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# An epidemiological study of sheep and goat pox outbreaks in the Sudan

**Ahmed Zein Elabdeen Mahmoud[1,2]*, Abdelmalik Ibrahim Khalafalla[3,4], Muaz Magzob Abdellatif[5,6]**

[1]Hail Veterinary Laboratory, Ministry of Agriculture. Saudi Arabia, [2]Ministry of Animal Resources and Fisheries, Khartoum, Sudan, [3]Camel Research Centre, King Faisal University, Saudi Arabia, [4]Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum, Sudan, [5]Department of Biology (Microbiology), Faculty of Arts & Sciences, Northern Borders University, Saudi Arabia, [6]Department of Microbiology, Faculty of Veterinary Science, University of Nyala, Sudan

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***Address for correspondence:**
Ahmed Zeinelabdeen Mahmoud, Hail Veterinary Laboratory, Ministry of Agriculture. Saudi Arabia. Postal Code; 81462, Saudi Arabia.
Email:ahmedzein78@yahoo.com

## ABSTRACT

Sheep pox (SP) and goat pox (GP) outbreaks occurred in the different geographic areas of Sudan and most strikingly, were highly species specific. Two outbreaks in Gedaref State in June 2013 affected no goats and outbreak in Khartoum state in March 2015 affected no sheep despite communal herding; affected goats were vaccinated with 0240 strain. Clinically, the disease was characterized by fever, depression, and eruption of generalized pox lesions. Mortality rate ranged between 5.2% and 6.7% with a mean of 6.1%. Isolation of viruses succeed on lamb testes cell culture at passage four; the diseases were diagnosed using virus neutralization test and polymerase chain reaction. SP and GP isolates grew well in lamb testes and vero cells. In Madin–Darby bovine kidney; however, both viruses induced slight cytopathic effect (CPE) that reached 60% in 9 days. On the other hand, both isolates induced no CPE in chick embryo fibroblast cells. Virus isolation attempts failed on chorioallantoic membrane of embryonated chicken eggs.

KEY WORDS: Capripox, Epidemiology, Isolation, PCR, Sudan

## INTRODUCTION

Sheep pox (SP) virus (SPPV), goat pox (GP) virus (GTPV), and lumpy skin disease virus are the members of *Capripoxviruses* (CaPVs) genus of the Poxviridae family. They are responsible for economically important poxvirus disease in sheep, goats, and cattle, respectively (Bhanuprakash *et al.*, 2006; 2010). SP is an OIE notifiable and transboundary disease of sheep and goats (Babiuk *et al.*, 2008a). It have major impacts on small ruminant production in Africa (north of the equator), the Middle East, central Asia, and the Indian subcontinent (Babiuk *et al.*, 2008a; Kitching and Carn, 2008b), due to the high morbidity and mortality associated with disease in susceptible sheep and goats (Garner *et al.*, 2000; Bhanuprakash *et al.*, 2005). In the Sudan, the presence of the disease had been confirmed since 1940 (Bennet *et al.*, 1944), and it has remained enzootic as well as in most part of Africa. It was known to be a seasonal disease associated with the cold winter (Muzichin and Ali, 1979). However, in the last few years, outbreaks of the disease were observed at different times of the year. GP has also been confirmed in the Sudan and caused severe disease in both goats and sheep (Rao and Bandyopadhyay, 2000).

Diagnosis of SP and GP is usually based on highly characteristic clinical signs, polymerase chain reaction (PCR), virus isolation, and virus neutralization test (VNT) although protocols exist for the use of enzyme-linked immunosorbent assay, western blotting, and several other assays (OIE, 2008). Socioeconomic factors preclude the use of test and slaughter policy and it is difficult to monitor animal movement. Vaccination is an economically feasible alternative to control by eradication of the disease (Rao and Bandyopadhyay, 2000).

This study details; the characteristics of SP and GP outbreaks in Gedaref and Khartoum state, Sudan, isolation and identification of viruses and properties of isolates in spectrum of cell cultures were investigated.

## MATERIALS AND METHODS

### Study Area

In June 2013, a pox-like disease was noted in sheep by their herder at Shott and Abu Garra regions, Gedaref State, Eastern Sudan, and in goats vaccinated with 0240 strain at Hillat Kuku, Khartoum State, Sudan in March 2015. Clinical signs and epidemiological data were recorded.

### Collection of Samples and Processing

Skin scabs were collected aseptically in 50% glycerol for virus isolation and without a preservative for PCR and vesicle swabs. Skin scabs were homogenized using sterile mortars and pestle with the aid of sterile sand and physiological saline, 20% suspensions were made and then centrifuged at 1000 rpm for 10 min. Vesicle swabs were resuspended in 2 ml of Glasgow modified Eagle's medium (GMEM) and then centrifuged at 1000 rpm for 10 min. Supernatant fluids were collected into sterile bottles and treated with antibiotics (1000 IU penicillin – 250 mg of streptomycin) and 5.000 IU of mycostatin; the supernatant fluids were left for ½-1 h at 4°C and then stored at −20°C until used.

### Virus Isolation

#### *Primary lamb testis (LT) cell culture*

Primary LT cell cultures were prepared from prepubertal lamb as described by Plowright and Ferris (1958).

#### *Virus propagation and adaptation*

Clinical sample suspensions (0.5 ml) were inoculated into semi-confluent LT in 25 $cm^2$ tissue culture flasks, incubated for 1 h at 37°C. Then, the inoculum was removed, and the semi confluent monolayer was washed twice with phosphate diluent and refed with GMEM monitored for 7 days for the appearance of cytopathic effect (CPE), the monolayer was then splitted using trypsin-versin solution, and the infected cells was reseeded again. This procedure was repeated for three passages; cultures were then harvested by freezing and thawing 3 times, centrifuged at 1000 rpm for 5 min. The supernatant was collected and preserved at −20°C till used.

#### *Hyper immune serum*

Hyper immune serum was kindly provided by the Central Veterinary Research Laboratories, Khartoum, Sudan.

#### *Virus neutralization*

The alpha neutralization procedure was performed as described by Beard (1980).

### PCR

#### *DNA extraction*

PUREGENE® DNA isolation kit (Gentra System, Minneapolis, USA) was used according to the manufacturer instructions. Briefly, 500 µl cell lysis solution was added to 100 mg scab in 2 ml microtube, and the mixture was homogenized, then 2 µl of proteinase K solution (20 mg/ml) was added to the lysate and the suspension incubated at 55°C for 60 min, followed by addition of 1.5 µl RNase solution (4 mg/ml). The procedure was then completed as described by the manufacturer.

#### *Positive and negative DNA controls*

SP vaccine (strain 0240) was used as positive control. Serial dilutions of the DNA were used to determine the optimum DNA concentration. Double distilled water (DDW) was used as negative control.

#### *PCR*

The PCR to amplify the *vap-gene* of CaPVs was as described by Ireland and Binepal (1998).

#### *Analysis of PCR product*

The amplicons were separated electrophoretically in 1.5% agarose gel (SIGMA) containing ethidium bromide (1 µl/40 ml agarose) (PROMEGA, Madison, USA, 10 mg/ml). 5 µl of 100 bp DNA Ladder (INVETROGEN) were loaded in the first slot of the gel. Then, 5 µl of the PCR products were mixed with 3 µl of dye and loaded on the rest of the wells.

#### *Cell culture spectrum*

Prepared LT and chicken embryo fibroblast (CEF) cells, vero and Madin–Darby bovine kidney (MDBK) cell lines kindly provided by the Veterinary Laboratories Agency, United Kingdom were inoculated with clinical samples, splitted, reseeded, and harvested as described previously.

#### *Inoculation of the chorioallantoic membrane (CAM)*

Viral isolates (200 µl) were inoculated on the CAM of chick embryos. Two blind passages were made for each before it was considered negative.

## RESULTS

### Clinical Signs

On clinical examination affected animals showed signs of a fever (40°C), labored breathing, depression, loss of appetite, eruptions on head, nostrils, lips, and areas of skin without wool. The vesicular stage was hemorrhagic with a tendency to generalize turned into

Table 1: Morbidity and mortality rates of CaPVs outbreaks

| Area | Animal species | Total animals | No. affected | No. dead | Mortality (%) |
|---|---|---|---|---|---|
| Shott, east river Atbara | Sheep | 400 | 30 | 25 | 6.3 |
| Abu Garra, South of Showak | Sheep | 550 | 45 | 37 | 6.7 |
| Hillat Kuku | Goats | 370 | 35 | 19 | 5.1 |
| Total | | 1320 | 110 | 81 | 6.1 |

CaPVs: *Capripoxvirus*

Table 2: Case fatality rate of CaPVs outbreaks

| Age group | Number of affected | Number of dead | Case fatality rate (%) |
|---|---|---|---|
| Young sheep (3-11 months) | 45 | 40 | 88.9 |
| Adult sheep (1-4 years) | 32 | 25 | 78.1 |
| Total | 77 | 65 | 84.4 |

CaPVs: *Capripoxvirus*

pustules and scabs (Figure 1). The disease affects all age, sex, and breed groups. Morbidity and mortality rates vary in accordance with the area (Table 1), more than 50% of mortality was reported in young animals (Table 2).

## Virus Isolation

### *LT*

Virus isolation onto LT yielded virus in 2 out of 4 tissue homogenates; the CPE appeared in passage four 7 days post-inoculation (PI) that reached 90% 7 days at the 6$^{th}$ passage.

### *VNT*

Isolates were identified as CaPVs by VNT and designated SP1O and GP1.

### *PCR*

All samples gave positive results for *vap-gene*. Bands were detected in the ethidium bromide stained gel that corresponds exactly to the expected size (192 bp) (Figure 2). No amplification product was detected when control negative (DDW) was used as a template.

## Growth of CaPVs in Cell Cultures

SP and GP isolate replicated in vero cells and produced 90% CPE at day 7 PI characterized by rounding of cells and destruction of the whole cell sheet (Figure 3). MDBK cells inoculated with SP1O and GP1 showed a few round cell formation up to 60% 9 days PI. No CPE was observed when SP1O and GP1 were inoculated onto CEF cells for 2 passages.

## Egg Inoculation

No lesions were produced by inoculation of the CAM with CaPVs isolates.

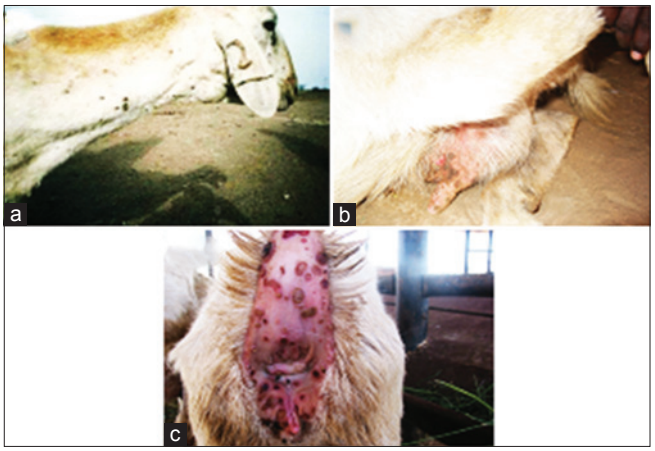


**Figure 1:** (a) Adult sheep affected by sheep pox showing pustules on the neck, (b) adult goat affected by goat pox (GP) showing scabs and ulcerations on the udder, (c) adult Saanen goat affected by GP showing ulcers, pustules, and scabs on the tail region

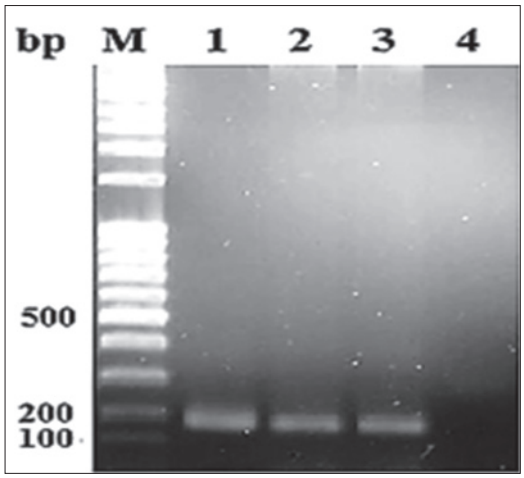


**Figure 2:** Ethidium bromide-stained agarose gel (1.5%). Lane M: 100 bp ladder, Lane 1: Control positive (vaccine strain 0240), Lane 2: Scab from goat, Lane 3: Scab from sheep, and Lane 4: Control negative (double distilled water)

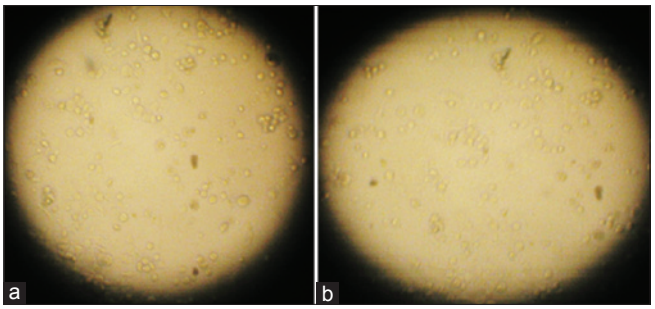


**Figure 3:** A vero cells showing rounding of cells at day 7 post-inoculation with, (a) sheep pox 1O, (b) goat pox 1

## DISCUSSION

Three natural outbreaks of CaPV disease have occurred in Sudan between 2013 and 2015. The most remarkable feature of these outbreaks was their species specificity.

Despite communal herding of sheep and goats, the outbreaks in Gedaref state caused clinical disease in sheep only while the outbreak in Khartoum state affects goats only. This suggested that the outbreaks were caused by a highly species-specific strain and the third by an equally species-specific GTPV strains. CaPVs are generally considered to be host-specific, leading to outbreaks in one preferred host, but recent records indicated that some strains of SPPV or GTPV could infect both sheep and goats. The GTPV or SPPV strains isolated from outbreaks were usually classified and designated based on the host from which the virus was isolated (Babiuk *et al.*, 2008a; Bhanuprakash *et al.*, 2006, 2010; Beard *et al.*, 2010). However, there are increasing indications that the classifying method is inaccurate (Babiuk *et al.*, 2009; Yan *et al.*, 2012). In the phylogenetic tree analysis, some CaPVs strains were found outside the group corresponding to their host of origin (Le Goff *et al.*, 2009; Lamien *et al.*, 2011). Recently, the causative agent of one outbreak in goats in India was confirmed as SPPV (Bhanuprakash *et al.*, 2010). In such cases, though it is not sufficient to confirm whether the CaPVs are separate entities with wider host spectrum, the possibility still exists that some strains naturally infect and cause disease in more than one animal species (Diallo and Viljoen, 2007).

Clinically, the disease was characterized by fever, labored breathing, depression and loss of appetite, eruptions on the head, nostrils, lips, and skin without wool. The vesicular stage which was hemorrhagic with a tendency to generalize followed by the development of pustules which later turned into scabs. These signs are similar to those described by Kitching and Taylor (1985). Mortality rate ranged between 5.2% and 6.7% with a mean of 6.1%. These mortality rates are lower than that described by Rao and Bandyopadhyay (2000) who reported 16% mortality rate in sheep in India, which may be due to the difference in herd's immunity, strain, and/or dose of the virus involved.

In the third outbreak, GP affected a Saanen herd imported from Holland where SP and GP are eradicated. The affected goats were previously vaccinated with 0240 strain. Infection of this vaccinated herd may points to a vaccination failure that may be due to poor quality vaccine and/or poor application procedure (Tizard, 2004). Similar observations of vaccination failure were previously reported (Sheikh-Ali *et al.*, 2004). Rao and Bandyopadhyay (2000) stated that live vaccine is the best long-term solution, but Sometimes, their usage is limited because of severe pock reaction and/or death of vaccinates due to the generalization of the disease.

In the investigated outbreaks all age, sex and breed groups were affected. However, more than 50% of deaths were reported in young animals in comparison to adult sheep. These findings are in line with previous outbreaks (Rao and Bandyopadhyay, 2000).

Two isolates were obtained when four clinical suspensions were inoculated onto LT culture at passage four. Mangana-Vougiouka *et al*. (1999) declared that SPPV isolation is difficult; it grows slowly or requires additional passages, even if cultured in most sensitive LT. Isolates grew well on LT cell culture with no difference in virus yield or type of CPE. Isolates produced characteristic pox CPE. These findings agreed with Adlakha *et al*. (1971), Joshi *et al*. (1994). In vero cells, both viruses produced more than 90% CPE within 7 days PI. In MDBK cells; however, both viruses induced slight CPE that reached 60% in 9 days. On the other hand, both viruses induced no CPE in CEF, results agreed with Ved Parkash *et al*. (1994) and Joshi *et al*. (1995).

Viral isolation attempts on the CAM of chick embryos failed. This result agrees with Onar (1972), Sen and Uppal (1972), Sharma and Dhanda (1972) and Bhatnagar and Gupta (1974), who unable to propagate it in chick embryos. SPPV isolates differ significantly in their growth behavior in embryonated chick eggs (Adlakha *et al.*, 1971; Tantawi and Al Falluji, 1979; Rao and Bandyopadhyay, 2000).

## CONCLUSION

Outbreaks of *Capripox* were recorded. Molecular characterization of capripoxvirus strains, with accompanying clinical data, would assist the development of accurate molecular designations of SPPV and GTPV, and help define the species specificity determinants of these viruses. The present situation highlights the need for vaccines that allow differentiation between infected and vaccinated animals. Since serological tests are the only ones which reveal past exposure to the viruses, vaccination prevents epidemiological investigations tracing back the introduction of the virus to the country, hampers surveillance for new outbreaks of the disease, and makes it very difficult to prove freedom from CaPV disease.

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