Chapter 9
Capripoxvirus and Orf Virus

Gnanavel Venkatesan, Amit Kumar, V. Bhanuprakash, V. Balamurugan, and Raj Kumar Singh

Abstract Capripox infections of small ruminants, namely goatpox and sheeppox, are OIE notifiable and transboundary animal diseases. Goatpox and sheeppox are prevalent in some parts of Africa, the Middle East, and Asia with occasional outbreaks in regions of Europe. The etiological agents, goatpox virus (GTPV) and sheeppox virus (SPPV), are indistinguishable serologically. However, they are differentiated by some of the molecular techniques. The diseases are characterized by fever, papules, nodular lesions on the skin, and sometimes internal organs and lymphadenopathy with high morbidity and mortality in affected animals. Contagious ecthyma (orf) is an economically important contagious disease of sheep, goat, and other ruminants with worldwide distribution. It is a local eruptive skin disease characterized by proliferative lesions on mouth and muzzle. The disease has zoonotic importance causing localized lesions in humans. The orf virus (ORFV) is the causative agent of this skin infection belongs to the genus Parapoxvirus. Also, it possesses the capacity to re-infect the host due to its epitheliotropic niche and encoded immunomodulators. Goatpox, sheeppox, and orf infections pose serious economic threat to the agricultural sector and livelihood of the farmers in endemic regions with a major impact on international trade. A prompt diagnosis along with well-planned vaccination and effective bio-security measures are main control measures to contain the infection in any endemic region. Development of recombinant protein-based serodiagnostic assays and rapid pen-side diagnostics that allow

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differentiation of GTPV, SPPV, and ORFV is the need of the hour for improved disease control.

Keywords Capripox infections · Goatpox virus (GTPV) · Sheeppox virus (SPPV) · Parapoxvirus · Contagious ecthyma · Genome · Diagnosis · Epidemiology

9.1 Prologue

Sheeppox and goatpox are collectively known as capripox, and orf are the pox viral diseases of mainly sheep and goats with high socio-economic threats (Malik et al. 1997; Bhanuprakash et al. 2011; Kumar et al. 2015; Madhavan et al. 2016). The causative agents, sheeppox virus (SPPV) and goatpox virus (GTPV), belong to genus *Capripoxvirus*, whereas orf virus (ORFV) belongs to genus *Parapoxvirus* of the family *Poxviridae* (Bhanuprakash et al. 2006; Hosamani et al. 2009; Kumar et al. 2015). Under mixed farming, where sheep and goats population are farmed together in a single platform, more than one or two viruses infect the same host in a simultaneous manner. The mixed infections of capripoxviruses (CaPVs) and ORFV or any other infectious agents may increase the severity of either of those infections (Hosamani et al. 2004a; Chu et al. 2011). Although the clinical sheeppox disease has been reported in first century A.D., the virus was first isolated by Borrel in 1902, whereas goatpox was reported from Norway by Hansen in 1879 (Rafyi and Ramyar 1959). Despite orf infection is long known in small ruminants by shepherds but was first described in 1787 by Steeb (Robinson et al. 1982) followed by elucidation of its contagious nature in 1890. Of late in 1923, the etiological agent to be different from vaccinia virus-based on physicochemical and immunological characteristics has been identified. Earlier classification of sheeppox and goatpox (SGP) based on animal species of origin has now been replaced by molecular methods of differentiation (Hosamani et al. 2004a; Venkatesan et al. 2012a, 2014a, b). Both sheeppox (SP) and goatpox (GP) infections are endemic in the Indian subcontinent, Central Asia, Middle East, and Africa, whereas orf is endemic in almost all parts of the world.

SPPV and GTPV cause similar kind of generalized disease showing the pyrexia, oculo-nasal discharge with typical pox nodules on the skin and internal mucosa (Babiuk et al. 2008) and the outbreaks are linked to a significant production loss due to high morbidity, a decrease in weight gain, damage to hide and wool, and trade barrier (Tuppurainen et al. 2017). In contrast to SGP infections, orf is a self-limiting skin disease causing typical localized proliferative cutaneous lesions over mouth and lips in sheep, goats, and also other wild ruminants. Both SP and GP are notifiable to OIE as the morbidity and mortality may be very high, up to 100% in naïve animals (Bhanuprakash et al. 2006). Orf is often considered as opportunistic pathogen associated with other viral diseases, especially *Peste des petits ruminants* (PPR) and Capripox infections (Hosamani et al. 2009).

Further, it is economically significant owing to its endemicity, ability to emerge in other host species, zoonotic potential, and occurrence of mixed infections (Hosamani et al. 2009). The disease has been described under various names viz., contagious pustular dermatitis, contagious ecthyma of sheep, sore mouth, scabby mouth, contagious pustular stomatitis (Nandi et al. 2011). Parapoxviruses (PPVs) including ORFV have unique ability to re-infect the same host, under the encoded immunomodulators subverting the host immune response (Hosamani et al. 2009) leading to short-term immunity as compared to other poxviruses. This book chapter describes the epidemiology of GTPV, SPPV, and ORFV infections and its molecular characterization, antigenic properties, host range, pathogenesis, clinical disease, diagnosis, and prevention/control measures.

9.2 Etiological Agents: Morphology and Genome Organization

SPPV and GTPV belong to genus *Capripoxvirus* of the family *Poxviridae*. Other member of the genus, lumpy skin disease virus (LSDV) affects cattle. Most of the SPPV and GTPV isolate show host-preference (Madhavan et al. 2016). The ORFV is the prototypic member of the genus *Parapoxvirus* belonging to the family *Poxviridae* (Nandi et al. 2011). Other important members in this genus are pseudocowpox virus (PCPV), bovine papular stomatitis virus (BPSV) of cattle, and parapoxvirus of red deer in New Zealand (PVNZ) of which except the last member, all are reported to be zoonotic. CaPVs are brick-shaped with complex symmetry and 300 × 270 × 200 nm size. The CaPV genome consists of covalently linked double-stranded DNA of 150–160 kbp length with inverted terminal repeats at the ends (Tulman et al. 2002). PPVs have several unique characteristics like distinct virion morphology, high G-C content, and presence of genes coding for immunomodulatory proteins. ORFV has a characteristic ovoid shape with 260 × 160 nm size. Genome is linear double-stranded DNA (134–139 kbp) with high G + C (63–64%) content in comparison to other poxviruses and encodes 132 proteins (Delhon et al. 2004).

The genomes of CaPVs appear to be more divergent as seen in orthopoxviruses (OPVs) in both sequence and size towards their termini (Madhavan et al. 2016). The genome possesses highest A-T content (73–75%) among poxviruses and encodes ~150 proteins. Both SPPV and GTPV share 96% nucleotide identity over the entire length of the genome and they share 97% similarity with LSDV (Tulman et al. 2002). All genes that are present in SPPV and GTPV also present in LSDV. However, there are nine genes that are intact in LSDV associated with virulence and host range functions, are fragmented in SPPV and GTPV genomes (Tulman et al. 2002). GTPV is more closely related to LSDV than to SPPV and they might have evolved from a common SPPV like ancestor (Hosamani et al. 2004a; Le Goff

et al. 2009). Several genes including P32, RPO30, and GPCR allow species differentiation of SPPV and GTPV (Madhavan et al. 2016) at molecular level.

In case of ORFV, most of the essential genes are organized in the conserved central region of the viral genome like VACV except VACV D9R and VACV F15R (Delhon et al. 2004). ORFV possesses all structural genes present in VACV except A36R, K2L, A56R, and B5R (Tan et al. 2009). Terminal ends of the genome are reported to be variable and encode for proteins that are involved in host–virus interaction, virulence, and pathogenesis (Hosamani et al. 2009). PPVs have evolved a repertoire of unique immunomodulatory or host range genes that encode factors targeting the host immune system (Bratke et al. 2013). Among these, CBP (chemokine-binding protein), GIF (GM-CSF inhibitory factor), VIR (viral interferon resistance), and dUTPase genes are homologs of VACV C23L, A41L, E3L, and F2L proteins, respectively (Hosamani et al. 2009; Fleming et al. 2015). DNA polymerase gene-based phylogeny of ORFV isolates shows the close genetic relatedness with *Molluscum contagiosum* virus (Fleming et al. 2015).

9.3 Epidemiology

9.3.1 Geographical Distribution

Currently, SGP infections are endemic in entire Southwest and Central Asia, the Indian subcontinent, and Northern and Central Africa. Occasional outbreaks have been reported from regions of Europe like Turkey, Greece, and Bulgaria (Bhanuprakash et al. 2011). Introduction of affected animals through trade is the major mode of spread in naïve areas (Madhavan et al. 2016; Tuppurainen et al. 2017). Capripox is endemic in India and reported in almost all geographical regions of the country (Bhanuprakash et al. 2006). ORFV infections are reported worldwide including American continent, Europe, Australia, the Indian subcontinent (Hosamani et al. 2009; Nandi et al. 2011). Reports have been documented from different states of India, including the North-Eastern region involving sheep and goats (Venkatesan et al. 2018b).

9.3.2 Host Range, Susceptibility, and Transmission

SPPV and GTPV show host specificity with more severe disease evident in the homologous hosts. But, some strains affect heterologous hosts with lesser severe disease (Bhanuprakash et al. 2010). Some strains have been reported to have a natural infection in both species, including SPPV infection in Makhdoom (India) (Bhanuprakash et al. 2010), GTPV infection in China (Yan et al. 2012), and Ethiopia (Gelaye et al. 2016). Some Middle-Eastern strains also have shown equal pathogenicity for sheep and goats (Kitching et al. 1986). CaPV cause systemic infection in

all ages but severe form is seen in the young animals (Bhanuprakash et al. 2006). Exotic breeds including European are more susceptible (Bhanuprakash et al. 2006). Infection is reported throughout the year. Evidence for the existence of CaPVs in wild ruminants is lacking (Tuppurainen et al. 2017). SPPV and GTPV are mainly transmitted by aerosol route, but it also occurs indirectly through other mucous membranes and abraded skin by contaminated feed and wool. Occasionally, the fomites and insects (Kitching and Mellor 1986) play the role in transmission. The virus can remain infective on hair or wool for as long as three months after infection and for a longer period in scabs (Bowden et al. 2008). Carrier stage is not seen in infected animals (Bhanuprakash et al. 2011). Goatpox has also been reported as a mixed infection with PPR or orf simultaneously and by co-infection (Saravanan et al. 2007; Malik et al. 2011).

Orf mainly infects goats and sheep but shows a wider host range to cattle, camelids, seals, reindeer, mule deer, Sichuan Takin, Japanese serows (*Capricornis crispus*), and Japanese deer (*Cervus Nippon centralis*) (Hosamani et al. 2009) and also identified in blackbuck associated with sarcoptic mange (Sharma et al. 2016). Some tentative species like chamois contagious virus, parapoxvirus of Japanese serow, musk ox, camels (Ausdyk virus), reindeer, seal, sea lions (King et al. 2012) and recently, PPV from the horse (Airas et al. 2016) are yet to be classified in the genus. ORFV primarily infects animals less than one year of age. The disease is seen throughout the year. Stressors such as transportation, pregnancy, and other factors act as predisposing factors (Nandi et al. 2011). ORFV is resistant to physical and chemical agents as identified in other poxviruses. It exists for years in dry and extreme cold conditions including dried scab (Venkatesan et al. 2012b). Dried grass or leaves predispose to skin abrasions around lips and mouth through which virus enters. Transmission mainly occurs through direct contact or indirectly through contaminated non-living objects or fomites (Venkatesan et al. 2011). Chronically infected animals may carry the virus and are responsible for reappearance within same flock and transfer between flocks (Hosamani et al. 2009; Nandi et al. 2011).

9.4 Pathogenesis, Immunity, and Clinical Disease

CaPV have tropism for skin, respiratory, and gastrointestinal tracts. Following primary multiplication, the virus disseminates to the blood via the draining lymph nodes. The cell-associated viremia composing of infected monocytes and macrophages leads to settlement of virus in skin and other tissues (Bowden et al. 2008) and virus sheds in conjunctival, nasal, and other secretions (Balinsky et al. 2008). Various virus, host, environmental, and biometeorological factors decide the severity of capripox infection in affected hosts (Bhanuprakash et al. 2006). In the field conditions, incubation of the disease is 6–12 days. Initially, animal shows high fever along with oedema of eyelids, oculo-nasal discharge followed by progressive appearance of skin lesions, especially on wool-free areas. Macules further develop into papules followed by scab formation (Madhavan et al. 2016) with swollen

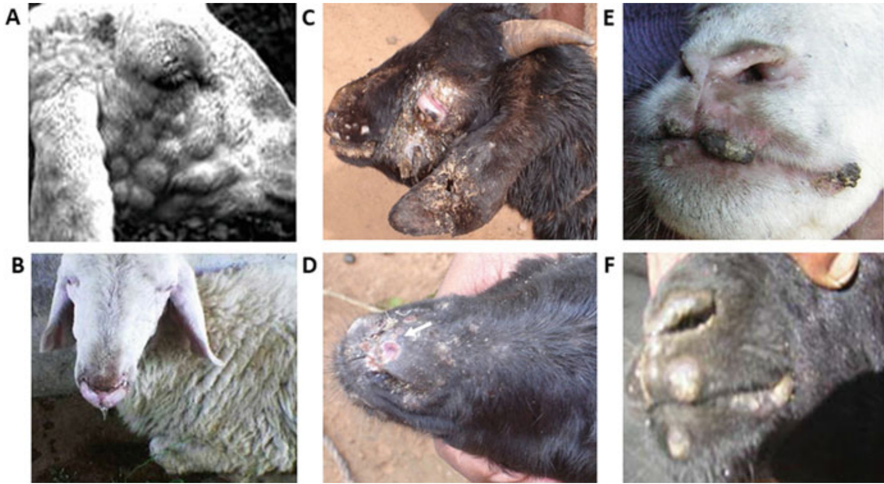

Fig. 9.1 Clinical picture of sheeppox (**a** and **b**), goatpox (**c** and **d**) and contagious ecthyma (**e** and **f**) infections in sheep and goats

pre-scapular lymph nodes. Nodular lesions may also be seen in lungs, digestive organs, liver, and other organs (Bhanuprakash et al. 2006). Visible skin nodular lesions over the head regions and frothy viscous nasal/mouth secretions are observed in sheep following sheeppox infection (Fig. 9.1a, b) and goatpox can cause severe erosions/ulcerations of skin lesions over nasal, ocular, and ear regions in goats which might be complicated with secondary bacterial infections (Fig. 9.1c, d) Virus shedding from mucosa is reported for up to 3–6 weeks following infection (Bowden et al. 2008). Although cell-mediated immunity (CMI) has predominant role following CaPV infection, humoral immunity also plays the part. Low level of neutralizing antibodies may be seen following mild disease or vaccination (Babiuk et al. 2009). Morbidity and mortality in adults may be moderate but, in young animals, these may reach 100% and 95%, respectively. Affected animals may recover in 4 weeks unless the secondary complications appear (Bhanuprakash et al. 2006).

Orf infection mainly starts following abrasions or breaks in the skin around lips and mouth, through which the virus enters and replicate in epidermal keratinocytes of skin as main predilection site (Nandi et al. 2011). Various virus-encoded immunomodulatory factors help the virus to replicate in skin environment. Clinically, orf is characterized by its proliferative skin lesions that may be painful, highly vascularized leading to bleeding. Lesions are seen mainly around mouth and lips either proliferative or nodular type growth in sheep and goats (Fig. 9.1e, f), but rarely seen in other regions (Hosamani et al. 2009). Mouth lesions cause inability to graze and suckle due to which young animals succumb to death causing high mortality in suckling kids and lambs (Venkatesan et al. 2018b). ORF induces short-lived immunity, in which CMI response plays the main role, whereas the role of antibody is unclear (Nandi et al. 2011). Orf in general is mild in affected animals but, severe in lambs and kids due to secondary complications (Hosamani et al. 2009). Both animal

and humans are susceptible to reinfection. Upon reinfection, lesions may be smaller and take less time to heal. Humans especially farmers, veterinarians, and zoo personnel contract the orf infection through direct contact with affected animal or contaminated fomite and is considered as occupational zoonosis (Nandi et al. 2011).

9.5 Economic Impact

Control of capripox and orf is important to boost the small ruminant sector in the developing countries (Babiuk et al. 2008; Nandi et al. 2011) as they cause tangible and intangible losses (Yeruham et al. 2007; Madhavan et al. 2016; Venkatesan et al. 2018b). Besides, they cause trade barrier on animals and their by-products from endemic regions inflicting indirect economic impact. An estimated loss of over INR 105 million in capripox outbreaks occurred in Maharashtra (India) state has been reported with average morbidity and mortality of 63.5% and 49.5%, respectively (Garner et al. 2000). Also, the extrapolated annual loss has been estimated to be INR 1250 million based on this data (Bhanuprakash et al. 2011). Though orf is of mild, it also inflicts economic loss due to severe morbidity in adults and high mortality in young ones and posing trade restriction on endemic countries (Hosamani et al. 2009).

9.6 Diagnostics

A tentative diagnosis of an acute form of SGP can be made based on typical clinical signs consisting of fever, pock lesions, swollen lymph nodes, and pneumonia (Rao and Bandyopadhyay 2000). Similarly, orf can be identified by typical proliferative lesions around mouth region (Hosamani et al. 2009). Nevertheless, the baffling clinical signs with other diseases involving skin, namely foot and mouth disease (FMD), bluetongue (BT), dermatophilosis/streptothricosis, mange, photosensitization, etc. emphasize precise clinical diagnosis, especially in low virulent strains (Bhanuprakash et al. 2006; Hosamani et al. 2009). Therefore, laboratory confirmation of capripox and orf by an array of routine serological and molecular tools is mandatory (Venkatesan et al. 2014a).

9.6.1 Conventional Techniques

Laboratory diagnosis of suspected cases is based on virus isolation, electron microscopy, and serological tests, namely SNT/VNT, FAT AGID, and ELISA (Rao and Bandyopadhyay 2000; Hosamani et al. 2009). Lesions from skin, lung, and lymph nodes collected during the first week of occurrence of clinical signs are preferable for

CaPV isolation and antigen detection, whereas mainly skin lesions around mouth are the main source of ORFV antigen.

1. *Electron microscopy*—Electron microscopy is usually used to detect the virus in tissue samples, but requires expertise and needs to be distinguished from OPVs by immunostaining. Negative electron microscopy readily differentiates ORFV from other poxviruses due to characteristic ovoid shape and criss-cross pattern of the virion (Hosamani et al. 2009).
2. *Virus isolation*—Primary lamb testes (PLT) and lamb kidney (PLK) cells are highly sensitive for primary isolation/adaptation of CaPVs and ORFV. In addition, Vero cells can also be used for CaPV isolation and continuous passaging to attenuate. A cytopathic effect like ballooning, rounding, increased refractivity, detachment, etc. is seen (Madhavan et al. 2016). CaPVs may require several blind passages for the appearance of CPE. Virus isolation of CaPV and ORFV is mostly tiresome and demands scientific skill as well. Laboratory animals do not support the growth of CaPVs (Bhanuprakash et al. 2011). PLK and PLT and OA3.Ts cells are most commonly used for isolation of ORFV (Plowright et al. 1959). Initial rounding, ballooning, and grape like clusters are typical CPE feature of ORFV infection (Nandi et al. 2011).
3. *Antigen detection assays*—Polyclonal antibody based antigen detection assays are common to detect soluble antigens and, therefore, lacks some degree of specificity against CaPV antigen. AGID (Rao and Negi 1997) and counter immunoelectrophoresis (CIE) (Sharma et al. 1988) have been used for diagnosis of capripox and orf, but they show serological cross-reactivity. Immunocapture ELISA using hyperimmune serum against the whole virus (Rao et al. 1997) or recombinant protein (Carn 1995) has been developed for the detection of CaPV antigen.
4. *Antibody detection*—Serological assays like VNT/SNT, AGPT, CIE, latex agglutination test (Rao et al. 1995), indirect fluorescent antibody test (IFA), whole antigen-based ELISA, and immunoblotting (Chand et al. 1994) have been developed but these cannot differentiate among CaPVs. CIE test can be employed to detect ORFV antibodies following infection and vaccination in targeted hosts (Venkatesan et al. 2011).

   i. Serum neutralization test (SNT)—It is the golden standard test for CaPV and ORFV specific antibodies detection and titration. But it is time-consuming, labour-intensive, difficult to interpret and requires handling of live virus (Venkatesan et al. 2018a). However, it is also useful to determine the antigenic relationship between CaPVs and assess the post-vaccination monitoring of antibody status (Bhanuprakash et al. 2006).
   ii. Western blotting using H3L homolog of CaPVs with sera to be tested is both sensitive and specific but tedious and expensive (Chand et al. 1994). It can differentiate CaPVs from ORFV. Western Blot analysis for ORFV is based on the presence of two immunogenic envelope proteins (39 and 22 kDa proteins) from serum samples (Czerny et al. 1997).

iii. ELISA—ELISA can be used to monitor the immune response of vaccinated and infected animals (Hosamani et al. 2004b). Whole inactivated capripoxvirus has been used as an ELISA antigen (Babiuk et al. 2009), but it is tedious and has bio-safety and security issues. Indirect ELISA based on expressed P32 antigen (Heine et al. 1999; Bhanot et al. 2009; Venkatesan et al. 2018a), ORF117 (Dashprakash 2013), ORF 095, and ORF 103 proteins (Bowden et al. 2009) have been developed. But, these assays are unable to detect low titer of neutralizing antibodies following vaccination or mild infection. Recombinant protein based validated immunodiagnostics like ELISA for CaPVs is still under development due to difficulties in selection of single immunodominant capripoxvirus antigen, its efficient expression in a heterologous host system and purification hamper its usage as a diagnostic. Therefore, no validated ELISA is commercially available for the detection of antibodies to CaPVs. There is a need of screening of immunogenic proteins of CaPVs for selection of suitable protein candidate with good expression level, efficient purification along with sensitive detection of low level of antibodies (Madhavan et al. 2016). Whole viruses as well as subunit antigens have been used in ELISA to detect ORFV antibodies significantly (Chin and Petersen 1995).

iv. Diagnostics using specific MAbs can be developed for rapid and sensitive detection of CaPVs antigen/antibody. Anti-ORF086 (Wang et al. 2015) and anti-ORFV059 MAbs (Li et al. 2012) have been reported as potential candidates for developing such diagnostics in past. A lateral flow immunochromatographic assay using two monoclonal antibodies against the ORF011 protein (Zhao et al. 2016) may be more suitable for field-level detection of orf infection.

9.6.2 *Nucleic Acid-Based Techniques*

In India, frequent outbreaks of SGP with orf often go misdiagnosed or unnoticed (Saravanan et al. 2007; Hosamani et al. 2009). Therefore, it is need of the hour to develop duplex/multiplex PCR targeting the detection of both CaPVs and orf simultaneously in single-tube reaction to enable early diagnosis of them occurring as single or mixed infection (Venkatesan et al. 2014a). These diagnostic tools and techniques can be handful in enzootic or naive regions during clinical surveillance of CaPV and ORFV.

1. *Conventional PCR for diagnosis of individual CaPV and orf infections*—several diagnostic PCR tools have been reported targeting P32 region of CaPV genome for its rapid detection (Ireland and Binepal 1998; Heine et al. 1999) and also DNA polymerase (*DNA pol*) gene has been targeted for PCR in past (Balamurugan et al. 2009). A highly specific semi-nested PCR targeting B2L (Inoshima et al. 2000) and diagnostic PCRs using DNA polymerase gene (Bora et al. 2011),

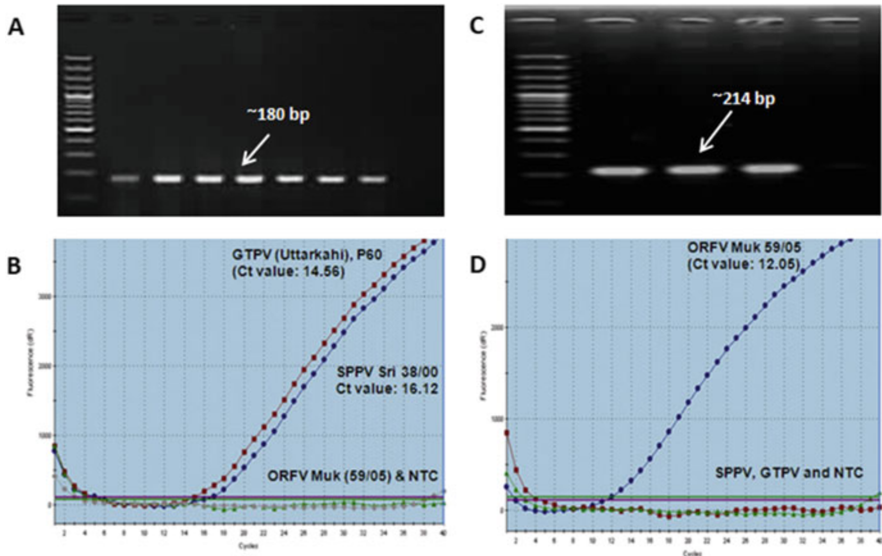

Fig. 9.2 Conventional PCR and TaqMan probe-based real-time PCR assays for rapid and sensitive detection of CaPV (**a** and **b**) and ORFV (**c** and **d**) DNAs

VLTF-1 gene (Kottaridi et al. 2006), VIR gene (Guo et al. 2004), A32L gene (Chan et al. 2009), and F1L gene (Hosamani et al. 2009) has been reported. *DNA Pol* gene-based PCR assays for sensitive diagnosis of CaPV and ORFV, respectively, amplifies approximately 179 bp and 214 bp fragments as observed in agarose gel analysis (Fig. 9.2a,c).

2. *Real-time PCR*—Several TaqMan quantitative polymerase chain reaction (qPCR) assays have been reported for detection of CaPV DNA targeting DNA pol gene (Balamurugan et al. 2009) and poly (A) polymerase (Balinsky et al. 2008). A novel qPCR using snapback primers has been developed for grouping of CaPVs (Gelaye et al. 2013). A genus-specific (Parapoxvirus) TaqMan qPCR has been reported targeting DNA polymerase gene (Das et al. 2016). Further, B2L (Gallina et al. 2006), ORF024 (Du et al. 2013), and DNA polymerase genes (Bora et al. 2011) have also been targeted for the same format. SYBR Green chemistry has also been attempted for the detection of ORFV and other PPVs (Venkatesan et al. 2012b; Zhao et al. 2013; Wang et al. 2017). DNA polymerase gene-based TaqMan probe real-time PCR for rapid, sensitive, and specific detection of CaPV (Fig. 9.2b) and ORFV (Fig. 9.2d) is shown.
3. *Real-time multiplex PCR (mPCR)*—TaqMan probe real-time duplex PCR targeting highly conserved DNA polymerase gene was reported to simultaneous detection of CaPV and its differentiation from ORFV from mixed infections (Venkatesan et al. 2014b). A dual hybridization probe qPCR assay has been reported for grouping of CaPV isolates (Lamien et al. 2011b).

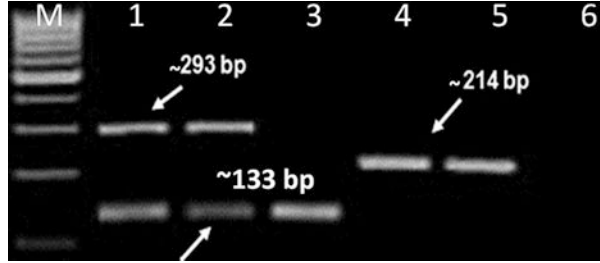

Fig. 9.3 Conventional multiplex PCR showing detection and differentiation of SPPV (293 and 133 bp), GTPV (only 133 bp), and ORFV (214 bp) in a single tube format

4. *Conventional mPCR*—A duplex PCR has been reported for simultaneous detection and differentiation of SPPV and GTPV (Fulzele et al. 2006; Zhao et al. 2017). A multiplex PCR for detection of SPPV and the differentiation of vaccine and field strains has also been reported earlier (Chibssa et al. 2018). Also, two sets of specific primers targeting different genes have been used in past for the same purpose (Zheng et al. 2007; Venkatesan et al. 2014a). RPO30 gene PCR has been developed for differentiation of SPPV and GTPV based on a 21-nucleotide deletion (Lamien et al. 2011a). The conventional mPCR which can amplify two fragments in case of SPPV (293 bp and 133 bp), whereas only one fragment in GTPV (133 bp) and ORFV (214 bp) is shown as Fig. 9.3.
5. *PCR-RFLP for differentiation of SPPV and GTPV*—Significant gene variations among different CaPV members identified by sequence analysis helped to develop a PCR-RFLP strategy in genotyping of SPPV and GTPV. Such a molecular tool using one or two specific restriction enzyme/s has been reported (Hosamani et al. 2004a; Venkatesan et al. 2012a). PCR amplification of P32 region of SPPV and GTPV isolates yield 1027 and 1024 bp amplicons, respectively, and RE digestion of PCR products yielding three fragments (300, 327, 400 bp in SPPV) and two fragments (327 and 697 bp in GTPV) has been reported. In addition, a PCR-RFLP targeting attachment gene PCR product (192 bp) using EcoRI restriction enzyme has been reported that can differentiate SPPV and GTPV upon digestion yielding two fragments of 129 bp and 63 bp for SPPV and whereas, it is single, i.e. 192 bp (Venkatesan et al. 2012a). In the same direction, the genomic RFLP methods using RE enzymes for differentiation of parapoxvirus strains and analysis of heterogeneity for groping of ORFV isolates (Robinson et al. 1982) have been reported.
6. *Isothermal amplification assays*—Among the different isothermal amplification assays, loop-mediated isothermal amplification (LAMP) provides a potential “ASSURED” policy for a diagnostic test to be deployable at field diagnostic settings. The LAMP could produce a highly specific and sensitive reaction (Notomi et al. 2000) which could be performed at a single temperature using a simple heating block and can be developed as POCT (point of care testing) in resource limited field settings (Venkatesan et al. 2015; 2016). LAMP assays targeting P32 (Murray et al. 2013), Poly (A) polymerase (Das et al. 2012), DNA polymerase genes (Venkatesan et al. 2015) of CaPVs have been reported for rapid detection of CaPV and also to differentiate them (Zhao et al. 2014).

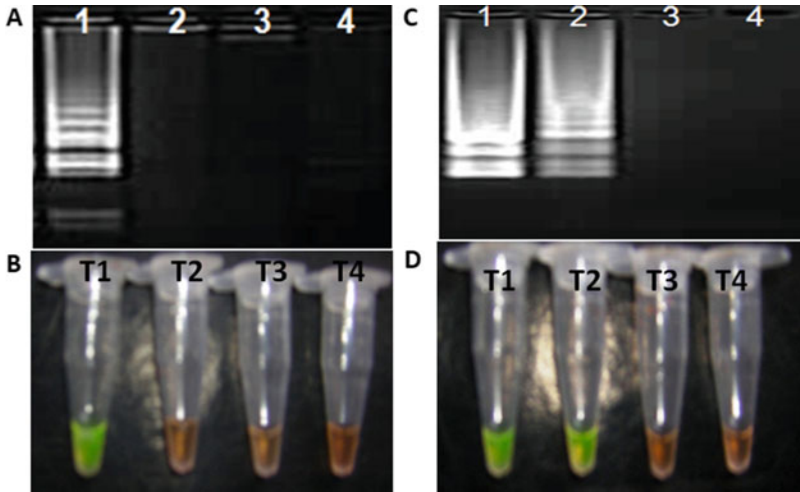

Fig. 9.4 DNA polymerase gene-based LAMP assays for simple and rapid detection of ORFV and CaPV gDNAs [Typical ladder-like pattern in agarose gel analysis and SYBR green I dye-based visual detection of ORFV DNAs (**a** and **b**) and CaPV DNAs (**c** and **d**) by respective LAMP assays] Panel (**a**) and (**b**): Lane 1: ORFV Mukteswar 59/05; Lane 2-3: Negative controls; Lane 4: No template control (NTC). Panel (**c**) and (**d**): Lane 1: SPPV-Srinagar; Lane 2: GTPV Uttarkashi; Lane 3: ORFV DNA, and Lane 4: NTC

These LAMP assays were specific and sensitive comparable to quantitative PCR assays. Similarly, LAMP assays targeting B2L gene (Tsai et al. 2009) and DNA polymerase gene (Li et al. 2013; Venkatesan et al. 2016) have been successfully done to detect ORFV. In similar line to LAMP, another isothermal amplification assay known as recombinase polymerase assay (RPA) using fluorescent probe was reported for rapid detection of ORFV (Yang et al. 2015). DNA polymerase gene-based LAMP assays for simple and fast identification of CaPV by characteristic ladder-like pattern in AGE (Fig. 9.4a) and visual detection using SYBR Green I dye (Fig. 9.4b) and the same pattern of identification for ORFV gDNAs (Fig. 9.4c,d) have been shown.

9.7 Prevention and Control

Active immunization of susceptible hosts against these infections is the best possible economical way of disease control in endemic regions (Madhavan et al. 2016). Vaccines used are either live or inactivated strains of SPPV or GTPV and protect homologous hosts with limited cross-protection potential (Hosamani et al. 2004b). For optimum protection, homologous vaccines are recommended using available indigenous strains (Rao and Bandyopadhyay 2000; Bhanuprakash et al.

2011). Although both inactivated and attenuated vaccines have been used for control of sheeppox, goatpox, and orf infections, live attenuated vaccines are preferred due to its long-lasting immunity.

(i) Live attenuated vaccines—In Africa and the Middle East, Kenyan sheep and goatpox (KSG) O-240 and RM65 strains are commonly used for control of capripox in sheep and goats with satisfactory results (Bhanuprakash et al. 2006, 2011). In India, attenuated SPPV strain (Romanian Fanar) is widely used to control sheeppox (Yogisharadhya et al. 2011). Another indigenous Ranipet strain of SPPV attenuated using ovine thyroid cells and lamb testes cells has been used in Tamil Nadu (Bhanuprakash et al. 2004). Recently, a new indigenous sheeppox vaccine using Srinagar strain of SPPV attenuated in Vero cells is found to be safer and more potent than RF and Ranipet strains (Yogisharadhya et al. 2011; Bhanuprakash et al. 2011). Similarly, a Vero cell-based homologous vaccine for goatpox, developed at IVRI, Mukteswar, is safe and potent inducing a protective immune response in goats on single immunization (Hosamani et al. 2004b). Till date, a vaccine that can confer solid immunity against ORFV infection is not reported. Vaccines derived from sheep ORFV strains may be less efficient to protect the infection in goats or *vice versa* (Musser et al. 2008). Till now, scarification is followed as the preventive measure in many countries to provide immunity. In India, live orf vaccine (Mukteswar 59/05 strain) using PLT cells has been reported as safe, efficacious, and potent in sheep and goats (Hosamani et al. 2009; Bhanuprakash et al. 2011). DNA vaccine expressing ORFV 011 and 059 chimeric proteins has been reported showing great improvement in immunogenicity and potency of vaccination. Therefore, ORF 011 encoding B2L protein can be an appropriate candidate for subunit vaccine development (Zhao et al. 2011).

(ii) Live attenuated combined vaccines—Vaccines using SPPV-RF and GTPV-Uttarkashi strains combined with Peste des petits ruminants (PPRV) Sungri/96 (Hosamani et al. 2006; Chaudhary et al. 2009) have been reported for simultaneous protection against Capripox and PPR in India (Bhanuprakash et al. 2011) as single shot vaccination strategy.

(iii) Recombinant vaccines—Capripox vectored PPR vaccines targeting immunogenic fusion or hemagglutinin genes have been reported as safe and potent against both capripox and PPR (Berhe et al. 2003; Chen et al. 2010). Currently, there are no vaccines that can differentiate infected from vaccinated animals (DIVA) available. For DIVA strategy, a non-essential immunogenic gene needs to be identified and targeted for development of a companion diagnostic test.

9.8 Conclusions and Future Perspectives

SGP and orf pose a socio-economic threat to small ruminants in enzootic countries like India. Possibility of future expansion of their geographical range into naive places due to animal trade is much possible. Mixed or co-infections that may enhance the severity of existing infection are common worldwide and cannot be ruled out during control program strategy. In developing countries like India, occurrence of these mixed type infections in sheep and goats is usually missed or un-diagnosed and no precise diagnostic approach(s) is available to identify them as SPPV or GTPV or ORFV or mixed viruses. In such a condition, early and rapid diagnosis of these targets using PCR as multiplex format in single-tube reaction will be handful (Venkatesan et al. 2014a) during implementation of control program. In addition to prompt and unequivocal diagnosis, a well-organized vaccination and effective bio-security measures are main control elements to contain the disease. Effective live attenuated vaccines, restriction of animal movement, properly implemented mass vaccination programs along with field-deployable diagnostic tests like LAMP assays in closed-tube format are necessary for control strategy. The routinely used conventional serological tests may not be sensitive and specific enough to detect vaccine or disease mounted immune response. Therefore, high-throughput ELISA with improved diagnostic performance is need of the hour. In case of capripox, identification of CaPV isolate(s) that are infective for both sheep and goats may be handful for developing single vaccine for both the species. In future, there is a need to develop DIVA compatible vaccine along with companion test and other molecular epidemiological tools to differentiate SPPV, GTPV, and ORFV in a robust and high throughput manner. Further, genetic characterization of virulence genes of ORFV isolates circulating in a geographical range will help in establishing molecular epidemiology and unravelling the immune evasion mechanisms of ORFV in target species.

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Conflict of Interest Authors declare that there is no conflict of interest.

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