Goats occupy a premier place in the livestock industry and contribute significantly to the world economy. India ranks second in the world goat population with 135.17 million sharing more than 20 % of global goat population (Livestock Census, Anon. 2012). The total goat population in Chhattisgarh is more than 3.2 million that comprises 21 % of the total livestock population in the state. The populations of goats are threatened by a number of health hazards, among the most notable of which is goatpox. Outbreaks of goatpox are being reported in different regions of India viz. Himachal Pradesh (Verma et al. 2011) and Maharashtra (Kadam et al. 2014) as well as in Chhattisgarh (Joshi, 2000). It is malignant pox disease of goats caused by Capripoxvirus of the family Poxviridae(Murphy et al. 1995). Diagnosis of goatpox is usually based on clinical signs followed by laboratory confirmation. Embryonated chicken eggs (Kadam et al. 2014) and primary cell cultures like lamb kidney and testes (OIE, 2012) are popular cultivation system used for sheeppox and goatpox (capripox) viruses. However, capripox virus can be successfully adapted in chicken embryo fibroblast (CEF) cells (Rao and Malik, 1982). Chinese hamster ovary (CHO) cells are one of the main cell line used in the production of...
recombinant therapeutics (Wurm, 2004). Although, CHO cells have restricted virus susceptibility as compared to BHK and vero cells (Berting et al. 2010), no systematic investigation of their virus susceptibility has been published. However, Schuenadel et al. (2012) has reported susceptibility of CHO cells for cow pox virus. In light of earlier reports, present investigation was undertaken to study the prevalence of goatpox in surrounding areas of Durg district of Chhattisgarh state followed by adaptation of capripox virus isolates from goats in heterologous cells viz. CHO and CEF cells.

**MATERIALS AND METHODS**

**Sample collection**

An outbreak of goatpox was attended in five different villages of Durg district and surrounding areas of Chhattisgarh, India. Skin scabs were collected in glycerin-saline from 250 goats which showed clinical signs suggestive of pox i.e. fever, generalized papules or nodules, vesicles, pneumonia and death and preserved in refrigerator until screening for capripox virus. Scabs were ground into paste with pestle and mortar using previously sterilized fine glass powder and suspended in phosphate buffered saline (PBS) (pH 7.0) making a 10% suspension. The suspension was put for three cycles of freezing and thawing and then the suspension was centrifuged for 15 min at 1500 rpm to remove large tissue particles and debris. Supernatant fluid was used as capripox virus antigen.

**Reference capripox virus antigen and raising of hyperimmune sera**

Sheeppox vaccine (BIO-MED private limited) containing attenuated virus was centrifuged for 15 min at 15000 rpm in the refrigerated centrifuge (Remi R,C) and then filtered through millipore syringe filter (0.45µ). 10^2.5TCID₅₀ titer of virus was used as antigen for preparation of hyperimmune sera. Hyperimmune sera were raised in rabbits as per protocol of Nour et al. (2012) with little modification. Serum was complement inactivated at 56°C for 30 min and stored in one ml aliquot at -20°C until used.

**Identification of capripox virus antigen and determination of prevalence rate**

Screening of capripox virus antigen in population was done by agar gel immuno diffusion (AGID) and countercurrent immune electrophoresis (CIE) test using hyperimmune sera raised in rabbits AGID was performed by taking 1% agarose in normal saline as per method described by Bambhani and Krishnamurty (1963). CIE was performed as per protocol described by Sharma et al. (1988). Electrophoresis was carried out by using a current of 8 mA per square cm area for 30 to 60 min Development of precipitation line was recorded as positive result. Failure to develop line for extended period of time (more than 72 hrs) was considered as negative result. Based on severity of clinical signs and laboratory confirmation by AGID; total prevalence rate, age wise prevalence and distribution of disease in various age group animals were determined (Table 1).

**Isolation of capripox virus on chorioallantoic membrane (CAM)**

Scab suspension was inoculated in to embryonated chicken eggs by CAM route as described by Cunningham (1966). The inoculated eggs, were incubated at 37°C in horizontal position and candled daily up to five days and observed for embryo mortality. Virus was harvested

| Table 1: Prevalence of goatpox in surrounding areas of Durg district of Chattisgarh |
|---------------------------------|----------|----------|----------|
| **Particulars**                 | **Below 6 months** | **6 months to 2 years** | **2 years and above** |
| Number of animals in population | 100      | 81       | 69       |
| Number of animals affected      | 84       | 58       | 43       |
| Age wise prevalence (%)**       | 84.00    | 71.60    | 62.32    |
| Distribution of disease (%)†    | 45.41    | 31.35    | 23.24    |

*Total prevalence = Total number of animals affected / Total number of animals in population.
** Number of animals affected in particular age group /Number of animals in particular age group.
†Number of animals affected in particular age group/Total number of animals affected.
by collection of infected CAM after appropriate chilling to avoid bleeding. Virus isolates of CAM were passaged serially in embryonated chicken eggs three to six times. On each passage, CAM was harvested after five days of incubation and examined for characteristic pock lesions and further confirmed by AGID. Capripox virus antigen from CAM homogenate was prepared by making 10% suspension of infected CAM in PBS.

PREPARATION OF CEF AND CHO CELL MONOLAYERS

CEF cells were prepared from 9 to 10 day-old chicken embryo as per standard procedure (Cunningham, 1966). The CHO cells were procured (Hi Media Laboratories Limited, Bombay) and prepared as per manufacturer’s instructions. Viability of CEF and CHO cells was checked using trypsin blue stain. Cells at concentration of 10^5 cells per ml were dispensed in Eagle’s minimum essential medium (EMEM) with Hank’s salts and L-glutamine without sodium bicarbonate (Himedia laboratories) with 5% serum for growth. The uniform cell sheets were formed after 36 to 48 hr of incubation and were used for inoculation.

Virus inoculation

Capripox virus isolated from embryonated chicken egg (ECE) through CAM route was used for preparation of virus inoculums in PBS for adaptation in cell culture. The growth medium was removed and monolayer was washed once with EMEM. Capripox virus isolate @ 10^6 EID_{50} / 0.1 ml was inoculated into 5 culture tubes, each tube received 0.2 ml of the inoculum. After 2 hrs of virus adsorption at 37°C, excess inoculum from tubes was discarded and monolayer was washed with 2 ml of EMEM to remove unadsorbed virus. Maintenance medium containing 2% serum was then added at the rate of 2 ml to all cell culture tubes. Five uninoculated tubes were kept as controls. The tubes after inoculation were incubated at 37°C and were examined daily for six days until maximum CPE was observed.

Confirmation of capripox virus isolates

Identity of CEF and CHO adapted capripox virus was confirmed by both AGID and CIE. Further, haemagglutinating property of capripox virus isolate was studied by haemagglutination (HA) test (Shakya et al. 2004) using 1% RBC suspension obtained from sheep, rabbit, dog and chicken blood and after 30 min of incubation at 28-30 °C, HA titer was calculated.

RESULTS AND DISCUSSION

Prevalence of goatpox

The present study reported 74% prevalence of goatpox using AGID test. Likewise, earlier reports on outbreak of goatpox (Joshi et al. 1999) in Durg revealed 74.67 % morbidity and 48.21 % mortality. Distribution and prevalence of disease in young animals, a particularly in kid (below 6 month) was more as compared to those between 6 to 24 months and 24 months and above (Table 1). In agreement with present findings, Das and Pradhan (2006) also reported higher morbidity (69%) in kids. In contrast, Venkatesan et al. (2010) reported less morbidity and mortality but higher (60%) case fatality rate due to goatpox. Earlier report (Joshi et al. 1999) and present findings strongly suggest that goatpox in kids in local areas of Durg has emerged as virulent strain and thus strict prophylactic measures in kids and pregnant goats are essential.

Pock lesions produced by capripox virus on CAM

The isolate in ECE was identified as capripox virus on the basis of characteristic pock lesions in third passage.

Table 2: Morphological changes induced by capripox virus on CAM in serial passages

<table>
<thead>
<tr>
<th>Level of passage</th>
<th>Morphology of the CAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Oedema and thickening</td>
</tr>
<tr>
<td>II</td>
<td>Oedema, thickening along with haemorrhage and necrosis</td>
</tr>
<tr>
<td>III</td>
<td>Oedema, necrosis along with minute pock with average size of 0.5 mm in diameter. Most of these lesion were distributed on CAM over an area just below the artificial air sac</td>
</tr>
<tr>
<td>IV</td>
<td>Minute pock with average size of 0.7 mm in diameter</td>
</tr>
<tr>
<td>V</td>
<td>Minute pock ranging between 0.7 to 1 mm in diameter</td>
</tr>
</tbody>
</table>
In first and second passage CAM showed thickening with oedematous swelling along with haemorrhages and diffuse areas of necrosis (Fig.1 and 2). Virus induced changes in CAM during subsequent passage in embryonated eggs are shown in Table 2. Pock lesion observed on CAM during present study is in accordance with reports of Joshi (2000).

**Table 3: CPE induced by capripox virus in CHO cell culture in serial passages**

<table>
<thead>
<tr>
<th>Cultivation system</th>
<th>Passage level</th>
<th>Rounding and Clump formation</th>
<th>Increase no. of refractile cells</th>
<th>Complete detachment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO cell culture</td>
<td>III</td>
<td>72 hr</td>
<td>96 hr</td>
<td>120 hr</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>72 hr</td>
<td>96 hr</td>
<td>120 hr</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>48 hr</td>
<td>72 hr</td>
<td>120 hr</td>
</tr>
</tbody>
</table>

Propagation of capripox virus in CEF and CHO cell monolayers

On adaptation to susceptible cell culture, the appearance of characteristic cytopathic effects (CPE) is strongly indicative of virus growth. In the present investigation, there was no CPE in both CHO and CEF cells inoculated with capripox virus during first two passages. On the third passage, CPE was observed in CHO cell but in CEF cells, it did not produce any CPE even up to fifth passage (Table 3).

There are not any earlier reports on adaptation of capripox virus in CHO cell, however Schuenadel *et al.* (2012) cultivated cow pox virus (a member of family Poxviridae) in CHO cell and observed that CHO cell is permissive for cow pox virus; can effectively multiply and produce characteristic CPE in CHO cells. Whereas, Ramsey-Ewin and Moss (1998) reported that CHO cells are readily killed by vaccinia virus within 2-3 days. CHO cells offer the advantages that they are easily genetically manipulated, can be adapted for large-scale suspension culture and can grow in serum-free and chemically defined media which ensures reproducibility between different batches of cell culture (Lai *et al.* 2013). Above facts encouraged to use CHO cells in present investigation.
CHO and CEF cell adapted capripox virus isolates at passage level I, II and III failed to agglutinate erythrocyte of all species tested. However, CHO adapted isolates of 4th and 5th fifth passage agglutinated only chicken erythrocyte and yielded HA titer of 1:256, which is in agreement with observation of Raof et al. (2005). However in a report by Shakya et al. (2004), goatpox virus failed to agglutinate the sheep, goat, pig, rabbit, dog, chicken and human type ‘O’ erythrocytes.

After formation of Chhattisgarh state in 2000, no new outbreaks were officially reported. However, present study reveals that the severity and extent of goatpox has increased and warrants immediate attention, particularly in the events of unknown history about vaccination against sheep and goatpox. Considering the severity of clinical symptoms, morbidity and distribution (particularly in kids) in current outbreaks, there is strong possibility about re-emergence of the disease in adjacent areas. This study offers a traditional, convenient and preliminary approach for diagnosis of capripox virus infection and help to formulate strategies on the basis of local field isolate of capripox virus modified by adapting it to suitable host system. Capripox virus isolate from goat was successfully adapted and propagated in CHO cell culture. Further studies would be required to investigate the infectivity titre of virus in CHO cell and to characterize capripox virus so as to develop diagnostics and candidate vaccine.

REFERENCES


