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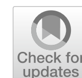
MINI-REVIEW



Recent advances in diagnostic approaches for orf virus

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Abstract

Orf virus (ORFV), the prototype species of the *Parapoxvirus* genus, is an important zoonotic virus, causing great economic losses in livestock production. At present, there are no effective drugs for orf treatment. Therefore, it is crucial to develop accurate and rapid diagnostic approaches for ORFV. Over decades, various diagnostic methods have been established, including conventional methods such as virus isolation and electron microscopy; serological methods such as virus neutralization test (VNT), immunohistochemistry (IHC) assay, immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA); and molecular methods such as polymerase chain reaction (PCR), real-time PCR, loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and recombinase-aided amplification (RAA) assay. This review provides an overview of currently available diagnostic approaches for ORFV and discusses their advantages and limitations and future perspectives, which would be significantly helpful for ORFV early diagnosis and surveillance to prevent outbreak of orf.

Key points

- *Orf virus emerged and reemerged in past years*
- *Rapid and efficient diagnostic approaches are needed and critical for ORFV detection*
- *Novel and sensitive diagnostic methods are required for ORFV detection*

Keywords Orf virus · Diagnostic approach · Conventional methods · Serological methods · Molecular methods

Introduction

Orf virus (ORFV) is a linear double-stranded DNA virus, belonging to the *Parapoxvirus* genus within the *Poxviridae* family (Yao et al. 2020). The genome of ORFV ranges from approximately 132 kb to 140 kb, encoding 132 genes. The relatively conserved central regions are essential for viral replication and morphogenesis while the terminal areas are crucial for virus virulence, pathogenesis, and immune evasion (Yu et al. 2020). ORFV is the pathogen of orf, which is a highly contagious, epitheliotropic, zoonotic disease. The disease mainly infects goats and sheep (Galante et al. 2019) and sometimes infects deer (Tryland et al. 2018), camels (Azwai et al. 1995), musk ox (Vikoren et al. 2008), and cats (Fairley et al. 2008; Frandsen et al. 2011). Clinical symptoms of orf include papules, vesicles, and growing scabs on the lips and muzzle of infected animals (Sahu et al. 2020). Although both cell-mediated and humoral immune responses have been demonstrated in infected hosts, cell-mediated immune response plays major role against ORFV. $CD4^+$ T cells, not $CD8^+$ cytotoxic T cells, are the predominant T cells in the infected skin in both primary and reinfection, playing crucial roles in eliminating the virus (Fleming et al. 2015). However, the humoral immune response does not play an important role in protecting animals from ORFV infection. The absence of ORFV-specific neutralizing antibodies in previously infected animals may be the main reason why hosts can be repeatedly infected (Koptopoulos et al. 1982). Furthermore, the ability of ORFV to repeatedly infect previously infected animals is largely attributed to multiple immunomodulatory proteins encoded by the virus that escape from host immune response (Bukar et al. 2021).

It has been reported that several orf outbreaks occurred in many countries in recent years (Bala et al. 2019; Da et al.

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2019; Peralta et al. 2018; Venkatesan et al. 2018; Zhong et al. 2019). Although ORFV infection leads to low mortality, it can result in great economic losses since lambs may not thrive due to appetite loss (Jamilu et al. 2018). Therefore, the rapid and effective diagnosis of ORFV is crucial and highly needed for disease control. At present, various diagnostic methods have been established for ORFV detection (Hosamani et al. 2009). Conventional diagnostic methods include clinical diagnosis, viral isolation, and electron microscopy. Serological diagnostic methods include virus neutralization test, immunohistochemistry assay, immunofluorescence assay, and enzyme-linked immunosorbent assay. Molecular-based diagnosis includes PCR, real-time PCR, loop-mediated isothermal amplification, recombinase polymerase amplification, and recombinase-aided amplification assay. So far, there are few published reviews focused on diagnostic methods for ORFV. Given this, this review is intended to systematically describe and discuss the features, advantages, and limitations of various available diagnostic approaches for ORFV (Table 1). I believe that ORFV could be rapidly and accurately diagnosed by clinical laboratories with the help of these data.

Conventional diagnosis

Clinical diagnosis

Clinically, ORFV infection mainly causes proliferative lesions on the skin of lips, oral mucosa, gums, tongues, and nostrils. As the disease progresses, erythema, vesicles, pustules, and scabs develop (Bukar et al. 2021; Wang et al. 2019a, b, c). It is difficult to differentiate ORFV from foot and mouth disease, bluetongue disease, peste des petits ruminants, and goat pox or sheep pox which cause similar clinical signs (Chu et al. 2011; Gelaye et al. 2016). Laboratory diagnosis especially molecular methods is always required for final identification.

Viral isolation

Viral isolation from clinical specimens is one of the most conventional ways for ORFV diagnosis. Various primary cells including primary bovine testis (BT) cells (Mercer et al. 2006), lamb testis (LT) cells (AlDaif et al. 2021; Kumar et al. 2014), ovine fetal turbinate (OFTU) cells (Khatiwada et al. 2021; Zhou et al. 2021a, b), goat skin fibroblast (GSF) cells (Pang et al. 2019, 2020), fetal lamb muscle (FLM) cells, and lamb kidney cells (McInnes et al. 2001) can be used for ORFV isolation. Among these, LT, OFTU, and GSF cells are currently the most widely used primary cells for ORFV isolation. However, there are some challenges such as requirement of large numbers of live animals and limited cell passages in using primary cells to isolate ORFV. To overcome these problems, researchers successfully develop two types of immortalized cell lines by transducing the large T gene of simian virus 40 to primary goat fibroblast cells and testis cells (Yamada et al. 2019). Furthermore, cell lines such as Madin-Darby bovine kidney (MDBK) cells (Wang et al. 2019a, b, c; Yamada et al. 2019) and Hela cells (Diel et al. 2010; Zhou et al. 2021a, b) are commonly employed for ORFV culture. Homogenates from scab materials are inoculated into permissive primary cell cultures or cell lines. Generally, obvious cytopathic effect (CPE) characterized by cell ballooning, rounding, degeneration, and detachment from the surface will appear after the third to fifth blind passages (Hosamani et al. 2009). Subsequently, plaque assays are conducted to get a monoclonal plaque caused by a single virus. Finally, the isolated virus is amplified, titrated, and stored at − 80 °C (Martins et al. 2021).

Compared with other methods, viral isolation is labor-intensive, time-consuming, and less sensitive and may require additional biosafety precautions, whereas it is still the gold standard for ORFV diagnosis.

Electron microscopy

Electron microscopy (EM) is one of the most direct and effective diagnoses for poxvirus infection in animals and humans (Kieser et al. 2020). Parapoxviruses including BPSV, PCPV, PVNZ, and ORFV can be differentiated from other poxvirus genera due to their ovoid shape and relatively small size (220–300 × 140–170 nm, around 260 nm × 160 nm for ORFV). Their most characteristic feature is a unique spiral crisscross-patterned tubule-like structure on the particle surface (Spyrou and Valiakos 2015). However, due to the similar size between ORFV and PCPV, it is hard to differentiate ORFV from PCPV. Despite its low sensitivity, EM remains an important tool for diagnosing ORFV infection.

Serological diagnosis

Serological methods including virus neutralization test, immunohistochemistry assay, immunofluorescence assay, and enzyme linked immunosorbent assay are widely used for various virus diagnoses (Haegeman et al. 2020; Rai et al. 2021). Due to their simplicity, low cost, and limited need for specialized devices or facilities, serological methods are frequently used for ORFV diagnosis.

Virus neutralization test

Virus neutralization test (VNT) is a technique for measuring virus-specific antibodies after natural infection or vaccination. In this approach, virus-specific antibodies neutralize

Table 1 Comparison of currently available diagnostic approaches for ORFV

Diagnostic approaches	Advantages	Disadvantages	References
Clinical diagnosis	Symptoms can be directly observed	Undiagnosable	(Bukar et al. 2021; Chu et al. 2011; Gelaye et al. 2016; Wang et al. 2019a, b, c)
Virus isolation	Gold standard for the diagnosis of ORFV	Complex, time-consuming, and insensitive operation, requiring other methods to assist verification; biosecurity measures required	(AlDaif et al. 2021; Diel et al. 2010; Khatiwada et al. 2021; Kumar et al. 2014; Pang et al. 2019, 2020; Wang et al. 2019a, b, c; Yamada et al. 2019; Zhou et al. 2021a, b
Electron microscopy	One of the most direct and effective methods; can be used as a supplement to other testing methods	Difficult to distinguish accurately; lower specificity and sensitivity; more expensive electron microscopy; requires technical staff and knowledge base	(Kieser et al. 2020; Martins et al. 2021; Spyrou and Valiakos 2015)
VNT	Convenience	Low sensitivity and specificity	(Hosamani et al. 2009; Kresic et al. 2020)
IHC	Detect the distribution and location of pathogens in tissues, organs, and cells	The accuracy is not high and relatively time-consuming, not suitable for clinical large-scale sample screening	(Martins et al. 2021; Fleming et al. 2015; Zhao et al. 2010)
IFA	Simple, rapid, specific, and intuitive operation; can be used for rapid on-site testing with low professional requirements for personnel and without expensive equipment	Focused antibody potency determination is subjective, requires special instruments and equipment, and is not suitable for early diagnosis	(Petrovan et al. 2019; Zhao et al. 2010, 2011)
LFIA	Can be used for rapid on-site testing with low professional requirements for personnel and without expensive equipment	Sensitivity and specificity need to be improved, not suitable for high throughput	(Zhao et al. 2016)
ELISA	Simple, fast, and relatively accurate results, suitable for clinical testing of a large number of samples	Prone to cross-reactivity; does not enable early diagnosis	(Babiuk et al. 2009; Bora et al. 2016; Fleming et al. 2017; Wong et al. 2020; Yogisharadhya et al. 2018)
PCR	Multiplex PCR for simultaneous detection of multiple viral nucleic acids	Sensitivity and specificity need to be improved; cannot be quantified; nucleic acid dyes are toxic and easily contaminated to people and the environment	(He et al. 2017; Sahu et al. 2022; Venkatesan et al. 2014a; Wang et al. 2019a, b, c; Zheng et al. 2007)
Real-time PCR	1000 times higher sensitivity than conventional PCR, no cross-reactivity with other viruses, high throughput	More expensive experimental instruments, high testing costs; need to be equipped with professional operators	(Bora et al. 2011; Du H et al. 2013; Gallina et al. 2006; Gelaye et al. 2017; Venkatesan et al. 2014b; Wang et al. 2017; Xu et al. 2019)
LAMP	Accurate, fast, inexpensive, can be amplified at very warm conditions (60 °C), suitable for grassroots	Polymerase is sensitive to temperature changes and prone to false positives; multiple primer pairs need to be designed	(Venkatesan et al. 2016b; Li et al. 2013; Malik et al. 2019; Tsai et al. 2009; Wang et al. 2013)
RPA	Faster, simpler, and shorter than LAMP (less than 20 min); can be run at constant temperature	Difficult to set up the system, many kinds of enzymes; vulnerable to aerosol contamination	(Fan et al. 2020; Yang et al. 2015, 2016)
RAA	Rapid, sensitive, and specific detection approach for ORFV clinical testing	Primer design is more demanding than ordinary PCR; easy to produce false positive; difficult to amplify larger fragments	(Xue et al. 2020; Wang et al. 2020)

viruses, which prevents cells from being infected by viruses (Kresic et al. 2020). However, serum neutralization test is not typically used for ORFV diagnosis since immune responses to ORFV infection are mostly cell-mediated and neutralizing antibodies are typically low in concentration. In general, titers ≥ 8 are considered to be positive in serum neutralization tests (Hosamani et al. 2009).

Immunohistochemistry assay

Immunohistochemistry (IHC) assay is an integral technique to utilize monoclonal and polyclonal antibodies for the detection of specific antigens in tissue sections. It has been widely used for clinical diagnosis of various diseases (Sukswai and Khoury 2019). Histopathological examination of orf virus infected skin reveals vascularization and the swelling of the keratinocytes in the stratum spinosum, reticular regeneration, and marked epidermal proliferation. The main histopathology of the underlying dermis are edema, marked capillary dilation, and infiltration of inflammatory cells (Martins et al. 2021; Fleming et al. 2015; Zhao et al. 2010). Upon infection of ORFV, a number of neutrophils, T cells, B cells, and DCs are accumulating in the lesion following primary infection and reinfection. $CD4^+$ T cells are the predominant T cells in the skin in both primary and reinfection, playing crucial roles in eliminating ORFV (Bukar et al. 2021).

Immunofluorescence assay

Immunofluorescence assay (IFA) is a technique to detect unknown antigens using fluorescence-labeled antibodies based on the principle of specific binding of antigens to antibodies. IFA has the characteristics of simple operation, fast speed, strong specificity, high sensitivity, and intuitiveness (Petrovan et al. 2019). Zhao and colleagues performed an indirect IFA on CPE-positive MDBK cells using rabbit anti-ORFV polyclonal antibody as the primary antibody and FITC-conjugated goat anti-rabbit IgG as the secondary antibody (Zhao et al. 2010). As a result, ORFV-infected MDBK cells appear green while the uninfected cells are not. In another experiment (Zhao et al. 2011), an indirect IFA employing the same FITC-conjugated secondary antibody is conducted to detect the expression of ORFV011 and ORFV059, two major immunodominant proteins of ORFV.

Lateral flow immunochromatographic assay

Zhao and colleagues establish a lateral flow immunochromatographic assay (LFIA) depending on monoclonal antibodies (MAbs) 5A5 and 6F2 against ORFV011 protein (Zhao et al. 2016). The MAb 5A5 is conjugated with colloidal gold, and the MAb 6F2 and goat anti-mouse IgG are sprayed onto a nitrocellulose membrane at positions designated test (T) and control (C), respectively. When ORFV complexed with colloidal gold-conjugated MAb 5A5, captured by MAb 6F2 at T line, a purple band would appear. Otherwise, the C line conjugated with goat anti-mouse IgG would be visible when samples contain no ORFV or a small amount below the limit of detection. The detection sensitivity of this test is $2.03 \times 10^{3.0}$ TCID50/ml. Although this method needs to be improved in sensitivity and specificity, the LFIA has a good performance in the rapid diagnosis of ORFV in the field, no need for well-trained technicians and expensive device.

Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) is a simple, rapid, and quantitative immunoassay to detect antigens or antibodies attached to a solid surface. In general, the ELISA method can be classified into direct, indirect, sandwich, and competitive types (Wong et al. 2020). Indirect ELISA is the most widely used diagnostic method to detect antibodies specific for ORFV in goats and sheep. Initially, heat-inactivated whole virus is used as the ELISA antigen for Capripoxvirus or ORFV diagnosis (Babiuk et al. 2009; Bora et al. 2016). However, it is not an ideal assay since it is expensive and difficult to produce large quantities of whole virus antigen. Furthermore, the virus may exist in the environment for a long time and may also cause operators to be infected. For these disadvantages, researchers develop an indirect ELISA based on C-terminal truncated recombinant F1L protein produced in *E.coli* for detecting ORFV-specific antibodies (Yogisharadhya et al. 2018). The established rF1L-ELISA with high specificity (92%) and diagnostic sensitivity (> 89%) may be used for serological surveillance of ORFV infection in goats and sheep. In another study, an indirect ELISA using purified ORFV075 scaffold protein as coating antigen is developed for anti-ORFV antibody detection (Fleming et al. 2017).

Molecular-based diagnosis

Molecular-based diagnostic methods depend on polymerase chain reaction to detect virus-specific DNA or RNA rather than viral antibodies or antigens (Zhou et al. 2022). Till now, a variety of molecular-based diagnosis including polymerase chain reaction, real-time PCR, loop-mediated isothermal amplification-based assay, RPA assay, and RAA assay are available for ORFV detection.

Polymerase chain reaction

Polymerase chain reaction (PCR) technology targeting specific DNA fragments of ORFV has been widely used

for ORFV diagnosis. F1L (ORFV059) gene and B2L (ORFV011) gene are the most commonly used targets for ORFV diagnosis while other conserved genes of ORFV can also be selected as candidates (Sahu et al. 2022; Wang et al. 2019a, b, c). First, the virus genome is extracted from clinical samples using a viral genome extraction kit. Then, PCR amplification is carried out with specific primers for target genes. Following that, the purified PCR products are cloned into vectors for Sanger sequencing.

In addition to single-gene PCR, several duplex and multiplex PCR approaches have been established and utilized for ORFV detection. For example, Zheng and colleagues develop a duplex PCR assay for simultaneously detecting Capripoxvirus (CaPV) (goatpox virus and sheeppox virus) targeting A29L gene and ORFV targeting ORFV059 gene (Zheng et al. 2007), respectively. For both CaPV and ORFV, the developed assay has a high specificity and sensitivity with a detection limit of 1 plaque forming unit (PFU). Similarly, researchers establish a multiplex PCR (mPCR) assay to detect sheeppox virus (SPPV), goatpox virus (GTPV), and ORFV (based on ORFV025 gene) (Venkatesan et al. 2014a). As little as 350 pg of viral genomic DNA or 10^2 copies of standard plasmid of individual targets or 10^3 copies of plasmid in a mixture of two or three viruses can be detected using this mPCR assay. Recently, He and colleagues develop a multiplex PCR assay for simultaneously detecting six DNA and RNA viruses including peste des petits ruminants virus (PPRV) (He et al. 2017), foot and mouth disease virus (FMDV), bluetongue virus (BTV), GTPV, SPPV, and ORFV (based on ORFV011 gene) from clinical samples from sheep and goats. In this assay, reverse transcription of viral RNA is performed in the first step, followed by mPCR execution of viral cDNA and DNA in the second step. This mPCR assay is highly sensitive with a detection limit of 100 pg of viral genomic DNA or RNA in a reaction involving in mixture of six viruses.

Real-time PCR

Since conventional PCR approaches are nonquantitative and time-consuming, sometimes resulting in nonspecific amplification, Wang and colleagues develop a SYBR Green I real-time method based on a 180 bp conserved region of B2L gene for ORFV diagnosis (Wang et al. 2017). It can detect as low as 20 copies of ORFV genomic DNA, about 1000 times higher than that of conventional PCRs. The test takes approximately 1.5 h and shows no cross-reactions with PRV, GTPV, and SPPV. However, a melting curve is necessitated to assess whether the product is the intended target. To overcome this problem, scientists develop a TaqMan-based real-time PCR assay that relied on amplification of a 70 bp fragment from B2L gene for ORFV diagnosis (Gallina et al. 2006). This assay is highly sensitive and reproductive, able to quantify virus solutions ranging from 1×10^1 to 1×10^6 TCID50/ml within 1 h. Moreover, Bora and colleagues establish a TaqMan real-time PCR assay by targeting the DNA polymerase gene (Bora et al. 2011). It is highly sensitive as the limit of detection of this assay is around 3.5 fg or 15 copies of ORFV genomic DNA. Similarly, researchers establish a TaqMan-based real-time PCR assay that relied on amplifying an 87 bp DNA fragment from ORFV024 gene (Du et al. 2013). The detection limit of this assay is 5 fg or 15 copies of ORFV genomic DNA.

For simultaneously detecting Capripoxvirus (CaPV) and ORFV, Venkatesan and colleagues develop a TaqMan-based real-time duplex PCR (drt-PCR) using two pairs of primers and two hybridization probes (Venkatesan et al. 2014b). This assay is specific only for targeted viruses other than BPXV, CMLV, PPRV, and BTV. As low as 20 copies for each of the standard plasmid and 35 fg of viral genomic DNA for CaPV and ORFV can be detected, respectively. In recent years, Xu et al. develop a multiplex TaqMan qPCR assay for simultaneously detecting four types of DNA and RNA viruses from clinical samples of goats and sheep (Xu et al. 2019). In this study, multiple primers and probes are used for detection of PPRV, FMDV, GTPV, and ORFV, respectively. The detection limits are 91.7, 169, 94.1, and 74.6 copies/μl for PPRV, FMDV, GTPV, and ORFV in a reaction involving four viruses, respectively. Moreover, a novel high-resolution melting (HRM) assay (Gelaye et al. 2017) for simultaneously detecting eight poxviruses belonging to *Orthopoxvirus* (CPXV, CMLV), *Capripoxvirus* (GTPV, SPPV, and LSDV), and *Parapoxvirus* (PCPV, BPSV, and ORFV) genera is established. The assay shows high sensitivity, specificity, and cost-effectivity for pox disease detection in a large variety of animals and humans. Compared with conventional PCRs, the real-time quantitative PCR assay has many advantages such as excellent sensitivity and specificity, less time and labor, and high-throughput ability. TaqMan-based real-time PCR is required to design and produce specific probes which are complicated and expensive while SYBR Green I-based real-time PCR is simple, cost-effective, and easy to perform in which melting curve is required to verify the intended products.

Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP), mostly depending on strand displacement DNA polymerase and specific primer sets, is an affordable, rapid, and accurate diagnostic approach that amplifies target genes under isothermal conditions, usually around 60 °C (Malik et al. 2019). To date, several LAMP assays have been established for ORFV diagnosis. For example, Tsai et al. develop a LAMP assay based on six primers targeting several conserved regions from the B2L gene (Tsai et al. 2009). The detection

limit of this LAMP assay is a single copy of standard plasmid, which is tenfold and 100-fold higher than nested PCR and PCR, respectively. After PicoGreen and ethidium bromide staining, fluorescent green and orange products can be visualized, respectively. Moreover, Li et al. develop a LAMP assay for ORFV detection by targeting the DNA polymerase gene (Li et al. 2013). For this LAMP assay, 45 min and 62 °C are determined to be the optimal time and temperature conditions, respectively. The LAMP assay has a similar sensitivity with real-time PCR, showing no cross-reactivity with SPPV, GTPV, or vesicular stomatitis virus. Venkatesan and colleagues (Venkatesan et al. 2016) also develop a specific and sensitive LAMP assay targeting DNA polymerase gene in which the optimal reaction conditions are at 65 °C incubation for 60 min. Furthermore, Wang et al. develop a LAMP assay based on the F1L gene with SYBR Green I dye for color inspection (Wang et al. 2013). The sensitivity of this assay is 10 copies of a standard plasmid, having no cross-reactions with either FMDV or Capripoxvirus.

LAMP can be carried out in a water bath or heat block under isothermal conditions, no requirements for a thermocycler. In contrast to PCR, repetitive denaturing and annealing process are not required and the inhibitory effects from substances in samples are significantly reduced. In conclusion, LAMP is a simple, sensitive, specific, rapid, and visualized field diagnostic approach for ORFV.

RPA assay and RAA assay

Since its initial development in 2006, the recombinase polymerase amplification (RPA) as a rapid isothermal molecular diagnostic method has been successfully used to detect various pathogens (Fan et al. 2020). This assay consists of a recombinase, a single-stranded DNA-binding protein (SSB), and a strand-displacing polymerase. Yang and colleagues develop a fluorescent probe-based RPA assay (ORFV exo RPA assay) targeting the DNA polymerase gene (Yang et al. 2015). This ORFV exo RPA assay is demonstrated to be highly specific with no cross-reactions with PPRV, FMDV, or Capripoxvirus, and the detection limit is 100 genome copies per reaction. Besides, Yang et al. develop a novel ORFV RPA-LFD assay by combining previously established RPA technology with a lateral flow dipstick (LFD) (Yang et al. 2016). This RPA-LFD assay is also highly specific and sensitive with a detection limit of only 80 copies per reaction. It can be used for ORFV diagnosis within 20 min with a wide range of temperature. In contrast to LAMP, RPA is simpler to run, requiring only a pair of primers, a lower temperature (37 °C to 42 °C), and a shorter run time (less than 20 min) while LAMP requires four or six primers, a higher temperature (62 °C), and a longer run time.

As a new isothermal amplification technology, recombinase-aided amplification (RAA) assay has been widely used for various pathogens' detection. Different from RPA assay, RAA assay consists of three core enzymes, recombinase UvsX, DNA polymerase, and SSB (Xue et al. 2020). Wang et al. successfully established a RAA assay for ORFV detection (Wang et al. 2020). This assay can be accomplished within 30 min and the detection limit is 10 copies per reaction, showing no cross-reaction other common DNA viruses. In conclusion, RAA assay is a rapid, sensitive, and specific detection approach for ORFV clinical testing.

Conclusion and perspectives

Currently, a range of diagnostic methods are available for ORFV detection. Clinical diagnosis can only be a preliminary diagnostic method for ORFV detection. Virus isolation remains to be the gold standard for ORFV detection despite this method being laborious, time-consuming, and less sensitive. Indirect ELISA is the most widely used serological diagnostic method due to its simplicity, relatively low cost, and high-throughput potential. Compared with these above diagnostic methods, molecular-based diagnoses including PCR, real-time PCR, and LAMP show higher sensitivity and specificity. The multiplex PCR or TaqMan-based real-time PCR makes it possible to simultaneously detect various pathogens, assisting to control the disease in time. Isothermal molecular diagnostic approaches including LAMP, RPA, and RAA assay are rapid, sensitive, and specific, suitable for ORFV clinical testing in field. It is expected that additional diagnostic methods such as duplex fluorescent microsphere immunoassay (FMIA) and droplet digital PCR (ddPCR) will be established in the near future for rapid diagnosis of ORFV (Ji et al. 2020; Kojabad et al. 2021). Additionally, commercial kits for ORFV and various pathogen diagnoses are quite needed.

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Declarations

Ethics approval No ethical approval was required as this is a review article with no original research data.

Conflict of interest The authors declare no competing interests.

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