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SOME BIOLOGICAL PROPERTIES OF CAMEL POX VIRUS

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SUMMARY

On the end of December 2001, mild local exanthemas and generalized pox-like lesions were observed in dromedary camels herd at Badrashin locality - Giza Governorate. Skin lesions (scabs&crusty materials) were scraped from the nose, upper and lower lips, upper and lower parts of the neck and the external sides of the hind limbs of affected camels (2-3 years old) then prepared aseptically for virus isolation on VERO cells and chorio-allantoic membrans (CAMs) of embryonated chicken eggs(ECE).

54 serum samples were collected randomly from apparently healthy camels found in the neighbouring villages,and tested by Serum Neutralization Test (SNT) and Agar Gel Precipitation Test (AGPT) for detection of specific neutralizing and precipitating antibodies of camel pox virus.

The results revealed the isolation of camel pox virus (CPV) from skin lesions on VERO cells and CAMs of ECE. The virus was identified by virus Neutralization test VNT and AGPT using reference camel pox hyperimmune serum. 8 out of 54 sera sample were positive by SNT with percent of 14.8 where as only 3 samples were positive in AGPT with percent of 5.6.

The isolated camel pox virus was designated as Badrashin isolate. The grouth behavior on VERO cells and ECE of the virus and its haemagglutinating activity were studied and compared with the previous Marsamatroh isolate. The results indicated that both isolates were considerably similar.

INTRODUCTION

Camel pox (CP) is a contagious eruptive skin disease caused by highly epithelio-tropic camel pox virus (CPV) belonging to genus Orthopoxvirus of Family Poxviridae (Wernery and Kaaden,1995). It is transmissable to man, thus creating a potential risk to handler's (Kriz, 1982. and Jezek, et al.1983).

The disease mostly affects young camels between 6-12 months of age, but when it occurs in a susceptible herds, all ages may be affected and the younger usually show more severe signs than older ones, causing high morbidity rate ranges from 25-100% in camels calves with mortality rate ranges from 5-25% in older camels with incubation period ranges from 10-15 days (McGrane&Higgins, 1985.; Khalafalla, et al. 1998.; Kinne, et al. 1998.; Pfeffer, et al. 1998.; Wernery & Zuchariah 1999, and Wernery, 2000).

Transmission of CP-disease occurs horizontally through direct contact between sick and healthy camels or by biting insects and by vectors such as tools and bridles (Richard, 1979 and Munz, 1992), but (Wernery, 2000) said that the most important vector seems to be dust originating from highly infecting scabs.

CP-epidemics generally occur every 3-5 years mostly during the raing wet seasons and often associated with the stress of weaning, immunological and nutritional status of the herds (Munz, 1992.; Al-Hendi, et al.1994.; Gitao, 1997.; Wernery et al. 1997 and Khalafalla, et al. 1998). It is still a mystry why camel pox disease is becoming more severe during the rainy seasons and usually follows a mild course in dry hot seasons. One explanation for this phenomenon could be that a larger population of insects build up during the rainy season, this enhancing a greater virus pressure and higher virus doses onto the camel population (Pfahler and Munz, 1989 and Wernery ,et al. 1997).

The onset of the disease is manifested by fever, weakness, papules and vesicles on the upper and lower lips, on nostriles and eyelids, as well as , oral and nasal mucosa with swallen head in some younger camels calves, the oedema may be so severe that the animal could not open his eyes,unable to see and wandered aimlessly, loss of condition occurred because the affected animal unable to eat or drink, these signs lasts for one week followed by crusts and scabs formation on the affected areas (Tantawi et al.1974.; Al-Faluji, et al. 1979.; Kriz, 1982.; Muntasir, et al, 1988.; Kenawy, et al. 1989 and Khalafalla, et al. 1998). Conjunctivitis with mucopurulent discharge were seen in some of the affected animals and in severe outbreaks the eruptive lesions are distributed over the entire body (Munz, 1992.; Pfehler and Munz,1989.; Wernery, et al 1997 and Pfeffer, et al. 1998). The pox lesions were detected in the internal organs mainly oesophagus, trachea and lung of the sick camels (Kinne, et al. 1998; Pfeffer, et al. 1998 and Wernery and Zuchariah, 1999). The recovered camels seem to aquire life long immunity to re-infection (Higgins, 1986),

CP-disease was initially described in India in 1909 (Leese,1909), subsequently it was reported in the Soviet Union in 1966 (Ramyer & Hassame 1972), Iran (Buxby, 1972) Egypt in 1974 (Tantawi,et al. 1974) Iraque (Al-Faluji, et al. 1979),

Yemen, Afganistan, and Pakistan (Odend Hal, 1983), Saudi Arabia (Hafez, et al, 1986), Morocco, Ethiopia, Oman and Sudan (Shommien & Osman, 1987).Niger(Nguyen, et al. 1989) and again in Saudi Arabia in 1992 (Hafez, et al, 1992) and in 1993 (Al-Hendi, et al. 1994) and in 1997 (Gabry, et al. 1997) and in UAE in 1992(Kaaden, et al. 1992) and again in UAE in 1996 (Pfeffer, et al, 1996a).

So, the disease was greatly incriminated as one of the major skin diseases of camels particularly in countries of Africa, Asia and the Middle East (Wernery, et al. 1997. and Wernery. 2000).

In the present study, clinical and virological investigations of a field problem of a disease causing skin eruption in camels in Egypt were carried out to isolate the causative agent (suspected CPV) and comparison of such isolate with the previous CPV strain. Also the pathogenesity on VERO cells, CAMs of ECE and haemagglutinating activities of the isolated CPV on human (O), sheep chicken, mice and G. pigs RBCs were studied.

MATERIALS & METHODS

[A] Materials:

1- Skin lesions: Scabs & crusty materials were removed from the nose, upper & lower lips, upper parts of the necks and the external sides of the hind limbs of dromedary camels (2-3 years old) persisted at Badrashin locatity- Giza Governorate (some of the affected camels showed mild local exanthemas and the others had generalized pox-like lesions). Samples were prepared for virus isolation on VERO cells and on CAMs of ECE.

2- Camel sera: A total of 54 sera sample was collected from apparently healthy camels located in the neighbouring villages to the infected area (no history of vaccination agianst CPV). The sera were stored at -20°C untill tested by AGPT and SNT for detection of specific CPV antibodies.

3- Tissue culture: VERO cells (a cell line derived from African green monkey kidney) were grown in Minimal Essential Medium (MEM) containing 5% foetal calf serum and antibiotics (100 Iu/ml penicillin + 100ug/ ml streptomyin). The same media but with 2% calf serum was used as maintenance media. VERO cells were used for virus isolation and in Neutralization test (NT).

4- Embryonated chicken eggs (ECE): 10-11 days old embryonated chicken eggs were used for virus isolation via CAMs . Control non inoculated eggs were also included.

5- Camel pox antigen: An attenuated camel pox vaccine produced by National Agriculture and Water Research Center, Riyadh, Saudi Arabia, from camel pox isolate designated Jouf-78 (Hafez, et al 1992). The antigen was used for detection of specific CP-antibodies in the collected sera by AGPT and SNT.

6- Camel pox hyperimmune serum: kindly supplied from Vaccine and Serum Production Research Institute- Abbasia. Egypt (SNT titer 1/64). The serum was used in AGPT and VNT

7- Marsamatroh CPV isolate : Scabs which collected from naturally infected camels at Marsamatroh Governorate and containing CPV particles detected by electron microscope (Maysa, et al., 1998).This isolate was used in studies the pathogenesity on VERO cells and CAMs and its haemagglutinating activities on human (O), sheep , chicken mice and G. pigs RBCs was compared with the new isolated CPV in the present study.

[B] Methods:

[I] Virus isolation :

1- **Preparation of skin lesions (scabs & crusty materials) for virus isolation:** The method of preparing the samples was carried out according to (Wernery, et al. 1997) as follows: Scabs & crusty materials were pooled and crushed in 8 ml sterile Phosphate Buffered Saline (PBS) and sterile sand, the mixture was centrifuged at 2000 r.p.m/20minutes then freezed and thawed twice, 1ml of the supernatant was incubated with 4ml MEM containing antibiotics for one hour at room temperature and overnight at 4°C then filtered through 0.45 um filter (Millipore), this filtrated solution was used for virus isolation on VERO cells and ECE via CAMs. The same method was carried out on scabs which collected from naturally infected camels at Marsamatroh Governorate).

2- **VERO cells:** The method of infecting the cell culture with the previously filtrated solutions was adopted according to (Wernery, et al. 1997).

3- **Embryonated chicken eggs (ECE):** The technique of eggs inoculation via CAMs with the previously filtrated solutions was applied according to (Pfeffer, et al.1996b).

[II] Identification of the isolated virus:

a) Neutralization test (NT): was performed on VERO cells according to (Pfeffer, et al. 1996 b).

At first the infectivity titer of the isolated virus was determined as follows: the infected VERO

cells that inducing CPE (the harvest of the 1rt passage of the virus) after they inoculated by the filtrated solution of scabs & crusty materials) was titrated.

Titration was performed through preparation of serial ten fold dilutions of the virus (isolate) from 10^{-1} to 10^{-8} in sterile MEM. Form each dilution (100 ul) was inoculated in 4 wells in a tissue culture plate containing confluent VERO cells, then incubated and examined daily for the presence of CPE. The end point was determined and the titer of the isolate ($TCID_{50}$) was calculated according to the method described by (Reed & Muench 1938).

The same method was applied on the infected VERO cells that inducing CPE (the harvest of the 1rt passage of Marsamatroh isolate) and it's titer was calculated.

SNT:

Serial-tenfold dilutions of the virus (isolate) was prepared in MEM, each dilution was mixed with an equal volume of a constant dilution of reference CP-hyperimmune serum (1/64), and inoculated into confluent VERO cells in tissue culture tubes. The cells observed daily untill complete neutralization was accrued (no CPE).

Neutralization Index (NI) was calculated (the difference between the virus titer (in log 10) in presence of diluent minus the titer in the presence of CP-hyperimmune serum. If NI is more than 1.5, the isolate is considered CPV (O.I.E. 1996).

(b) Agar Gel Precipitation Test (AGPT):

The inoculated VERO cells that gave CPE (the harvest of the 1rt passage) and the CAMs had pock lesions (the harvest of the 3rd passage) as well as 10% suspension of crushed scabs & crusty materials (collected from Badrashin locality) were tested against reference CP-hyperimmune serum by AGPT according to (O.I.E. 1989).

[III] Camel pox antibodies detection:

1) **AGPT** : was applied according to (O.I.E. 1989) on the collected sera against attenuated CP-antigen.

2) **VNT:** Was conducted for detection and titration of specific neutralizing CP- antibodies in camels serum samples according to (Pfeffer, et al. 1996 b), and the titer was calculated according to (Reed & Muench, 1938).

[IV] Haemagglutination activities of Badrashin and Marsamatroh CP- isolates on RBCs of various species:

Haemagglutintion test:

Blood of human (0), sheep, chicken, mice, and G.

pigs were used in this test which carried out according to (Wernery, 2000) as follows:
50 ul of diluent (PBS) was dispensed into all wells of microtiter plate (U-shape).

50 ul of the harvest VERO cells inducing CPE was dispensed into the first well of the plate.

Two-fold serial dilutions were applied ($^1/_2$, $^1/_4$, $^1/_8$,...., ..., $^1/_{1024}$).
50 ul of 0.5% G. pig RBCs was added to each well of the plate, then gentile shaking, the plate was covered and incubated at room temperature.

The end point was calculated the highest dilution of the virus isolate which haemagglutinate G. pig RBCs.

The test was applied as mentioned before with all types of RBCs and with the Marsamatroh isolate. Neumber of HA units/ml of the virus were determined with each type of RBCs mentioned before.

RESULTS

(1) Clinical finding :

On the end of December 2001, mild local exanthemas and generalized pox-like lesions were observed in dromedary camels hard at Badrashin locality. Giza Governorate. The skin lesions were observed definitely on the nose, upper & lower lips , upper & lower parts of the neck and the internal & external sides of the limbs of the affected camels.

(2) Isolation of the causative agent :

(a) On VERO cells :

The cytopathic effect (CPE) appear on the 1rt passage at 5 days post-inoculation in the form of polynucleited gaint cells (syncytia).

(b) On CAMs of ECE:

Pock lesions were observed clearly on the 3rd passage at 6 days post-inoculation as proliferative small whitish spots.

(3) Identification of the isolated virus (designated as Badrashin isolate):

The harvest of the 1rt passage on VERO cells (Badrashin isolate) had infective titer $10^{5.4}$ $TCID_{50}$/ml. The harvest of the 1rt passage on VERO cells (Marsamatroh isolate had infective titer $10^{5.3}$ $TCID_{50}$/ml.

(a) By VNT:

Complete neutralization was occurred (no CPE) 5-days post-inoculation in VERO cells that inoculated with the virus-serum mixture (virus isolate titer equal $10^{3.5}$ $TCID_{50}$/ml) , i.e the neutralizing index (NI) is more than 1.5.

$NI = 10^{5.4} - 10^{3.5} = 10^{1.9}$ $TCID_{50}$ /ml.

(b) By AGPT: By testing the inoculated VERO cells that showing CPE and 10% homogenate of CAMs having pock lesions against reference CP-hyperimmune serum a definite precipitin lines were observed and by testing a 10% suspension of crushed scabs & crusty materials (which collected from Badrashin locality) in sterile PBS against CP-hyper immune serum precipitin line was also detected.

(4) Camel pox antibodies detection :

The results of AGPT & SNT which carried out on 54 serum samples which collected from apparently healthy camels (located in neighbouring villages to the infected camels) revealed that 3 out of 54 were positive by AGPT with percent (5.6%) and 8 of them were positive with percent (14.8%) by SNT (Table.I).

(5) Growth behavior of Badrashin and Marsamatroh CPV isolates on VERO cells and CAMs of ECE:

The harvest of the 1st passage of VERO cells (Badrashin isolate) was inoculated into VERO cells inducing CPE 5 days post inoculation in the form of giant cells formation & pock lesions were observed 6 days post inoculation as proliferative small whitish spots. Also from the filtrated solution that prepared from scabs of infected camels with Marsamatroh strain, VERO cells and CAMs of ECE were inoculated. VERO cells induced CPE on the 1rt passage, 6 days post-inoculation in the form of cells rounding, gaint cells formation, also, pock lesions were observed clearly on CAMs on the 3rd passage, 5 days post-inoculation as diffuse opaque thickening with slight haemorrhagic spots.

Table (I) : Results of AGPT & SNT which carried out on 54 serum samples collected from apparently healthy camels at Badrashin locality.

Tested Sera	AGPT				SNT						
	+ve	+ve %	Total -ve	-ve %	+ve			Total +ve	+ve %	Total -ve	-ve %
					1/10	1/20	1/40				
54	3	5.6%	51	94.4%	5	2	1	8	14.8%	46	85.2%

Table (II) : Haemagglutnating activities of Badrashin and Marsamatroh CPV isolates on RBCs of various species.

Animal spcies	RBCs of different species				
	Human (0)	Chicken	G. pigs	Mice	Sheep
HA titer of Badrashin isolate	512	256	256	-vc	-ve
HA titer of Marsamatroh isolate	512	256	128	-vc	-ve

(6) Haemagglutinating activities of Badrashin & Marsamatroh CPV isolates on RBCs of various species.

Results are expressed in Table (II). In these results HA of the two isolates was equal with human (0) and chicken RBCs (512 or 2^9 and 256 or 2^8 respectively. G. pigs RBCs gave HA titer 256 or 2^8 with Badrashin isolate and HA titer 128 or 2^7 for Marsamatroh isolate. Sheep and mice RBCs have no haemagglutination with the two islates.

DISCUSSION

Pox-like exanthema in camels can be caused by an infection with camel pox virus, camel contagious ecthyma virus or camel papilloma virus (Munz, 1992. and Wernery and Kaaden , 1995). Current standared methods for identification of the causative agents are electron microscope and virus isolation (Mayer and Czerny, 1990), but (Pfeffer, et al. 1996b) said that the most sensitive method was the polymerase chain reaction (PCR) followed by virus isolation and electron microscope respectivelly, while (Tantawi, et al. 1974.; Kenawy, et al. 1989.; Kaaden, et al. 1992.; Wernery, et al. 1997.; Khalafalla, et al. 1998 and Wernery & Zuchariah, 1999) reported that the diagnosis of camel pox was based on the history, clinical signs, and the course of the disease, i.e, the presence of the various forms and sizes of skin lesions on the different parts of the infected animal body in association with systemic involvement including anorexia and fever suggest that the disease was camel pox.

In the present study we depend on the clinical finding where, mild local exanthemas were shown on some of camels and generalized pox-like lesions (papules &scabs) on others. The skin lesions were found definitly on the nose, upper & lower lips, upper & lower parts of the necks, and the internal & external sides of the limbs of affected camels, simillar signs were previously ob-

served by (Tantawi, et al. 1974:, Muntasir, et al.1988:, Kenawy, et al. 1989:, Munz, 1992:, Pfeffer, et al. 1996a:, Wernery, et al. 1997:, Khalaffalla, et al. 1998:, Maysa , et al. 1998, Wernery & Zuchariah, 1999.; and Zaitoun, et al 2000.).

Trials were conducted for isolation of the causative agent on VERO cells and on embryonated chicken eggs (ECE). The virus was found to be able to replicate on the 1rt passage of VERO cells inducing cytopathic effect (CPE) characterized by polynucleited gaint cells (syncytia) 5 days post-inoculation, this result coinicided with the pervious reported studies (Kaaden, et al. 1992.; Klopries, et al. 1995.; Renner-Mueller, et al. 1995.; Wernery & Kaaden, 1995.; Pfeffer, et al. 1996b.; Munz. et al. 1997.; Wernery, et al., 1997. and Wernery, 2000). Also, it was found to be able to replicate on the CAMs of ECE (10-11 days old) producing proliferative small whitish pock lesions on the 3rd passage at the 6th day post-inoculation as previously reported (Marennikova, et al. 1974.; Nakano& Bingham, 1974.; Tantawi et al. 1974.; chauhan and Kauskik, 1987.; Al-Hendi, et al. 1994.; Munz, et al, 1997.; Maysa, et al. 1998. and Zaitoun, et al. 2000).

The identification of the isolated virus was obtained through: (a) VNT, where complete neutralization was occurred (no CPE) in VERO cells that inoclated with the virus - serum mixture and gave isolate titer equal $10^{3.5}$ $TCID_{50}$/ml with neutralizaing index NI= 1.9 i.e more than 1.5. this specfic neutralization and according to (O.I.E. 1996) that reported if NI is more than 1.5, the isolate considered CP-virus, i.e the harvest of the 1rt passage on VERO cells contained CP-virus, these findings coincided with (Al-Hendi, et al. 1994; Munz, et al 1997 and Pfeffer et al. 1996). (b) AGPT: By testing the inoculated VERO cells that showing CPE and 10% homogenate of CAMs having pock lesions against reference CP-hyperimmune serum, a definite precipitin lines were observed, also, by testing a 10% suspension of crushed scabs & crusty materials in sterile PBS (without any other treatment) against reference CP- hyperimmune serum precipitin line was also detected. The detection of CP -antigen by AGPT using reference CP-hyperimmune serum indicate infection and confirm the identity, similar results were previouslly obtained by (Borisovich 1974.; Marennikova , et al. 1974.; Nikano and Bingham, 1974, Tantawi, et al. 1974.; and Maysa, et al. 1998).

On the other side and from Table (I) the presence of specific precipitating & neutralizing antibodies in serum samples of apparently healthy camels (3 out of 54 were positive by AGPT with percent (5.6%) and 8 of them were positive with percent (14.8%) by SNT) without history of vaccination and without developing the disease, indicated that camels can be infected subclinically.

Precipitating antibodies only detected by AGPT in boostered or recent infected animals because this test needs high quantity of antibodies to react with solid antigen forming a definite precipitin line. So, with the absence of vaccination program, the detected precipitating antibodies indicated recent infection with CPV.

Concerning the comparison which carried out between the isolated CPV (Badrashin isolate) with other isolate (Marsamatroh CPV isolate) for their pathogenesity on VERO cells and on CAMs of ECE, it can be noticed the successful propagation of these isolates (Badrashin CPV isolate & Marsamatroh CPV isolate) on VERO cells inducing CPE on the 1rt passage at 5th, 6th day post-inoculation with virus titer measuring $10^{5.4}$ and $10^{5.3}$ $TCID_{50}$/ ml respectively, the CPE was characterized mainly by giant cell formation for the two isolates, i.e the two isolates are nearly similar in their pathogenesity on VERO cells (Pfeffer, et al.1996a). Also, it is obvious that these isolates are also similar for their pathogenesity on CAMs of ECE inducing pock lesions characterized mainly by haemorrhagic oedematoes small spots usually on the 3rd passage, in average 5 days post - inoculation (Renner-Mueller, et al. 1995).

We studied haemagglutinating activities of the isolated CPV strains on erythrocytes of different species, it is clear from Table (II) HA of the two isolates was equal with human (0) and chicken RBCs (512 or 2^9 and 256 or 2^8 respectively. G. pigs RBCs gave HA titer 256 or 2^8 with Badrashin isolate and HA titer 128 or 2^7 for Marsamatroh isolate. Sheep and mice RBCs have no haemagglutination with the two isolates. These results agreed with (Wernery, 2000) who denote that CP-virus RBCs receptors are found in the aforementioned animal species, which abscent in non-haemagglutinating RBCs of other species.

Camel pox disease occurs wherever camel husbandary is persisted (Munz, et, al. 1986 and Wernery, 2000), an exeption is the Australian dromedary population , where sofar camel pox has not been reported (Hafez, et al. 1992). Between 23-24 million camels are distributed in Africa, Asia, India and the Middle East, about 150,000 camel are found in Egypt (General Organization for Veterinary Services statistics. 2001).

From the obtained results, we can find that the isolation of CPV from clinically diseased camels persisted in Giza Governorate and detection of CP- antibodies in apparentally healthy camels located in some villages belonging to Giza Governorate. This isolation and detection of CP-antibodies with the abscence of applying a vaccination programme against CP- disease in Egypt give an indication for the prevalence of the dis-

ease and that means its enzootic in Egypt. Therefore, it would be of great value for the epidemiology of CP to compare the many CP strains isolated in order to prepare an effective and safe vaccine because uptill now, nomads or farmers and camel owners sometimes protect their calving by dissolving scabs from affected animals in milk and rubbing the mixture on the calves scarified lips and even veterinarians treat the affected camels symptomatically and this leads to the persistence of the virus. Another need to compare the many CP-strains isolated is: The disease causes great economic losses in camel breeding areas in term of high morbidity and relatively high mortality rates particularly in younger, reduction of milk yield in lactating she-camels, loss of weight in association with debilitation in camels of all ages, and the lowest price of the clinically diseased camels with pox virus infection in the markets (Fenner, et al. 1989.; Munz, 1992. and Kinne, et al. 1998).

In conclusion we present data about some biological characteristics of two CPV isolates on VERO cells and on CAMs of ECE and their haemagglutinating effects on different species of RBCs, the results revealed that the two CPV isolates are nearly similar, This will be extremely important for our future vaccination programme especially when all CPV isolates are very or nearly similar in their biological properties and in this case there is no need to change the vaccine strain.

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