# Chapter 7
# Buffalopox Virus


**Amit Kumar, Gnanavel Venkatesan, and Raj Kumar Singh**

**Abstract** Buffalopox is a highly contagious, emerging and re-emerging zoonosis that affects buffaloes with occasional involvement of cows and humans. Since the first recorded incidence of buffalopox infection from undivided India in 1934, several Indian states and other countries of Indian subcontinent have reported regular outbreaks. The aetiological agent, buffalopox virus (BPXV) was later isolated and confirmed as a separate entity from the vaccinia virus or cowpox virus. BPXV is considered as an Indian variant of vaccinia like viruses (VLVs). It shares genetic, biological and serological identity with VACV. In animals, the infection is characterized by localized pock lesions on teats and udder with complications of mastitis and is often associated with high morbidity and productivity losses. Rarely, a generalized form involving several body parts is also observed. Epidemiologically, the disease spreads through milkers with zoonotic involvement. The disease has been declared as important occupational zoonosis by Joint FAO/WHO Expert Committee on Zoonosis. Present-day human population born after discontinuation of smallpox vaccination program lacks antibodies to orthopoxvirus infections like BPXV. Recently, concurrent involvement of buffaloes, cows and human in India and nosocomial infections in burn patients in Pakistan raises serious economic and public health concerns. There is a need for detailed systematic study on viral epidemiology along with whole-genome sequencing of BPXV isolates from different geographical areas and development of rapid, specific and sensitive diagnostics for confirmatory diagnosis of BPXV. Here, epidemiology, clinical aspects of BPXV infection and diagnostic procedures along with control measures are described.

**Keywords** Buffalopox virus · Diagnosis · Epidemiology · Vaccinia-like-virus · Zoonosis

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## 7.1 Prologue

Buffalopox is a contagious emerging and re-emerging zoonosis affecting buffaloes with occasional involvement of cows and humans. The first recorded incidence of buffalopox disease was reported from undivided India (Sharma 1934). In the early 1950s, buffalopox infection in animals was believed to be caused by vaccinia virus (VACV) or cowpox virus (CPXV) because of its similar clinical proximity, giving rise to synonyms like variola, variola-vaccinia, cowpox-variola, etc. to describe the disease. Later, the definitive isolation of the causative agent, buffalopox virus (BPXV), was confirmed as a separate entity from North India (Singh and Singh 1967). Further biological, physicochemical and serological characterization of BPXV revealed it as a distinct entity, most closely related to VACV (Singh and Singh 1967; Kataria and Singh 1970; Baxby and Hill 1971; Lal and Singh 1973). Later, electron microscopic studies were undertaken which also demonstrated the resemblance of BPXV with VACV (Bloch and Lal 1975; Sehgal et al. 1977). To cultivate the virus, researchers used chorioallantoic membrane of chick embryo and several tissue cultures (Lal and Singh 1977). Apart from natural infection, the experimental host range of BPXV in different farm and laboratory animals has been determined (Singh et al. 1996). Since 1934, the zoonotic nature of BPXV has been demonstrated. Some buffalopox outbreaks have been contemporary to vaccinia virus vaccination programs and smallpox outbreaks, while other buffalopox outbreaks continued even after the cessation of the anti-smallpox vaccination program. The origin of BPXV is still obscure, for which several hypotheses have been proposed. 'Vaccine-escape hypothesis' suggests that during the initial production of VACV vaccines in the buffalo-calf skin in India and Indonesia, VACV probably got adapted to buffaloes via several passages to become a separate entity which is now naturally maintained in a transmission cycle involving buffaloes (Hobday et al. 1961). Thus, BPXV is considered as an Indian vaccinia-like virus (VLV). Similar outbreaks caused by other VLVs, namely Cantagalo virus, Aracatuba virus, Guarani virus, etc., have been reported in dairy cows and humans in Brazil (Kroon et al. 2011). The disease is prevalent in many states of India, Pakistan, Nepal, Bangladesh, Egypt, Indonesia, Italy and Russia (Singh et al. 2007b). Buffalopox infection in animals is characterized mainly by localized pock lesions on teats and udder with complications of mastitis and is often associated with high morbidity and productivity losses in terms of decreased milk yield, severe mastitis, hide damage and reduced draught capacity of the affected animals (Bhanuprakash et al. 2010). The disease has been declared as an important occupational zoonosis by joint FAO/WHO Expert Committee on Zoonosis (FAO/WHO 1967). Apart from frequent outbreaks in buffaloes in the Indian subcontinent, simultaneous involvement of cows and human in recent outbreaks is of economic and public health concern. Present-day human population born after cessation of smallpox vaccination program lacks protective antibodies and is, therefore, more susceptible to orthopoxvirus (OPV)

infections like BPXV and other Brazilian VLVs. In this chapter, BPXV has been described with reference to molecular characterization, antigenic properties, epidemiology, host range, pathogenesis, clinical disease, zoonosis, diagnosis and prevention strategies.

## 7.2 Buffalopox Virus

The aetiological agent, BPXV, is classified as a vaccinia-like virus or variant of VACV in genus *Orthopoxvirus* (OPV) of the subfamily *Chordopoxvirinae* in the *Poxviridae* family (King et al. 2012) based on its biological, physicochemical and serological properties (Singh and Singh 1967; Kataria and Singh 1970; Baxby and Hill 1971; Lal and Singh 1973). The BP4 (Hisar) strain isolated by Singh and Singh (1967) has been regarded as the reference virus strain for BPXV.

### *7.2.1 Morphology and Physicochemical Characteristics*

The mature virion of BPXV resembles VACV in electron microscopic studies with the size of 280–330 nm × 200–250 nm (Bloch and Lal 1975). The developmental forms are similar to those of VACV. The virion is brick-shaped with complex symmetry and produces small, abundant, irregular, eosinophilic and granulated intra-cytoplasmic B-type inclusions (Singh and Singh 1967). The virus shows resistance to the ether but is susceptible to heat, acid, bile salts, chloroform and pH (Singh and Singh 1967; Lal and Singh 1977).

### *7.2.2 Genome Organization*

BPXV is presumed to have a similar genomic organization to that of VACV, i.e., double-stranded DNA genome of ~200 kbp size with covalently closed termini. Till date, the complete genome sequence of BPXV is not available. Whole-genome RFLP (restriction fragment length polymorphism) analysis did not reveal a significant difference between VACV and BPXV (Dumbell and Richardson 1993). Although BP4 strain differed from other 12 BPXV isolates from Maharashtra state (India) in above study. A number of BPXV genes encoding for structural, non-structural and host-range proteins have been characterized, namely VACV homologues of H3L, A27L, D8L (Singh et al. 2006b), B5R (Singh et al. 2007b; Bera et al. 2012), K1L (Barua et al. 2011), H4L (Singh et al. 2007a), C17L/B23R (Singh et al. 2008) and E3L, K3L, C7L (Bera et al. 2012). These genes showed 98–99% nucleotide and amino acid sequence identity to VACV. The D8L protein showed K163T substitution in all BPXV isolates as compared to VACV. C18L gene

encoding ankyrin-repeat protein is used specifically to differentiate BPXV from VACV and other OPVs, as BPXV shows only <77% nucleotide and <67% amino acid identity with VACV. BPXV C18L gene encodes only 50 amino acids length protein as compared to 150 amino acids in VACV and shows separate clustering from VACVs (Singh et al. 2008). Sequence analyses of haemagglutinin (HA) gene of isolates from cows in India confirmed the aetiological agent to be BPXV with a higher sequence identity with modified vaccinia Ankara strain than Brazilian VLVs (Yadav et al. 2010). Brazilian VLVs characteristically show a deletion of six codons at position 248–253 in HA protein (Kroon et al. 2011). Whole-genome sequencing of BPXV will provide a clearer picture of origin, evolution and virulence determinants.

### *7.2.3 Antigenic and Serological Properties*

Numerous antigens of BPXV have been recognized by conventional serological tests such as protein soluble antigens (LS antigens), nucleoprotein antigens (NP antigens), haemagglutinin (HA) and factors responsible for infectivity (Kataria and Singh 1970; Baxby and Hill 1971; Lal and Singh 1973). Virion polypeptides of different purified BPXV isolates using SDS-PAGE gel electrophoresis are 15–26 (14.2–347 kDa size) (Maan and Kalra 1995; Singh et al. 2006a) depending on the specific isolate. Western blot analysis revealed 15 immunodominant proteins specific to BPXV (Anand Kumar and Butchaiah 2004).

BPXV shows serological cross-reactivity with other OPVs especially VACV by serum neutralization test (SNT) (Singh and Singh 1967; Kataria and Singh 1970), immunodiffusion (Lal and Singh 1973), double immunodiffusion (Baxby and Hill 1971) and complement fixation test (Kataria and Singh 1970; Lal and Singh 1973). BPXV shows no cross-reactivity with fowlpox virus, swinepox virus and sheeppox virus (Lal and Singh 1973). BPXV shows haemagglutination activity with sheep, rabbit, guinea-pig and chicken RBCs (Baxby and Hill 1971) but does not agglutinate RBCs of camel, buffalo calf and goat (Kataria and Singh 1970).

## 7.3 Epidemiology of Disease

### *7.3.1 Geographical Distribution*

Since 1934, buffalopox has been reported from several Indian states including Maharashtra, Haryana, Punjab, Uttar Pradesh, Gujarat, Andhra Pradesh, Karnataka, Kerala, etc. (Singh and Singh 1967; Dumbell and Richardson 1993; Venkatesan et al. 2010; Gurav et al. 2011; Goyal et al. 2013) (Table 7.1). In the Maharashtra state of India, outbreaks of buffalopox were reported from 1976, 1985–1987, 1992–1996, 2003, 2008–2009 (Table 7.1). Dhule, Kolhapur, Beed districts of Maharashtra state

**Table 7.1** Major Buffalopox outbreaks in India in chronological order

| Year of outbreak | Place | Comments | Zoonotic involvement | Reference |
|---|---|---|---|---|
| 1934 | Lahore (then, undivided India) | Buffaloes and cows with generalized infection along with localized infection in humans | Yes | Sharma (1934) |
| 1935 | Bhiwani (then, Hisar district), Haryana (then Punjab) | Buffaloes with eye and ear lesions | No | Bhatia (1936) |
| 1952 | Guntur, Andhra Pradesh | Generalized infection in buffaloes with high infection rate, no disease in in-contact cows | No | Ramakrishnan and Ananthapadmanabhan (1957) |
| 1966 | Hisar, Haryana | Generalized lesions in buffaloes with localized lesions on hands in humans | Yes | Singh and Singh (1967) |
| 1975–1976 | Aarey Milk colony, Goregaon, Bombay | Several outbreaks with high morbidity in animals involving death of buffalo calves, localized lesions in humans | Yes | Mathew et al. (1978) |
| 1976 | Dhule, Maharashtra | Animals from several villages showed localized lesions, fever and localized lesions in humans | Yes | Ghosh et al. (1977) Sehgal et al. (1977) |
| 1978 | Bareilly, Uttar Pradesh | Large population of buffaloes affected with localized lesions on ear, eyes and udder; humans with localized infection | Yes | Mehrotra et al. (1981) |
| 1978 | Bithari Chainpur, Bareilly district, Uttar Pradesh | Large population of buffaloes affected with ear and/or eye lesions with secondary complications like otorrhoea and conjunctivitis and high morbidity rate | No | Mallick and Dwivedi (1982) |
| 1986 | Rethoura, Bareilly district, Uttar Pradesh | Large population of buffaloes affected with ear or eye lesions, low morbidity rate | No | Mallick (1988) |
| 1985–1987 | Ratnagiri, Beed, Dhule, Pune, Sholapur districts of Maharashtra | 18 scab samples collected | No | Dumbel and Richardson (1993) |

(continued)

**Table 7.1** (continued)

| Year of outbreak | Place | Comments | Zoonotic involvement | Reference |
|---|---|---|---|---|
| 1992–1996 | Dhule, Jalgaon, Beed districts of Maharashtra | Udder and teats lesions in buffaloes, cows; localized lesions in humans | Yes | Kolhapure et al. (1997) |
| 1997 | Thotlavalluru, Krishna district (Andhra Pradesh) | Buffaloes with atypical form involving only brisket region | | Babu et al. (1998) |
| 1997 | Nasik, Maharashtra | Both localized and generalized skin lesions in animals, localized lesions in young humans not vaccinated for smallpox | Yes | Raut et al. (1997) |
| 2003 | Aurangabad, Maharashtra | Lesions on udder, teats, hindquarters; local lesions on forehand, fingers, fever, lymphadenopathy in humans | Yes | Singh et al. (2006c) |
| 2006 | Thotapalli Gudur, James Garden, Nellore (Andhra Pradesh) | Localized lesions in buffalo calves along with human cases | Yes | Bhanuprakash et al. (2010) |
| 2006 | Sardar Krishinagar, Gujarat | Localized infections in humans | Yes | Bhanuprakash et al. (2010) |
| 2008–2009 | Solapur and Kolhapur districts, Maharashtra | Lesions on udder and teats in buffaloes with human involvement | Yes | Gurav et al. (2011) |
| 2009 | Kolhapur, Maharashtra | Large population of buffaloes affected with lesions on udder and teats, localized lesions in humans | Yes | Venkatesan et al. (2010) |
| 2011 | Batnor, Meerut (Uttar Pradesh) | Simultaneous localized infection of buffaloes, cows and humans with corneal opacity in two humans | Yes | Goyal et al. (2013) |
| 2014 | Hisar | Laboratory-acquired infection in human | Yes | Riyesh et al. (2014) |

(India) have been endemic regions for buffalopox. Dhule, being a large cattle market, acts as foci for the spreading of many infectious diseases including buffalopox into nearby areas. Outbreaks of buffalopox have also been reported from many other countries of the world including Pakistan (Maqsood 1958), Egypt (Tantawi et al. 1979), Italy (Oreste and Sabastini cited by Hutyra et al. 1946), Russia (Ganiev and Ferzaliev 1964), Indonesia (Mansjoer 1951), Nepal and Bangladesh (Singh et al. 2007). The disease is presently associated with increasing outbreaks in buffaloes (Venkatesan et al. 2010). Four outbreaks of BPXV have been reported in India between 2006 and 2008 (Bhanuprakash et al. 2010).

### 7.3.2 Host Range

Naturally, water buffaloes are the primary host for BPXV with occasional involvement of cows and humans (Ghosh et al. 1977; Yadav et al. 2010; Gurav et al. 2011). Cows and buffaloes in the same herd can be infected simultaneously (Yadav et al. 2010; Goyal et al. 2013). The disease has not been reported from African buffalo (*Syncerus caffer*) (Singh et al. 2012). Experimentally, a wide host range involving buffalo calves, cow calves (Singh et al. 1996), rabbits (Singh et al. 1996), guinea pigs (Singh and Singh 1967; Singh et al. 1996; Kumar et al. 2016), BALB/c and Swiss white infant mice (Dogra et al. 1978; Singh et al. 1996; Kumar et al. 2015) are susceptible, whereas adult mice (BALB/c and Swiss white), hamsters, sheep, goat and fowl are refractory to buffalopox infection (Singh and Singh 1967; Singh et al. 1996). Transmissibility of BPXV between multiple species, including buffaloes, cows and humans, may lead to the emergence of BPXV with altered pathogenicity.

### 7.3.3 Transmission

The disease is reported to occur in sporadic and epidemic forms in domestic/commercial farms. The spread of BPXV into naive areas is predominantly associated with animal movement through trade. Animal-to-animal transmission of BPXV usually occurs through close contact, especially during calf feeding, contaminated milkers' hands and milking machine. Mechanical transmission by flies and insects has also been suspected (Muraleedharan et al. 1989). Animal handlers and milkers get the infection while milking or close contact with lesions, which is further spread to naïve animals of the same farm or distinct farms. Human-to-human transmission of BPXV has been suspected in India (Singh et al. 1996; Kolhapure et al. 1997) but the same has been confirmed in Brazilian VLVs (Oliveira et al. 2014). BPXV (Singh et al. 2006c) and Brazilian VLVs (Abrahao et al. 2009b) have been isolated from milk indicating possible food-borne transmission. Till date, reservoir host/s for BPXV is still not known. Although some rodent species have been suspected to be involved in the transmission of BPXV in Namakkal district of Tamil Nadu state,

India (Nedunchelliyan et al. 1992). Peridomestic rodents have been suspected of transmitting the bovine vaccinia infection from wild to domestic animals in Brazil (Abrahao et al. 2009a). Further, the circulation of BPXV in wild animals in India still needs to be explored.

## 7.4 Immunopathobiology

Pathogenesis of BPXV has been experimentally studied in susceptible hosts like rabbits, suckling mice and buffalo calves. After intradermal inoculation, the virus undergoes local multiplication at the site of entry in the skin and moves to regional lymph node followed by WBC-associated primary viremia (Rana et al. 1985; Chandra et al. 1985). Following viremia, virus disseminates to target organs, namely lungs, liver and spleen. Multiplication of virus in target organs causes secondary viremia, which is responsible for secondary lesions in the gonads, intestines, stomach, kidney, etc. Suckling mice following inoculation show symptoms like paralysis of hind legs, staggering gait and circling followed by death (Dogra et al. 1978). Usually, generalized skin lesions are not seen in rabbits, guinea pigs and suckling mice. Following experimental inoculation in buffalo calves, symptoms like lacrimation, mucopurulent nasal discharge and diarrhoea were observed. After the appearance of primary skin lesion, the virus is detected in the regional lymph node on the 2nd day followed by viremia at 4th day and multiplication in target organs at 5th day. Secondary viremia starts at 6th day causing a secondary rash, between day 6th–8th on the lips, tongue, neck, perineum region and around the nostrils and eyes. The experimental infection had a course of 13–15 days (Rana et al. 1985). Antibodies are detected after 12 days following experimental infection. Both humoral and cellular immune responses have been shown to play a role in buffalopox infected rabbits (Chandra et al. 1990), mice (Kaushik and Pandey 1980) and guinea pigs (Kalra et al. 1976). Role of antibodies in poxvirus control and recovery from secondary infection is well known. Passive transfer of anti-BPXV serum (Kaushik and Pandey 1980), anti-A27L serum (Kumar et al. 2015), anti-H3L serum (Kumar et al. 2016) and combined A27L and H3L antibody cocktail (Kumar et al. 2017) has shown 87%, 60%, 80% and 100% protection, respectively. Antibodies to intracellular mature virion (IMV) proteins like A27L and H3L probably directly neutralize the initial virus inoculum as well as progeny IMV virions released from lysed cells and also inhibit maturation of IMV to intracellular enveloped virion (IEV). Complete cross-protection has been demonstrated in buffaloes between BPXV and VACV (Ramakrishnan and Ananthapadmanabhan 1957) and BPXV and CPXV (Tantawi et al. 1979).

## 7.5 Clinical Disease in Animals

Buffaloes of all ages and both the sex are affected, but the clinical disease is more severe in young and old animals. After an incubation period of 4–6 days (Ghosh et al. 1977), animal shows anorexia, the rise in temperature and lacrimation, followed by the development of skin lesions. Clinically, buffalopox occurs in two forms, namely mild localized and severe generalized. A severe generalized form involving several body parts like udder, teats, vulva, thigh and abdomen (Ramakrishnan and Ananthapadmanabhan 1957; Sreemannarayana and Ramachandraiah 1999) is rare and uncommon nowadays. Buffalopox occurs mainly in localized mild form affecting mainly udder and teats (Fig. 7.1), sometimes inguinal region and thighs in milking animals (Sharma 1934; Singh and Singh 1967) or parotid region, ear and eyes in draught animals (Venkatesan et al. 2010). Lesions on udder and teats lead to thickening of teats, stenosis of teat ducts followed by secondary bacterial complications. Approximately 50% of buffalopox affected animals develop mastitis leading to a reduction in milk yield. Severe cases may involve a permanent reduction in milk yield (Singh et al. 2006c). Suckling calves may contract the disease from the affected dam and develop lesions around muzzle including purulent gingivitis. After the involvement of eyes and ears, frequent complications like otorrhoea, conjunctivitis and corneal opacity are observed (Venkatesan et al. 2010). An atypical form involving only brisket region has been reported in BPXV outbreak in Thotlavalluru village of Krishna district in Andhra Pradesh, India (Babu et al. 1998). Pock lesions pass through successive stages such as roseolar, papular, vesicular, pustular and desquamative. Temperature returns to normal after pustules subside. Recovery usually takes 3–4 weeks in uncomplicated cases. Recovered animals show life-long immunity. Morbidity in affected animals is as low as 5–10% to as high as 65–70%. Death is seen sometimes in calves (Mathew et al. 1978).

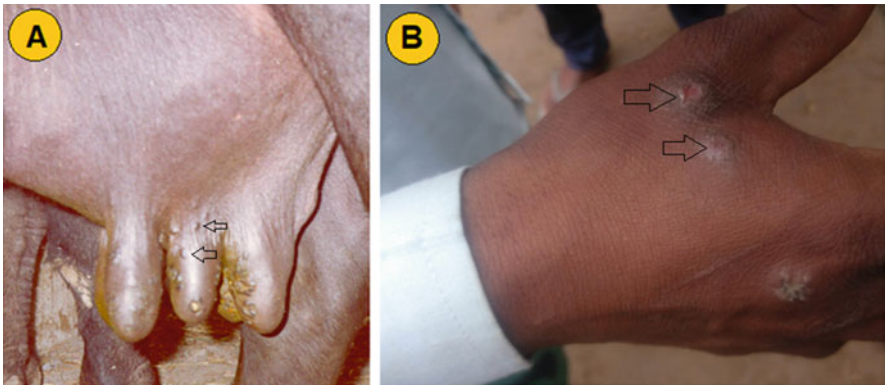


**Fig. 7.1** Localized lesions of buffalopox infection. (**a**) Teat lesions in buffalo (**b**) lesions on hand in human (indicated by arrows)

## 7.6 Buffalopox in Humans

The joint FAO/WHO Expert Committee on zoonosis has listed buffalopox as an important emerging, re-emerging zoonosis. Humans in close contact with infected animals like milkers, animal attendants, veterinarians, laboratory personnel handling the virus, etc. are particularly susceptible to buffalopox infection. Milking of infected animals is one of the major modes of spread. After an incubation period of 3–19 days, affected humans show symptoms like fever, general malaise, regional lymphadenopathy followed by appearance of localized pock lesions on forearms, forehead and face (Kolhapure et al. 1997; Venkatesan et al. 2010; Gurav et al. 2011; Goyal et al. 2013) (Fig. 7.1). The generalized BPXV infection in humans has not been reported except one case of an immunodeficient patient with a history of Darier's disease in Telangana, India (Prasad et al. 2009). Zoonotic outbreaks are frequent in endemic regions of Maharashtra state (India), namely Dhule, Beed, Jalgaon, Nasik, Kolhapur and Aurangabad districts of Maharashtra (Dumbell and Richardson 1993; Kolhapure et al. 1997; Singh et al. 2006c; Venkatesan et al. 2010; Gurav et al. 2011). In recent years, it has been observed that buffalopox is running a more severe course in humans, especially those who have not been immunized for smallpox in the past. During 1992–1996 outbreak of buffalopox in Dhule (Maharashtra state, India), in addition to affected humans and in-contact milkers, neutralizing antibodies against BPXV were also detected in young individuals who have neither history of clinical pox disease nor history of contact with buffaloes and never been vaccinated against smallpox (Kolhapure et al. 1997; Venkatesan et al. 2010). In some outbreaks, a few children who had no previous contact with infected animals showed clinical manifestations raising suspicion for possible man-to-man transmission (Kolhapure et al. 1997; Gurav et al. 2011). BPXV infection in buffaloes, cows and humans simultaneously in the same space and time has been reported in Meerut (Uttar Pradesh, India) in 2011 (Goyal et al. 2013). The outbreak also involved two human cases with corneal opacity. There has also been a report of nosocomial infections of buffalopox from Karachi, Pakistan, in burn patients and paramedical staff which proved an efficient mode of indirect transmission of an OPV (Zafar et al. 2007). Laboratory-acquired BPXV infection has been seen on both smallpox vaccinated (Baxby and Hill 1971) and non-vaccinated humans (Riyesh et al. 2014).

## 7.7 Diagnostics

Although clinical characteristics of buffalopox might be helpful in symptomatic diagnosis, clinical lesions of various other vesicular diseases such as cowpox, pseudocowpox and bovine herpesvirus mammillitis may potentially mislead the diagnosis. Laboratory investigations are, therefore, essential for establishing a definitive diagnosis. Buffalopox infections can be diagnosed by conventional, serological (antigen and antibody detection) and molecular techniques. Suitable specimens like

scabs, vesicle fluids, skin biopsy tissues and serum can be collected. After making 10% (w/v) suspension of scab material using phosphate-buffered saline (pH 7.4), homogenate suspension is freeze-thawed three times followed by clarification at 1500 × g for 10 min. The supernatant is filtered through 0.45 μm membrane filters after addition of antibiotics such as penicillin and streptomycin and stored for further use either for DNA extraction or isolation of the virus in cell culture.

### *7.7.1 Conventional Diagnosis*

Conventional techniques like electron microscopy by negative staining of vesicular fluid, or micro-sectioning of scab; virus isolation in embryonated eggs via chorio-allantoic membrane (CAM) route, cell culture, inoculation into experimental animals like rabbits or infant suckling mice have been used for primary diagnosis (Singh et al. 2007a). By electron microscopy, it is easy to differentiate OPVs from other viruses, but differentiation at species level within OPVs is not possible. On the chorioallantoic membrane of embryonated chicken eggs, BPXV produces two types of non-haemorrhagic pocks 48–72 h post-infection—greyish flat type and white raised type (Singh and Singh 1967). BPXV replicates in a wide range of cell cultures including Vero, $BHK_{21}$, RK-13, primary hamster kidney cells, HeLa cells, CEF, pup kidney cell culture, etc. Vero cells are commonly used for isolation of BPXV. In cell culture, rounding, clumping and increased refractivity of infected cells is seen at 24 hpi (hours post-infection) followed by pin-point degenerative foci at 42 hpi, and microplaque formation followed by complete detachment after 72–96 hpi (Fig. 7.2). Clinical samples may require two blind passages to give cytopathic effect (CPE). The virus produces plaques on RK-13 and CEF cells.

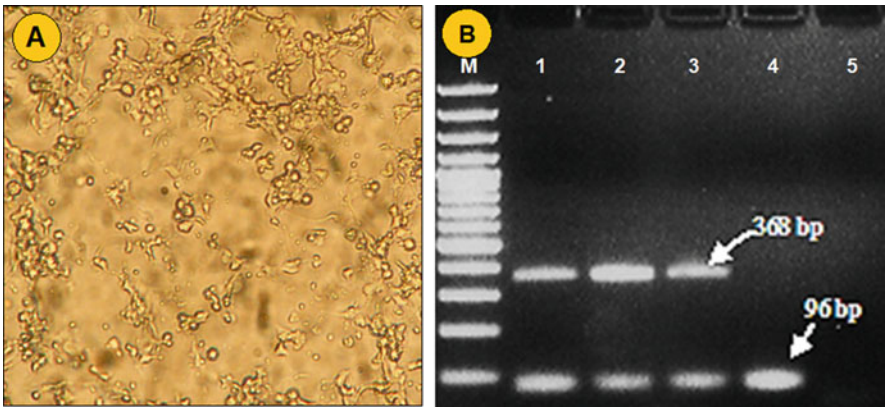


**Fig. 7.2** (**a**) Cytopathic effect of BPXV 48 h post-infection showing rounding, clumping of infected cells. (**b**) Duplex PCR based on C18L and DNA polymerase genes (Singh et al. 2008) showing 368 bp amplified product specific for BPXV and 96 bp amplified product specific for OPV. Lane 1: BPXV-Vij96 (vaccine virus); Lane 2: BPXV-Pune (buffalo); Lane 3: BPXV-Pune (human); Lane 4: Camelpox virus; Lane 5: No template control

### 7.7.2 Antigen Detection

Serological tests like agar gel immunodiffusion (AGID) (Paul et al. 1984) and counter-immunoelectrophoresis (CIE) using a reference antigen and its hyperimmune serum can also be used. CIE test identifies either unknown soluble antigen in suspected pox lesions or antibody present in the serum samples using standard/reference positive serum or antigen, respectively. Fluorescent antibody test (Kaushik and Pandey 1981), immunoperoxidase test (IPT) (Grover et al. 1980; Ghildyal et al. 1986; Mohanty et al. 1989c) and immunoenzymatic (Grover et al. 1980; Kaushik and Pandey 1981) techniques can be employed for detection of BPXV in cell culture or formalin-fixed tissues. Serological assays like AGPT, CIE and IPT tests fail in the accurate diagnosis of the disease because of antigenic cross-reactivity with other OPVs. Moreover, these techniques are less sensitive, laborious and time-consuming.

### 7.7.3 Antibody Detection

Serum neutralization test (SNT) and whole antigen-based indirect ELISA have been developed for detection of buffalopox antibodies in experimentally infected rabbits (Ghildyal et al. 1986) and vaccinated/infected buffaloes (Mohanty and Rai 1990). Whole antigen-based indirect ELISA has been found to be more sensitive than SNT and CFT. The whole antigen-based ELISA and SNT require handling of the live virus which is risky, laborious and uneconomical to produce the diagnostic whole viral antigen in bulk posing a public/biosecurity threat. A recombinant antigen-based diagnostic assay will be a better alternative for reliable post-outbreak sero-surveillance in the countries where BPXV or VACV-like agents are endemic. Positive serological findings in humans have to be carefully interpreted in light of previous smallpox vaccination. Due to the advancement in gene expression technology, the production of recombinant viral proteins has become relatively easier and more efficient. In the past, various VACV proteins have been expressed in different expression systems such as bacterial, mammalian, yeast and insect cells to assess potential diagnostic and immunoprophylactic potential. Two IMV proteins of BPXV, namely A27L and H3L, have been expressed in the prokaryotic system and evaluated for their diagnostic potential in ELISA (Kumar et al. 2015, 2016).

### 7.7.4 Molecular Diagnosis

PCR assays targeting HA (Ropp et al. 1995; Damaso et al. 2007) and ATI genes (Meyer et al. 1997) are used for the detection and differentiation of OPVs. Several molecular tests have been described for the detection and differentiation of BPXV.

TaqMan probe-based quantitative real-time PCR targeting C18L gene and duplex PCR based on C18L and DNA polymerase genes can be used to differentiate BPXV from other OPVs (Singh et al. 2008). C18L and DNA polymerase genes based duplex PCR amplifies 96 bp amplicon for all OPVs and 368 bp amplicon only BPXV (Fig. 7.2).

RAPD-PCR can also be used for differentiation of BPXV (Singh et al. 2007a). However, these techniques need specialized equipment leading to the high cost of diagnosis at field conditions. Therefore, there is an urgent need for sensitive, specific, rapid and user-friendly diagnostic tool like loop-mediated isothermal amplification (LAMP) assay in less equipped field diagnostic laboratories for timely identification of BPXV infection and its differentiation from other OPVs in the target population to initiate control measures quickly.

## 7.8 Prevention and Control

India and other South Asian countries are endemic to buffalopox. BPXV affected animals should be segregated from apparently healthy animals with separate arrangement for milking of infected animals by separate attendants. The difficulty in restricting animal movement especially between villages during trade, socio-cultural issues and economic considerations restricts efficient control of BPXV in India. Application of biosecurity measures such as segregation of infected animals, use of disinfectants in sheds, etc. is the only means of containing the spread of infection. Uncomplicated buffalopox infections are self-limiting with a course of 3–4 weeks, but animals need to be treated symptomatically along with treatment for secondary bacterial infections. For teat/other skin lesions, antiseptic lotions/ointments can be applied, whereas mastitis cases are treated with parenteral antibiotics. Local antibiotic ointment for eye and/or ear lesions along with parenteral antibiotics is generally used to control secondary infections. Human cases are treated with antipyretics, anti-inflammatory drugs and broad-spectrum antibiotics. Efficacy of certain drugs effective against OPV infections, viz. ST-246, cidofovir, STI-571, CMX001, etc., needs to be evaluated for BPXV.

Initially, the vaccinia lymph vaccine has been tried for BPXV (Mathew et al. 1978). Later, homologous ethylamine, formalin and beta-propiolactone inactivated BPXV vaccine was tried, but unsuccessful with lack of protection in rabbits and buffaloes (Mohanty et al. 1989a). Protection by using CEF attenuated buffalopox virus BP4 strain (passage-70) in rabbits (Dogra and Sharma 1981) and Vero cell-adapted buffalopox virus BP4 strain (passage-40) in buffaloes (Mohanty and Rai 1989) has been demonstrated. In Pox Virus Laboratory, IVRI Mukteshwar (India), a live attenuated vaccine has been developed using Vero cell-adapted BPXV (Vijayawada 96 strain, passage-50). This vaccine has been found to be safe and potent in in-house trials. This vaccine needs further large-scale field validation. However, there is a need for alternative immunoprophylactic strategies involving the use of DNA and subunit vaccines because of safety and zoonotic potential of the virus. Out

of ~200 proteins encoded by VACV, few proteins including H3L, A27L, B5R, D8L and L1R proteins have been shown to induce neutralizing antibodies. Several VACV proteins have been expressed in different expression systems and tested for immunogenicity and protective efficacy in animal models, individually or in combination (Hooper et al. 2003; Berhanu et al. 2008). Antibodies against surface proteins from both IMV and extracellular enveloped virion (EEV) forms are important for protective immunity. So, combined subunit vaccine using different IMV and/or EEV proteins in the form of polyvalent formulations might be effective for better protection than individual proteins. Immunogenicity of two IMV proteins of BPXV, namely A27L and H3L, has been evaluated in laboratory animals (Kumar et al. 2015, 2016). However, work on BPXV and other VLVs proteomics is scanty. Further, the nature of immunogenic viral proteins involved in eliciting immune response of these viruses in farm animals and human is yet to be known.

## 7.9 Conclusion

Buffalopox is an emerging and re-emerging zoonosis of public health importance. The possibility of human-to-human transmission may be a serious concern to veterinarians and WHO. Considering the zoonotic significance and widespread incidence of buffalopox infection in the Indian subcontinent, a detailed systematic study is warranted to carry out epidemiological investigations and appropriate control measures. Whole-genome sequencing of BPXV isolates from different geographical area will provide a clear picture of the origin of BPXV.

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