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Orf Virus: A New Class of Immunotherapy Drugs

Ruixue Wang and Shuhong Luo

Abstract

Orf is one of the most widespread viral diseases worldwide, usually benign and self-limiting, and mainly affects not only sheep and goats, but also various other ruminants and mammals. The causative agent, orf virus (ORFV) is a member of the genus parapoxvirus, owing to its zoonotic importance and ability to cross-infect other species sporadically. ORFV encodes virulence and immunomodulatory factors that interfere with host inflammatory effect and antiviral immune mechanisms and induces a transient and complex cytokine response, initially represented by Th1-related cytokines followed by Th2-related cytokines. The ORFV has evolved several mechanisms to survive in the presence of the immune system, resulting in repeated infections. Currently, ORFV has been developed as vaccines in veterinary field. The unique host immune escape ability obtained by ORFV has made it one of the important candidates for prevention and treatment of various diseases (including chronic viral diseases, tumor, and liver fibrosis).

Keywords: immunoregulation, immunotherapy, orf virus, vaccine, oncolysis virus

1. Introduction

Orf was first discovered in Europe in 1920. At present, the main geographical distribution of the pathogen is not clear, which is considered to be prevalent worldwide since orf exists in all areas where sheep exists. The United States of America, Germany, Korea, Japan, India, Argentina, Malaysia, Egypt, and China have reported the occurrence and prevalence of the disease, which has brought a certain degree of loss to the sheep industry. The causative agent, orf virus (ORFV), also known as contagious pustular stomatitis (contagious ecthyma) virus, belongs to the parapoxvirus genus of poxvirus family and causes nonsystemic cutaneous disease by mainly infecting sheep and goats. In recent years, the cases of human, camel, yak, red squirrel, cat, domestic reindeer, etc. infected by ORFV have been reported. This indicates that the host range of the virus is expanding.

After being infected by ORFV, the infected animals begin with the appearance of erythema on the lips, tongue, nose, and breast of sheep, then develop into papules, blisters, and pustules, and finally form crusts, characterized by proliferative inflammation (**Figure 1**). The course of the disease is mostly an acute infection, healed within 1-2 months, but there are also cases of chronic persistent infection records. The disease rarely causes animal death unless host immunosuppression or secondary infection occurs, but there are also reports of a high mortality rate of 93% in young goats.

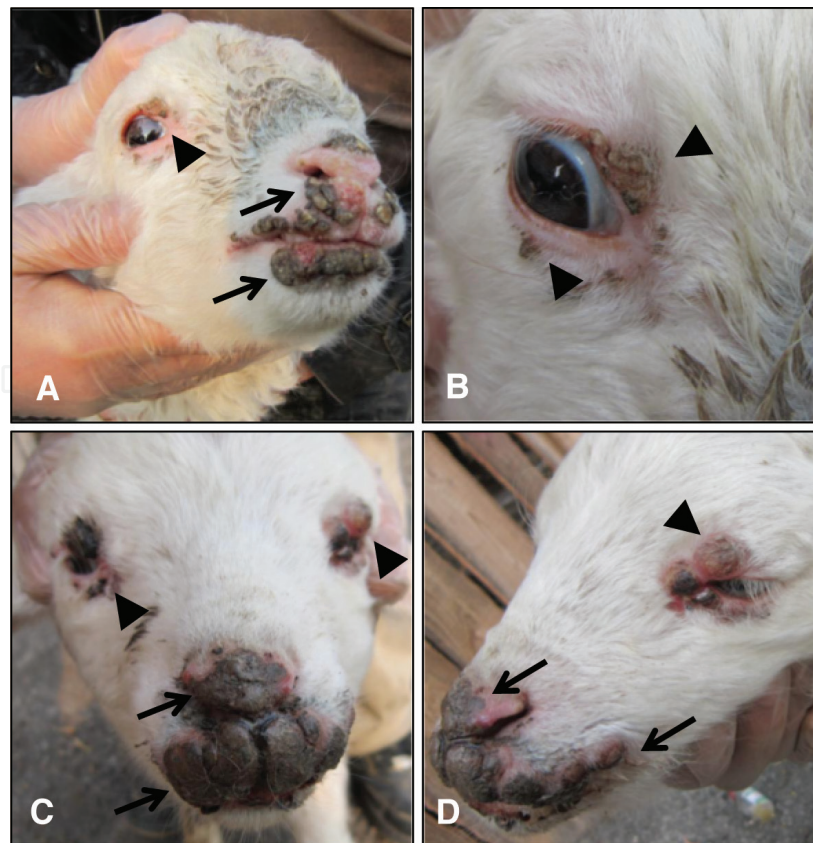


Figure 1.

Typical clinical signs of ORFV infection in sheep (cited from reference [1]). Proliferative skin lesions of two infected sheep around the mouth, nostrils (arrows), and the eyelids (arrowheads).

2. ORFV genome biology

ORFV belongs to the subfamily parapoxvirus of the poxvirus family. Other members of this genus include pseudovaccinia virus (PCPV), bovine papular stomatitis virus (BPSV), and parapoxvirus of red deer in New Zealand (PVNZ). The mature ORFV particles are 250–280 nm in length and 170–200 nm in width, and elliptical and coiled shaped, while the immature virus particles are conical, brick-shaped, and special coiled spherical particles (**Figure 2**). The surface of the virus particles showed a characteristic braided helical structure of cross-arranged around the long axis of the virus particles for eight-shaped winding. There are other ways of winding, and the virus particles encapsulated outside the capsule. ORFV replicates and matures in the cytoplasm, encoding polymerases associated with virus replication and transcription.

ORFV is a linear double-stranded (ds) DNA virus with a genome size of 134–139 kb. The average G + C content of the virus genome was approximately 64%. The content of G + C in the ORFs in the central coding region of the genome is not very different, but the ORFs in the two ends of the genome are very different, which are even less than 50% in some regions, such as ORFV127. Mercer believes that the terminal is based on the conservation of the genome sequence (OVSA00, OVIA82, and NZ2) and the transcriptional initiation of the gene in three strains of the virus. Even though the G + C content of seven ORFs in ORFV102–104, 109–112 is quite different from the genome average G + C content, and the homology of the encoded proteins is low, but the G + C content between different strains is very close [2]. The content of G + C in BPSV genome terminal variant region is similar, too, which is a marker of poxvirus members. Like other members of the poxvirus family, it has a large central coding region in the middle of the genome and an inverted terminal repeat (ITR) at both ends, which had covalently closed terminal hairpin structures (**Figure 3**).

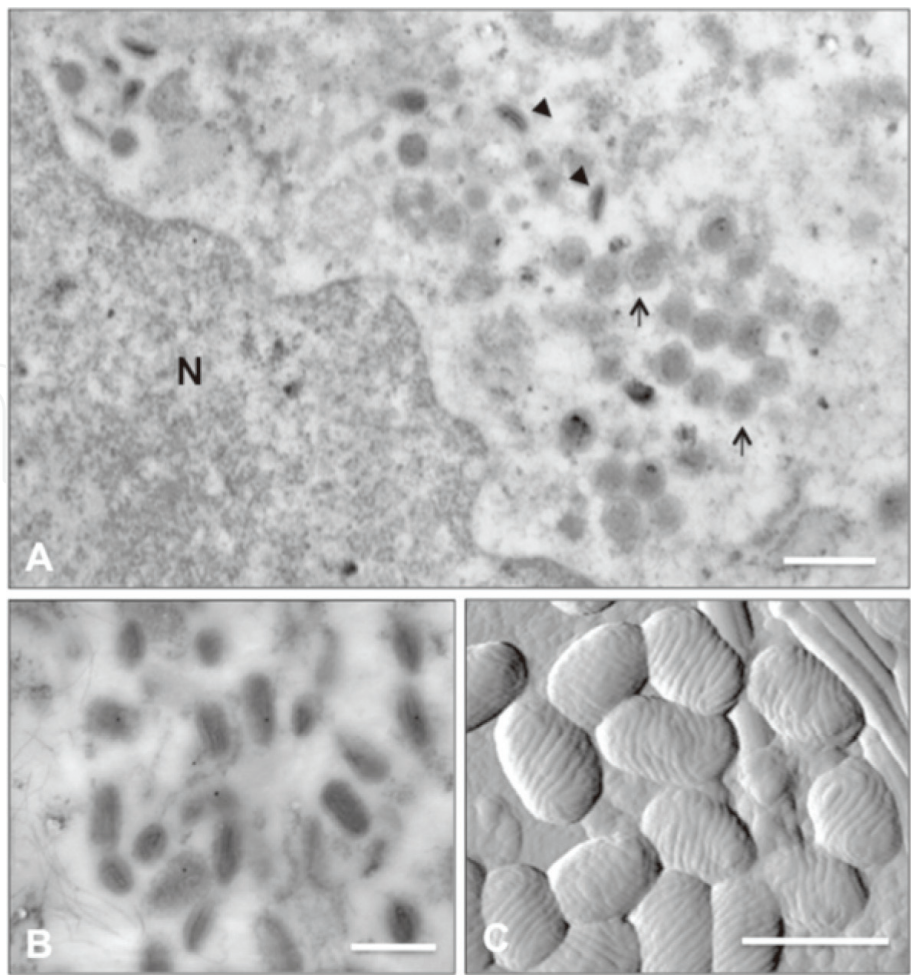


Figure 2.
The electron microscopy of the ORFV (cited from reference [1]). The predominantly immature virions (arrows and arrowheads) (A) and intracellular mature virions (B) under transmission electron microscopy. (C) The extracellular virions under atomic force microscopy. Scale bars = 500 nm.

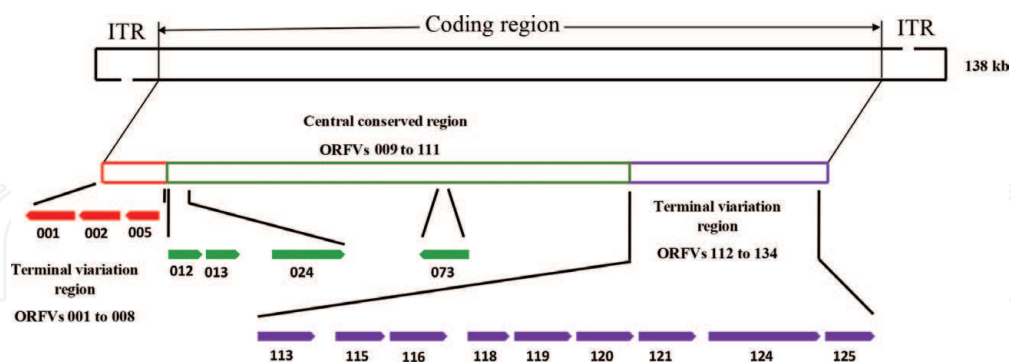


Figure 3.
The structure of the genome of ORFV strain NA1/11. The genome of ORFV is 138 kb, encoding 132 genes, and includes highly variable terminal regions, responsible for virus virulence and pathogenesis, and relatively conserved central regions with a high GC content for viral replication and virus morphogenesis. There are 16 novel genes unique to parapoxvirus, with putative virulence-host range functions.

There are many studies on the whole gene sequencing and analysis. The first genome of two strains (SA00 and IA82) was sequenced in 2003 by the Mei Dao Animal Disease Center of the US Department of Agriculture. OVSA00 was identified as the reference sequence of the virus. Mercer submitted the full genome sequence of the NZ2 strain in 2006, and McGuire sequenced strain (D1701) and submitted it to GenBank. Luo submitted the full genome sequence of the ORFV

Strain	Species of origin	Country of origin	No. of predicted genes	Genome size (bp)	ITR size (bp)	G + C content	Geninfo identifier	Reference
D1701	Sheep	Germany	288	134,038			325073632	[3]
YX	Goat	China	132	138,231	3446	63.8	913203877	[4]
NP	Goat	China	124	132,111	2426	63.8	913204143	[4]
GO	Goat	China	132	139,886	3964	63.6	913204010	[4]
SJ1	Goat	China	129	139,112	4153	63.6	913204268	[4]
HN3/12	Sheep	China	132	136,643	2794	63.7	1240685690	[2]
SA00	Goat	USA	132	139,962	3936	63.4	40019123	[5]
NA1/11	Sheep	China	134	137,080	3020	63.6	632123481	[6]
IA82	Sheep	USA	132	137,241	3092	64.3	40019122	[5]
NZ2	Sheep	New Zealand	132	137,820	3389	64.3	74230714	[7]

Table 1.
Summary of complete genomic sequence data of 10 ORFV strains.

strains of China. Up to now, NCBI has included 10 complete genome sequences of ORFV (**Table 1**).

Delhon et al. conservatively estimated about 130 coding genes in the ORFV genome [5]. Through analysis of the genome sequence of NZ2 strain, 132 possible coding genes were found in the genome of this virus strain. Transcription initiation elements (TAAAT) existed before the coding regions of the two genes and were found in BPSV. Similar conserved sequences also exist, but these two genes only exist in parapoxvirus but not in orthopoxvirus. Mercer et al. checked the ORFs of 24 genes of ORFV, which showed high interspecific variability mainly in the two terminal variant regions [7]. Many genes located in the core region have been identified. The ORFV050 gene, similar to L4R of vaccinia virus (VACV), encodes the DNA-binding virion core protein VP8 [8]. ORFV057 encodes protein OH1, analogous to the VACV structural protein VH1, that can dephosphorylate phosphatidylinositol 3, 5-bisphosphate, and plays a role in virion maturation [9]. The ORFV011 (B2L) gene, a homolog of the F13 L gene of VACV, encodes a major envelope protein of 42 kDa, which is thought to be a lipase. Additionally, the viral A32L gene (ORFV108) encodes an ATPase involved in virion DNA packaging [10]. Virulence genes, coding genes related to host pathogenesis and immunoregulatory genes are located in the ITR regions of the ORFV genome, such as ORFVs 007, 020, 112, 117, 119, 125, and 132.

3. The immunomodulatory ability of ORFV

3.1 ORFV and immunomodulatory ability

ORFV is widely recognized as having a powerful host immunoregulatory function. ORFV can quickly mediate humoral and adaptive immune responses. After being infected with ORFV, many cells of the innate immune system are activated and induce the secretion of chemokines and cytokines. Neutrophils, natural killer (NK) cells, and dendritic cells (DC) are recruited at the site of infection. In the early stage of infection, ORFV mainly induces the Th1-type immune response. Peripheral immune cells secrete IFN- γ , TNF- α , IL-6, IL-8, IL-12, IL-18, and then Th2-type immune response appears, mainly inducing secreting of IL-4, IL-10, IL-1 receptor antagonists (IL-1RA). The conditioning of complement and antigen-presenting-cell (APC)-mediated antigen presentation are important steps to activate the immune response.

ORFV and some of its encoded proteins have a good immunomodulatory function. Homologous alignment analysis of the host sequence and the viral gene sequence has identified that some ORFV genes have corresponding immunoregulatory functions, including coding for IL-10 homologous proteins, chemokine binding proteins, secretory inhibitors of GM-CSF and IL-2, vascular endothelial factor (VEGF), and interferon resistance protein. The main targets of IFN resistance genes are host cytokines, chemokines, NF- κ B signaling pathway, and apoptosis pathway. The synergistic effect of these proteins has strong immunomodulatory effects on ORFV.

ORFV is the only virus that contains the gene encoding IL-10 found in the poxvirus family. vIL-10, a 21.7 kDa protein with remarkable homology to IL-10, which is encoded by ORFV127 gene, plays an important role in immunosuppression through inhibition of cytokine syntheses, such as TNF- α and IL-8, IFN- γ [11], suppression of the maturation and functionality of DC [12, 13], blockage of Th1 cell activation indirectly through weakening the antigen processing, and presentation ability of APC. The direct role of the vIL-10 gene in virulence was demonstrated using an ORFV lacking the IL-10 gene, which showed attenuated properties in animal experiments [14], while vIL-10 can exert immunostimulatory effects by inducing moderate compensatory immune activation [15].

One of the characteristics of ORFV infection is the proliferation of capillaries and the increasing of permeability in the dermis, which is caused by viral VEGF. The deletion of VEGF gene leads to vascular permeability reduction, inhibition of epidermal cells and inactivation of VEGF receptors (VEGF-2) [16, 17]. Viral VEGF, sharing 16–27% of amino acid identity with its homolog VEGFs (VEGF-A, VEGF-B, VEGF-C, VEGF-D), has the same function as VEGFs: promoting the proliferation of epidermal cells, inducing the proliferation of host vascular endothelial cells and increasing the permeability of capillary vessel wall. Interestingly, VEGF variants are observed in different strains of ORFV. Despite such variation existing, the functional domains of the protein exhibit conserved structure. Studies reported that a recombinant virus strains lacking VEGF gene reduced vascular changes characteristic of natural infections, with less proliferation of blood vessels and dermal edema, pustule, and scab formation in ORFV pathogenesis [18].

In the early stage of ORFV infection, the orf virus interferon resistance protein (OVIFNR) encoded by ORFV20 binds to the viral replication intermediates and prevents the termination of IFN-induced virus-carrying protein translation. OVIFNR shares 31% sequence similarity with the E3L protein of VACV, and the C-terminal region with the binding activity of dsRNA (or viral replication intermediates) was the necessary region to prevent the antiviral activity of IFN and associated with pathogenicity and host tropism. OVIFNR eliminates the antiviral effect of IFN through the synthesis of the dsRNA-dependent protein, like protein kinase (PKR) [19]. The dsRNA-activated PKR is one of the main antiviral proteins induced by IFN. Activated PKR phosphorylates the translation initiation factor eIF2- α and impairs protein synthesis to inhibit viral replication [20]. OVIFNR not only compete with PKR to bind to viral replication intermediates but also inhibit the activity of PKR, thus preventing host cell interference from terminating the translation of viral proteins.

The ORFV007-encoded dUTPase clusters with mammalian counterparts and is more similar to mammalian dUTPases than to dUTPases from other poxviruses [21]. The virulence of ORFV with the 007-gene deletion is significantly lower than that of natural ORFV.

Chemokine-binding protein (CBP), the coding product of ORFV112, has similarities in structure and function with CBP II of orthopoxvirus and rabbit poxvirus. It can bind and inhibit chemokine and prevent chemokine-receptor

interaction. When chemokines bind to their receptor, the G protein-coupled receptor, the white blood cells were recruited and activated in viral infection. In addition, chemokines interact with glycosaminoglycan (GAG) and establish a gradient liquid phase that guides leukocytes through the endothelium into tissues. ORFV-CBP and CBP-II have high affinity to some CC chemokines, such as monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1 (MIP1), and regulated on activation, normal T cell expressed and secreted (RANTES), which can produce chemotactic effects of both nucleus/macrophage and T lymphocyte toward inflammation. Although ORFV encodes a number of secreted anti-inflammatory factors, the deletion of the CBP gene severely attenuated viral virulence and pathogenesis [22].

GIF is encoded by ORFV117, expressed in the late stage of infection, and has the dual activity of inhibiting host GM-CSF and IL-2, thus inhibiting host immune activity [23]. The gene is conserved in different ORFV strains and also exists in other parapoxvirus strains, but the amino acid sequence similarity between ORFV and BPSN is only 40%. However, the function of GIF in virulence and pathogenesis is not yet known.

3.2 ORFV and immune evasion

After infection, sheep produces antibodies to four or five immunodominant antigens [24–26]. Murine monoclonal antibodies recognizing 42 kDa envelope proteins, the 10 kDa putative fusion protein, and 65 kDa antigens have been described that can discriminate between the different parapoxvirus species [27, 28]. In spite of an apparently normal immune response to infection, sheep can be repeatedly infected, suggesting that, in common with other large DNA viruses, ORFV has evolved an immune evasion strategy [29, 30].

ORFV infection stimulated hyperplasia of epidermal cells and capillaries growth with increased vascular permeability, which allows increased virus replication and formation of scabs on wound healing. Scabs are rich in virus particles and provide temporary refuge for viruses to escape from immunization. The antiviral effect of IFN is the first line of defense against viral infection; ORFV evades immune clearance by inhibiting IFN-stimulated genes expression mediated by the JAK/STAT signaling pathway [31]. In addition, ORFV also can induce apoptosis mediated by CD95 pathway [15] or inhibit the pro-inflammatory NF- κ B signaling, a crucial regulator of host innate immune responses. For pathogens, interfering with the activation of NF- κ B is a particular strategy against host defense mechanisms. The regulation of NF- κ B includes the regulation of I κ B in the cytoplasm, and post-translational phosphorylation, acetylation, and methylation in the nucleus. The ORFV 002, 024, 073, 119, and 121 genes have been reported that play roles in NF- κ B pathway regulation [32–38].

ORFV002 is an early and late stage virus gene, mainly located in the nucleus. ORFV002-encoded protein can inhibit the activation of NF- κ B pathway induced by TNF- α and ORFV virus infection, which may through interfering the interaction between NF- κ B-p65 and P300 in the nucleus block the acetylation of NF- κ B-p65 Lys310 when phosphorylation occurs at ORFV002 Ser 276 [32, 33]. The 52 amino acids of ORFV002 N terminal may interact with protein S100A4 [34].

The ORFV024-encoded protein combines with LAGE3 to inhibit the phosphorylation of IKKs complex and then affects the phosphorylation of NF- κ B-p65, inhibits the host immune cells to secrete some important cytokines, and regulates the host's immune response [35].

ORFV119 blocks the NF- κ B signaling largely in a pRb-dependent manner, by inhibiting IKK complex activation early in infection [36]. ORFV119 interacted

with TNF receptor-associated factor 2 (TRAF2), an adaptor protein recruited to signaling complexes upstream of IKK in infected cells, in a LxCxE motif-dependent manner, which leads to inhibition of NF- κ B signaling.

The ORFV073 protein, 188 amino acids with a molecular weight of 21.8 kDa, whose protein at 149, 160, and 166 locations contained three predicted and partially overlapping nuclear localization signals, is located in nucleus during viral replication and be related to gene expression regulation. When the ORFV073 gene was deleted in the ORFV genome, the expression of chemokines and other pro-inflammatory genes was significantly increased, and most of the gene expression changes were regulated by the NF- κ B transcription factor family [37].

After infected with an ORFV121 gene deletion mutant, NF- κ B-mediated gene transcription was increased, while the expression of ORFV121 in cell cultures significantly decreased NF- κ B-regulated reporter gene expression, suggesting that NF- κ B inhibitor binds to and inhibits the phosphorylation and nuclear translocation of NF- κ B-p65 in the cell cytoplasm, thus providing a mechanism for the inhibition of NF- κ B-p65 phosphorylation and nuclear translocation [38].

4. Diagnosis and treatment

At present, there is no international standard for the diagnosis of amniotic aphthous ulcer, mainly based on the typical clinical symptoms and laboratory tests to diagnose. As shown in **Figure 4**, current laboratory diagnoses include PCR, ELISA, electron microscopy, histopathology, Western blotting.

The orf is mainly observed by the morphology of virus particles. A human case of orf was identified by transmission electron microscopy in a 20-year-old woman with two painful pruritic lesions on her left index finger [39]. Under the transmission electron microscopy, multiple typical ORFV particles existed with brick-shaped morphology, consisting of a central DNA-containing core surrounded by a bilayered capsid.

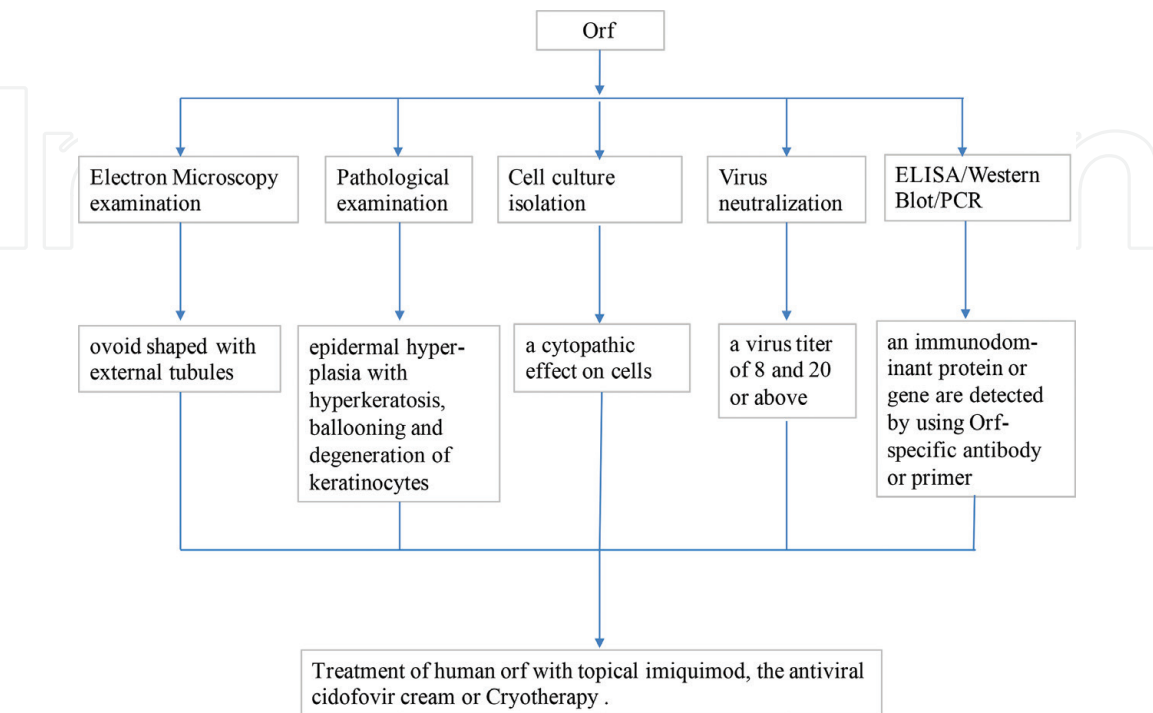


Figure 4.
The diagnosis methods and treatment of orf.

Histopathological features of the orf lesion include vacuole and swelling of keratinocytes, interstitial degeneration, marked epidermal hyperplasia, microswelling in the epidermis, aggregation of neutrophils, DC, T cells and B cells in the subcutaneous tissue, and formation of the crust. Eosinophilic inclusion bodies were also evident in the infected cytoplasm, but not at all stages of infection. There are mononuclear cells infiltrating into the dermis, such as phagocytes, lymphocytes, and eosinophils. In secondary infection, a dermal infiltration of neutrophils appeared [40].

A variety of primary cells and cell lines can be used to isolate ORFV. Primary lamb testicular cells and primary lamb kidney-trophoblast cells, which were originally used by Plowright et al., are the most common in the prozona cells [41]. The primary fetal lamb muscle cells [42] and turbinate cells [5], fetal bovine lung cells [43], Madin-Darby bovine kidney cells [44], and vero cells [45] were also used. ORFV can induce cytopathy in these cells after inoculation of the first generation or blindly transmitted for one to two generations. The common cytopathic effects are aggregation, fusion, shrinkage, and cell detachment. Eosinophils in the cytoplasm can be observed by staining of infected cell lines. Parapoxvirus culturing, in general, is considered to be difficult, with a need for many passages before observing cytopathic effects, such as ballooning, wounding, degeneration of cells, etc.

Serum neutralization tests, commonly used for the detection of antibodies, are not considered to be the method of choice for primary diagnosis, as immunity to ORFV is mainly cell-mediated, and neutralizing antibodies are usually at small concentrations. Suspected serum with a titer of 8 and 20 or above is considered as positive for orf infection, in serum neutralization test and complement-fixation test, respectively [46].

Indirect ELISA with a purified virus as coating antigen, peroxidase complex protein A, G and chimeric A/G as secondary antibodies can be used to detect antibody levels in different animals [47]. The method has been successfully applied to detect the virus in camels [48], lambs [49], and humans [50] suspected to be infected with the disease.

A 40 kDa immunogenic protein has been found in the positive sera of infected animals by Western blotting. Similarly, two proteins of approximately 22 and 20 kDa have been found by this method.

Different PCR methods can be used for rapid diagnosis of ORFV. A conventional PCR assay based on amplification of the ORFV B2L gene, a homolog of the F13 L gene of VACV, encodes a major envelope protein of 42 kDa, which is supposed to be a lipase, and was used to detect parapoxvirus species. A duplex PCR assay using A29 gene (413 bp) and H3L gene (708 bp) has the potential to differentiate capripoxviruses from ORFV [51]. A single-step PCR method was applied for the rapid differential diagnosis of ORFV infections [52]. Primers targeting the A32L gene, besides, the complete sequences of another two viral genes were also investigated: the B2L, and E3L genes, which encodes a dsRNA-binding protein. A conventional PCR assay combined with DNA sequencing can be used to distinguish among the different parapoxvirus species [53]. A sensitive and specific SYBR Green I real-time PCR assay was performed to quantitatively detect ORFV [54].

Restriction fragment length polymorphism (RFLP) of the genome is a powerful tool for analyzing the molecular characteristics of poxvirus, which can potentially distinguish different strains of amniotic stomatitis virus. Restriction enzyme fragments are obtained by enzyme (EcoRI, BamHI, and HindIII) digestion. Commercialized kits with random amplified polymorphic DNA have been used to distinguish virus strains from large numbers of species. Loop-mediated isothermal

amplification targeting B2L, DNA polymerase, and F1L genes have been developed and proven to be effective diagnostic tools [55, 56].

The ORFV has a strong ability to adapt to the external environment and has a strong resistance, even after a year in the sheep pen around, the virus still has a strong infectivity. But the virus is sensitive to temperature changes, suitable for the humid environment, and can be killed at 60°C in 30 min. Besides, the use of antiviral drugs has been applied in human and animal orf infections with satisfying results, such as Cidofovir [57, 58].

The virus is virulent for about a year when it is added to 50% glycerol saline and stored at 4°C. In the actual feeding process, generally choosing to use 20% hot grass and wood ash solution, 10% lime milk, 2% sodium hydroxide solution, and 1% acetic acid for disinfection can kill the virus, while using 2% sodium hydroxide solution to kill the virus in 5 min. The treatment for human orf is often focused on secondary infection. Previous reports have described speeding up the healing process with topical imiquimod [59] and the antiviral cidofovir cream [60]. Cryotherapy has also been used successfully to treat orf cases, especially in immunocompromised patients [61].

5. Clinical applications

ORFV is widely recognized as a virus with powerful host immunoregulatory function, but neutralizing antibody in ORFV infection is rare [27], and passive transfer of antibody-rich colostrum or serum does not protect lambs from infection [62, 63].

In veterinary medicine, ORFV is widely used as a new type of preventive and immunomodulatory preparation. Activated or inactivated ORFV preparations have a dose-dependent immunomodulatory function. Therefore, based on ORFV, drugs for a variety of infectious animal diseases have been developed (Baypamun; Zylexis). For example, Baypamun was used to suppress stress-related infections in horses, and clinical data showed a significant 40% reduction in the incidence of stress-related infections in the medication group [64]. Its therapeutic effect has also been verified in other animals, such as the treatment of bovine herpesvirus type 1, chronic stomatitis or infectious peritonitis in cats, and breast tumors in dogs. Inactivated ORFV can induce spontaneous regulation of cytokine responses in mice, such as up-regulation of Th1 cytokines (IL-12, IL-18, and IFN- γ), activation of CD14 and TLR-mediated monocyte activation, and release of anti-inflammatory Th2-related cytokines.

5.1 Antiviral preparations

Activated or inactivated ORFV makes many kinds of animals to fight different viral diseases. The ORFV has significant antifibrous activity in CCL4-mediated liver fibrosis [65]. The inactivated ORFV agents with a low dose (only 500,000 virus particles) are more effective in transgenic mice than the standard 3TC for HBV infection [66]; thus, it can be used as the candidate antiviral agent for the treatment of human HBV. Inactivated ORFV has anti-HCV activity in vitro and transgenic mice model [67]. In addition, ORFV can prevent the recurrence of fatal herpes simplex virus (HSV) and recurrence of genital herpes in Guinea [68].

ORFV can be used as a carrier to produce new animal recombinant vaccines. Recombinant ORFV vector induces an antiviral response in various animals. Recombinant pseudorabies virus glycoprotein gC or gD can be used to prevent

infection in mice and pigs [69, 70]. ORFV recombinant with protein P40 can induce the immune protection of rats from infection, and effectively eliminated the Borna disease virus in the brain [71]. ORFV recombinant classical swine fever virus protein E2 can also make pigs immune to classical swine fever virus (CSFV) [72]. Recombinant ORFV expressing hemagglutinin (HA) or nucleoprotein (NP) of highly pathogenic avian influenza virus H5N1 protects mice against H5N1 and H1N1 influenza viruses [73]. A recombinant virus strain D1701-VP1 of rabbit blast virus VP60 gene expression induced the infected cells releasing goblet-like particles to protect the rabbit from ORFV attack [74].

5.2 Oncolytic virus

Live or inactivated ORFV induces antitumor immune responses in multiple tumor models. Fiebig et al. reported for the first time that inactivated ORFV has antitumor effects in a variety of tumor metastasis models, such as mouse-transplanted malignant melanoma B16F10 and human breast cancer MDA-MB-231 models [75], and found that NK cells play an important role in the antitumor of ORFV. After neutralizing IFN- γ , the antitumor effect disappeared, while the anti-NK-1.1 antibody partially weakened the antitumor activity of ORFV by inhibiting the activity of NK and NKT cells. Inactivated ORFV inhibited tumor growth in a mouse MDA-MB-231 tumor model without NK and lacking functional T and B lymphocytes. Whether inactivated or active ORFV is used to treat mouse tumor models, NK cells play an important role in antitumor. A study by Rintoul further confirmed that ORFV inhibits tumor growth of melanoma and colorectal cancer, and proved that ORFV could play an antitumor role by activating NK cells and stimulating their secretion of cytokines IFN- γ and granzyme B [76]. Tai et al. found that surgery mediated the dysfunction of NK cells [77]. Intraoperative injection of ORFV improves the function of NK cells, thereby reducing intraoperative metastasis and prolong survival. Recently, a study of the virus strain CF189, which is the high similarity with the ORFV virus strain NZ2 obtained by homologous recombination, showed that CF189 effectively kills three negative breast cancer cells with time and dose dependence [78].

6. Conclusion

ORFV causes orf, a nonsystemic, highly contagious, ubiquitous disease of sheep and goats [79], which is characterized by maculopapular and proliferative lesions affecting the skin around the mouths, nostrils, and teats. Virus virulence and immunomodulation genes of ORFV contribute to combat local inflammatory response, innate immunity (including apoptosis, NK cell activation, and antiviral response), and immune adaptation. Therefore, ORFV has been used in veterinary medicine as preventive and therapeutic immunomodulatory agents. Moreover, live or inactivated ORFV preparations exhibit immunomodulatory effects, with therapeutic efficacy demonstrated for various diseases, including infectious diseases and tumors.

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Conflict of interest

The authors declare that no conflict of interest exists.

Author details

Ruixue Wang^{1,2} and Shuhong Luo^{1*}

1 Department of Laboratory Medicine, School of Stomatology and Medicine, Foshan University, Foshan, Guangdong Province, P.R. China

2 Department of Basic Medical Sciences, School of Stomatology and Medicine, Foshan University, Foshan, Guangdong Province, P.R. China

*Address all correspondence to: sluo815@gmail.com

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References

- [1] Li W, Ning Z, Hao W, Song D, Gao F, Zhao K, et al. Isolation and phylogenetic analysis of orf virus from the sheep herd outbreak in northeast China. *BMC Veterinary Research*. 2012;**8**:229. DOI: 10.1186/1746-6148-8-229
- [2] Chen H, Li W, Kuang Z, Chen D, Liao X, Li M, et al. The whole genomic analysis of orf virus strain HN3/12 isolated from Henan province, central China. *BMC Veterinary Research*. 2017;**13**(1):260. DOI: 10.1186/s12917-017-1178-1
- [3] McGuire MJ, Johnston SA, Sykes KF. Novel immune-modulator identified by a rapid, functional screen of the parapoxvirus ovis (Orf virus) genome. *Proteome Science*. 2012;**10**(1):4. DOI: 10.1186/1477-5956-10-4
- [4] Chi X, Zeng X, Li W, Hao W, Li M, Huang X, et al. Genome analysis of orf virus isolates from goats in the Fujian province of southern China. *Frontiers in Microbiology*. 2015;**6**:1135. DOI: 10.3389/fmicb.2015.01135
- [5] Delhon G, Tulman ER, Afonso CL, Lu Z, de la Concha-Bermejillo A, Lehmkuhl HD, et al. Genomes of the parapoxviruses ORF virus and bovine papular stomatitis virus. *Journal of Virology*. 2004;**78**(1):168-177. DOI: 10.1128/JVI.78.1.168-177.2004
- [6] Li W, Hao W, Peng Y, Duan C, Tong C, Song D, et al. Comparative genomic sequence analysis of Chinese orf virus strain NA1/11 with other parapoxviruses. *Archives of Virology*. 2015;**160**(1):253-266. DOI: 10.1007/s00705-014-2274-1
- [7] Mercer AA, Ueda N, Friederichs SM, Hofmann K, Fraser KM, Bateman T, et al. Comparative analysis of genome sequences of three isolates of Orf virus reveals unexpected sequence variation. *Virus Research*. 2006;**116**(1-2):146-158. DOI: 10.1016/j.virusres.2005.09.011
- [8] Wang H, Jiang J, Ding R, Wang X, Liao M, Shao J, et al. Identification and characterization of Orf virus 050 protein proteolysis. *Virus Genes*. 2017;**53**(3):400-409. DOI: 10.1007/s11262-017-1430-6
- [9] Segovia D, Haouz A, Porley D, Olivero N, Martinez M, Mariadassou M, et al. OH1 from Orf virus: A new tyrosine phosphatase that displays distinct structural features and triple substrate specificity. *Journal of Molecular Biology*. 2017;**429**(18):2816-2824. DOI: 10.1016/j.jmb.2017.07.017
- [10] Yogisharadhya R, Bhanuprakash V, Venkatesan G, Balamurugan V, Pandey AB, Shivachandra SB. Comparative sequence analysis of poxvirus A32 gene encoded ATPase protein and carboxyl terminal heterogeneity of Indian orf viruses. *Veterinary Microbiology*. 2012;**156**(1-2):72-80. DOI: 10.1016/j.vetmic.2011.10.021
- [11] Imlach W, McCaughan CA, Mercer AA, Haig D, Fleming SB. Orf virus-encoded interleukin-10 stimulates the proliferation of murine mast cells and inhibits cytokine synthesis in murine peritoneal macrophages. *The Journal of General Virology*. 2002;**83**(Pt 5):1049-1058. DOI: 10.1099/0022-1317-83-5-1049
- [12] Chan A, Baird M, Mercer AA, Fleming SB. Maturation and function of human dendritic cells are inhibited by orf virus-encoded interleukin-10. *The Journal of General Virology*. 2006;**87**(Pt 11):3177-3181. DOI: 10.1099/vir.0.82238-0
- [13] Lateef Z, Fleming S, Halliday G, Faulkner L, Mercer A, Baird M. Orf virus-encoded interleukin-10 inhibits maturation, antigen presentation

and migration of murine dendritic cells. *The Journal of General Virology*. 2003;**84**(Pt 5):1101-1109. DOI: 10.1099/vir.0.18978-0

[14] Fleming SB, Anderson IE, Thomson J, Deane DL, McInnes CJ, McCaughan CA, et al. Infection with recombinant orf viruses demonstrates that the viral interleukin-10 is a virulence factor. *The Journal of General Virology*. 2007;**88**(Pt 7):1922-1927. DOI: 10.1099/vir.0.82833-0

[15] Kruse N, Weber O. Selective induction of apoptosis in antigen-presenting cells in mice by Parapoxvirus ovis. *Journal of Virology*. 2001;**75**(10):4699-4704. DOI: 10.1128/JVI.75.10.4699-4704.2001

[16] Savory LJ, Stacker SA, Fleming SB, Niven BE, Mercer AA. Viral vascular endothelial growth factor plays a critical role in orf virus infection. *Journal of Virology*. 2000;**74**(22):10699-10706. DOI: 10.1128/JVI.74.22.10699-10706.2000

[17] Ueda N, Wise LM, Stacker SA, Fleming SB, Mercer AA. Pseudocowpox virus encodes a homolog of vascular endothelial growth factor. *Virology*. 2003;**305**(2):298-309. DOI: 10.1006/viro.2002.1750

[18] Wise LM, Savory LJ, Dryden NH, Whelan EM, Fleming SB, Mercer AA. Major amino acid sequence variants of viral vascular endothelial growth factor are functionally equivalent during Orf virus infection of sheep skin. *Virus Research*. 2007;**128**(1-2):115-125. DOI: 10.1016/j.virusres.2007.04.018

[19] Tossici-Bolt L, Fleming JS, Conway JH, Martonen TB. An analytical technique to recover the third dimension in planar imaging of inhaled aerosols--2 estimation of the deposition per airway generation. *Journal of Aerosol Medicine*. 2007;**20**(2):127-140. DOI: 10.1089/jam.2007.0577

[20] Li H, Zhu X, Zheng Y, Wang S, Liu Z, Dou Y, et al. Phylogenetic analysis of two Chinese orf virus isolates based on sequences of B2L and VIR genes. *Archives of Virology*. 2013;**158**(7):1477-1485

[21] Cottone R, Buttner M, McInnes CJ, Wood AR, Rziha HJ. Orf virus encodes a functional dUTPase gene. *The Journal of General Virology*. 2002;**83**(Pt 5):1043-1048. DOI: 10.1007/s00705-013-1641-7

[22] Fleming SB, McCaughan C, Lateef Z, Dunn A, Wise LM, Real NC, et al. Deletion of the chemokine binding protein gene from the parapoxvirus Orf virus reduces virulence and pathogenesis in sheep. *Frontiers in Microbiology*. 2017;**8**:46. DOI: 10.3389/fmicb.2017.00046

[23] Deane D, McInnes CJ, Percival A, Wood A, Thomson J, Lear A, et al. Orf virus encodes a novel secreted protein inhibitor of granulocyte-macrophage colony-stimulating factor and interleukin-2. *Journal of Virology*. 2000;**74**(3):1313-1320. DOI: 10.1128/JVI.74.3.1313-1320.2000

[24] McKeever DJ, Jenkinson DM, Hutchison G, Reid HW. Studies of the pathogenesis of orf virus infection in sheep. *Journal of Comparative Pathology*. 1988;**99**(3):317-328. DOI: 10.1016/0021-9975(88)90052-7

[25] Yirrell DL, Reid HW, Norval M, Howie SE. Immune response of lambs to experimental infection with Orf virus. *Veterinary Immunology and Immunopathology*. 1989;**22**(4):321-332. DOI: 10.1016/0165-2427(89)90168-2

[26] Chand P, Kitching RP, Black DN. Western blot analysis of virus-specific antibody responses for capripox and contagious pustular dermatitis viral infections in sheep. *Epidemiology and Infection*. 1994;**113**(2):377-385. DOI: 10.1017/S0950268800051803

- [27] Czerny CP, Waldmann R, Scheubeck T. Identification of three distinct antigenic sites in parapoxviruses. *Archives of Virology*. 1997;**142**(4):807-821. DOI: 10.1007/s007050050120
- [28] Housawi FM, Roberts GM, Gilray JA, Pow I, Reid HW, Nettleton PF, et al. The reactivity of monoclonal antibodies against orf virus with other parapoxviruses and the identification of a 39 kDa immunodominant protein. *Archives of Virology*. 1998;**143**(12):2289-2303. DOI: 10.1007/s007050050461
- [29] Alcamí A, Koszinowski UH. Viral mechanisms of immune evasion. *Immunology Today*. 2000;**21**(9):447-455. DOI: 10.1016/S0167-5699(00)01699-6
- [30] Haig DM. Subversion and piracy: DNA viruses and immune evasion. *Research in Veterinary Science*. 2001;**70**(3):205-219. DOI: 10.1053/rvsc.2001.0462
- [31] Harvey R, McCaughan C, Wise LM, Mercer AA, Fleming SB. Orf virus inhibits interferon stimulated gene expression and modulates the JAK/STAT signalling pathway. *Virus Research*. 2015;**208**:180-178. DOI: 10.1016/j.virusres.2015.06.014
- [32] Ning Z, Zheng Z, Hao W, Duan C, Li W, Wang Y, et al. The N terminus of orf virus-encoded protein 002 inhibits acetylation of NF-kappaB p65 by preventing Ser(276) phosphorylation. *PLoS One*. 2013;**8**(3):e58854. DOI: 10.1371/journal.pone.0058854
- [33] Diel DG, Luo S, Delhon G, Peng Y, Flores EF, Rock DL. A nuclear inhibitor of NF-kappaB encoded by a poxvirus. *Journal of Virology*. 2011;**85**(1):264-275. DOI: 10.1128/JVI.01149-10
- [34] Chen D, Zheng Z, Xiao B, Li W, Long M, Chen H, et al. Corrigendum: Orf virus 002 protein targets ovine protein S100A4 and inhibits NF-kappaB signaling. *Frontiers in Microbiology*. 2017;**8**:160. DOI: 10.3389/fmicb.2017.00160
- [35] Diel DG, Delhon G, Luo S, Flores EF, Rock DL. A novel inhibitor of the NF- κ B signaling pathway encoded by the parapoxvirus orf virus. *Journal of Virology*. 2010;**84**(8):3962-3973. DOI: 10.1128/JVI.02291-09
- [36] Nagendraprabhu P, Khatiwada S, Chaulagain S, Delhon G, Rock DL. A parapoxviral virion protein targets the retinoblastoma protein to inhibit NF-kappaB signaling. *PLoS Pathogens*. 2017;**13**(12):e1006779. DOI: 10.1371/journal.ppat.1006779
- [37] Khatiwada S, Delhon G, Nagendraprabhu P, Chaulagain S, Luo S, Diel DG, et al. A parapoxviral virion protein inhibits NF-kappaB signaling early in infection. *PLoS Pathogens*. 2017;**13**(8):e1006561
- [38] Diel DG, Luo S, Delhon G, Peng Y, Flores EF, Rock DL. Orf virus ORFV121 encodes a novel inhibitor of NF-kappaB that contributes to virus virulence. *Journal of Virology*. 2011;**85**(5):2037-2049. DOI: 10.1371/journal.ppat.1006561
- [39] Peng F, Chen Z, Zheng SY, Li HM, Du J, Zhang JZ. A case of Orf identified by transmission electron microscopy. *Chinese Medical Journal*. 2016;**129**(1):108-109. DOI: 10.4103/0366-6999.172606
- [40] Vikoren T, Lillehaug A, Akerstedt J, Bretten T, Haugum M, Tryland M. A severe outbreak of contagious ecthyma (orf) in a free-ranging musk ox (*Ovibos moschatus*) population in Norway. *Veterinary Microbiology*. 2008;**127**(1-2):10-20. DOI: 10.1016/j.vetmic.2007.07.029
- [41] Plowright W, Ferris RD. Studies with rinderpest virus in tissue culture.

II. Pathogenicity for cattle of culture-passaged virus. *Journal of Comparative Pathology*. 1959;**69**(2):173-184

[42] McInnes CJ, Wood AR, Nettleton PE, Gilray JA. Genomic comparison of an avirulent strain of Orf virus with that of a virulent wild type isolate reveals that the Orf virus G2L gene is non-essential for replication. *Virus Genes*. 2001;**22**(2):141-150

[43] Inoshima Y, Murakami K, Wu D, Sentsui H. Characterization of parapoxviruses circulating among wild Japanese serows (*Capricornis crispus*). *Microbiology and Immunology*. 2002;**46**(8):583-587. DOI: 10.1111/j.1348-0421.2002.tb02738.x

[44] Guo J, Zhang Z, Edwards JF, Ermel RW, Taylor CJ, de la Concha-Bermejillo A. Characterization of a North American orf virus isolated from a goat with persistent, proliferative dermatitis. *Virus Research*. 2003;**93**(2):169-179. DOI: 10.1016/S0168-1702(03)00095-9

[45] Tedla M, Berhan N, Molla W, Temesgen W, Alemu S. Molecular identification and investigations of contagious ecthyma (Orf virus) in small ruminants, North west Ethiopia. *BMC Veterinary Research*. 2018;**14**(1):13. DOI: 10.1186/s12917-018-1339-x

[46] Zarnke RL, Dieterich RA, Neiland KA, Ranglack G. Serologic and experimental investigations of contagious ecthyma in Alaska. *Journal of Wildlife Diseases*. 1983;**19**(3):170-174. DOI: 10.7589/0090-3558-19.3.170

[47] Inoshima Y, Shimizu S, Minamoto N, Hirai K, Sentsui H. Use of protein AG in an enzyme-linked immunosorbent assay for screening for antibodies against parapoxvirus in wild animals in Japan. *Clinical and Diagnostic Laboratory Immunology*. 1999;**6**(3):388-391

[48] Azwai SM, Carter SD, Woldehiwet Z. Immune responses of the camel (*Camelus dromedarius*) to contagious ecthyma (Orf) virus infection. *Veterinary Microbiology*. 1995;**47**(1-2):119-131. DOI: 10.1016/0378-1135(95)00055-F

[49] McKeever DJ, Reid HW, Inglis NF, Herring AJ. A qualitative and quantitative assessment of the humoral antibody response of the sheep to orf virus infection. *Veterinary Microbiology*. 1987;**15**(3):229-241

[50] Yirrell DL, Vestey JP, Norval M. Immune responses of patients to orf virus infection. *The British Journal of Dermatology*. 1994;**130**(4):438-443. DOI: 10.1016/0378-1135(87)90077-0

[51] Zheng M, Liu Q, Jin N, Guo J, Huang X, Li H, et al. A duplex PCR assay for simultaneous detection and differentiation of Capripoxvirus and Orf virus. *Molecular and Cellular Probes*. 2007;**21**(4):276-281. DOI: 10.1016/j.mcp.2007.01.005

[52] Chan KW, Hsu WL, Wang CY, Yang CH, Lin FY, Chulakasian S, et al. Differential diagnosis of orf viruses by a single-step PCR. *Journal of Virological Methods*. 2009;**160**(1-2):85-89. DOI: 10.1016/j.jviromet.2009.04.025

[53] Inoshima Y, Morooka A, Sentsui H. Detection and diagnosis of parapoxvirus by the polymerase chain reaction. *Journal of Virological Methods*. 2000;**84**(2):201-208. DOI: 10.1016/S0166-0934(99)00144-5

[54] Wang Y, Yang K, Bai C, Yin D, Li G, Qi K, et al. Development of a SYBR Green I real-time PCR for the detection of the orf virus. *AMB Express*. 2017;**7**(1):21. DOI: 10.1186/s13568-016-0322-9

[55] Tsai SM, Chan KW, Hsu WL, Chang TJ, Wong ML, Wang CY. Development of a loop-mediated isothermal

amplification for rapid detection of orf virus. *Journal of Virological Methods*. 2009;**157**(2):200-204. DOI: 10.1016/j.jviromet.2009.01.003

[56] Li J, Song D, He W, Bao Y, Lu R, Su G, et al. Rapid detection of orf virus by loop-mediated isothermal amplification based on the DNA polymerase gene. *Archives of Virology*. 2013;**158**(4):793-798. DOI: 10.1007/s00705-012-1526-1

[57] De Clercq E. Cidofovir in the therapy and short-term prophylaxis of poxvirus infections. *Trends in Pharmacological Sciences*. 2002;**23**(10):456-458. DOI: 10.1016/S0165-6147(02)02091-6

[58] Dal Pozzo F, Andrei G, Lebeau I, Beadle JR, Hostetler KY, De Clercq E, et al. In vitro evaluation of the anti-orf virus activity of alkoxyalkyl esters of CDV, cCDV and (S)-HPMPA. *Antiviral Research*. 2007;**75**(1):52-57. DOI: 10.1016/j.antiviral.2006.11.010

[59] Erbagci Z, Erbagci I, Almila Tuncel A. Rapid improvement of human orf (ecthyma contagiosum) with topical imiquimod cream: Report of four complicated cases. *The Journal of Dermatological Treatment*. 2005;**16**:353-356

[60] De Clercq E. Clinical potential of the acyclic nucleoside phosphonates cidofovir, adefovir, and tenofovir in treatment of DNA virus and retrovirus infections. *Clinical Microbiology Reviews*. 2003;**16**:569-596. DOI: 10.1128/CMR.16.4.569-596.2003

[61] Degraeve C, De Coninck A, Senneseael J, Roseeuw D. Recurrent contagious ecthyma (Orf) in an immunocompromised host successfully treated with cryotherapy. *Dermatology*. 1999;**198**:162-163. DOI: 10.1159/000018095

[62] Buddle BM, Pulford HD. Effect of passively-acquired antibodies and

vaccination on the immune response to contagious ecthyma virus. *Veterinary Microbiology*. 1984;**9**(6):515-522. DOI: 10.1016/0378-1135(84)90013-0

[63] Mercer AA, Yirrell DL, Reid HW, Robinson AJ. Lack of cross-protection between vaccinia virus and orf virus in hysterectomy-procured, barrier-maintained lambs. *Veterinary Microbiology*. 1994;**41**(4):373-382. DOI: 10.1016/0378-1135(94)90033-7

[64] Horohov DW, Breathnach CC, Sturgill TL, Rashid C, Stiltner JL, Strong D, et al. In vitro and in vivo modulation of the equine immune response by parapoxvirus ovis. *Equine Veterinary Journal*. 2008;**40**(5):468-472. DOI: 10.2746/042516408X322111

[65] Nowatzky J, Knorr A, Hirth-Dietrich C, Siegling A, Volk HD, Limmer A, et al. Inactivated Orf virus (Parapoxvirus ovis) elicits antifibrotic activity in models of liver fibrosis. *Hepatology Research*. 2013;**43**(5):535-546. DOI: 10.1111/j.1872-034X.2012.01086.x

[66] Weber O, Siegling A, Friebe A, Limmer A, Schlapp T, Knolle P, et al. Inactivated parapoxvirus ovis (Orf virus) has antiviral activity against hepatitis B virus and herpes simplex virus. *The Journal of General Virology*. 2003;**84**(Pt 7):1843-1852. DOI: 10.1099/vir.0.19138-0

[67] Paulsen D, Urban A, Knorr A, Hirth-Dietrich C, Siegling A, Volk HD, et al. Inactivated ORF virus shows antifibrotic activity and inhibits human hepatitis B virus (HBV) and hepatitis C virus (HCV) replication in preclinical models. *PLoS One*. 2013;**8**(9):e74605. DOI: 10.1371/journal.pone.0074605

[68] Ons E, Van Brussel L, Lane S, King V, Cullinane A, Kenna R, et al. Efficacy of a Parapoxvirus ovis-based immunomodulator against equine herpesvirus type 1 and

Streptococcus equi equi infections in horses. *Veterinary Microbiology*. 2014;**173**(3-4):232-240. DOI: 10.1016/j.vetmic.2014.07.015

[69] van Rooij EM, Rijsewijk FA, Moonen-Leusen HW, Bianchi AT, Rziha HJ. Comparison of different prime-boost regimes with DNA and recombinant Orf virus based vaccines expressing glycoprotein D of pseudorabies virus in pigs. *Vaccine*. 2010;**28**(7):1808-1813. DOI: 10.1016/j.vaccine.2009.12.004

[70] Fischer T, Planz O, Stitz L, Rziha HJ. Novel recombinant parapoxvirus vectors induce protective humoral and cellular immunity against lethal herpesvirus challenge infection in mice. *Journal of Virology*. 2003;**77**(17):9312-9323. DOI: 10.1128/JVI.77.17.9312-9323.2003

[71] Henkel M, Planz O, Fischer T, Stitz L, Rziha HJ. Prevention of virus persistence and protection against immunopathology after Borna disease virus infection of the brain by a novel Orf virus recombinant. *Journal of Virology*. 2005;**79**(1):314-325. DOI: 10.1128/JVI.79.1.314-325.2005

[72] Voigt H, Merant C, Wienhold D, Braun A, Hutet E, Le Potier MF, et al. Efficient priming against classical swine fever with a safe glycoprotein E2 expressing Orf virus recombinant (ORFV VrV-E2). *Vaccine*. 2007;**25**(31):5915-5926. DOI: 10.1016/j.vaccine.2007.05.035

[73] Rohde J, Amann R, Rziha HJ. New Orf virus (Parapoxvirus) recombinant expressing H5 hemagglutinin protects mice against H5N1 and H1N1 influenza A virus. *PLoS One*. 2013;**8**(12):e83802. DOI: 10.1371/journal.pone.0083802

[74] Rohde J, Schirrmeier H, Granzow H, Rziha HJ. A new recombinant Orf virus (ORFV, Parapoxvirus) protects rabbits against lethal infection with rabbit

hemorrhagic disease virus (RHDV). *Vaccine*. 2011;**29**(49):9256-9264. DOI: 10.1016/j.vaccine.2011.09.121

[75] Fiebig HH, Siegling A, Volk HD, et al. Inactivated orf virus (Parapoxvirus ovis) induces antitumoral activity in transplantable tumor models. *Anticancer Research*. 2011;**31**(12):4185-4190

[76] Rintoul JL, Lemay CG, Tai LH, Stanford MM, Falls TJ, de Souza CT, et al. ORFV: A novel oncolytic and immune stimulating parapoxvirus therapeutic. *Molecular Therapy*. 2012;**20**(6):1148-1157. DOI: 10.1038/mt.2011.301

[77] Tai LH, de Souza CT, Belanger S, Ly L, Alkayyal AA, Zhang J, et al. Preventing postoperative metastatic disease by inhibiting surgery-induced dysfunction in natural killer cells. *Cancer Research*. 2013;**73**(1):97-107. DOI: 10.1158/0008-5472.CAN-12-1993 80

[78] Choi AH, O'Leary MP, Chaurasiya S, Lu J, Kim SI, Fong Y, et al. Novel chimeric parapoxvirus CF189 as an oncolytic immunotherapy in triple-negative breast cancer. *Surgery*. 2018;**163**(2):336-342. DOI: 10.1016/j.surg.2017.09.030

[79] Spyrou V, Valiakos G. Orf virus infection in sheep or goats. *Veterinary Microbiology*. 2015;**181**(1-2):178-182. DOI: 10.1016/j.vetmic.2015.08.010