Isolation and Molecular Characterization of Camel Pox Virus

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SUMMARY

Camel pox one of the economic disease of dromedary infected camels. The skin eruption one of the characteristic lesion which appear in various stages of the infection. The samples were collected from camel flock in Southern Sinai [sera and skin lesions (scabs)]. The camel pox virus (CPV) were isolated on chorioallantoic membrane (CAM), and Vero cells. The isolated virus was identified by Agar Gel Precipitation Test (AGPT), electron microscope (E.M.) and Polymerase Chain Reaction (PCR). E.M. considers one of the rapid and specific methods of laboratory confirmation. E.M. was done on CAM samples with characteristic pock lesions, and detection of a typical brick shape of orthopox virus in the cytoplasm of the infected cells. Applying PCR technique considers the faster and more sensitive molecular advanced technique for diagnosis of CPV.

INTRODUCTION

Camel-pox was greatly incriminated as one of the major skin disease of camels, it is a highly transmissible and contagious disease. The disease occurs more frequently and more severely in young animals (Gitao, 1997 and Muhammad et al., 1998). Camel-pox virus (CPV) is DNA epitheliotropic virus, the etiological agent of camel- pox (CP), belongs to family pox-viridae, genus orthopoxvirus. The viral infectious disease has been reported through areas of middle east, Africa, Asia and southern parts of Russia and India. Outbreaks of CPV infection can be responsible for sever economic losses in these countries (Sophie et al., 2007 and O.I.E., 2008). The incubation period is
usually 9-13 days. The clinical manifestations of (CP) range from in apparent and mild local infections, confined to the skin, to moderate and severe systemic infections (Wernery and Kaaden, 2002). The disease is characterized by fever, enlarged lymph nodes and skin lesions.

In generalized form pox lesions may cover the entire body, pox lesions can be found in the mucous membranes of the mouth and respiratory and digestive tracts (Kritz, 1982 and Wernery and Kaaden, 2002). The morbidity rate of CP is variable and depends on whether the virus is circulating in the herd. The incidence of the disease is higher in males, than females and the mortality rate is greater in young (25% - 100%) than in adults (5- 28%), (Mayer and Zerny, 1990). Transmission is by either direct contact between infected and susceptible animals or indirect infection via a contaminated environment. Virus is secreted in milk, saliva and ocular and nasal discharge. Dried scabs shed from the pox lesions contain live virus for at least 4 months and contaminate the environment (Wernery et al., 1997). CPV is very host specific and dose not infect other animal species. Field reports of mild skin lesions in humans associated with CP have been made (Coetzer, 2004). In Egypt studies concerning CPV are somewhat few. Tantawi et al. (1974) isolated (CPV) for the first time in Fayoum Governorate, Kenawy et al. (1989) in Sharkia Governorate, Nawal and Ahmed (1997) and Maysa et al. (1998) in Matrouh Governorate and Zaitoun et al. (2000) in Assuit Governorate. The aim of the present work to deal with camels that suffered from skin lesions suspected pox virus in Southern Sinai Governorate, Egypt, by trials of virus isolation on chorioallantoic membrane (CAM) of specific pathogen free embryonated chicken eggs, propagation of the isolates on tissue culture cell line (Vero cells) and Identification of the isolates by Agar gel precipitation Test, Electron microscopical technique and molecular identification by polymerase chain reaction (PCR).

**MATERIALS AND METHODS**

(1) Samples:

**a- Skin lesions:**

A number of camels were reported to have skin lesions in southern Sinai governorate. Dried scabs were collected from 5 selected cases showed skin lesions (at different stages of development) and kept in sterile vials contain glycerol buffer to be used in this study. The collected scabs were ground in a sterile mortar according to (Kenawy et al., 1989).
b- Serum samples:  
5 serum samples were collected from 5 camels with skin lesions and 20 samples were collected from the contact apparently healthy camels.

(2) Hyperimmune serum:  
Hyper immune serum of camel pox virus was obtained from animal Health research institute (AHRI) Virology Department, Dokki, Cairo.

(3) Camel- pox virus:  
Obtained from (AHRI) Virology Department, Dokki. Cairo.

(4) Virus isolation:  
**a- Specific pathogen free (SPF) embryonated chicken eggs (ECE):**  
SPF-ECE (11-12) days old were obtained from National Laboratory for Veterinary Quality Control on Poultry Production (NLQP), Dokki, Giza, Egypt, were inoculated by the prepared samples via CAM route according to (Robinson and Balassu, 1981).

**b- African green monkey kidney cells (Vero):**  
It was kindly supplied and propagated at virology department, (AHRI), Dokki, Cairo.

The CAM suspensions obtained from infected SPF, ECE with characteristic pock lesions of (CPV) were inoculated into Vero cells after filtration through 0.45 μm filter.

(5) virus Identification:  
**a- Agar Gel precipitation test (AGPT):**  
It was carried out according to (Kitching et al., 1986), the isolated virus was identified as CPV using Hyper immune serum, kindly supplied from (AHRI), virology department, Dokki, Cairo.

**b- Electron microscopy (E. M):**  
Samples from CAM Pock lesions were fixed in 3% glutardldehyde + 10% formaline, processed and sectioned for transmission electron microscopy in Electron Microscopical Center of Veterinary Hospital according to Frances and Anderson (1987).

**C- Polymerase chain reaction (PCR):**  
The received samples were tested by PCR, Briefly, DNA was extracted from each sample by using DNA extraction QiaAmp DNA kit (Qi A Gen, Valencia, Calif, USA). Samples were amplified using PCR Reddy Mix PCR master Mix (Thermo, UK).

PCR assay was done as described by Meyer et al. (1994), 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 (CPV), is 881 bp. The test was applied on PCR thermal cycles machine (AB1, 2720).

(6) Serological investigation by AGPT:
All sera collected were screened against the identified locally isolated CPV from AHRI, Virology department, Dokki. Cairo. According to method described by (Tantawi et al., 1974).

RESULTS

(1) Virus propagation on CAM of SPF-ECE:
The virus was grown on the CAM and produced characteristic Pock lesions (circular, opaque, white enlarged areas) on the 5th days post inoculation at the second passage. The virus grow readily at 37ºC Fig (1).

(2) Virus propagation on Tissue culture:
The virus replicate on Vero cells after 3 blind passages of the filtrated virus isolated on CAM of the SPE-ECE and produce the cytopathic effect CPE of (CPV) after 4-5 days post inoculation the CPE was characterized by cell rounding, aggregation and detachment of the cell sheet Fig. (2).

(3) Agar Gel precipitation test AGPT:
A clear precipitation lines (positive results) appear between the isolated virus and the CPV hyper immure serum.

(4) Electron microscopy:
The ultrastructure of E. M. clarified the CPE in nucleus which appears has uneven distribution of chromatin which accumulated in many corners of the nucleus in inner surface of the nuclear membrane. Progeny virus develops in the cytoplasm in association with aggregates of dense fibrous or granular material and some immature particles appear spherical (Fig., 3). CPV has a typical brick shaped appearance with irregularly tubular surface proteins appear in the cytoplasm of infected cells (Fig., 4).

(5) Polymerase Chain Reaction (PCR):
The five tested samples showed the characteristic PCR positive bands of 881 bp size fragment of camel-pox virus (Fig., 5).

(6) Serological investigation by AGPT:
The AGPT was applied to detect the immune status of the infected and contact apparently healthy camels.
The results revealed that 100% of infected camels (5 samples out of 5 infected camels) were positive reactors and 60% of contact camels (12 samples out of 20 contact camels) were positive reactors.
Fig. (1): Chorioallantoic membrane showing pock lesions characteristic for CPV.

Fig. (2): A- Non infected Vero cells. 
B- Infected Vero cells showing CPE characterized by cell rounding, aggregation and detachment of cell sheet.
Fig. (3): The ultrastructure of EM clarified the CPE in nucleus and cytoplasm (X 10.000).

Fig. (4): The CPV has a typical brick-shaped appearance with irregularly tubular surface proteins appear in the cytoplasm of infected cells (X 100.000).
DISCUSSION

The presence of the various forms and sizes of skin eruptions on the different parts of the infected camels rarely associated with systemic involvement including pyrexia, anorexia and fever may suggested that the suspected disease was camel pox (CP). The complain from camels flock in southern Sinai during 2008, the characteristic lesions on the mouth and nostrils suspect a camel pox virus infection. They all passed through the typical stages of pox viral disease (papule, vesicles, pustular scabs and ulceration) with elevation of temperature 40-41ºC in some cases. Such suspicion was primarily supported by the appearance of the characteristic Pock lesions on the chorioallantoic membrane (CAM) of the infected SPF fertile eggs. The pock lesions appear clear after the second passages as opaque, white, circular, enlarged areas with slight thickening in the CAM, most of the inoculated eggs still containing living embryos 5 days post inoculation (PI), these coincidence with (Tantawi et al., 1974) who isolated CPV for the first time in Egypt in Fayoum Governorate and Kenawy et al. (1989) in Sharkia Governorate, Chauhan and Kaushik (1987) reported the pock lesions characteristic for (CPV) on CAM as small, opaque and circular, Kaaden et al. (1989) reported the same results, Alhendi et al. (1994) reported that non of inoculated eggs died until
they were all opened, 5 days post inoculation and CAM showed whitish lesions. **Nawal and Ahmed (1997)** showed that the virus (CPV) grow easily on the CAM of ECE. (embryonated chicken egg) and produced pock lesions on the 5th day PI. **Maysa et al. (1998)** found that CPV was able to replicate on CAM of ECE producing pock lesions resembling buffalo pox virus. **Zaitoun et al. (2000)** found that (CPV) a characteristic pock lesions on CAM of ECE (multiple, small, opaque and circular). These isolates blindly pass aged on T. C the specific cells from our choice were Vero cell line. The isolates could be filerated by 0.45 µm disposable filter the filterable material were inoculated on T.C. for cells after 3 blind passages and produce the CPE of CPV after 4-5 days PI. The CPE was characterizing at first by cell rounding, aggregation and finally detachment of the cell sheet. These results agree with **(Nguyen et al., 1989)** who isolated strains of CPV which had a strong CPE in different cell lines, **(Otterbein et al., 1996)** isolated two CPV strains, which were serially passage on Vero cells, **Nawal and Ahmed (1997)** reported that the isolated CPV from Matrouh Governorate replicated in Vero cells and produce CPE at the 4th day PI. **Wernery et al. (1997)** isolated CPV in Vero cells and Dubca cell lines, **(Khalafalla et al., 1998)** isolated seven viruses in Vero cells from outbreaks of CPV in Eastern Sudan, **EL-Harrak and Loutfi (2000)** reported that isolated CPV from dromedaries in moracco was passaged eight times in Vero cells and **Aboul Soud et al. (2004)** studies the growth of CPV in Vero cells. The viral isolate tested against hyper-immune serum of CPV that showed clear precipitation lines, these results were similar to those obtained by **Kenawy et al. (1989), Kalafalla et al. (1998), Maysa et al. (1998) and Aboul Soud et al. (2004).** The growth of camel pox virus (CPV) on a cell culture can be confirmed by Electron microscopical examination (EM) and polymerase chain reaction (PCR). Transmission electron microscopy (TEM) is a rapid method to demonstrate CPV in tissue samples, however it is the best method for distinguish clinical cases of orthopoxviruses and parapox viruses **(Munz, 1992)**. EM findings proved that orthopox viral infection was probable cause of pox in our camels. Which has a typical brick- shaped appearance with irregularly arranged tubular surface proteins which present free in the cytoplasm of the cells **(Andrews et al., 1987)** noted that E.M. detection of the virus particles in pox lesions was one of the important diagnostic tools of the disease. The electron microscopi-
cal pictures were similar to those obtained by Munz et al. (1986) and coincidence with that of Mayer and Zerny (1990). The distribution and morphological features of the virus particles similar to those obtained by Kaaden et al. (1992) and Munz (1992). The results also agree with that of Pfeffer et al. (1996) and Pfeffer et al. (1998). CPV was isolated in Saudi Arabia and identified by E. M. (Abu El Zein et al., 1999). CPV can be diagnosed in crusts collected from suspected camels using CAM of infected ECE, tissue culture propagation and identification by Electron microscope, however these method are laborious and time consuming, today assays based on advanced technology that can serve diagnosis of animal infectious disease like PCR that provide easy to handle tools for virus diagnosis, requiring only minute amounts of specimen, we therefore apply this technique to specific detection of CPV.

PCR assay was done using the primer pair: 5'-AAT-ACA-AGG-AGG-ATC-T-3' and 5'-CTT-ACC-TTT-TTC-TTC-CTC-3'. The gene sequence encoding the A-type inclusion protein (ATIP) will be amplified. The size of the PCR product, specific for the camel-pox virus (CPV), is 881 bp. These results agree with those obtained by Meyer et al. (1994) and results recorded by Caroline and Geoffrey (2002), Afonso et al. (2002) and O.I.E. (2008).

The serological investigation revealed that all sera (5) collected from camels which had pox infection showed positive results in AGPT, while 12 serum samples out of 20 serum samples from symptomless contact camels gave positive results. These positive results may be attributed to subclinical infection as these camels in close contact with diseased camels. These results similar to those obtained by Tantawi et al., (1974) who showed the presence of antibodies against CPV in the sera of camels using AGPT, Chauhan and Kaushik (1987) who found that 25.8% of serum samples were positive reactors, Nawal and Ahmed (1997), applied AGPT and detect CPV antibodies in 100% of infected camels sera and 67% in contact camels sera.

CONCLUSION

Our conclusion that CPV one of the infectious disease affected camel endemic in Egypt, from time to time there is a field problem among camel flocks mostly in desert governorate like Matrouh (1997), recently Sinai (2008). The characteristic lesions and clinical sings appear on skin one of diagnostic tools for suspicious of camel pox which supported and confirmed by lab diag-
nosis include virus isolation and identification, sero-diagnosis, E. M. and advanced technology like PCR.

Although there is a vaccination policy against camel pox, by using local producing attenuated camel pox vaccine supplied by (Veterinary Serum and Vaccine Research Institute, there is few cases appear among camel flocks, the further studies must be submitted to determine the role of insect climatic condition, nature of soil, efficacy of vaccination and other factors play a role of transmission.

REFERENCES


Frances W. and Doane Nan


Wernery, U.; Kaaden, O.R and
العزل والتصنيف الجزيئي لفيروس جدرى الجمال

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الملخص العربي

أوضحَت الدراسة بأن جرذ الجمال من الأمراض المستوطنة في مصر، وظاهرة من حين إلى آخر، وتمييز بإصابات عالية في الجمال على هيئة أفات جلدية متعددة المراحل والمميزة لمرض جرداء الجمال، وقد تم عزل الفيروس المسبب في مجموعة أورثو على بيش مخصص في الأغشية الجنينية وكذلك خلايا الزرع النسيجي (Vero) والطريقة الإلكترونية وأختيار البلاسته المتسجل. وأوضحَت نتائج فحص عينات السيرام من الجمال المصاب بإختبار الأجراي التي تسببت في أصابات الفيروس من خلال مناعة مضادة الفيروس.

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