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Camel Pox: Isolation and Characterization in and Around Jigjiga of Somali Regional State, Eastern Ethiopia

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Abstract: Purposive study has been conducted for isolation and characterization of camel poxvirus from clinically ill camels. This study was carried out in and around Jigjiga of Somali Regional State from November 2013 to April 2014. The main objective of the study was isolation and characterization of camel poxvirus. A purposive sampling method was used to collect sample of skin scraping from camels that showing clinical signs of camel pox, with a typical clinical signs of which varies from mild localized to generalized pox lesions and sampled for laboratory analysis. The area is difficult to collect sample since the pastoralists move from place to place to search water and feed for animals. The twenty-two samples were collected from camels showing clinical signs at watering point and grazing area. After that, the samples were transported to National Veterinary Institute (NVI) for laboratory procedures. Viral isolation was done from tissue sample processed and cultured on Vero cell culture and among 22 samples 17 of them showed cytopathic effect (CPE) 10-11 days of post inoculation. Then DNA extraction and Polymerase chain reaction (PCR) has been followed for virus isolation and characterization purpose. Among 17 samples 12 samples were showed that positive to poxvirus. For diagnosis of camel pox clinical signs are not enough thus, diagnosis should be supported by laboratory tests.

Key words: Camel · Camel Pox · Poxvirus · DNA and Vero Cell

INTRODUCTION

Camel poxvirus (CPV) is a highly contagious viral disease affecting mostly young animals and is characterized by pustular eruptions on the skin and mucous membranes. Ethiopia possesses more than two million camel population and ranked third after Somalia and Sudan in Africa and fourth in the world India included [1]. In Ethiopia, camels are found in eastern and northern arid and semi-arid areas of the country, mainly Borena, Ogaden and Afar regions. Camels are kept by nomadic pastoralist and provide milk, meat and draught power even in severe condition where other livestock have difficulty of surviving. Therefore, camels are extremely important for livelihood of the pastoralist communities and their cultural life [2].

Camel pox is one of the important diseases of camel in the world [3]. It is characterized by high morbidity and a relatively high mortality rate in young animals, adult lactating females have a reduction in milk production and animals of all ages may lose weight and suffer debilitation [4]. Camel pox is caused by *Orthopoxvirus camel* virus, which belongs to genus *Orthopoxvirus,* sub family chordopoxivirinae and belongs to the family poxviridae, it is wide spread infectious viral disease of old world camelid. New world camelids are also susceptible [5]. Camel pox is very host specific and does not affect other animals only one suspected case of human camel pox involving mild skin lesion has been described [6].

Based on sequence analysis, it has been determined that the camel poxvirus is the most closely related to variolavirus, the aetiological agent for small pox.

The Incubation period of poxvirus is about 3-15 days, the onset of skin lesion and fever is 3 days. The lesion begins as erythematous macules then to papules and vesicles, which rupture and make pustules, due to secondary infection or dry to form crusts, lesion may take up to 4 -6 weeks to heel, lesion begin in head then to neck and may be generalized to whole body [7].

The morbidity rate of camel pox is variable and depends on whether the virus is circulating in the herd. The incidence of disease is higher in males than females

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and the mortality rate is greater in young animals than in adults. Themortality rate in adult animals is between 5% and 28% and in young animals between 25% and 100% [8].

Transmission is by either direct contact between infected and susceptible animals or indirect infection via a contaminated environment.

Several diagnostic methods are available and, where possible, more than one should be used to make a confirmatory accurate diagnosis of disease. The fastest method of laboratory confirmation of camel pox is by the demonstration of the characteristic, brick-shaped orthopoxvirions in skin lesions, scabs or tissue samples using transmission electron microscopy (TEM). In addition, the paraffin-embedded samples can be stored for a long period of time, enabling future epidemiological, retrospective studies. Camel poxvirus may be propagated on the chorioallantoic membrane (CAM) of embryonated chicken eggs. After 5 days, characteristic lesions can be observed on the CAM. Camel pox virus shows typical cytopathic effect on a wide variety of cell cultures [9].

Intracytoplasmic, eosinophilic and inclusion bodies are characteristic of poxvirus infection and may be demonstrated in infected cells using haematoxyline and eosin staining. The presence of viral nucleic acid may be confirmed by polymerase chain reaction and different strains of camel pox virus may be identified using DNA restriction enzyme analysis. An antigen-capture enzyme-linked immune sorbent assay (ELISA) for the detection of camel pox virus has been described. A wide range of serological tests are available to identify camel pox. The tests used for the detection of the antibodies against camel pox virus include neutralization, agar gel precipitation, haemagglutination, haemagglutination inhibition, complement fixation, fluorescent antibody and antibody-capturing ELISA [10].

No successful treatment, but isolation of infected cases and supportive treatment (vitamin C) to increase body immunity against the virus and some antibiotic to avoid secondary bacterial infection, for example (Oxytetracycline) and antipyretic (NSAIDs). For the control and prevention of camel poxvirus, inactivated or live attenuated vaccine, vaccinia virus strains every 6 or 12 months before season of outbreak (usually from September to October) is used [11]. Therefore the objective of the study was to isolate and characterize the camel poxvirus from apparently sick camels in outbreak conditions.

MATERIALS AND METHODS

Study Area: The study was conducted from November 2013 to April 2014 in and around Jigjiga town. Jigjiga is a capital city of Somali Regional State found at Eastern Ethiopia. It is located 635 km from Addis Ababa. Somali Region covers the rangeland of southeastern part of Ethiopia. The total area estimated is about 382, 000 square kilometers. It is bounded to the North with Djibouti and Afar region, to Eastern and Southeast with Somalia and to the West by Oromia regional state. The climate of Somali regional state is arid and semi-arid. Rainfall is bimodal with an average precipitation of less than 200 mm, in the South East to some 600-700 mm in the North and West. Inarea bordering the high lands, there are four seasons locally known as "Gu, Dary, Kerent and Jilal" representing April to June, July to September, October to December and January to March respectively.

Study Animal: The study was carried on local breeds of camel in presumed camel pox outbreak in the area. The camels with camel pox clinical signs and lesions including fever, enlarged lymph node, skin lesion and lesion on the mouth where sampled [12].

Study Design: Purposive sampling type of camel pox outbreak study was conducted on field, watering point and grazing areas for isolation and characterization of camel pox in outbreak scenario from Somali region of Ethiopia by collecting skin biopsy (tissue scrapping) at the febrile stage of the disease.

Sample Collection and Transportation: Camel herds were observed to check the presence of pox lesions. Samples for virus isolation were taken from camels with typical pox lesions characteristics. Active skin lesions, healing skin scab as well as blood from actively sick camels were collected for the purpose of virus isolation and characterization on Vero cell cultures and/or direct detection of the virus by polymerase chain reaction (PCR) techniques.

Samples for virus isolation were collected, which is skin biopsy (tissue scrapping) at the febrile stage of the disease. A minimum of 2 g of skin biopsies (scabs) was minced with a disposable blade or sterile scissors and forceps and was collected for virus isolation. Tissue samples collected for virus isolation were placed in a virus transport medium, such as Tris-buffered tryptose broth,

kept at 4°C for transportation in icebox [13], to National Veterinary Institute, Bishoftu for virological and molecular analysis purpose.

Study Methodology

Viral Isolation

Tissue Sample Processing: Suspected clinical samples were taken from deep freeze and put it into an incubator at 37°C for 1 hourto warm the sample. Then 1 gram of tissue sample was taken and washed thoroughly in the mortar by phosphate buffered saline (PBS) that had antibiotics for each samples. The samples were washed about three times (3x) by PBS in every step and discarded. Then the tissue samples were crushed by scissor in the mortar and also further crushed by pistol and mortar until tissues were not visible. Then 9 ml of PBS with antibiotics was added to the mortar by using graduated pipette. PBS and tissues were mixed thoroughly in the mortar by pistol. Then the solutions were transferred into each test tube and labeled carefully. The test tubes were centrifuged at 3500 rpm for 10 minutes. The supernatants were taken from the test tubes by using syringe. Then bacterial and fungal agents [13] injected into the bottle with filter paper by using 0.04 µl size to avoid contamination.

Vero Cell Culturing: A 0.5ml of the filtered solution was individually inoculated on sub-cultured monolayer Vero cells after centrifugation of specimen at 4000 rpm for 20 minutes in tissue culture flask. The flask was then incubated at 37°C for 60 minutes to allow the virus to absorb on to the cell culture in humidified incubator and examined daily for Cytopathic Effect (CPE), which is observed as giant, multinucleated, Syncytium formation and detachment of cells. Each sample inoculated in Vero cells and un-inoculated flasks used as negative controls were included in each run. Each sample in the virus isolation was passaged three times in cell culture before declaring specimen negative. Those inoculated tissue sample which contained the virus caused specific morphological changes or cytopathic effects to the cell line [9].

Characterization of the Virus

DNA Extraction: DNA extraction using commercial kits, Qiagen, DNeasy, Blood and Tissue Kit were used for the extraction of DNA from the vero cell cultures showing cytopathic effect (CPE). Extractions were performed according to the manufacturer's recommendations. DNA extracts were kept frozen at -20°C until tested using PCR [14]. A generic PCR assay described by Meyer *et al.* [15], was used to detect the presence of Ortho Pox Virus (OPV) sequences in DNA from the vero cell culture showing Cytopathic effect (CPE). DNA amplification was carried out using camel pox specific primer pair of a forward primer (Campox F) having a sequence of 5' to 3' AAT ACA AGG AGG ATC T and a reverse primer (Campox R) having a sequence of 5' to 3' CTT AAC TTT TTC TTT CTC in volume of master mix for each samples containing 35 µl RNAase free water, Dream Taq buffer 5 µl, 1 µl of each dNTP, 1.5 µl of $MgCl_2$ (25 mM), 1 µl of each primer, 0.5 µl Taq DNA polymerase. The Prepared master mixs are placed into PCR tubes and 5 µl of DNA extract was added in to each PCR tube, then the mixture was centrifuged to homogenize. The samples (PCR tubes) were incubated in a thermal cycler and adjusted at First cycle: At 95°C (initial denaturation step) for 5 minutes, Second cycle: At 94°C for 1 minute, At 55°C for 1 minute, At 72°C for 2 minutes, final elongation at 72°C for 7 minutes [13].

Preparation of Gel and Gel Electrophoresis: Gel powder of 2 gm was weighed and mixed with TAB buffer of 198 ml to make 1% gel. Then it was melted in micro-oven and the solution was left to cool in 50°c bath water, swirling the flask occasionally to cool. Then the end of the casting tray was sealed with two layers of tape and placed the combs in the gel casting tray. Poured the melted solution into the casting tray and let cool until it was solid. Then carefully pulled out the combs and removed the tape. The gel was placed in the electrophoresis chamber (electrophoresis) and waited for 30 minutes at room temperature. Enough tris borate EDTA (TBE) Buffer was added so that there will about 2-3 mm of buffer over the gel [14]. Loading the gel was followed by added 2 ml of leading buffer to each PCR product and mixed them thoroughly. Then the gel was placed on to electrophoresis dish. Then lµl mixtures of the leading buffers and the sample primers complex were brought and negative control was put at right side. Then the marker, ladder, or reference was put which is 1000 base pair (bp) at the first and the last comb space or well. After that the dish was connected with power from electrophoresis machine and waited for one hour and 42 minutes [13]. The gel was removed from the casting tray by using glove and placed into the staining dish. Then warmed staining was mixed to gel and gel was allowed to stain for

30 minutes until the entire gel was becoming dark blue. Then pour off the stain and the gel and staining tray were rinsed with water to remove residual stain. The gel image was captured using UV camera in darkroom. The size of the positive PCR product (band) specific for the camel-pox virus (CPV) is 881bp [14].

Data Analysis: The raw data was entered and managed in Microsoft Excel worksheet and descriptive statistics is utilized to summarize the data. The data were analyzed using SPSS version-17 statistical software. Kappa statistic was used to determine the level of agreement between the tests used. A statistical significance of p -value < 0.05 and 95% Confidence level was taken. A kappa statistic value of < 0 as less than chance agreement, 0.01-0.20 as slight agreement, 0.21-0.40 as fair agreement, 0.41-0.60 as moderate agreement, 0.61-0.80 as substantial agreement and 0.81-0.99 as almost perfect agreement was taken [16].

RESULTS

Field Observation Result: During the study, detail physical examination was undergone. A as a result out of 450 camels were examined during the study period, only twenty two (22) camels were found with typical clinical signs of camel pox, that varies from mild localized to generalized pox lesions. Among the camels exhibiting clinical features of camel pox, ten of the camels were males and twelve were females with age of the camel ranging from 1 to 5 years and they were obtained from Fafan and Gursum.

Virus Isolation Result: Infected vero cells showing characteristic, plaque-type cytopathic effect (CPE) showing foci of rounded cells, detachment of cell sheet, giant cell and syncytia formation under inverted micros cope were considered positive [17], Vero cell culture 17 samples showed CPE 10 - 11 days post inoculation and five (5) samples were remained without showing CPE. The cells are then harvested to undertake Polymarase chain reaction after 80%-90% of the cell sheet shows cytopathic effect.

Polymerase Chain Reaction Result: Samples that showed CPE were processed by PCR and gene sequence, encoding the A type Inclusion protein (ATIP) was amplified. The size of the PCR product of 881 bp, specific for The camel poxvirus was obtained in 12 of the samples.

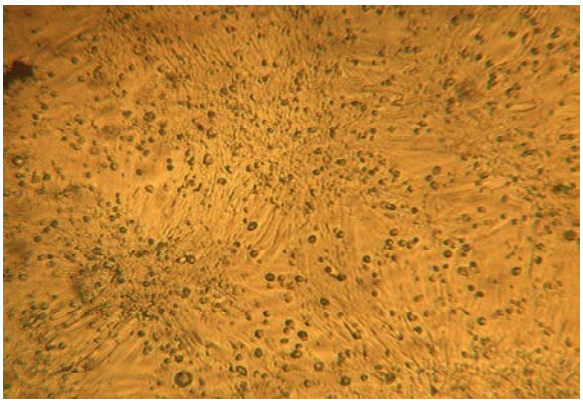

Fig. 1: Infected Vero Cells Showing Cytopathic Effect

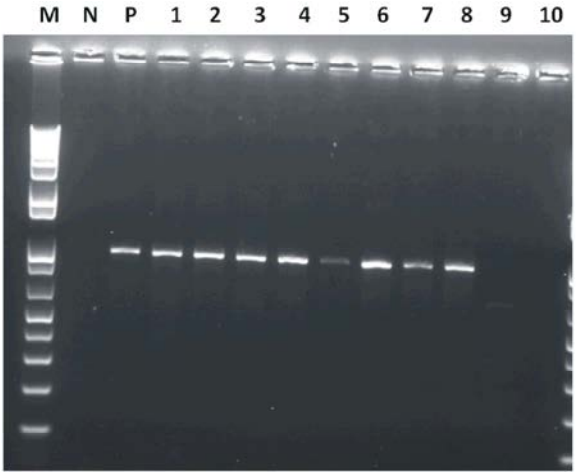

Fig. 2: Amplification of ATIP gene -881 bp by PCR.
M- Molecular marker-100pb (Fermentas);
N- Negative control without template (No amplification)
P- Positive control (around 881 bp)
1-8- samples positive for camel pox (around 881 bp)
9 and 10 samples negative for camel pox

Table 1: Camel pox infection with regards to risk factors

Risk factors		No. and % of Samples	No. and % CPE Positive	No. and % PCR Positive
Origin	Fafan	16 (72.7)	11 (68.7)	7 (43.7)
	Gursum	6 (27.3)	6 (100)	5 (83.3)
Age	Young	11 (50)	7 (63.6)	6 (54.5)
	Adult	11 (50)	10 (90.9)	6 (54.5)
Sex	Male	10 (45.5)	6 (60)	3 (30)
	Female	12 (54.5)	11 (91.7)	9 (75)

Table 2: Cross-tabulation of CPE and PCR results

Analysis Type	Kappa Value	P-value
Measure of agreement between CPE and PCR	0.330	0.078

DISCUSSION

Nomadic pastoralist to ensure food security by providing meat, milk production and serves as means of transportation and drought power even in severe condition where other livestock have difficulty of surviving keeps camels. Long lactation and ability to maintain milk production over long dry spells are important facets of camel production; therefore, camels are extremely important for livelihood of the pastoralist communities and their cultural life [2]. In the current study typical, clinical signs of camel pox were observed in infected camels with localized to generalized lesions. This is also supported by the idea of Gitao [18] which states that a typical camel pox virus infection cause localized and generalized lesions and OlE [13], that states camel pox is characterized by fever, local or generalized pox lesions on the skin and in the mucous membranes of the mouth, respiratory and digestive tracts. The clinical manifestations range from in apparent infection to mild, moderate and, less commonly, severe systemic infection and death.

Camel pox infection is usually commenced with numerous papules that cover the entire body of the camel. However, the present study also revealed that not all camels with clinical signs have the poxvirus. Because the papules of disease can be confused with an insect bite, ringworm infection and another non-specific conditions. As a result only 17 out of 22 (77.3%) of camels were truly infected by the virus, which shows the characteristic of plaque-type cytopathic effect (CPE). Consequently, it has been observed that diagnosis of camel pox, which only based on clinical signs, could lead to 22.7% of misdiagnosis. Camel poxvirus propagation (tissue) and identification of cytopathic effect that is characterized by showing foci of rounded cells, cell detachment and giant cell formation and syncytia that appear as soon as 24 hours post-inoculation [13].

The diagnosis of the suspected camel poxvirus in direct prepared samples was made by conventional polymerase chain reaction (PCR), which is the cardinal laboratory technology of molecular biology. PCR performed according to the method described by Paxson *et al.* [17], where specific 500 bp amplification products for Camel poxvirus were obtained. This finding is also has in agreement with those of Khalafalla and Mohamed [19] and Hanan *et al.* [20] in using PCR in diagnosis of camel pox virus where 881bp were identified as type of camel pox specific primer.

The PCR correctly identify twelve (12) out of twenty-two (22) (54.5%) positive cultures. PCR based pathogen detection is rapid and can detect much smaller quantities of pathogen than many other tests. With the PCR, it is possible to specifically address a particular DNA sequence and to amplify this sequence to extremely high copy numbers Anthony and Garret Weighted kappa [16], Quinin *et al.* [21]. It is also independent of host response and can distinguish vaccination from pathogen.

The kappa statistic is used to measure the agreement between two tests, cytopathic effect (CPE) and polymerase chain reaction (PCR) (Table 2 above) that shows the statistic value of kappa is 0.330 which shows fair agreement of two tests [16].

Although PCR based tests have great potential in pathogen detection, laboratory technicians and researchers should consider the limitations of PCR testing. A negative PCR test result will be generated due to absence of genetic material in the sample of an infected and clinically affected individual, the amount of DNA polymerase chain reaction and difference in primer-template, concentration ratio purity or quality of DNA templates [16].

In Ethiopia, despite of its significant contribution to the livelihood of the pastoralist and agro-pastoralist society, camel is one of the neglected domestic livestock by scientific community. Because regular vaccination, animal health service deliveries, promotion programs and research agendas are usually excluding camels. The few previously conducted studies mainly concentrated on the prevalence of specific diseases based on short time surveying and a limited sample size. The depth of information on camels and camel production has not been adequate to solve its multifaceted problems [1].

CONCLUSION AND RECOMMENDATION

Camel is one of the most important animals in the study areas and plays a central role in the socio-economic activities of the pastoralist and agro-pastoralist communities. Studies find out that the characteristic lesions and clinical signs are not enough for diagnosis of the disease, thus, special attention should be given on the epidemiological investigation and an integrated approach in the control of camel pox virus is required to obtain the maximum benefit of camel herding.

Therefore, to conduct comprehensive study and conduct diagnostic evaluation of PCR and other diagnostic methods of camel pox, it needs strong attention of the governmental and non- governmental Organizations (NGOs) support to promote programs and research agendas like National Veterinary Institute that initiates the Camel pox project.

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