Research Article

Orf in India: Opportunities and challenges

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Abstract | Orf virus is the most studied poxvirus next to Vaccinia virus that has been used as vaccine candidate for small pox infection in humans. This virus causes contagious form of disease, mainly but not limited to sheep and goats, which lead to high economic impact in developing countries including India. The causative agent, Orf virus (ORFV) belong to the genus *Parapoxvirus* (PPV) of the family *poxviridae* produces a typical papillomatous, cauliflower like external growth around mouth regions and rarely the lesions are found in other parts of the body and internal organs. Another characteristic feature of ORFV including other PPVs is the ability to re-infect the same host and conferring short term immunity in contrast to other pox viruses and also produce localized skin lesions in humans who are in contact with infected animals and their products. Unique and specific virulence genes located at termini of ORFV genome with their encoded products are attributed to host immune evasion, short-term immunity both in natural infection post-vaccination in target species and repeated host susceptibility. However, these genes are variable among ORFV isolates/strains as they are not located in central part of genome and some of them are either virus specific namely Viral interferon resistant (VIR) and GM-CSF/IL2 inhibition factor (GIF) genes and or host specific namely vascular endothelial growth factor (VEGF-E) and viral IL-10 genes. ORFV can cause high morbidity in adults along with considerable mortality in young suckling animals and showing an expanding host range even to marine species make this contagious virus, an important animal pathogen globally. Being zoonotic and endemic in most parts of the developing world, it is necessary to prevent and control the infection using latest molecular diagnostics and effective vaccine along with anti-viral therapeutics. This short commentary deals with current knowledge and opportunities on ORFV and future challenges as control perspectives in India.

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Introduction

Among many contagious viral diseases affecting sheep and goats, Orf or sore mouth is one among them and cause a debilitating disease in domesticated and several wild small ruminant species (Nandi et al., 2011; Venkatesan et al., 2018). The etiological agent, Orf virus (ORFV) is the prototypic member of the genus *Parapoxvirus* of subfamily *Chordopoxvirinae* and family *Poxviridae*. The genus *parapoxvirus* include pseudocowpox virus (PCPV), bovine papular stomatitis virus (BPSV) of cattle and parapoxvirus of red deer in New Zealand (PVNZ) and most of them are zoonotic. Some tentative species like chamois contagious virus, parapoxvirus of Japanese serow, musk ox, camels (Ausdyk virus), reindeer, seal and sea lions (King et al., 2012) and recently a novel poxvirus (Airas et al., 2013) from horse are tentative members of this genera. ORFV is an enveloped, large dsDNA virus of ~135 kbp in size replicating in the cytoplasm (Hosam-

ani et al., 2009) encoding approximately 132 proteins, with some variations among the species or isolates. The central conserved region of ORFV (ORF009 to ORF111) which encodes the proteins is essential for viral replication, virion assembly and morphogenesis. ORFV including other PPVs are found to be closely related to *Molluscum Contagiosum virus* (MOCV) based on the high GC content (62-65%) (Delhon et al., 2004). The contagious and zoonotic nature of ORFV and its ability to re-infect the same host and emerge in new host species make the virus is of interesting and challenging for developing countries.

Epidemiology, infection and immunity

Contagious ecthyma is enzootic in most parts of the world. In India, it has been reported in almost all states of the country (Mondal et al., 2006; Hosamani et al., 2009; Venkatesan et al., 2011; Nandi et al., 2011) including North-eastern regions (NER) namely Meghalaya, Assam (Bora et al., 2012) and Tripura (Venkatesan et al., 2018) a bordering state to Bangladesh and its geographical range is expanding to other NE regions also. Although the Orf is endemic in various countries, not much attention has been given as the disease is not included in OIE notifiable terrestrial disease list (Gelaye et al., 2016), it is a group 2 risk pathogen of OIE listed. ORFV mainly infects sheep and goats and has been reported in other animals including camels, alpacas, squirrels and seals (Hosamani et al., 2009) and in several wild animal species namely reindeer, musk ox, mule deer, white-tailed deer, pronghorn fawns and wapiti calves (Nandi et al., 2011) and recently in free range black buck (Sharma et al., 2016) associated with sarcoptic mange showing its emerging host range. Sporadic cases of PPV in camels have been reported (Nagarajan et al., 2010) and other countries (Oryan et al., 2017). Infection occurs through skin abrasions in-contact with contaminated field causing localized lesions mostly confined to epithelium of oral mucosa, skin of lips and around the nostrils and scab lesions shed to contaminate environment. The contaminated field will act as source of infection to same or other susceptible hosts when they habitat or graze the environment again (Venkatesan et al., 2011). The disease usually lasts for 3-4 weeks and subsides in 1-2 months with shedding of scabs without leaving any scar (Nandi et al., 2011) and persistently infected carrier sheep may be source of infection within or between the flocks. The disease occurs as mild form but with significant productivity losses due to high morbidity. Mortality rate is rare in adults but it varies from 10 to 90% in lambs and kids (Mondal et al., 2006) due to starvation and secondary bacterial infections (Venkatesan et al., 2011). Mixed infections of ORFV with other pathogens like PPRV (Saravanan et al., 2007), CaPV (Venkatesan et al., 2014a; b), Mycoplasma (Chu et al., 2011), Streptococcus-Staphylococcus (Chi et al., 2017) and sarcoptic mange (Sharma et al., 2016) have been reported. Humans contract the infection through direct contact with affected sheep and goats as occupational zoonosis or by contaminated fomites and produce benign self-limiting lesions restricted to fingers, hand and forearm and rarely severe in case of immuno-compromised patients (Nandi et al., 2011). Immunity developed against ORFV is short-lived and it can able to re-infect the host successfully by its immune evasion strategy (Haig et al., 1997) due to the presence of dispensable virulence genes located at termini of the genome. They help the virus to replicate and survive in specific host immune environment. Among these, CBP, GIF, VIR and dUTPase genes are of vaccinia virus (VACV) homologues proteins (C23L, A41L, E3L and F2L respectively) and indicate their origin from the ancestral poxviral genes. Because of the close relationship between the virus and its host during the course of evolution, some of the virulence genes had been "captured" from the host like dUTPase, vIL-10, VEGF, anaphase promoting complex analog (PACR) and anti-apoptotic factors (Flemimg et al., 2015).

Genetic and phylogenetic analysis of circulating ORFV in India

Genetic characterization of ORFV strains or isolates from diverse geographical origin targeting various genes of ORFV genome will provide deep insight into phylogenetic relationship and genetic variations among circulating strains. This knowledge will help to develop novel molecular diagnostics, subunit vaccines and design suitable anti-viral agents. B2L (ORF011, an immunogenic envelope protein) gene is most frequently targeted in genetic analysis and development of molecular diagnostics for PPV including ORFV (Inoshima et al., 2000; Hosamani et al., 2006; Venkatesan et al., 2012; Bora et al., 2011; Venkatesan et al., 2018). B2L Sequence analysis of Indian ORFV isolates from sheep and goats has revealed a high nucleotide (nt) at ~97.3–100% level and amino acid (aa) at ~96.3–98.9% level of identities among themselves. North-Eastern Indian ORFV isolates have shown close genetic relationship with North Indian isolates with particular reference to ORFV-Shajahanpur/04

(Bora et al., 2012; 2015; Venkatesan et al., 2018). In addition to B2L, other genes including ORF 059 (F1L), ORF 020 (E3L/VIR), ORF 117 (Guo et al., 2004; Hosamani et al., 2007), ORF032 and ORF 080 have also been used but less frequently (Chi et al., 2017). A32L gene encoding ATPase based molecular epidemiology of ORFV isolates in India have shown the heterogeneity of circulating virus isolates or strains (Yogisharadhya et al., 2012). The inherent short immunity and ability to re-infect the same host can be attributed to the presence of several terminally located virulence genes including viral interferon resistance genes (VIR), NF-kB inhibitors, chemokine binding proteins (CBP), GM-CSF/IL-2 inhibitory factor (GIF), dUTPase, poxviral anaphase promoting complex (PACR)/ring H2 protein, vascular endothelial growth factor (VEGF), viral Interleukin-10 (vIL-10), inhibitor of apoptosis etc. The conserved functionality of these genes due to presence of some functional conserved domains in spite of significant sequence variations is responsible for the activity of encoded virulence factors and establishing them as promising candidates for further studies in near future (Karki et al., 2017).

Current developments in Orf virus research

Diagnostics: The conventional diagnosis of Orf is done on the basis of its characteristic acute and proliferative skin lesions and differential diagnosis from goatpox, sheeppox, FMD, and bluetongue diseases (Venkatesan et al., 2011; 2018). However, a specific and sensitive diagnosis is imperative to eliminate the chances of misdiagnosis and provide a confirmatory identification in case of co-infection or mixed type of infections involving other pathogens and it will enable the correct therapeutic management to be initiated. Laboratory confirmatory diagnosis is usually done by virus isolation in primary lamb/kid cells and identification by negative electron microscopy to differentiate the ORFV from other poxviruses (Nandi et al., 2011). Several common serological assays (Hosamani et al., 2009) including ELISA using either conventional or recombinant antigens have been established to detect ORFV antibodies (Yogisharadhya et al., 2017). However, molecular detection of ORFV using PCR and related formats namely multiplex PCR and real-time PCRs has been reported (Nandi et al., 2011; Venkatesan et al., 2012; Venkatesan et al., 2014a; b) as highly sensitive and quick diagnostics. Among the genes of ORFV, B2L is targeted more frequently for identification of ORFV as conventional and real-time PCRs (Inoshima et al., 2000; Hosamani et al., 2006; Das et al., 2016). As an alternate to PCR/real-time PCR system in field oriented diagnosis, LAMP assay is proved to be a successful system in various infectious diseases (Notomi et al., 2000). LAMP assays targeting B2L gene (Tsai et al., 2009; Venkatesan et al., 2016), F1L gene (Wang et al., 2016) and DNA polymerase gene (Venkatesan et al., 2015) have been successfully developed and evaluated to detect ORFV in several small ruminant species including Japanese serows (Inoshima et al., 2016). Comparison of different LAMP assays targeting different structural/non-structural genes should be done to select one candidate LAMP for sensitive and specific detection of PPVs including ORFV with high diagnostic performance. Open LAMP system that requires post-reaction manipulation should be replaced with closed tube methods involving different dyes to avoid carry over contamination in diagnostic settings. A closed tube LAMP assay in field applicable simple format will be handful in clinical surveillance and differential diagnosis of Orf occurring alone as well as mixed infections involving other pathogens namely PPRV, BTV, FMDV and CaPV.

Vaccines/anti-viral therapeutics: Vaccination is the ultimate feasible procedure to control and eradicate any infectious disease from an endemic country like India. Till now, there is no vaccine that can give 100% protection against ORFV available and the conventional vaccines or methods including dermal scarification using infected scab are found to be ineffective or provide only short term immunity (Bhanuprakash et al., 2012). Till now, scarification is followed as the preventive measure in many countries. Interestingly, ovine and caprine ORFV strains may not provide complete protection to each other (Musser et al., 2008). In India, live attenuated Orf vaccine (Mukteswar 59/05 strain) attenuated in primary lamb testis (PLT) cells has been developed and is found to be safe, efficacious and potent in sheep and goats (Bhanuprakash et al., 2012) by limited clinical trials. ORFV-D1701 is a sheep-derived attenuated strain obtained after 135 passages in cell culture is also approved in many countries to be used as live vaccine for Orf (Bhanuprakash et al., 2012). In addition to conventional vaccines, recombinant DNA approach involving immunogenic genes of ORFV namely F1L in VACV (Bhanuprakash et al., 2012) and GTPV (Zhang et al., 2014) as vector has been demonstrated. A highly conserved and immuno-dominant envelope protein

namely B2L was found to elicit immune response as an appropriate candidate for vaccine development (Yogisharadhya et al., 2017). The demerits associated with ORFV vaccine development can be overcome by using antiviral agents like nucleoside/nucleotide analogues and their derivatives (Hosamani et al., 2009). Among all the analyzed antiviral agents, cidofovir is found to be potential for parapoxviruses including ORFV (Nettleton et al., 2000) and effectively used in topical or oral route for human as well as animal cases (Scagliarini et al., 2007). As ORFV has large genome size that can accommodate foreign genes and its inherent immunomodulatory property, it is targeted for development of novel recombinants for developing diagnostics and viral vectored vaccines. Further, attenuated ORFV strain can stimulate a short term non-specific immunity termed as paramunity inducers (commercially available as Baypamun® or Zylexis®) and can be used to potentiate immunity against several viral infections and cancers. (Ziebell et al., 1997).

Future challenges and perspectives

Orf as a contagious enzootic disease and zoonotic nature can cause economic impact in developing countries including India where sheep and goats are major livestock species to poor marginal farmers. It has also the potential threat to expand its host range including wild animals which could be a spill over source to domestic species. Despite its endemicity to India, there is no information available on proper surveillance, epidemiology and future control strategies for the disease. Besides, wide host range, the zoonotic potential, short-term immunity, and the unique immune evasion property of the virus are interesting characteristics to be studied in detail. However, Orf is an ignored viral disease in most of the developing countries including India and the impact of the disease in terms of direct and indirect losses it inflicts should be determined. As ORFV is stable in all types of agro-climatic conditions, it spreads very easily leading to frequent outbreaks in same regions or even within the same affected flocks. In field conditions, the lesions are often confused with other important diseases like capripox, bluetongue, foot and mouth disease and it necessitates developing a rapid and sensitive field applicable tests including LAMP assay for specific detection of ORFV. Also, a recombinant antigen based ELISA for ORFV in sheep and goats could be a choice to differentiate them from other similar lesion causing agents and also useful in sero-surveillance of the disease in other species including wild animals. As ovine and caprine origin strains of ORFV are not conferring protection against each other, there is a need to develop a differentiation PCR to identify the origin of ORFV and identify strains that can protect both sheep and goats. Genetic characterization of ORFV isolates/strains from different outbreaks targeting structural/non-structural genes will help to establish epidemiological distribution of the ORFV in India by identifying genetic relatedness or variation among circulating virus isolates/strains in diverse geographical areas. ORFV provides short-term immunity as compared to other members of *Poxviridae* due to presence of immunomodulatory or virulence associated genes. Detailed characterization of these virulence genes may unravel the mechanism of immune evasion strategy of ORFV and may provide co-evolutionary process of virus with host in the process of developing the resistance to immunity and persistency in same infected hosts in future. Dispensable sites within the genome can be targeted to develop vectored vaccines and gene-deleted/marker vaccines. Whole genome NGS sequencing of ORFV strains/isolates including vaccine will bridge the gap the existing knowledge on genetic evolutionary relationship of circulating ORFV in India and also provide insight in development of novel diagnostics, vaccines and antivirals for PPV.

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Authors Contribution

Gnanavel Venkatesan collected, reviewed and drafted the manuscript and AK helped in drafting and revising the manuscript.

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