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Epidemiology and Characterization of Camel Poxvirus in Northwest Costal Area of Egypt

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Abstract: Camel pox (CPV) is a highly contagious viral disease affecting mostly young animals and is characterized by popular, pustular eruptions on the skin and mucous membranes. Samples were collected from camel's herds of Matrouh and Alexandria Governorates. The isolated virus was isolated on chorioallantoic membrane (CAM) and Vero cells. Virus Neutralization Test (VNT) and Polymerase Chain Reaction (PCR) identified the isolated virus as camelpox. The overall morbidity and mortality rates were 52.4% and 5.7% respectively. In conclusion, vaccination, improved management and further education of herd owner about the etiology of camelpox have a paramount impact in reducing the circulation of CPV in camels. Further studies must be done to produce a local efficient vaccine.

Key words: Camelpox · VNT · PCR · Epidemiology

INTRODUCTION

Camel pox is a highly contagious viral skin disease, occurs in the dromedary and the Bactrian camel [1, 2]. The camelpox virus (CPV) causes a proliferative skin disease that primarily affects younger animals [3-5]. Camelpox virus (CPV) is a member of the genus *Orthopoxvirus* (OPV), subfamily *chordopoxvirinae* of the family *Poxviridae* a group of large double-stranded DNA viruses that replicate in the cytoplasm [6]. The disease is characterized by fever, enlarged lymph nodes and skin lesions. Skin lesions appear 1-3 days after the onset of fever, starting as erythematous macules, developing into papules and vesicles and later turning into pustules. Crusts develop on the ruptured pustules. These lesions first appear on the head, eyelids, nostrils and the margins of the ears. In the generalized form, pox lesions may cover the entire body. Skin lesions may take up to 4-6 weeks to heal. In the systemic form of the disease, pox lesions can be found in the mucous membranes of the mouth, respiratory and digestive tracts [7, 8]. Camelpox is diagnosed based on clinical signs, epizootiological and pathological findings [9], isolation of virus in chicken eggs and cell lines, a wide range of serological tests are available to identify camelpox. The tests include neutralization, agar gel precipitation, antibody-capturing ELISA [8, 10], polymerase chain reaction (PCR) and restriction enzyme protocols for detection and differentiation of species of the genus OPV were established [11]. During the past ten years, reports have appeared on outbreaks of camel pox from Egypt, Sudan, Niger, Ethiopia, Kenya, Morocco, Somalia, Mauritania Iraq, United Arab Emirates, Oman, Yemen, Bahrain, Saudi Arabia, Iran, Pakistan, India and the former USSR [12, 13]. The disease is endemic in these countries and a pattern of sporadic outbreaks occurs with a rise in the seasonal incidence usually during the rainy season [14].

The aim of the present work was to deal with camels that suffered from skin lesions suspected to be pox virus in Matrouh and Alexandria Governorates, Egypt, by trials of virus isolation and identification. AS well as describe the epidemiologic and clinical features of the disease.

MATERIALS AND METHODS

The Herd and Area of Study: The camel herd of Desert Research Center, Alexandria, Egypt was composed of 78 animals of different ages and sexes. The animals were kept

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in a fenced-land. Animal movement was allowed to graze within the boundaries of the research station. The station's management provided water and feed. While investigated camels of Matrouh Governorate (63 animals) were, mostly grazing animals belong to private owners.

Samples

Skin Lesions: During winter 2009, number of camels were reported to have nodular and proliferative skin lesions in Matrouh and Alexandria Governorates (Desert Research Center Station). According to the history, these animals had not been vaccinated against CPV infection. Dried scabs were collected from 10 selected cases showed different stages of skin lesions and kept in sterile vials contain glycerol buffer till used in the study. The collected scabs were ground in a sterile mortar according to Kenawy *et al*. [15]

Serum Samples: Forty serum samples were collected from camels with skin lesions and 20 samples were collected from the contact apparently healthy camels.

Hyperimmune Serum (HIS): Hyperimmune serum of camelpox virus was prepared in rabbits (2-3 kgms) according to the method described by Aboul Soud [16].

Camel Pox Virus: Camel pox virus (CPV jouf-78 strain) was kindly obtained from Pox vaccine production and Research Dept. Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

Virus Isolation

Specific Pathogen Free (SPF) Emberyonated Chicken Eggs (ECE): Fertile SPF-ECE (11-12) days old were obtained from Newcastle disease Dept., Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt. Emberyonated chicken eggs were inoculated by the prepared samples via chorio-allantoic membrane (CAM) route according to Robinson and Balassu [17].

Tissue Cultures: African green monkey kidney cells (Vero) were kindly supplied and propagated at Pox vaccine production and Research Dept., Veterinary Serum and Vaccine Research Institute (VSVRI). The CAM suspension obtained from infected SPF, ECE with characteristic pock lesions of (CPV) were inoculated into Vero cells after filtration through 0.45-µm filter according to Kaaden *et al*. and Abdel Bakey *et al.*[18, 19]

Virus Identification

Virus Neutralization test (VNT): VNT was conducted as described by Abdel Bakey *et al*. [20] to specify the virus isolate as a camelpox virus.

Polymerase Chain Reaction (PCR): Five samples from camels with skin lesions were tested by PCR, Briefly; DNA was extracted from each sample by using DNA extraction QiaAmp DNA kit (Qi A Gen, Valencia, California, USA). The Samples were amplified using PCR Reddy Mix PCR master Mix (Thermo, UK). PCR assay was applied as described by Meyer *et al*. [21] allows the detection and differentiation of species of the genus orthopoxvirus because of the size differences of the amplicons. Using the primer pair: 5`-AAT-ACA-AGG AGG-ATC-T-3`-and 5'-CTT AAC-TTT-TTC-TTT-CTC-3` the gene sequence encoding the A type inclusion protein (ATIP) will be amplified. The size of the PCR product, specific for the camelpox virus is 881 bp. The test was applied on PCR thermal cycles machine (AB1, 2720).

Serological Investigation by Neutralization Test (SNT): All collected sera were screened against the identified locally isolated CPV kindly supplied from (VSVRI) according to method described by Beard [22]. The neutralization index (NI) was calculated according to Reed and Muench [23]. NI = VT (virus titer)-SVT (serum virus titer).

Treatment: A combination of long acting Oxytetracyclin (10mg/kg. b. wt.) twice a week at 3 day's interval and antihistaminic (1 ml/30 kg. b. wt.) at 24 hours of interval, to protect diseased animals against secondary bacterial infection. A local antiseptic solution (Betadine) was used as wound dressing.

RESULTS

Clinical Manifestation: The affected camels were febrile, depressed, prostrated and anorexic. They lost weight and were emaciated because of restricted suckling and grazing due to extensive mouth lesions. The sick animals suffered from purities as manifested by loss of hair on affected parts due to rubbing against hard objects. Papules vesicles, ocular lacrimation and thick scabs present on the lips and nostrils Figures (1 to 4). The prevalence rate of camelpox virus was 42.2 and 65.1% at Alexandria and Matrouh respectively (Table 1). By age, the morbidity and

Fig. 1: Young camel showing pustules on nostrils, upper, lower lips and eyelids.

Fig. 2: Vesicles on the inner side of the upper lip.

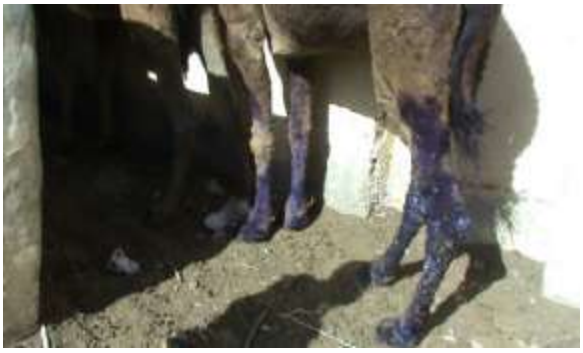

Fig. 3: Young male camel with pox lesion on all 4 limbs.

Fig. 4: Male camel-calf with sever pox lesion (eye and buccal cavity).

Table 1: Prevalence of Camelpox virus (CPV) according to examined area by clinical examination

		Infected		Negative	
Area	Examined	No.	%	No.	%
Alexandria	78	33	42.4	45	57.6
Matrouh	63	41	65.1	22	34.9
Total	141	74	52.4	67	47.5

mortality rates were highest in the less than one year age group 80 and 12.7% respectively, while the lowest morbidity rate was in the adult group 22.0% and mortality rate was nil (Table 2). Considering the sex, the morbidity and mortality rates were higher in females than in males 57.8 and 5.9 and 38.5 and 5.1% respectively (Table 3). Abortion was occurred in three of 27 pregnant she-camels (11.1%) of Desert Research Center Station.

Propagation of the Isolated Virus on CAM of ECE: Isolated virus was inoculated on CAM of SPF-ECE 9-11day old for 10 successive passages resulting in the characteristic pock lesion of CPV (circular, opaque, white enlarged areas) as illustrated in fig. 5.

Table 2: Prevalence of Camelpox virus Infection according to age by clinical examination

Age	No. of animals	morbidity	%	Mortality	%
Less than 1 year	55	44	80	7	12.7
More than1 year to 5 years	36	19	52.7	1	2.8
More than 5 years	50	11	22.0		
Total	141	74	52.4	8	5.7

Table 3: Prevalence of CPV according to gender.

Gender	No. of animals	morbidity	%	Mortality	%
Male	39	15	38.5	2	5.1
Female	102	59	57.8	6	5.9
Total	141	74	52.4	8	5.7

Table 4: Neutralization index of diseased and contact camels

Area	Numbers of diseased camels	Neutralization index (NI)	Number of symptomless camels	Neutralization index (NI)
Alexandria	4	1.5	1	0.9
	4	1.6	2	0.7
	5	1.7	2	0.8
	4	1.8	3	0.6
	3	1.9	2	0.5
Total	20	10		
Matrouh	3	1.5	2	0.6
	3	1.6	2	0.8
	4	1.7	1	0.7
	4	1.8	1	0.5
	6	1.9	3	0.3
	1	0.4		
Total	20	10		

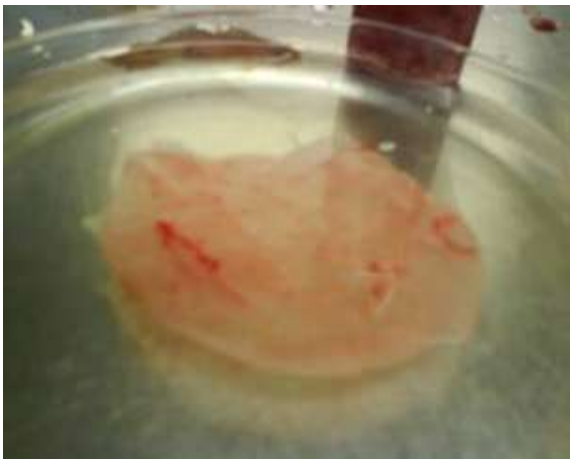

Fig. 5: Numerous pock lesions post CAM inoculation.

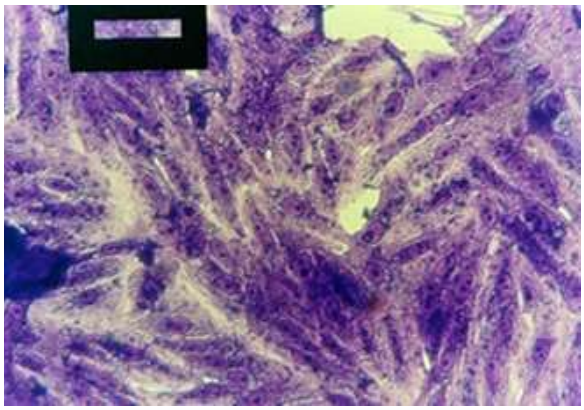

Fig. 6: CPE of camelpox virus after 48-hour post infection (high power).

Propagation Isolated Virus in African Green Monkey Cells (Vero Cell Lines): Isolated CPV was inoculated on Vero cell line, the isolated CPV was produce the cytopathic effect CPE of CPV after the third passage

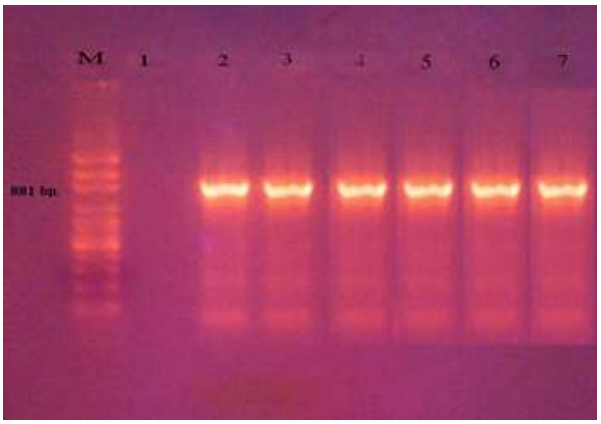

Fig. 7: Polymerase Chain Reaction (PCR) to detect camelpox virus.
Lanes M, 100-bp molecular weight marker; 1, Negative control; 2, camel positive control (881 bp); 3-7, Camelpox positive examined samples.

4-5 days post inoculation the CPE was characterized by cell rounding, cytoplasmic elongation, multinucleated giant cell formation, aggregation and detachment of the cell sheet (Fig. 6).

Application of Virus Neutralization Test in Vero Cell Line for the Propagated Isolated Camelpox Virus (Alfa Procedure): VNT shows reduction of virus titer with 2 $\log_{10}TICD_{50}$/ml with neutralizing index of 2.0. For both isolated virus passages no. (5) and (10).

Polymerase Chain Reaction: The examined five samples showed the characteristic PCR positive bands of 881 bp. size fragment of camelpox virus (Fig. 7).

Serological Investigation by Neutralization Test: The results of SNT applied on collected serum samples were in table 4.

The neutralization index (NI) of positive samples was more than or equal 1.5. The infection rate was 100%.

Treated animals recovered within 2 to 3 weeks from the onset of the disease.

DISSCUSION

Poxviridae is a very large family of ds-DNA-containing viruses that infect mammals, birds and insects. Viruses that infect vertebrate are classified in the subfamily chordopoxvirinae [24].

Pox is the most frequent infectious viral disease of the camel and therefore the most widely reported [8].

In this study, the typical clinical sings of CPV were observed, where affected camels showed signs varied from acute (camel calves) to mild infection in adults, as the disease possibly manifested itself as two or more clinical forms [8, 25].

In this study, the morbidity and mortality rates of CPV were 52.4 and 5.7% respectively. Nawal *et al.* [26] reported the isolation of CPV from Matrouh Governorate and Dafalla and Elfadil [27] in previous study reported a 41% and a mortality of 3.6% Jazan region, Saudi Arabia. However, Salem *et al.* [28] recorded a 100% morbidity rate and a zero mortality. The morbidity and mortality rates vary according to the geographic location, season and the strains of camelpox viruses. Epidemics usually occur in regular cycles dependent on the rainy season and relationship of the density of the insect population to the number of immune camels in the population [8, 29]. The clinical signs observed in this study demonstrate that camelpox virus can affect camel of both sexes at any age; however, camels of less than one year and between 1-5 year were the age group commonly affected 80% and 52.4%, respectively.

Deaths attributed to CPV occurred mainly in ages less than one year 12.7%, however, a zero mortality were recorded in animals over five years old. This coincides with reports from other investigation of Dafalla and Elfadil [27] and Sharawi *et al.* [29]. Three of 27 pregnant she-camel (11.1%) were aborted. Hafez *et al.* [30] reported Abortion in CPV infected she-camels.

CPV can be isolated and propagated in chick embryos and large variety of cell culture including Vero cells [11, 31]. Inoculation of materials from suspected camelpox lesions in CAM and Vero cells were done successfully where distinct pock lesion and characteristic cytopathic effects were observed on CAMs and Vero cells, respectively [13, 32, 33].

Virus neutralization test (VNT) was used in this study both for identification of the isolated virus as well as a diagnostic test of CPV in tested sera. The results confirmed the identity of CPV isolates used in the present study using the hyperimmune serum against CPV [34, 35].

The results of SNT on tested serum samples of infected camels gave a neutralization index more than or equal 1.5; which indicates a cross-reaction between the used virus (jouf-78) and antibodies of serum of infected camels. Similar results obtained by Tantawi *et al*. [31] and Sheikh Ali *et al*. [36].

On the other hand, Murphy *et al*. [37] reported 95% positive cases in Sudan, which was confirmed in 72.5% by Khalafalla and Mohamed [34].

Pfeffer *et al*. [38] found prevalence between 88% and 100% in 1,000 dromedaries.

The initial diagnosis of the suspected CPV in direct prepared samples was made by conventional PCR according to the method described by Meyer *et al.* [21] where specific 881 bp. amplification products for CPV was obtained. These findings agree with those of Meyer *et al*. [28], Salem *et al*. [29], Sheikh Ali *et al*. [36], Sharawi *et al.* [39], Yousif *et al.* [40] and Bassiouny [41].

CONCLUSION

Along with a vaccination program, improved management strategies could diminish the prevalence of the disease. Further education of herd owners about the etiology of camel pox, strict separation of diseased and healthy young camels, improved health care including long acting antibiotics, improved hygiene, general supportive treatment and control measures would be of immense value to curtail the infection in the field and have a paramount importance in reducing the circulation of CPV in camels.

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