

## Research Article



# Immunogenic Proteins of Capripox Virus: Potential Applications in Diagnostic/Prophylactic Developments

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**Abstract** | Capripoxvirus (CaPV) infections are highly contagious and OIE notifiable viral diseases of sheep, goats and cattle. They are endemic in most parts of the globe associated with significant production losses due to high morbidity, high mortality rate and animal trade restrictions. Though several diagnostics including molecular tools are available, recombinant protein based diagnostic assays namely ELISA is safer and robust to handle large sample size and also to minimize labor/time. However, the genus *Capripoxvirus* encodes putative 147 proteins in their genome, among which some of them are reported as potential immunogenic candidate genes. Selection and use of such candidate immunogenic proteins from an array of genes located at different structures of a mature virion are the real challenge and time consuming task for researchers. Nevertheless, identification of candidate gene(s) using advanced bioinformatic tools will ease the process and can select the suitable protein(s) to use in the development of specific and sensitive diagnostic assays and also effective vaccine candidates which are vital elements in control and eradication of any infectious disease from an endemic country. In this review, we describe different structures of mature pox virion with reference to vaccinia virus (VACV), list of immunogenic candidate genes presents in CaPV genome and their potential use in diagnostic/vaccine developments.

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## Introduction

Sheeppox (SP) and goatpox (GP) are highly contagious and devastating viral systemic disease of sheep and goats whereas Lumpy skin disease (LSD) is disease of cattle. All three diseases are classified by the OIE as a notifiable disease (OIE, 2016) based on the risk it poses to animal health and the agricultural economy. They are considered economically important transboundary diseases of livestock. The causative agents of SP and GP are sheeppox virus (SPPV) and goatpox virus (GTPV)

that belong to the *Capripoxvirus* genus within the subfamily *Chordopoxvirinae* of the family *Poxviridae* (Madhavan et al., 2016). The other member of this genus is lumpy skin disease virus (LSDV). Both SP and GP are endemic mainly in central and northern Africa, central Asia and parts of China (Bowie et al., 2000; Babiuk et al., 2008; Bowden et al., 2009; Kitching and Carn, 2008). The disease is associated with high morbidity (70–90%) and mortality up to 49.5% (Garner et al., 2000). Young animals show more severe disease, and mortality in kids may be as high as 100% (Rao and Bandyopadhyay, 2000). The disease is

characterized by high fever, generalized pox lesions on the skin and mucous membranes and enlargement of all the superficial lymph nodes. Goatpox is considered as the important constraint in the international trade of animals and their products (Babiuk et al., 2008; Madhavan et al., 2016).

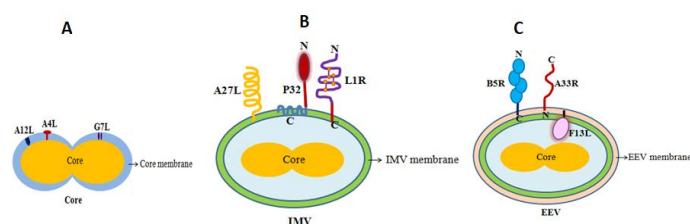
The viral particle with a complex symmetry contains linear double-stranded DNA, ~150 Kbp size. The virion is enveloped and brick shaped with 300×270×200 nm in size (King et al., 2011). These three viruses show 96–97% similarity in nucleotide and amino acid sequence over their entire length (Black et al., 1986; Tulman et al., 2002; Zeng et al., 2014). They cannot be differentiated by serological methods. Diagnosis of capripoxvirus infection is based on detecting the viral antigen or antibody including virus isolation; counter immunoelectrophoresis (CIE), serum neutralization test (SNT) and nucleic acid detection methods including visual detection LAMP assay (Bhanuprakash et al., 2011; Madhavan et al., 2016). The impeccable advancements in gene expression technology have made easier the process of diagnostic/prophylactic antigen production and purification in an efficient manner.

In the past, researchers made attempts to express and purify various CaPV proteins using bacterial, mammalian and yeast cells and assess the potential utility of these expressed antigens in various diagnostic assays including ELISA. However, several problems associated with the right identification and selection of a single immunogenic gene or its combination impedes the process of recombinant ELISA development and its utility. In general, poxviruses typically produce two infectious forms, namely extracellular enveloped virions (EEV) and intracellular mature virions (IMV) each with specific protein composition (Chung et al., 2006). It could be a significant achievement if the immunodominant CaPV proteins were identified from the IMV/EEV/core part of the virus and developed into an ELISA format having comparable diagnostic efficacy to SNT/VNT. Development of such a robust assay is need of the hour for high throughput screening of infected and vaccinated serum samples from target hosts namely sheep, goats and cattle particularly during control and eradication programs. In this mini-review, we discuss the different virion structures associated with encoded immunogenic proteins, varying degrees of host interaction and their potential implications in

diagnostic/prophylactic measures.

### Immunogenic proteins of vaccinia virus

Among poxviruses, Vaccinia virus (VACV) has been studied in depth at both genomic and proteomic levels. VACV encodes about 200 proteins, but only few IMV proteins (A27L, L1R, D8L, H3L and A17L), EEV proteins (B5R, A33R, A34R, A36R and A56R), and core proteins (A4L and A10L) have been shown to be immunogenic (Ramirez et al., 2002). The schematic of putative immunogenic proteins and its location in different infectious structures of the mature virion is shown as Figure 1. Of the two infectious forms, the most potent neutralizing antibodies were found against the IMV form (Ichihashi and Oie, 1996). Experimental evidence shows that at least five of these proteins (H3L, A27L, B5R, D8L and L1R) elicit protective neutralizing antibodies in mice (Rodriguez et al., 1987; Wolffe et al., 1995; Galmiche et al., 1999; Hsiao et al., 1999) and one protein (A33R) induces a protective, but non-neutralizing antibody response (Galmiche et al., 1999). As there is a significant evolutionary distance between CaPVs and orthopoxviruses (OPVs), it is not possible to directly predict whether the CaPV orthologs will also elicit neutralizing antibodies. Therefore, it is necessary to study in detail about an array of VV orthologs present in CaPV for secondary structural characteristics including antigenicity and hydrophilicity by using bioinformatic tools and selection of candidate gene(s) to express and evaluate in immunological assays like ELISA.



**Figure 1:** Schematic representation of membrane structures and associated putative immunogenic proteins in a mature poxvirus (Panel A: Core; Panel B: Intracellular mature virion; Panel C: Extracellular enveloped virion).

### VACV homologs in SPPV and GTPV

CaPV members including GTPV possess ~150 kb dsDNA genome coding for ~147 putative proteins that are likely to be involved in replication, structure, assembly, virulence and host range (Moss, 2001; Zeng et al., 2014). During replication process in hosts, CaPV produces 147 putative proteins of varying size ranges from 53–2027 amino acids in length. Some

of them are immunogenic to produce neutralizing antibodies after infection while some proteins elicit non-neutralizing antibodies though immunogenic in nature. The presence of a large number of proteins in virions presents challenges to the identification of immunomodulatory proteins of CaPV. Targeting of immunogenic proteins is helpful in the development of specific and sensitive diagnostic assays and effective vaccine candidate that helps in control and eradication of the disease from any country (Tuppurainen et al., 2017; Bhanuprakash et al., 2011). During pox virus infection, intracellular mature virion (IMV) and extracellular enveloped virion (EEV) are produced, which are the two major infectious forms of poxviruses. IMVs are assembled in the cytoplasm and are composed of a core particle containing the genome and numerous enveloped enzymes. At least 11 proteins are included in the envelope of IMVs (Franke et al., 1990; Smith et al., 2002). IMV is the major form that accumulates in the cytoplasm; they may be released from the cell by cell lysis or alternatively can be wrapped in two additional trans-Golgi-derived membranes and released from infected cells by budding as EEV. The outer envelope membrane of EEV contains several unique glycosylated and non-glycosylated proteins involved in virus entry. Some of the encoded proteins are reported to be located at the core of the viral genome and partially exposed to host immunity. The outer membrane of these structures harbors large numbers of proteins among them few are immunogenic in nature (Table 1).

#### Immunogenic proteins of core region

**A4L:** ORF 095 gene of CaPV, a vaccinia virus A4L homolog is reported as the highly conserved immunodominant acidic core protein of 39 kDa size synthesized at a later stage of infection. It was found to be highly antigenic in vaccinia virus as demonstrated by immunization of mice with recombinant A4L protein that has mounted strong humoral immune responses (Demkowicz et al., 1992). Multiple sequence alignments of CaPV ORF095 and its corresponding phylogeny did not reveal specific differences among the vaccine and virulent strains of GTPV and SPPV indicating that the A4L may not play a role in virulence or attenuation process. Immunogenicity and diagnostic potential of A4L homolog of CaPV have been evaluated in indirect ELISA for the detection of specific antibodies in infected animals (Bowden et al., 2009). It has also been reported that a siRNA construct targeting GTPV

A4L has shown to inhibit the replication of GTPV in Vero cells (Zhao et al., 2012). A4L homologs have been reported in other pox viruses namely myxoma virus (MYXV) M093L (Van Vliet et al., 2009) and fowlpox virus (FWPV). FWPV A4L homolog is also an immunodominant protein and shows the same localization as VACV A4L (Boulanger et al., 1998) possessing highly charged domains at each end of the protein and multiple copies of a 12-amino-acid serine-rich repeat sequence in the middle of the protein (Boulanger et al., 1998). The predicted CaPV A4L protein characteristics demonstrated that this protein possesses a high antigenic index with good hydrophilicity and surface probability spanning the amino acid residues indicating a potential use of this protein as candidate diagnostic/prophylactic (Madhavan et al., 2016).

**A12L:** The core protein A12L encoded by VACV is ~25 kDa in size and plays a major role in cleavage processing associated with virion morphogenesis and assembly (Yang and Hruby, 2007). CaPV ORF103 encodes VACV A12L homolog has been expressed and evaluated in indirect ELISA along with CaPV A4L for the detection of *Capripoxvirus* specific antibodies in infected animals (Bowden et al., 2009). However, there is still a need to evaluate this core protein homolog for its ideal protein characteristics using an array of bioinformatic tools and apply for diagnostic/prophylactic development.

**G7L:** G7L (~42kDa) is reported as a major core protein of VACV in addition to A4L and A12L expressed late during viral replication and interacts with the A30L protein to stabilize it to play a role in virion morphogenesis (Szajner et al., 2003) similar to A12L homolog. VACV G7L is reported to be highly conserved among all members of the *Chordopoxvirinae* subfamily. SPPV homolog, G7L has been expressed in the prokaryotic system and the immunogenicity has been evaluated (Bowden et al., 2009). Detailed genetic characterization using different GTPV and SPPV isolates and *in-silico* analysis of protein characteristics of G7L are necessary before its selection and application as candidate diagnostic/vaccine antigen.

#### Immunogenic proteins of IMV

**L1R:** L1R is (~25kDa) myristylated protein associated with IMV membrane and produced in late infection. C-terminal of L1R has a hydrophobic transmembrane

**Table 1:** List of immunogenic proteins present at different structures of mature virion and their potential utility/applications.

| Virus structure | Pro-teins | Immunogenicity/diagnostic potential reported                                                                                             |                                                                                                                            | Potential utility                                                                                             |
|-----------------|-----------|------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
|                 |           | VV                                                                                                                                       | CaPV                                                                                                                       |                                                                                                               |
| Core            | G7L       | NA                                                                                                                                       | Bowden et al., 2009                                                                                                        | Potential diagnostic antigen, Individually or combination with other proteins                                 |
|                 | A4L       | Demkowicz et al., 1992                                                                                                                   | Bowden et al., 2009; Chervyakova et al., 2016                                                                              | Good candidate for ELISA , Individually or combination with other proteins                                    |
|                 | A12L      | NA                                                                                                                                       | Bowden et al., 2009                                                                                                        | Potential diagnostic antigen, Individually or combination with other proteins                                 |
| IMV             | A27L      | Rodriguez et al., 1987; Lai et al., 1991; Demkowicz et al., 1992; Berhanu et al., 2008; Rudraraju and Ramsay, 2010; Ramirez et al., 2002 | Chervyakova et al., 2016; Dasprakash et al., 2019                                                                          | Potential diagnostic antigen, Individually or combination with other proteins, anti-A27L serum/MAb production |
|                 | P32/H3L   | Lin et al., 2000; Davies et al., 2005; McCausland et al., 2010                                                                           | Heine et al., 1999; Xiong et al., 2008; Fang et al., 2009; Venkatesan et al., 2018; Chen et al., 2008; Bhanot et al., 2009 | Good candidate for ELISA                                                                                      |
|                 | L1R       | Hooper et al., 2000, 2003, 2004; Fogg et al., 2004; Heraud et al., 2006                                                                  | Chervyakova et al., 2016                                                                                                   | MAb production, Potential diagnostic antigen, Individually or combination with other proteins                 |
| EEV             | A33R      | Galmiche et al., 1999; Fogg et al., 2004; Hooper et al., 2004; Chen et al., 2007                                                         | Chervyakova et al., 2016                                                                                                   | Good candidate for ELISA                                                                                      |
|                 | B5R       | Galmiche et al., 1999; Berhanu et al., 2008; Benhnia et al., 2009; McCausland et al., 2010                                               | Zheng et al., 2009                                                                                                         | Potential diagnostic antigen, Individually or combination with other proteins                                 |
|                 | F13L      | NA                                                                                                                                       | Bowden et al., 2009; Kumar et al., 2019                                                                                    | Potential diagnostic antigen, Individually or combination with other proteins                                 |

**Note:** IMV: Intracellular mature virion; EEV: Extracellular enveloped virion; NA: Not available.

domain (Franke et al., 1990; Ravanello and Hruby, 1994; Wolffe et al., 1995). L1R has a role in the formation of infectious IMV, virus entry and penetration (Wolffe et al., 1995; Ichihashi and Oie, 1996). L1R Protein is another major target of neutralizing antibodies (Wolffe et al., 1995; Ichihashi and Oie, 1996). The L1R protein contains six cysteine amino acid residues that have a role in intramolecular disulphide bond formation (Su et al., 2005). Vaccination of mice with plasmid encoding L1R gene elicit neutralizing antibodies but not shown a protective immune response against the lethal virus. However, mice immunized with DNA encoding L1R with other immunogenic genes (A33R, B5R and A27L) provide sufficient protection against lethal vaccinia virus (Hooper et al., 2000, 2003, 2004; Fogg et al., 2004; Heraud et al., 2006). Sequence analysis of the CaPV L1R gene using different Indian GTPV and SPPV isolates revealed that it is highly conserved and showing more than 99% and >96% identity both at nucleotide and amino acid levels, within species and between species, respectively (Karki et al., 2018).

It has been observed that SPPV L1R expressed in prokaryotic system has induced a potent neutralizing antibody response (Chervyakova et al., 2016) and may be a potent candidate for novel subunit vaccine development (Xiao et al., 2007). The conserved and immunogenic nature of CaPV L1R may prove to be a potential candidate for developing molecular diagnostics including recombinant protein based assays and prophylactics.

**P32/H3L:** Among IMV proteins, P32 protein is a homolog of VACV H3L a most immunodominant protein (~35kDa), present on the surface of IMV of all poxviruses (Lin et al., 2000). The C-terminal domain of H3L has a hydrophobic membrane tail that mediates the insertion of H3L into IMV membranes post-translationally (da Fonseca et al., 2000; Davies et al., 2005). In VACV, H3L has a role in virus attachment to cells by binding to cell surface glycosaminoglycans and also takes part in virion assembly (Lin et al., 2000). It has been reported that recombinant H3L protein induced high titers neutralizing antibodies

in the immunized mice (Davies et al., 2005). In a similar line, the immunogenicity of buffalopox virus H3L has been evaluated and found to be protective in laboratory animals in passive protection studies (Kumar et al., 2016, 2017). MAbs against H3L can protect laboratory animals as passive immunity (McCausland et al., 2010). As a potent diagnostic antigen compared to VACV antigens, H3L was found to be a sensitive and specific candidate for diagnostic ELISA. In the CaPVs, ORF074 coding for P32 is reported as the VACV H3L homolog present in all the members namely SPPV, GTPV and LSDV as a major antigenic determinant (Chand et al., 1994; Tulman et al., 2002). CaPV P32 protein has been expressed in prokaryotic (Heine et al., 1999; Xiong et al., 2008; Fang et al., 2009; Venkatesan et al., 2018) and eukaryotic system (Chen et al., 2008; Bhanot et al., 2009). In the past, P32 protein has been targeted for detecting capripox-specific antibodies employing indirect ELISA (Heine et al., 1999; Venkatesan et al., 2018) and as capripox antigen by immunocapture ELISA (Carn, 1995). However, problems associated with the level of expression, purification and stability in the prokaryotic expression system urge researchers to use eukaryotic expression systems including insect cells to overcome those associated problems.

**A27L:** A27L is a major envelope ~14kDa fusion protein located in the IMV membrane of VACV and present in all other pox viruses (Vazquez et al., 1998). This highly conserved IMV protein directly mediates the virus interaction with cell surface heparan sulphate (Hsiao et al., 1998) and also indirectly through fusion with A17L as a complex and thereby, it plays an important role in virus assembly, morphogenesis and release (Kumar et al., 2015). It has been reported that it possess coiled-coil helical region to form coiled coil trimers (Rodriguez et al., 1987; Sodeik et al., 1995). A27L is the best-characterized and most promising proteins to develop protective immune responses against vaccinia virus. A27L has a role in IMV-cell attachment, virus-to-cell and cell-to-cell fusion and microtubule transport (Chung et al., 2006; Van Vliet et al., 2009). Immunogenicity and protective efficacy of A27L have been evaluated in mice and it induces virus-neutralizing antibodies to protect against lethal VACV challenge (Demkowicz et al., 1992; Berhanu et al., 2008; Rudraraju and Ramsay, 2010). MAb against A27L protein provides passive protection by neutralizing the virus (Ramirez et al., 2002). Immunogenicity, passive protection efficacy and

diagnostic potential of buffalopox virus A27L have been evaluated (Kumar et al., 2015). CaPV A27L has been characterized and found to be conserved in nature (Dasprakash et al., 2015). Antibodies against CaPV A27L protein possess efficient neutralizing activity (Chervyakova et al., 2016). An indirect ELISA using rA27L protein of CaPV has been optimized for detection of antibodies against GTPV in infected animals (Dasprakash et al., 2019) and may prove to be a sero-monitoring and sero-surveillance tool used during different phases of control and eradication programs after thorough validation. Also, A27L could be a suitable candidate antigen for producing polyclonal or monoclonal antibodies to capture test antigen in detection assays (Stern et al., 2016).

### *Immunogenic proteins of EEV*

**A33R:** Among EEV proteins, A33R is the outer envelope type II integral transmembrane glycosylated protein having both N and O glycosylation (~23-28kDa) (Payne, 1992; Roper et al., 1996; Roper et al., 1998). A33R mainly gets localized to the golgi apparatus and helps in EEV formation and is present as a dimer unit. Also, A33R is heavily phosphorylated at serine residues and coordinates the incorporation of A36R into intracellular enveloped virion (IEV) membranes and, subsequently, the production of actin tails (Wolffe et al., 2001). It plays an essential role in efficient cell-to-cell spread of viral particles. VACV A33R protein has been expressed in *E. coli* and insect cells (Fogg et al., 2004) and found to be immunogenic. Vaccination with recombinant A33R protein or DNA provided protection in experimental animals (Hooper et al., 2003; Heraud et al., 2006; Sakhatskyy et al., 2008). Antisera against A33R are protective *in vivo* (Hooper et al., 2004; Chen et al., 2007). As there is a large genetic divergence between OPVs and CaPVs, the A33R gene of CaPV shows very less nucleotide identity between them. Antibodies against CaPV A33R protein possess efficient neutralizing activity (Chervyakova et al., 2016). The potential use of CaPV A33R as a diagnostic antigen in antibody/antigen detection assays is yet to be explored.

**B5R:** In contrast to A33R, the other significant EEV membrane protein B5R (~42kDa) is but type I membrane protein that is glycosylated at N-terminal and palmitylated (Isaacs et al., 1992; Payne, 1992; Smith et al., 2002). This glycosylated protein is highly conserved among many strains of VACV as well as other poxviruses (Engelstad et al., 1993). The

ectodomain of B5R presents four conserved short consensus repeats (SCR) that activate the complement system (Takahashi-Nishimaki et al., 1991; Engelstad et al., 1992). B5R is reported to mount the neutralizing antibodies (Law and Smith, 2001; Hooper et al., 2003) and antisera against B5R possess efficient EEV neutralizing activity (Benhnia et al., 2009). Further, anti-B5R MAbs shown protective efficacy against VACV and other OPXVs like monkeypox and variola virus (Benhnia et al., 2009; McCausland et al., 2010). Immunogenicity of GTPV homolog, B5R has been evaluated in combination with other protective immunogens (Zheng et al., 2009). However, CaPV B5R homolog like A33R needs further study to prove its worth as a diagnostic/prophylactic agent for capripoxvirus infections.

**F13L:** Among EEV membrane proteins of VACV, F13L is the most abundant protein of ~37kDa size with a palmitoylated component (Grosenbach et al., 1997). It is synthesized mainly at the later stage of virus replication and involved in viral envelopment and egress. F13L is reported to have phospholipase activity due to the presence of a conserved HKD motif that belongs to the members of the phospholipase D family. It has also been found to have lipid binding ability (Koonin, 1996; Sung et al., 1997). The F13L gene is a homolog to the Orf Virus (ORFV) B2L gene and the potential utility and immunogenic nature of rB2L of ORFV has been reported (Yogisharadhya et al., 2017). There are reports that ORFV B2L can elicit neutralizing antibodies in mice (Zhao et al., 2011). Further, GTPV F13L has been expressed in prokaryotic system (Bowden et al., 2009; Kumar et al., 2019) and diagnostic potential of F13L homolog of CaPVs have been evaluated in indirect ELISA (Bowden et al., 2009).

## Conclusions and Recommendation

CaPVs cause highly contagious infections in target hosts namely sheep, goats and cattle. Its endemicity in many countries worldwide causes a significant economic loss to the farmers. Recombinant protein based diagnostics including ELISAs are pivotal in control and management of CaPVs in association with an effective vaccine. Also it will be a handful tool in sero-monitoring and sero-surveillance of CaPV in endemic areas following vaccination. ELISA will be much faster, economical and easier to perform compared to VNT or other nucleic acid-based assays.

Due to the complex structure of the virion and presence of a large number of proteins in poxviruses, it is difficult to identify the most suitable candidate protein and use in developing serological assay like ELISA. This selection warrants a thorough screening and analysis from an array of genes of the CaPV genome. Targeting the immunogenic proteins from different structures of CaPV will help in developing high throughput, sensitive and specific diagnostics. Individual or in the combination of more than one recombinant proteins may be imperative for the development of such assays or vaccine that helps in prevention of infectious diseases caused by members of CaPVs in livestock.

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## Authors Contribution

Gnanavel Venkatesan conceptualized, executed and corrected the final version of the review article. Anand Kushwaha and Amit Kumar have written the draft. Aparna Madhavan, Golmei Poulinlu and Durga Goswami have collected the necessary data for this review.

## Conflict of interest

There are no conflicts of interest among the authors

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