SOME VIROLOGICAL STUDIES ON CONTAGIOUS PUSTULAR DERMATITIS VIRUS IN SHEEP

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ABSTRACT

Orf virus(Contagious pustular dermatitis virus) isolates obtained during the time period in 2008 and 2009 from crusted scab lesions of sheep from different Egyptian Governorates (Sharkia, Ismailia, Cairo and South of Sinai). Suspected samples of skin and lip of the affected lambs were collected and sent to the lab for laboratory diagnosis. Orf virus (Contagious pustular dermatitis virus) was isolated on chorioallantoic membrane (CAM) with characteristic lesions . The virus correctly identified by Agar- gel-precipitation test (AGPT), Electron microscope (EM) and viral DNA was further identified by polymerase chain reaction (PCR), which consider a suitable technique for rapid and accurate diagnosis .

INTRODUCTION

Contagious pustular dermatitis (CPD) virus is an exanthemas viral disease that is widespread in sheep and goats. It has been recorded since the late 1800s and reported from most sheep or goat-raising areas including those in the Middle East, the United States, Africa, Asia, Alaskan, South America, Europe, Canada, Newzeland and Australia ***(Couch, 1983)***. It is known by a variety of names around the world

including Orf virus, contagious ecthyma, infectious labial dermatitis, Sore mouth and scabby mouth ***(James et al., 2006).*** The disease caused by a virus in the family *poxviridae* and genus *parapoxvirus* ***(Matthews, 1982).*** The virus is one of the largest viruses; the brick-shaped 170 to 260 by 300 to 450-nm-diameter capsid contains a linear non segmented double-stranded DNA genome of approximately 140 kilobases that is surrounded by a layer of lipid that is not a true envelope ***(Episito and Fenner, 2001 and Moss, 2001).*** CPD is a common epitheliotropic viral disease of sheep, goat and wild ruminants and characterized by formation of papules, nodule or vesicles that progress into thick crusts or heavy scabs on the lips, muzzle, nostrils, eye lids and ears. It may affect udder ***(winter et al., 1999)***. The virus was previously isolated on chorio allantoic membrane (CAM) of emberyonated chicken egg and identified by agar gel immunodifusion test and Electron microscop. The development of PCR methods for the molecular detection of parapox DNA has met the demands for specific and sensitive laboratory diagnosis of the disease ***(Mazur et al., 2000).*** Conventional PCR method that is based on amplification of the B2L (the major virus envelop protein gene) has been used for the detection of ORF virus by *PCR* ***(Hosamani et al., 2006)***. The B2L gene is an important parapox virus (PPV) molecular target and several PPV B2L nucleotides and amino acids sequences are available in Gen Bank. Moreover, detection of the B2L gene is the most sensitive methods for virus detection because it harbors epidemiologically relevant sequence information (***Hosamani et al., 2006)***.

The aim of the present work: Isolation of the Orf virus from clinically infected sheep on chorio-allantioc membrane of developing embryos and identification of the isolated virus by different serological tests and electron microscope and molecular identification using polymerase chain reaction (PCR).

MATERIALS AND METHODS

1. Samples:

Skin lesions (Scabs) were collected from clinically diseased lambs from different Egyptian governorates (Sharkia, Ismailia, Cairo and South of Sinai) during 2008 -2009. The total number of samples were 26(twenty six) scab samples (7 from Sharkia governorate, 5 from Ismailia governorate, 8 from Cairo governorate and 6 from south of Sinai governorate). The samples kept in sterile vials containing glycerol - buffer. The collected scabs were ground in sterile morters, then homogenate as 10% suspension in maintenance media, then centrifugated at 3000 rpm for 10 minutes, then the supernatant fluid stored at -20°C till used for virus isolation.

2. Virus Isolation On Emberyonated Chicken Eggs (ECE):

Specific pathogen free (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 scabs via chorio-allantoic membrane rout (CAM) according to ***(Robinson and Balassu, 1981).***

3. Hyper Immune Serum:

Anti-CPD serum was obtained from (AHRI) virology department, Dokki, Cairo.

4. Control positive:

Localy identified Orf virus was obtained from (AHRI) virology department, Dokki, Cairo.

5. Agar Gel Precipitation Test (AGPT):

It was carried out according to ***(Cheesbrough, 1984).***

6. Transmission electron microscopy (TEM):

For electron microscope study, pieces of infected CAM were processed accordiog to the method of ***(Tuppurainen, 2004).***

7. Molecular Characterization of CPDV Local Isolates:

The received CAM samples were tested by PCR, briefly DNA was extracted from each samples using DNA extraction Qia Amp DNA kit (Qiagen, Germany) and amplified using a PCR kit (thermo, Uk) The reactions were carried out accordng to ***(Jaek et al., 2009).*** The primer sets and their numbered according to the Orf virus sequences were illustrated in Table (1). It was designed in Metabion Company, Germany.

Table (1): The olignucleotide primers for PCR:

Primer name	Sequence (5`- 3`)	No. of nucleotides	Amplified produce
OVS-1-3	AGG CGG TGG AAT GGA AAG A	19	708 bP
OVS.-2-3	CCA GCA GGT ATG CCA GGATG	20	

The amplification reaction:

It was performed using the following temperature profile: initial denaturation at 95°C for 15 min, then 40 cycles of 95 °c for 1 min, 56°C for 1 min, and 72°C for 2 min, then a final extension step at 72°C for 10 minutes on thermocycler (Biometra, Germany), to reveal the PCR products which consists of fragment of (708 bp)for (B2L) gene of Orf virus. The amplified DNA reaction is loaded into 1.5% agarose gel stained with ethidium bromide stain (o.5 ug/ml) (molecular biology grade) electrophoresis was visualized by ultraviolet trans illumination. The DNA marker used was wide range PCR marker (ABgene, Germany).positive and negative control virus also included.

RESULTS

The suspected cases in the present study were collected from 26 affected lambs with signs of loss of appetite, unwilling to eat or walk, nodules, pustules and scabs on upper and lower lips, gums and mouth commisure with high morbidity rate between young animals and low mortality in different Egyptian governorates (sharkia, Ismailia, Cairo and South of Sinai).

(1) Isolation of the suspected CPD virus from affected animals in SPF fertile chicken eggs:

Twenty six prepared skin lesions from clinically infected animals were inoculated via CAM onto ECE, 20 samples induced lesions on CAM by the 3rd passage as shown in Table (2) and Fig. (1). The lesions were characterized by thickening, oedema and hemorrhage of the CAM.

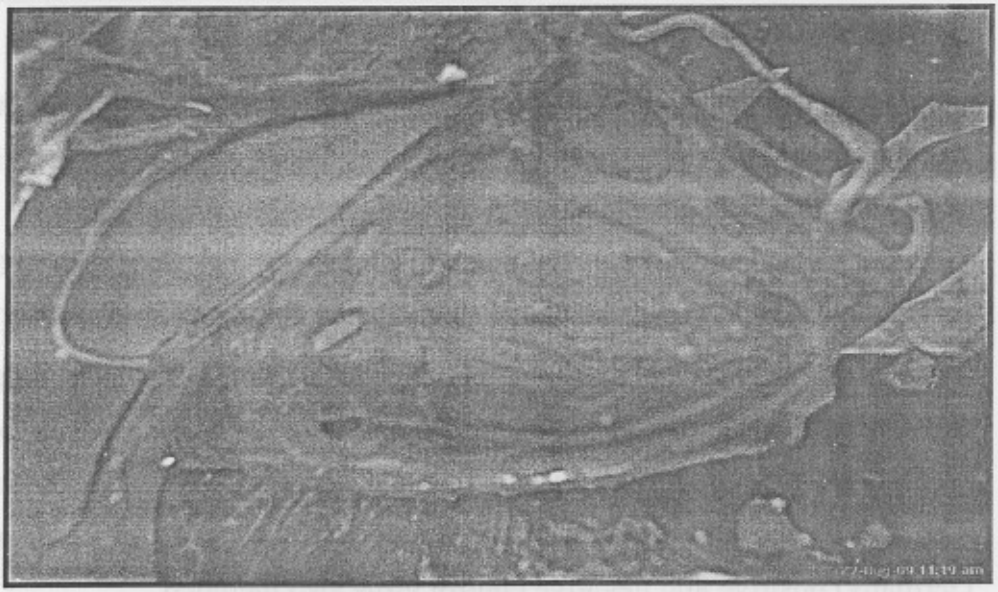

Fig. (1): Chorioallantoic membrane showing charactrestic lesions for CPDV

(2) Serological identification of the suspected CPDV isolates using AGPT:

In Table (2), out of 20 isolates, 14 samples showed positive precipitation Lines Against specific hyperimmum serum

Table (2): Detection of CPD virus by AGPT.

Locality	No. of collected samples	Positive propagated samples	Positive AGPT samples
Sharkia	7	5	3
Ismailia	8	6	4
Cairo	5	4	3
South of Sini	6	5	4
Total	26	20	14

(3) Identification of Suspected CPDV isolates by E.M:

The CAM isolates examined and revealed that the negatively stained Orf virus particles were detected by E.M, which revealed the ovoid-shaped morphology of Orf virus with surface tubules form criss cross design **(Fig. 2:A)** and characteristic of ball of wool appearance of Orf virus **(Fig. 2:B).**

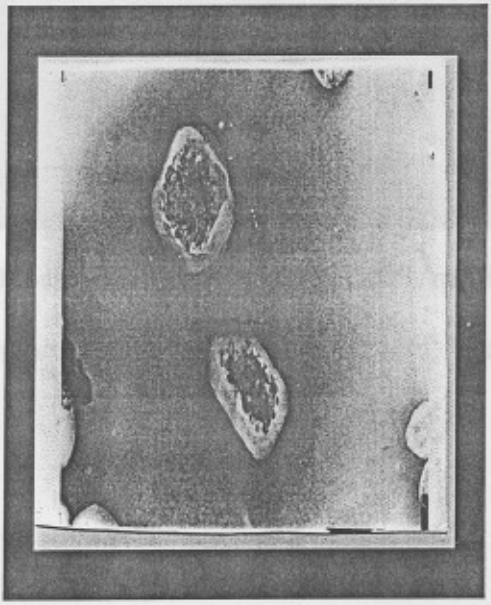

Fig. (2) A: Ovoid – shaped viral particle of CPD virus.

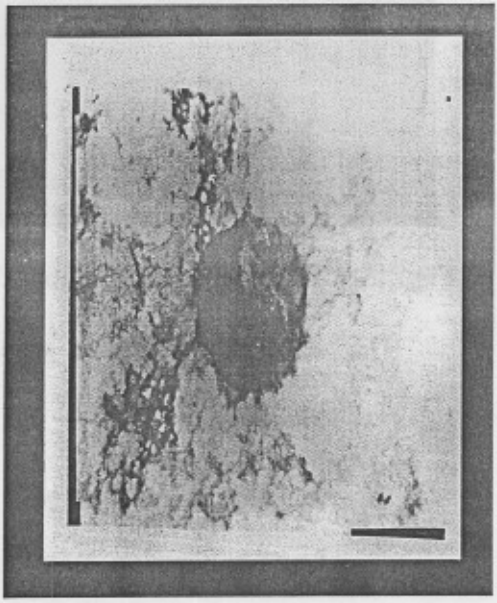

Fig. (2)B: A charactrestic ball of wool appearance of CPD virus.

(4) Molecular Characterization Orf The Suspected CPDV Isolates:

The examined suspected isolates, showed characteristic positive bands of 708 bp size fragment parallel to an equal molecular weight marker band which revealed primers and products specificity to CPDV (Fig. 3).

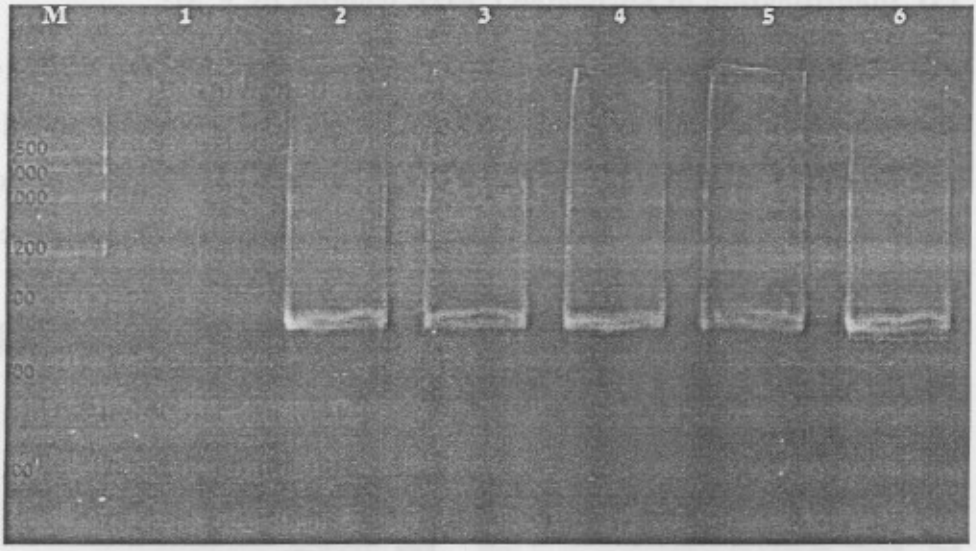

Fig. (3): PCR product of about 708 bp PCR products were analyzed by electrophoresis and visualized by ultraviolet rays with ethidium bromide.

M: wide range marker.

Lane 1: negative control.

Lane 2: positive control.

Lane 3 to 6: positive samples.

DISCUSSION

Our work directed towards isolation and identification and molecular characterization of the locally isolated CPD virus in sheep in different Egyptian governorates during 2008 and 2009.In many farms in different Egyptian governorates,The disease was clinically suspected, then the laboratory diagnosis achieved by viral isolation on CAM of spf-

.ECE. inducing signs in CAM thickening, oedema and hemorrhage (Fig. 1). These results were in agreement with ***(Sanjay and Joshi 1995, Ahmed et al., 2001and EISA et al., 2003)***. The isolated virus on CAM were identified serologically by AGPT also ***(Eisa et al., 2003 and Dilip, 2004)*** described that the lab. Confirmation of Orf virus is based on detection of virus speific antigen by AGPT and showed that AGPT is one of the serological tests used for Orf diagnosis. The non serological mean for confirmation of the isolated virus is by E.M. All examined isolates showed the characteristic ovoid shapes verion of Orf virus (**Fig2: A**) and a characteristic ball of wool appearance of Orf virus (**Fig2:B**) .

The E.M results confirm our diagnosis and In complete agreement with ***(Buchan 1996 and Paiba et al., 1999)*** who stated that Orf virus is cylindrical virus measuring 200x160 nm, its surface tubules form along criss cross design ,that seem in negatively stained preparation by E.M. ***(Olson et al., 2004)*** confirm the clinical diagnosis of Orf virus by performing the E.M with negative staining of the crusts or small biopsy, it demonstrate the classic ovoid cross-hatched versions ***(Robinson and Balassu, 1981)*** reported that the virus appears by E.M as a ball of wall, ***(Allworth et al., 1987)*** said that the diagnosis of Orf generally made by clinical basis but can be confirmed by E.M ***(Guo et al., 2003)*** demonstrate the characteristic ovoid shape of verion, ***(Mandal et al., 2006)*** said that the characteristic features that distinguish parapox virus from other pox genera are the ovoid version shape and the criss- cross pattern on the particle surface ***(Jae et al., 2009)***, stated that E.M examination revealed the oval shape of morphology of parapoxvirus, the size of observed virus was approx. 150-200 nm. E.M as a

morphodiagnosis mean combined with clinical information is in most cases sufficient to permit a provisional diagnosis but because E.M is not suitable for screening large no. of samples, many molecular methods have been developed on the basis of nucleic acid complication techniques. To access accurate diagnosis of orf virus without virus culture and E.M study design, PCR provides easy to handle tools, require minute amounts of specimen, rapid, sensitive, specific for ORF virus. PCR was therefore verified as specific for Orf virus for molecular characterization of the local obtained CPDV isolates. We apply PCR assay for detection of virus in CAM. The primers used in PCR assay were to amplify (708 bp) located in the B2L of the CPDV genome. The results obtained by this assay confirmed the presence of CPDV in the harvested CAM, the amplified target region of the CPDV genome revealed strong visible band corresponding to the molecular wt. marker specific band which confirmed primers and fragment specificity **(fig. 3)** Our results also resemble that of ***(Torfason and Gunadottir, 2002)*** who said that PCR can definitively identifies a parapoxvirus as orf virus ***(Einor and sigrum, 2007)*** stated that the sensitivity in PCR seems to be good or excellent (95.7%).

(Mazur et al 1991) characteriz CPD virus on molecular level in order to allow quick epidemldogical survey of CPDV in Brazil eliminating the need for time-consuming and expensive viral propagation in cell culture. ***(Guo et al.,2003)*** performed PCR for preliminery screening of the scab samples as a diagnostic method ***(Inoshima et al. 2000,Hosamani et al., 2006, Zheng et al., 2007 ,Chan et al., 2009 and Jae et al., 2009)*** identified the viral DNA of orf virus using PCR

technique. In conclusion orf infection is recorded in Egypt and no vaccination has been implemented to control this disease, clinical diagnosis and E.M has been used routinely to identify orf virus in Egypt. Recently PCR methods have been used to detect the virus, so we confirm the previous conclusion that E.M and PCR studies demonstrated that the outbreak of a contagious disease causing erosions, papules, pustuls, ulcers and scabs in the mouth caused by parapox-virus (Orf virus) and PCR is suitable for screening large no. of samples at molecular level in a short time if compared with other methods.

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بعض الدراسات الفيرولوجية عن فيروس الالتهاب النفطى المعدى فى الاغنام

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بعد ظهور عدد من حالات الاصابات الجلدية فى الاغنام فى بعض محافظات جمهورية مصر العربية (الشرقية والاسماعيلية والقاهرة وجنوب سيناء) تم تجميع عينات من القشور الجلدية من الحملان المصابة وارسالها الى المعمل للتشخيص المعملى تم عزل فيروس الالتهاب النفطى المعدى فى الاغنام على اجنة البيض المخصب مع وجود التغيرات المصاحبة للفيروس وتم التعرف على الفيروس المعزول باستخدام اختبار الاجار الترسيبى والفحص بالميكروسكوب الالكترونى وتم التعرف على الجين المسبب للفيروس باستخدام اختبار البلمرة المتسلسل الذى يعتبر طريقة مناسبة للتشخيص السريع والدقيق .