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Review Article
Capripoxviruses of Small Ruminants: Current Updates and Future Perspectives

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Abstract

Sheeppox and goatpox collectively known as capripox are systemic infectious diseases of small ruminants affecting agricultural economy and livelihood of the farmers in endemic regions. Capripox diseases are notifiable to Office International des Epizooties and enzootic in many parts of Africa, Middle East and Asia including Indian sub-continent along with possibility of incursion into Europe from Middle-East regions as transportation of infected animals. They cause high morbidity (75-100%) and mortality (50-100%) in affected sheep and goats depending on virulence of strains, host immunity, breed and environmental factors. They incur severe economic losses to livestock industry affecting small and marginal farmers of developing world. In enzootic countries like India, prompt and unequivocal diagnosis along with well-organized vaccination and effective bio-security are main control measures to contain the disease. It is necessary to update knowledge on sheeppox and goatpox diseases of small ruminants and recent developments in diagnostics and vaccines including recombinant DNA approaches needed to control them. In future, there is a need to develop DIVA vaccines with companion serological assay along with molecular epidemiology tools such as differentiation of sheeppox and goatpox along with development of robust high-throughput diagnostics. In this study, the current geographical distribution, economic impact, epidemiology, updates on available diagnostics and vaccines to control capripox were reviewed along with future perspectives.

Key words: Sheeppox, goatpox, small ruminants, enzootic, epidemiology, diagnostics, vaccines, control, eradication

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INTRODUCTION

Sheeppox and goatpox are OIE notifiable and economically important transboundary diseases of sheep and goats, respectively. The causative agents, sheeppox virus (SPPV) and goatpox virus (GTPV) are members of the genus *Capripoxvirus* of the family Poxviridae along with Lumpy Skin Disease Virus (LSDV) of cattle, the other member of the genus[1]. Clinically, these infections are characterized by generalized pox lesions throughout the skin and mucous membranes, persistent fever, lymphadenitis and often a focal viral pneumonia. Sheeppox and goatpox are enzootic in Africa, particularly to the North and West of the Sahara, in Middle and Far-East and in Indian sub-continent (Fig. 1). Outbreaks of sheeppox and goatpox occur frequently in India incurring economic losses to the sheep and goat industry with average morbidity and mortality rates of 63.5 and 49.5%, respectively[2]. These diseases pose economic impact directly by causing high morbidity and mortality and indirectly by reduced reproductive performance and quality of wool and meat posing non-tariff barriers on international trade[3]. Capripoxviruses (CaPVs), although indistinguishable by serological means, can be differentiated by molecular tools such as restriction endonuclease analysis[4] (REA) and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)[5]. The majority of SPPV and GTPV strains show a host preference with severe disease in heterologous host compared to milder disease in heterologous host, but some strains do cause disease in both sheep and goats[6,7]. In the Indian sub-continent, sheeppox and goatpox appear to be caused by separate viruses and no single virus that can cause disease in both sheep and goats in a mixed flock under natural conditions has thus far been identified[5]. Therefore, it may not be justified to refer sheeppox and goatpox as a single disease. Although, clinical signs are indicative of the disease but a laboratory confirmation is necessary for unequivocal diagnosis and studying epidemiology due to cross-infectivity[8]. Prophylaxis using live attenuated vaccines is the choice of control measure as the immunity is long lasting. This study emphasizes on disease epidemiology, pathogenesis, diagnosis including recombinant protein based techniques, vaccines and control strategies that would help in updating the scientific knowledge on capripox diseases of sheep and goats.

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Goatpox was reported first time by Hansen in 1879 from Norway[9] while sheeppox was originated in Central Asia and spread to many Western countries[10]. In India, goatpox was reported for the first time in India in 1936 from Indian Veterinary Research Institute, Izatnagar (then, Imperial Institute of Veterinary Research). Later goatpox was reported from most of the states which includes Haryana[11], Uttar Pradesh[12], Orissa[13], Madhya Pradesh[14,15], Tamil Nadu[16] and Maharashtra[17]. An outbreak of sheeppox was first reported in Tamil Nadu, then from Bombay[18] and Mysore[19]. Sheeppox has been reported from almost all the states of the country[7,20-27]. Both sheeppox and goatpox are reported in all the states and there are no confirmed reports of capripox infections in North-Eastern region of India so far (Fig. 2). Goatpox has also been reported as mixed infection with PPR or orf

Fig. 1: Global distribution of sheeppox and goatpox (Grey areas)

Fig. 2: Map showing geographic distribution of sheeppox and goatpox in Indian states (Grey areas)

simultaneously or by co-infection in goats was reported[15,28,29]. Globally, the geographical range of sheeppox and goatpox includes Africa above the equator, Middle East, Turkey, Iran, Iraq and most Indian sub-continent including China and occasional outbreaks occur in regions of Europe surrounding the Middle-East like Turkey, Greece and Bulgaria[2]. The most recent outbreaks occurred in Vietnam in 2005, Azerbaijan in 2009, Turkey, Bulgaria and Mongolia[30] in 2008 and 2009 and Morocco[31] in 2014.

ECONOMIC IMPACT

A CaPV disease outbreak would potentially cause considerable economic losses due to disruption of trade in livestock and livestock products, as well as costs associated with disease control and eradication[32,33]. They are associated with significant production losses due to high morbidity, decrease in weight gain, abortion, damage to wool and hides, high contagiousness and high mortality rate[34]. In India, annual losses in Maharashtra state alone was estimated as Rs. 107.5 million due to CaPV infections with average mortality and morbidity and extrapolated annual loss in the country due to capripox in sheep and goats will be INR 1250 million[35]. It was calculated that it took 6 years for a flock in India to recover from an outbreak of sheeppox with 49.5% mortality rate[35]. In countries for which CaPV is exotic, the economic costs would be substantial due to trade restrictions and comparable to foot and mouth disease outbreaks[33,36]. Economic analysis for losses arising out of sheeppox have indicated that some variables such as number of adult animals affected, number of days of illness and flock size are significantly influencing the losses[37]. New outbreaks of sheeppox, goatpox and lumpy skin disease (LSD) in previously free regions are immediately notifiable diseases under OIE guidelines. In addition, CaPVs are listed by United States Department of Agriculture as potential animal biological welfare agents[34].

ETIOLOGY AND GENOMIC ORGANIZATION

Capripoxvirus (CaPV) genus belongs to sub-family Chordopoxvirinae and family Poxviridae. It comprises of sheeppox virus (SPPV), goatpox virus (GTPV), lumpy skin disease virus (LSDV) which cause disease in sheep, goats and cattle, respectively[38]. These viruses are considered as reportable agents to World Organization for Animal Health (OIE) due to their potential for significant economic impact on livestock industry.

Virions are brick shaped, enveloped with complex symmetry and about 300×270×200 nm in size. Double stranded genomic DNA is about 154 kbp size[1] with less variable central region bounded by two identical Inverted Terminal Repeats (ITR) at the ends[39-41]. Within subfamily Chordopoxvirinae, CaPVs have highest A-T content i.e., 73-75%. An extensive DNA cross hybridization between species of the genus is reported[42]. Viral genome shares 147 putative genes which encode proteins of 53-2,027 amino acids in size likely involved in replication, structure, virulence and host range functions. The SPPV, GTPV and LSDV exhibit 96% nucleotide and amino acid identity over their entire length[40,43] and 9 LSDV genes with probable virulence and host range

functions are disrupted in SPPV and GTPV. Both SPPV and GTPV are likely derived from LSDV like ancestor but they possess specific nucleotide differences suggesting that both are phylogenetically distinct. The coding region of CaPV genome has 1-156 ORFs in which central ORFs (024-123) are conserved genes involved in replication and transcription mechanisms[44] whereas, the terminal ORFs (001-023 and 124-156) are variable in nature involving in host immune evasion and host-range functions[39]. Recent hypothesis suggests that GTPV and LSDV are more closely related to each other than to SPPV and they are emerged from a common ancestor close to SPPV based on the phylogenetic studies on different genomic segments[5,45,46]. Moreover, GTPV and LSDV lineages show more diversity than SPPV due to split from common ancestor and differences in selection pressure experienced in different host species[46].

PHYSIOCHEMICAL PROPERTIES

CaPVs are generally resistant to drying and freezing-thawing. Sensitivity of heat differs between isolates[8]. Infectivity of virus is sensitive to ether, trypsin, chloroform and formalin[47,48]. Virus can be destroyed at 56°C for 2 h or 65°C for 30 min but remains viable in wool for 3 months. High alkaline and acidic pH is detrimental to SPPV. Infectivity of virus gets affected by repeated freezing and thawing[49].

EPIZOOTIOLOGY AND TRANSMISSION

Both SPPV and GTPV are considered as host specific. But, some strains affect heterologous hosts[7,48]. Goatpox and sheeppox affect goats and sheep of all ages, both sexes and all breeds, but are more common and severe in young and old animals. Young and exotic animals are more susceptible than adult and indigenous breed[48], respectively. European breeds are more susceptible to infection with Capripoxviruses[50]. But in India an outbreak has been reported among indigenous breeds with high mortality and morbidity than exotic breeds[26]. Virus transmission can occur through infected aerosols due to nasal discharge, conjunctival secretions, saliva, urine and faeces from infected animals and direct abraded skin contact or indirectly through mechanical transmission by vectors[51]. The extensive involvement of skin and associated viral load can result in mechanical transmission by insects[32,52]. *Stomoxys calcitrans* is capable of transmitting sheeppox and goatpox mechanically under experimental conditions[53]. Animals are most contagious before neutralizing antibodies develop, which occurs approximately a week after the onset of clinical signs. Virus can persist on the wool or hair for as long as three months after infection and possibly longer in scabs[52]. There is no carrier stage in infected animals[2,48]. In a susceptible flock, morbidity is 75-100% and mortality is 10-58% depending on the virulence of the isolate[48] and can reach up to 100% in naïve animals. Global climate change could make impact on further spread of these diseases into naive geographic regions. The spread of CaPVs into new areas is predominantly associated with the increase of illegal animal movement through trade[54] as well as inadequate or breakdown of veterinary services[55].

HOST SPECIFICITY

CaPVs are generally considered to be host specific[56]. Some strains found to infect both sheep and goats while, most SPPV and GTPV isolates show distinct host preferences with more severe disease evident in the homologous host[6,48,57]. Usually, nomenclature of SPPV, GTPV and LSDV is based on animal species from which the virus was first isolated but cross-species transmission may complicate the situation. Sheeppox and goatpox are impossible to be distinguished in physical and serological assays due to their structural and antigenic relatedness but possible at molecular level targeting host range genes, virulence genes and immunodominant genes[5,46,58,59]. Some African[60] and Middle-Eastern[61] strains have equal pathogenicity for sheep and goat. Presence of SPPV signature residue of P32 gene in goatpox virus infecting goats in India is seen[7]. The GTPV associated with disease in sheep was confirmed in China by P32 and RP030 gene based species-specific PCR methods[58]. Two natural outbreaks of highly species specific SPPV and GTPV occurred in Mongolia in 2006 and 2008 and the causative strains were different from previously archived GTPV outbreak strain of 1967. The SPPV field strains from Mongolia were identical to previously published P32 gene sequences of SPPV, including strains from India[30]. The GTPV field strains from Mongolia were identical to GTPV strains from China and a recent outbreak in Vietnam[6]. In a study, CaPV outbreaks in sheep and goats in Ethiopia were solely associated with GTPV[62]. Neither sheeppox nor goatpox has been reported in sheep or goats in South Africa where LSDV is endemic. Surprisingly, there is no evidence for existence of CaPVs in wild ruminants[63]. Both the diseases are not considered as zoonotic agents as there is no clear evidence of these malignant diseases causing infection in humans[64]. However, there are two incidences of goatpox in human reported merely based on clinical signs during goat pox outbreak in India[65] and Sweden[66]. However, these two incidences did not involve any virus isolation and serological response that can prove them to be differentiated from either orf or vaccinia virus infections.

PATHOGENESIS AND CLINICAL SIGNS

Capripox is acute febrile and highly contagious transboundary viral disease of sheep and goats[3,67]. In natural cases, the diseases have an incubation period of 1-2 weeks with mild to severe clinical signs in infected sheep and goats depending on breed of host and strains of *Capripoxvirus*[68]. Initial clinical signs include high fever, coughing, salivation, edema of eyelids, diarrhoea, emaciation, arched back followed by progressive development of erythematous macules, vesicles, papules, pustules and scabs on skin with 50% of the skin surface involved. Lesions may also develop on mucous membrane and on internal organs. However, the lesions are restricted to a few nodules under the tail and are thus only detected on close examination particularly in enzootic countries like India[48].

The SPPV and GTPV have tropism for skin, lung and discrete sites within mucosal surfaces of oro-nasal tissues and gastrointestinal tract with lesser extent, lymphoid tissue. Tropism of SPPV and GTPV for the skin as well as minor involvement of liver and spleen suggests that pathogenesis of capripox is closely resemble smallpox and monkeypox[3,69-71]. The SPPV and GTPV disseminate from primary lesion to draining lymph node and then to systemic circulation with localization to skin and other tissues by infected monocytes and macrophages[72]. Cell-associated viremia starts at the time of lesion occurrence and lasts until the time of sero-conversion when host antibodies can neutralize the virus[57]. Virus shedding from mucosal surface for up to 3-6 weeks is reported after the onset of fever[3]. The disease pathogenesis is associated with both viral and host factors and the control of virus replication by the host likely determines the clinical outcome[73].

The animal may recover in 3-4 weeks, with permanent depressed scars unless secondary bacterial infections including pneumonia. Post-mortem lesions include tracheal congestion, bullet-shaped nodules in lungs and increased quantity of blood tinged pleural fluid. Microscopically, epidermal changes include hyperkeratosis, ballooning and degeneration of proliferating epithelial cells and appearance of "Sheeppox" cells[48]. There is congestion, red hepatization, coagulative necrosis surrounded by a zone of inflammatory reaction and thickening of the interlobular septae in lungs[8].

IMMUNITY

Unique characteristic of poxvirus that most progeny virus remains inside the infected cells with release of few virions leads to low amount of extracellular virus[74]. Circulating antibody derived through natural infection or vaccination limits spread of virus in the animals but it does not prevent replication at the site of infection[32]. Local cell to cell spread of infection effectively protects the virus from circulating antibodies. Poxvirus infection or vaccination induce strong or long lasting immunity through involvement of both humoral and cell mediated immunity[75]. Cellular counterpart is the most remarkable feature of pox viral immunity[76] although humoral immunity also plays a role[77]. The level of neutralizing antibodies does not correlate to immune status of a previously infected or vaccinated animal[77]. Animals with mild disease or after vaccination may develop only low levels of neutralizing antibodies. Animals vaccinated at the age of 6 months were immune to challenge even after 52 months of vaccination[78] although further studies are needed to investigate the long-term persistence of CaPV antibodies post-vaccination. There is no stage of viral persistence following exposure in sheep and goats[48,63]. Vaccinated or recovered animals are immune to exposure of virulent virus and considered to be life-long against invading virus from field outbreaks[2]. Passively transferred colostral or maternal antibodies protect against generalized infection[48].

DIAGNOSIS OF CAPRIPOX

Diagnosis of capripox is done clinically on basis of signs and lesions, host species affected and post-mortem findings as described earlier. However, it should be differentiated from contagious ecthyma, bluetongue, foot and mouth disease, dermatophilosis/streptothricosis, mange (e.g., psoroptic mange/sheep scab), photosensitization or urticaria, peste des petits ruminants, parasitic pneumonia, multiple insect bites and caseous lymphadenitis[8,38] as they cause similar kind of skin lesions in affected hosts. Therefore, laboratory confirmation using conventional including antigen/antibody based tests and molecular diagnostics is necessary to conclude the outbreaks.

Virus isolation: Skin papule, lung lesion and lymph node are the preferable material for virus isolation and antigen detection and are to be sampled on 1st week of occurrence of clinical signs prior to the development of neutralizing antibodies[8]. Blood and buffy coat are ideal source of isolation during viremia stage or before or within 4 days of generalization[48]. The Primary Lamb Kidney (PLK) and Primary Lamb Testis (PLT) are the most commonly used cells for primary isolation/adaptation of CaPVs[79,80]. Virus can also be grown in established cell lines such as Vero cells[81,82], MDBK cells[83,84] and OA3Ts cell lines[85]. Both SPPV and GTPV will

produce similar cytopathic effects like ballooning, high refractility, rounding, intra-cytoplasmic inclusion bodies, chromatin fragmentation, detachment and plaque formation[47]. Electron microscopy can be used to identify capripox virion in skin lesions but to be distinguished from orthopox viruses by immunostaining[86]. Different CaPVs vary in their growth on chorio-allantoic membrane. Laboratory animals do not support growth of CaPVs[2,48].

Antigen detection assays: Mostly antigen detection assays are based on polyclonal antibodies to detect soluble antigens of CaPVs. Agar gel precipitation test (AGPT)[87], counter-immuno electrophoresis (CIE)[88] and immuno-capture ELISA[89] based on hyperimmune serum against whole virus[90] or recombinant protein[91] were introduced for the detection of capripox virus antigen. Agar gel immuno-diffusion and fluorescent antibody test are difficult to interpret because of the presence of common antigen between capripox and parapoxviruses[92] and lack monoclonal specific antibody against *Capripoxvirus* specific antigen. An immunohistochemical detection of sheeppox virus antigen in naturally infected lamb tissues was also established[93].

Antibody detection assays: Serology can identify GTPV and SPPV as Capripoxviruses, but cannot distinguish these two viruses from each other. There are several methods available to detect poxvirus induced antibodies[8]. Serological tests include CIE[88], virus neutralization, AGPT, latex agglutination test[94], indirect fluorescent antibody test (IFA), ELISA[95] and immunoblotting[96]. Virus neutralization is the most specific serological test, but it is not sensitive. However, it is used to study the antigenic relationship between SPPV, GTPV and LSDV and assess the post-vaccinal immune status[48]. The AGID and indirect immunofluorescence test are less specific due to cross-reactivity with antibody to other poxviruses. Western blotting using the reaction between the P32 antigen of CaPVs with test sera is both sensitive and specific, but is expensive and difficult to carry out. The ELISA is widely used to detect antibodies which are more sensitive than neutralization test[97] as neutralizing antibody titers are usually low in pox virus infection. So, it can be used in monitoring the immune response of immunized animals and diseased animals[98]. Avidin-biotin ELISA and whole antigen based indirect ELISA have been developed to detect antibodies in infected animals[2,99]. Sucrose gradient-purified inactivated SPPV antigen based I-ELISA has been reported for detection of antibodies to SPPV, GTPV and LSDV[100]. To enhance the specificity, indirect ELISA based on recombinant P32 antigen produced in *E. coli* cells[50] and yeast[101] had also been reported. Western blot analysis based on the antibody response to P32 antigen of CaPVs to differentiate them from ORFV was established[96]. Development of monoclonal antibody based diagnostics would pave the way for specific and sensitive detection of Capripoxviruses in future.

Nucleic acid detection methods: Differentiation from similar diseases is necessary for disease surveillance and eradication but it is not possible by conventional virological or biochemical or serological methods. Several molecular techniques have been developed in recent past that are capable for differentiating Capripoxviruses in highly specific and sensitive manner[28,29]. The PCR based diagnostic techniques and genome sequencing has provided sensitive and powerful technique for the identification of infection. The PCR tests have been developed for the detection of CaPV[5,50,67,102-107]. A duplex PCR was reported for simultaneous detection and differentiation of *Capripoxvirus* from mixed infection using A29L gene of *Capripoxvirus*[106]. A highly sensitive multiplex PCR targeting DNA binding phosphoprotein (I3L) coding gene of CaPV and DNA polymerase (E9L) gene of ORFV has been developed for detection and differentiation of sheeppox, goatpox and orf viruses from clinical samples[28]. In addition to the above, full length[5] and partial P32[108] gene based PCR-RFLP, attachment and fusion gene specific duplex PCR[109], G-protein coupled receptor binding gene based real-time PCR[110] and genomic sequencing[40] are capable of differentiating the Capripoxviruses. The real-time PCR assay provides a rapid sensitive, reliable and robust high-throughput test for CaPV than conventional PCR[111,112]. It can detect CaPVs prior to onset of clinical disease and is comparable to established method of virus isolation for preclinical detection of CaPV[113]. The TaqMan based real-time duplex PCR was developed for simultaneous detection, differentiation and quantitation of *Capripoxvirus* genomes in mixed infection targeting the highly conserved DNA polymerase gene of viral genome[29]. Real time PCR assay using dual hybridization process for genotyping of CaPVs has been developed[110]. Loop-mediated isothermal amplification (LAMP) assay is simple to use, inexpensive, highly sensitive and is well suited for the diagnosis of capripox in less well equipped laboratories and in rural settings where resources are limited[114]. The LAMP assays targeting different structural genes like P32[115,116] and non-structural genes like Poly (A) polymerase[117], DNA polymerase[118] of CaPVs have been optimized and evaluated for rapid, specific and sensitive detection of members of CaPVs and to differentiate SPPV and GTPV[119]. Performance of these LAMP assays were comparable to highly sensitive PCR/quantitative PCR assays with no

apparent cross reactivity with other related viruses. A new kind of real-time PCR assay which is cost-effective and cross platform compatible is useful for genotyping of CaPVs using snapback primers and DNA intercalating eva green dye[120]. Whole genome/gene(s) sequencing and analysis of CaPVs have shown distinct variations at both nucleotide and amino acid levels that can be exploited to genotype these species[5,40,46,121]. An automated mobile PCR system including DNA extraction followed by an established real-time PCR had been evaluated for rapid detection of CaPV DNA in endemic field settings[122]. These molecular tools and techniques can be handful for effective clinical surveillance of CaPV in endemic or disease free regions.

RECOMBINANT DNA TECHNOLOGY IN DIAGNOSTICS

The use of recombinant DNA (rDNA) technology in animal disease diagnosis has revolutionized the rapidity and sensitivity of various diagnostic assays. Use of rDNA technology for cloning and subsequent expression of specific gene of interest of particular virus in an appropriate expression system will circumvent the difficulties associated with the production of large quantities of diagnostic antigens/agents[123]. Recombinant antigen based diagnostics for CaPVs are under development in the recent past but the difficulties in finding out a single immuno-dominant CaPV antigen, expressing it in a heterologous host system and its purification hampers its usage as a diagnostic antigen in large scale screening for sero-monitoring and outbreak management. Attempts have been made at using whole inactivated CaPV as an ELISA antigen; however, this was found to be impractical in a routine diagnostic laboratory setup as virus cultivation is too labour intensive and requires bio-containment facilities. It would be a significant advance if the immuno-dominant CaPV antigens were identified and utilized in an ELISA format, with test sensitivity and specificity at least comparable to those of the virus neutralization assay. Several capripox proteins with diagnostic potential have been expressed in prokaryotic and eukaryotic systems (Table 1). Capripox structural protein, P32, expressed as fusion protein in prokaryotic[50,97,124] and eukaryotic system[101,125] has been used as trapping antigen in indirect ELISA. It has also been employed in latex agglutination test[126]. However, problems associated with expression level of the full-length P32 antigen in *E. coli* due to toxicity of the expressed hydrophobic product and instability of expressed protein[50] are considered as limitations in development of P32 based commercial ELISA for sero-evaluation. Synthetic P32 peptide based indirect ELISA has been developed for detecting SPPV and GTPV antibodies in animals[127]. Another CaPV protein, ORF117 is a fusion protein present mainly on intra-cellular mature virion (IMV) is homolog of vaccinia virus A27L protein and mediates the virus interaction with cell surface heparan sulphate[128]. Further, ORF 117 protein had

Table 1: List of recombinant CaPV proteins tested for their diagnostic potential

Proteins	Functions	Expression systems	Uses	Comments	References
P32	Intracellular mature virion protein involved in viral attachment	Prokaryotic	Indirect ELISA	Developed for detection of antibodies to LSDV in bovines	Carn *et al.*[97]
		Prokaryotic	Indirect ELISA	Developed for detection of antibodies to sheeppox and goatpox but toxic due to hydrophobicity of expressed protein	Heine *et al.*[50] and Zhang *et al.*[124]
		Prokaryotic	LAT	Developed for detection of antibodies to capripox	Muinamia *et al.*[126]
		Eukaryotic	Indirect ELISA	Developed for detection of antibodies to sheeppox and goatpox viruses	Bhanot *et al.*[101] and Chen *et al.*[125]
		Peptide	Indirect ELISA	Developed for detection of antibodies to sheeppox and goatpox viruses	Tian *et al.*[127]
ORF 117	Intracellular mature virion protein involved in viral fusion	Prokaryotic	Indirect ELISA	Protein is available in high level in native soluble form but less sensitive for detection of CaPV specific antibodies in vaccinated animals	Dashprakash *et al.*[129]
ORF 095	Virion core protein	Prokaryotic	Indirect ELISA	For detection of CaPV specific antibodies in infected animals but unable to detect antibodies in sheep or goats following vaccination	Bowden *et al.*[130]
ORF 103	Virion core protein	Prokaryotic	Indirect ELISA	For detection of sheeppox and goatpox specific antibodies in infected animals but unable to detect antibodies in sheep or goats following vaccination	Bowden *et al.*[130]

been expressed in prokaryotic system and evaluated for its diagnostic potential[129] in recent past. Though, the protein was expressed at high level in native soluble form, A27L based ELISA was less sensitive for detection of CaPV specific antibodies in vaccinated animals[129]. Further, sheeppox and goatpox viruses induce poor sero-conversion following vaccination or infection in target hosts and regular conventional serological tests may not be sensitive enough to detect specific antibodies following vaccination or infection[2,100]. A cluster of other open reading frames like 095 and 103 of CaPV are tested and expressed in prokaryotic system for exploiting diagnostic potential in ELISA[130]. Sequencing of more CaPV genomes would help to predict other immunogenic proteins that elicit neutralizing antibodies and would be used for development of improved diagnostics.

VACCINES

Exposure to SPPV and GTPV results in strong and long lasting immunity against reinfection. In India and other endemic developing countries, vaccination is considered as an economical and sustainable mean of disease control[78,131]. Most commonly used vaccines against sheeppox and goatpox are attenuated live or inactivated strains of SPPV or GTPV. Cross protection among sheep and goats due to sheeppox and goatpox vaccine or vice versa is partial[78,98]. For optimum protection, homologous vaccine are recommended based on indigenous or locally prevalent strain for either sheeppox or goatpox[8,23]. Inactivated vaccines do not provide adequate and long lasting immunity. However, an inactivated SPPV vaccine would provide a safe and valuable tool to protect livestock against SPPV, particularly during the first incursion of the virus in the previously disease-free country[132]. In this line, an inactivated SPPV vaccine using Roumanian Fanar (RF) strain showed potential to replace attenuated vaccine to control and prevent sheep pox in disease-free or endemic countries[132].

Live vaccine is the better choice for long term immunity against CaPVs[78]. Susceptible animals can be vaccinated by single vaccine through intradermal or subcutaneous route with OIE recommended safe dose of GTPV vaccine ($10^{2.5}$ TCID50)[38]. Heterologous cell systems like bovine fetal muscle cells[32] and baby hamster kidney cells[56,77] have been used for development of attenuated capripox vaccines. Vero cell attenuated GTPV provided complete protection against virulent challenge. Immunity to poxvirus is considered predominantly cell-mediated and the immune status of animals does not correspond to serum neutralizing antibody titers[32]. A single vaccine using 0240 strain of Kenyan sheep and goatpox virus (KSG) which protected both sheep and goat against virulent strains of CaPV[77] has been used in many countries in Middle East and Africa with satisfactory results.

With regard to sheeppox and goatpox vaccines in India, currently SPPV-RF strain attenuated by serial passages in primary lamb testes (PLT) cells is used extensively for sheeppox[133]. Another indigenous strain SPPV-Ranipet which is attenuated in ovine thyroid cells and LT cells is used in Tamil Nadu (India)[134]. A new Vero cell adapted vaccine using indigenous SPPV (Srinagar) strain has been developed and is safe and potent than other vaccine viruses available in India[133]. Recently, an indigenous live attenuated Vero cell based goatpox vaccine has also been developed in India. Vaccine produces durable protective immunity for a period of 52 months[78]. Combined vaccines consisting of Vero cell derived SPPV-RF and Peste des petites ruminants (PPRV)[135] Sungri/96 as well as GTPV-Uttarkashi and PPRV-Sungri/96 strains[136] are available to combat capripox and PPR in India[78].

Capripox vectored recombinant PPR vaccines (rCPV- PPR) using F or H genes have been developed and shown effective protection against both capripox and PPR[137-140]. Currently, no vaccines with a differentiation of infected from vaccinated animals (DIVA)-component are commercially available against CaPVs. Also, RNA interference has been shown to down regulate the expression of ORF 095 which inhibits replication of GTPV on Vero cells[141].

CONTROL AND ERADICATION STRATEGY

Socio-economic and political stability, availability of veterinary services and adequate infrastructure and logistic supports are essential for implementing effective control programs[2]. Capripox free countries maintain their disease free status by restriction on import of livestock and animal products from affected areas. Infected animal products should be decontaminated before entry in to non-enzootic regions. In the case of disease free countries, the implementation of a radical slaughter policy, restricted animal movement coupled with ring vaccination will help in elimination of the disease[32]. Control of capripox is possible by application of appropriate diagnostics, use of effective vaccine(s) and animal management to restrict movement of animal and their products to prevent introduction into naive areas[2]. After specific diagnosis, active mass immunization is the main approach to control capripox infection. Inadequate infrastructure in most of developing countries is one of the major elements that conflict with effective implementation of building herd immunity.

CONCLUSION AND FUTURE PERSPECTIVES

Sheeppox and goatpox are OIE notifiable and transboundary diseases of developing world and there is always a constant threat of spreading of CaPVs into new geographic regions of non-enzootic nature, specifically into South-East Asia and Europe whereas, LSDV into the Middle-East by trade of infected animals and their products such as wool and hides, as well as through the movement of insect vectors. Effective live attenuated vaccines and improved diagnostic tests may facilitate in initiating effective control measures. Well-organized vaccination campaigns, using sufficient coverage and effective vaccines should be monitored by active surveillance especially for the detecting viruses or their antigens by field applicable molecular tests like LAMP assays or lateral flow devices, because serologic tests cannot differentiate between infection and vaccination. As these viruses induce poor sero-conversion following vaccination or infection in target hosts, regular conventional serological tests may not be sensitive enough to detect specific antibodies following vaccination or infection. Therefore, development of high-throughput ELISA with enhanced sensitivity and specificity in the form of multiple immunogenic epitope/antigen(s) based ELISA or competitive ELISA using monoclonal antibodies is required. These diagnostic tools will be used to respond sudden outbreaks, monitoring/surveillance of the diseases and study the epidemiology of the disease in endemic regions. Also, there is need to identify CaPV isolates that are infective for both sheep and goats, so that a single vaccine may be developed to protect both species. Further, it should be studied to estimate actual prevalence and possible risk factors associated with spread and maintenance of these viruses in sheep and goats. It will help in modeling disease patterns and identifying high risk geographical regions for proper implementation of control measures.

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