion produced from the wrapped virion form, wrapped from two additional membranes derived from the *trans*-Colgi after losing its outermost membrane during egress (Tan et al., 2009). By producing these extracellular virion particles, *Orf virus* is able to spread from cell to cell.

The general genomic structure of *Orf virus* (~138 kbp long and 132 genes) is similar to that of other poxviruses. The central area of the genome consists of essential functional genes involved in viral replication and morphogenesis of mature virions and extracellular virions, while factors associated with virulence, pathogenesis, immune evasion/modulation and host range are encoded in the terminal parts that represent the 20% of the viral genome (Buttner and Rziha, 2002). Inverted terminal repeats (ITR), of ~3 kpbs in length, which are identical but oppositely oriented sequences, are found at the two ends of the genome and the two strands of DNA are covalently closed with hairpin loops of 100 bp in size. *Orf virus* strains are characterised by an extend heterogeneity; even different strains exhibit diverse restriction fragment profiles (Robinson and Lyttle, 1992) and comparison of independent isolates of the virus have revealed a high degree of interspecies genetic variation (Mercer et al., 2006).

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3. Epidemiology and pathogenesis

Orf virus is the causative agent of contagious ecthyma ('contagious pustular dermatitis', 'sore mouth', 'scabby mouth', 'Orf'), a disease with worldwide distribution and significant financial importance. The disease affects primarily sheep and goats (usually more severe in the latter species), but it has also been reported in camels and camelids, members of the Cervidae family and various other ruminants (e.g. chamois, serows, tahr, steenboks); dogs, cats and squirrels can also been affected. It is also a zoonosis, affecting usually people who come in direct or indirect contact with infected livestock (e.g. farmers, veterinarians). The virus has been found to survive for up to 17 years in environments with dry climate and remain viable on the wool of animals and contaminated material for significant periods.

Virus transmission is done through contact, entering via damaged skin and replicating in epidermal cells.This usually occurs during grazing and through the abrasions developed on the lips, nostrils and mouth by the dried feeds. The viral replication leads to oedematous and granulomatous inflammation of dermal cells. Typical lesions are initially erythematous spots followed by formation of papules, vesicles, pustules with a yellowish creamy appearance and scabs that finally become dry and shed with no scar remaining. This development pattern takes place in a period of one to two months (Nandi et al., 2011).

When Orf affects the teats of lactating ewes or does, changes in the local defence mechanisms of can occur potentially predisposing the animals to mastitis (Mavrogianni et al., 2006; Mavrogianni and Fthenakis, 2007). In fact, the virus encodes immunomodulatory factors that interfere with host inflammatory effect or/and anti-virus immune mechanisms. These are interleukin-10, which suppresses cytokine production by activated macrophages, and another protein that inhibits the biological activity of granulocyte macrophage-colony stimulating factor and interleukin-2 (Haig and McInnes, 2002). The granulocyte macrophage-colony stimulating factor plays a significant role in activating neutrophils and macrophages and improving chemotactism and peroxidase production from neutrophils, while interleukin-2 normally enhances phagocytic action of macrophages (Sordillo et al., 1997); however, the activity of macrophages in udder is depressed in Orf infections (Mavrogianni et al., 2006).

4. Clinical presentations

In lambs or kids, after approximately a week of incubation period, an initial rise in temperature is accompanied by development of skin lesions at the area of mouth, lips and nose, with the previously described development pattern. Lesions can also be seen in the buccal cavity (tongue, gums, hard palate), occasionally also in the oesophagus or the abomasum of affected animals. Lesions are more severe in young (<2 month-old) animals, when they can extend to the skin of face, feet, flanks, scrotum and peri-anal area (Reid, 2000; De la Concha-Bermejillo et al., 2003; Guo et al., 2003). Lesions can expand into the oral cavity (buccal mucosa, tongue and palate) in the form of papules that become ulcerated and covered with exudate (McElroy and Bassett, 2007).

Lambs and kids suffer, because of restricted suckling and grazing (Chan et al., 2007), as Orf lesions are painful. Severe lesions of udder skin may result also to abandonment of the offspring, as suckling becomes painful. In the summer, myiasis can be a complication of the lesions (Housawi and Abu Elzein, 2000). Foot lesions may also be severe, especially when coronets of finishing lambs are affected; co-infections with *D. congolensis* may result in the so-called 'strawberry foot rot' (Cooper et al., 1970; Yeruham et al., 2000). Dual co-infection with *Papilloma virus* and *Sheep pox virus* infection has also been reported (Yeruham et al., 1998; Wilson et al., 2002).

In ewes, lesions are observed mainly in the body of the teat and around the teat orifice, as well as at the udder skin and, less often, in the inguinal area and the thigh (Nandi et al., 2011). Affected ewes may subsequently develop mastitis caused by *Mannheimia haemolytica* or staphylococci (Watt, 1983; Reid, 1991; Mavrogianni et al., 2006).

In adult animals, lesions can also be found in the genital organs (rams: preputial orifice, ewes: vulva and skin-vaginal junction) (Gouletsou and Fthenakis, 2010; Billinis et al., 2012). The venereal form of the disease is characterised by appearance of papules, vesicles and ulcers on the skin of vulva of ewes and the preputial orifice of rams (De la Concha-Bermejillo et al., 2003). Invasion by *F. necrophorum* can cause severe complications and necrosis. In rams, ulcerative lesions characteristic of Orf in the prepuce are accompanied with inability to mount the ewe, loss of libido and incomplete erection (Fthenakis et al., 2001).

In flocks where the disease occurs for the first time, morbidity rates can be up to 70%. Mortality however, is usually low (<1%), although increased rates (up to 90%) have been reported in lambs after secondary bacterial infections (Zhao et al., 2010). These usually involve staphylococci, streptococci, corynebacteria, and less often *Dermatophilus congolensis* and *Fusobacterium necrophorum*. However, the disease is usually self-limiting with varying clinical features and in some animals may remain subclinical.

5. Diagnosis

Clinical signs and lesions are in most cases characteristic of the disease and can help differentiate the disease from similar ones causing postular lesions. Other diseases causing postular lesions in the face are: pox, foot and mouth disease, blue-tongue disease, peste-des-petits-ruminants, sarcoptic mange, chorioptic mange and staphylococcal dermatitis (Wilson et al., 2002; Watson, 2004). Due to the nature of some of these diseases, it is important that the final diagnosis is achieved with high accuracy. Any case of doubt should be referred to the state veterinary services, as some of the above diseases are notifiable, with a quick transmission potential and severe production losses. It is recommended that a laboratory diagnosis is always undertaken, in order to support the clinical diagnosis. A variety of laboratory techniques are available.

Isolation of the virus in cell cultures has been reported to be successful in a variety of primary and continuous cell lines, e.g. primary lamb testis, kidney, foetal lamb muscle and turbinate cells, foetal bovine muscle and lung cells, MDOK, MDBK and Vero cells (Mercer et al., 1994; Inoshima et al., 1999; McInnes et al., 2001; Guo et al., 2003; Delhon et al., 2004; Klein and Tryland, 2005). Parapoxvirus culturing, in general, is considered to be difficult, with a need for many passages before observing cytopathic effects, e.g. ballooning, wounding, degeneration of cells (Kruse and Weber, 2001; Vikoren et al., 2008).

Serum neutralization test is not considered to be the method of choice for primary diagnosis, as immunity to *Orf virus* is mainly cell-mediated and neutralising antibodies are usually at small concentrations (Haig and Mercer, 1998). Titres ≥8 are considered positive in serum neutralisation tests (Zarnke et al., 1983). Antibody detection by ELISA has been applied with success in lamb serum samples (McKeever et al., 1987; Yirrell et al., 1994; Azwaiet al., 1995), applying peroxidase conjugated protein A or G or chimeric A/G (Inoshima et al., 1999). Indirect IFA based on a panel of monoclonal antibodies against *Orf virus* have been used (Lard et al., 1991; Rosenbusch and Reed, 1983; Inoshima et al., 2001; Kanou et al., 2005) with the results indicating significant antigenic cross reactions between *Orf virus*, *Pseudocowpox virus* and *Bovine popular stomatitis virus* (Lard et al., 1991; Rosenbusch

and Reed, 1983), although six monoclonal antibodies against *Orf virus* were found capable of differentiating the virus from other viruses (Inoshima et al., 2001).

Diagnosis of *Orf virus* infections by PCR is based on the amplification of B2L (Inoshima et al., 2000) or VIR (Kottaridi et al., 2006a) genomic region. A duplex PCR assay using A29 gene (413 bp) and H3L gene (708 bp) has the potential to differentiate capripox viruses from *Orf virus* (Zheng et al., 2007), which is a particular significance in the differential diagnosis of the disease. Real time PCR has also been developed based on B2L gene, encoding major envelope antigen, to identify and quantify *Orf virus* in clinical samples and to differentiate from related viruses (Gallina et al., 2006).

Loop-mediated isothermal amplification is a simple technique that amplifies specific DNA sequences with high sensitivity under isothermal conditions. Assays targeting B2L, DNA polymerase and F1L genes have been developed and proven to be effective diagnostic tools (Tsai et al., 2009; Li et al., 2013; Wang et al., 2013). The use of restricted fragment length polymorphism, by employing various restriction enzymes for digestion of viral DNA for molecular characterisation of different strains of parapox viruses, genome typing and determining the virus heterogenicity has also been demonstrated (Rafii and Burger, 1985).

Phylogenetic analysis is an important tool for investigating relationship between various isolates (Klein and Tryland, 2005). Various genomic regions with a strong phylogenetic signal have been recognised and used in phylogenetic studies, e.g. the VIR or the B2L genes (Inoshima et al., 2000; Kottaridi et al., 2006b). In a recent study, it was found that differences may be observed between strains based on their geographical origin, although these could not be associated with pathogenicity of the strains (Billinis et al., 2012). Few phylogenetic studies have been performed worldwide regarding *Orf virus* (Abrahao et al., 2009; Oem et al., 2009; Zhang et al., 2010). However, molecular correlation of strains with geographical origin could be usefully employed as an epidemiological tool during outbreak investigations, detecting origin of exposure. A recent example indicates the introduction and circulation in India of distinct *Orf virus* strains of Chinese origin (Kumar et al., 2014). Also, high heterogeneity among isolates within a relatively small distance heightens the need for better surveillance (Chi et al., 2013). Moreover, molecular investigation of isolated strains should be implemented in a wider scale from all over the world, in order to obtain more secure conclusions regarding possible correlation of strain differences and clinical presentations.

6. Treatment

Control of contagious ecthyma outbreaks is based on standard hygiene practices established in the infected flocks/herds. Every animal with clinical signs should be kept separately from other animals in the farm. Because of the zoonotic nature of the disease, farmers and veterinarians should wear protective gloves and facemasks when dealing with sick animals, as well as during vaccination. Introduction of new animals in an infected farm should be done with caution and after keeping them in quarantine until control measures have been undertaken. An important part of disease control has to do with the disinfection programmes implemented. The virus can survive for years in the environment, thus all premises of animal housing as well as all potentially infected equipment and materials should be disinfected using incineration and anti-viral agents.

Special treatment should also be performed regarding secondary bacterial infections that commonly coexist with the disease. Use of antibiotics can help and should be applied for this purpose. Locally, an antibiotic ointment can be applied (Nandi et al., 1999), whilst, local application of preparations that contain $KMnO_4$ and boric acid may also be helpful (Rao et al., 1994). Levamisole has been proposed for use as immunostimulant (Wilson et al., 2002). Supportive treatment of young animals in bad state due to received feed intake should be performed by administering glycose solution by means of oesophagal intubation.

In recent years, the use of anti-viral drugs has been applied in human and animal Orf infections with satisfying results. Cidofovir has been tried as an anti-*Orf virus* for treatment of human Orf, with promising results, although current costs can be prohibitive for using it in lambs (De Clercq, 2002; Dal Pozzo et al., 2007). An experimental preparation containing sucralfate and cidofovir, in spray form, was shown to have efficacy in lambs, as it combined the antiviral activity of cidofovir and the wound healing properties of sucralfate (Sonvico et al., 2009).

7. Vaccination and other prevention measures

Vaccination remains the best, if not the only option, for efficient control of contagious ecthyma. Vaccines against Orf should be used where infections has arisen and vaccinated animals should be kept separated from unvaccinated ones. Even newborns with no maternal antibodies can obtain a sufficient protective immunity if vaccinated up to four days after birth. Attenuated vaccines are usually preferred because of improved efficacy. Autologous vaccines can be produced by dissolving scab material in saline-penicillin/streptomycin solution and inoculating after scarification in the inner thigh. In a vaccinated flock, disease will re-emerge only after unvaccinated animals become dominant, years after the vaccination of the flock. Attenuated tissue culture vaccines have been proved to be important for reduction of the severity of symptoms in infected flocks (Nettleton et al., 1996; Bath et al., 2005). However, it has also been proven that occasionally the vaccine virus strain may cause a disease, whilst at the same time failing to confer strong immunity and thus prevent re-infections (Buddle et al., 1984). Recently, a wild virus strain attenuated through serial passages on primary chicken embryo fibroblast tissue cultures was used successfully as a vaccine, being able to block the normal cause of the disease and induce rapid recovery (Mercante et al., 2008). An older commercial vaccine with scab material is available with effective immunity lasting for two to three years. A vaccine containing a caprine strain of the virus has been shown to be more effective when used in goats than a commercial vaccine licenced only for sheep (Musser et al., 2008).

8. Public health significance

Contagious ecthyma cannot be underestimated regarding its zoonotic potential, as many human cases have been reported worldwide, occurring more often in farmers and veterinarians (Arranz et al., 2000; Georgiades et al., 2005; Nourani and Maleki, 2006; Turan et al., 2013; Nougairede et al., 2013). Recently, a case report of *Orf virus* infection in a hunter indicated possible transmission by game (Kitchen et al., 2014). Incubation period is of a few days duration (usually 3–7) and the disease usually occurs as a single papule on a finger, hand or other body part. Symptoms may include feel of pain, lymphangitis and adenitis or, less frequently, fever or malaise. The disease is usually self-limiting and can heal with no treatment in three to six6 weeks, however large lesions are seen in immunosuppressive patients (Degraeve et al., 1999).

Prevention measures for avoiding human infection include mainly the avoidance of any contact of abraded or cut skin with infected animals and relevant crusts, scabs, wool and hides using the appropriate protective clothes and equipment. Disinfectants should be used, and all infected should be kept far from healthy

animals and exposed hands should be thoroughly washed after treating animals.

9. Concluding remarks

Even though contagious ecthyma does not seem to be a serious disease with light to moderate and self-limiting clinical presentation, its economic impact can be very significant, especially in endemic areas, where the disease has spread in a great extent. Implementation of a contagious ecthyma vaccination in management schemes is still not widely applied by the farmers; nevertheless, that is an important measure that could greatly benefit them if added, as economic losses will be reduced and production will be improved in the long-term, minimising greatly the impact of the disease.

Conflict of interest

The authors declare no conflict of interest.

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