Molecular detection of Contagious Pustular Dermatitis Virus (CPDV)isolatedfrom infected Sheep and Goats inQaliubeya Governorate, Egypt

El-Habbaa, A.S.

Department of Virology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt,

Abstract

Contagious Pustular Dermatitis Virus (CPDV) of genus parapoxvirus, subfamily Chordopoxvirinae, familyPoxviridae, causesContagiousPustular Dermatitis in sheep and goats worldwide and in Egypt. Forty samples of skin lesions from suspected 30 sheep and 10 goats from different areas of Qaliubeya governorate were subjected tovirusisolation on embryonated chicken eggs (ECE), antigen detection using agar gel immune-diffusion test (AGIDT) and indirect-Immunofluorescence assay (IFA) and molecular detection of viral DNA using PCR. Positive results were showed in 36 of samples (28/30 of sheep samples and 8/10 of goat samples)upon isolation on ECE by the 3rd passage and 22 of samples (17/28 of sheep isolates and 5/8 of goat isolates) using AGIDT and indirect-IFA. PCR detection showed positive results with 30 /40 of samples before isolation (23/30 and 7/10 of sheep and goat samples, respectively) while it showed positive results with 26 /36 of samples upon isolation (20/28 and 6/8 of sheep and goat samples, respectively). It was concluded that AGIDT and indirect-IFA were suitable methods for CPDV detection but PCR was most specific and sensitive diagnostic tool for rapid detection of CPDV.

Introduction

Contagious Pustular Dermatitis Virus (CPDV) is the prototype of genus parapoxvirus, sub-family Chordopoxvirinae, familyPoxviridae.It primarily causes Contagious Pustular Dermatitis (CPD) in goats, sheep, and other ruminants worldwide (**Lojkic et al., 2010**). CPD is also known as ORF, contagious ecthymaandscabbymouth**(Haig and Mercer, 1998)**. It is characterized by maculopapular then vesicular pustules on the skin around the lips, mouth muzzle, nostrils, teats, and oral mucosa in sheep and goats.The economic loss of the disease is due to unthriftinessof lambs 3-6 months of age and severely affected adult animals**(Radostits et al., 2008)**.

The disease is endemic around the world (**Haig and Mercer, 1998 and Billinis et al., 2012).**In Egypt CPDV infection occur wherever sheep and goats are found since it was recorded for the first time **(Sabban et al., 1961),**thenseveral outbreaks of variable severity were recorded up till now **(Gabry, 1987, Ahmed et al., 2001, Eisa et al., 2003,Shemies, 2006and Mahmoud et al., 2010).**The viral genome consists of linear double-stranded DNA that measures 138 kbp in length and contains 132 putative genes most of them are located in the central part of the genome (**Mercer et al., 2006).**The late transcription factor (VLTF-1) gene has been successfully used for the detection, molecular characterization and phylogenetic analysis of ORFV [**Mahmoud et al., 2010**].

Laboratory diagnosis of CPDV is based on virus isolation on emberyonatedchickeneggthen identification using electron microscopy, agar gel immune-diffusion (AGIDT) test, immunofluorescence assay (IFA) and Enzyme– Linked Immunosorbent Assay (ELISA) [**Mangana et al. 2000,Christine et al., 2006,Abu Elzein**

and Housawi, 2009 and Mahmoud et al., 2010].Polymerase chain reaction (PCR) presented a reliable laboratory diagnostic tool for diagnosis of CPDV infections[**Sullivan et al., 1994, Kottaridi et al., 2006andHosamani et al., 2007].** PCR was also successfully used fordetectionand molecular characterization of Egyptian CPDVisolates**[Mahmoud et al., 2010**].

The aim of this work is trying to isolate CPDV from the skin samples (scabs and nodules),obtained from suspected sheep and goats in Qaliubeyagovernorate, in emberyonated chicken eggs (ECE)thenidentificationof the isolates using AGIDT, IFAanddetection of CPDV using PCR.

2. Material and Methods

2.1. Samples:

Fortysampleswere collected from grassing sheep and goats (30 from sheep and 10 from goats) fromdifferent localities in Qaliubeyagovernorate. Animals were suspected with CPDV infection due to the presence of skin lesions in the form of scabs and nodules on lips especially at its comissuresandgums. Scab samples were collected on 50% glycerol buffered saline then kept in 4°C until samples prepared. A 10% suspension of scab in phosphate buffered saline containing 5% of stock antibiotic solution were homogenized with sterile sand in mortars, frozen and thawed for three successive timesthen centrifuged at 3,000 ×g for 10minutesandsupernatantswere stored at -20 °C until used for virus isolation and PCR detection **(OIE, 2008)**.

2.2. Emberyonated Chicken Egg (ECE):

A total of 360 one day old specific pathogen free ECE were obtained from KoomOsheem farm, El-Fayom governorate, incubated at egg incubator for9-11 days old and used for CPDV isolation on chorio-allantoic membrane (CAM) (**Tantawi et al., 1979**).

2.3. Reference CPDV::

It was an ECE adapted virus with a titer of 5 $\log_{10}EID_{50}$/ml, obtained from virology department, Animal Health Research Institute, Dokki, Egypt. It was used as positive control for both serological tests and PCR.

2.4. Reference CPDV antiserum:

Rabbit anti-serum against CPDV wasobtained from virology department, Animal Health Research Institute, Dokki, Egypt. It was used for Agar Gel Immuno-Diffusion test (AGIDT) and indirect Immuno-FlourescenceAssay (IFA).

2. 5. Anti- sheep and anti- goat IgG Conjugates:

Anti-sheep and anti-goat IgGs conjugated with fluorescinisothiocynate, developed in rabbits were supplied by Sigma immune chemicals and used for indirect Immuno-FlourescenceAssay.

2.6. Agar Gel Immuno-Diffusion test (AGIDT):

Itwas used for detection of CPD viral antigen using reference CPDV antiserum according to **Davis and Otema (1981)**.

2.7. Indirect Immuno-FlourescenceAssay(indirect-IFA):

Impression smears from CAM of the specific pathogen free ECE inoculated with the viral samples showing positive results were used for detection of CPD antigen using indirect-IFA. The technique was performed using reference CPDV antiserum and anti-sheep and anti-goat IgGs conjugated with fluorescinisothiocynate according to **Soad et al. (1996)**.

2.8. DNA extraction:

DNA was extracted from the prepared samples of scabs and from the inoculated ECE with pock lesions by using QIAamp DNA Mini kit (QIAGEN) according to the

manufacturer's instructions, then the extracted DNA was eluted with100 µl elution buffer and stored at – 20 °C until used for PCR.

2.9. Polymerase chain reaction (PCR):

PCR was conducted in a 50 µl reaction mixture comprising 25µl 2 X ReddyMix™ PCR Master mix containing: [75 mMTris–HCl (pH8.8), 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.01% Tween20, 0.2 mM each of dATP, dCTP, dGTP, dTTP, 1.25 U Thermoprime Plus DNA polymerase and red dye for electrophoresis (ABgene)]; 20 pmol from each forward and reverse primer, 2 µl template DNA and appropriate amount of PCR grade water. The primers employed were VLTF-1 gene-F (5´-CCT ACT TCT CGG AGT TCA GC-3´) and VLTF-1 gene-R (5´-GCA GCA CTT CTC CTC GTA G-3´) according to (**Kottaridi et al., 2006)**. The reaction mixture was subjected to 40 amplification cycles under the following conditions: denaturation at 95 °C for 10 seconds, annealing at 47°C for 10 seconds, extension at 72°C for 15 seconds. The amplification cycles were preceded by a denaturation step at 95 °C for5 minutes and followed by an extended elongation step at 72 °C for10 minutes.

2.10. Detection of the PCR product:

Amplification products were analysed on 1.5% agarose gel, stained withGelRed™ Nucleic Acid Gel Stain, 10,000X in water (BIOTREND)and then visualized under UV trans-illuminator. A TrackIt™ 100 bp DNA ladder(Invitrogen) was used as DNA base pair marker.

3. Results:

3.1. Disease picture of suspected cases:

Grazing flocks of sheep and goats from different localities in Qaliubeya Governorate were suspected for CPDV infection during February to September 2012. Animals from both species and sexes between 20 days up to 2 yearsagewere showing nodules, pustules and scabs on lips, mouth commisures and gums. Theysuffered loss of appetite, illthriftness and unwilling to eat or nurse and walk (Fig. 1and 2).

Fig. (1): Nodule and scabs on the upperlip of CPD suspected sheep.

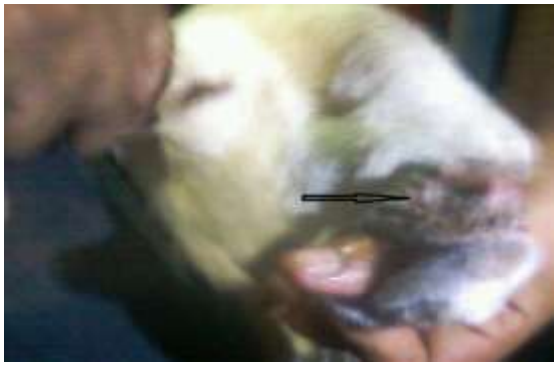

Fig. (2): scabs on the upperlipof CPD suspected goat.

3.2. Isolation on Emberyonated Chicken Egg:

Inoculation oftheprepared samples from skin lesions on CAM of ECE were positive in

28/30 samples from sheep (93.33%) and 8/10samples from goats (80.00%) by the 3rdpassage (Tables 1 and 2). The resulted signs on inoculated CAM are thickening and presence of pock lesions on the CAM at 6 -7 days post-inoculation.

Table (1): isolation of CPDV suspected samples on Emberyonated Chicken Egg (CAM)

Species	Total number of samples	Number of positive samples		
		1st passage	2nd passage	3rd passage
Sheep	30	11	23	28
Goats	10	2	5	8
Total	40	13	28	36

Positive samples showed thickening and presence of pock lesions on the CAM

3.3. Serological detection of suspected CPDV isolates using Agar Gel Immuno-Diffusion Test (AGIDT):

Detection of CPD antigen in the suspected isolates using reference CPDV antiserum revealed that 22/36 (61.11%) isolates by embryonated chicken egg inoculation were positive for the presence of CPD antigen by AGIDT in 17/28 for isolated sheep samples (60.71%) and 5/8 for isolated goat samples (62.50%), (Table 2). The positive result of AGIDT appeared as precipitin line between the CPDV antigen of the isolate and specific reference CPD antiserum.

Table (2): Detection of CPDV isolates from using AGID and IFA

Species	Number of collected samples	Positive samples					
		On ECE Isolation		AGIDT		Indirect IFT	
		Number	Percent	Number	Percent	Number	Percent
Sheep	30	28	93.33%	17	60.71%	17	60.71%
Goats	10	8	80.00%	5	62.50%	5	62.50%
Total	40	36	90.00%	22	61.11%	22	61.11%

3.4. Serological detection of suspected CPDV isolates using Indirect Immuno-FlourescenceAssay (indirect-IFA):

Detection of CPDV proteins in impression smears from the CAM of ECE with suspected isolates using reference CPDV antiserum and anti-sheep and anti-goat IgG conjugates revealed that 22/36 (61.11%) isolates by ECE inoculation were positive for the presence of CPD antigen by indirect-IFT in 17/28(60.71%) for isolated sheep samples and 5/8(62.50%) for isolated goat samples, (Table 2). Positive result of indirect-IFA appeared as specific yellow green emission form the rounded cells lining the CAM in the impression smears examined under the fluorescent microscope (Fig.3).

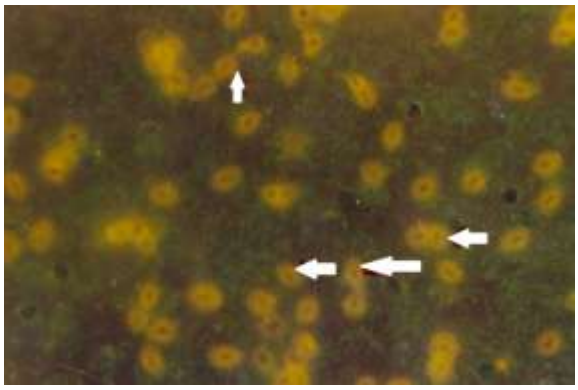

Fig. (3): Specific yellow green fluorescence of rounded cells infilterated between and under epithelial cells lining CAM impression smears.

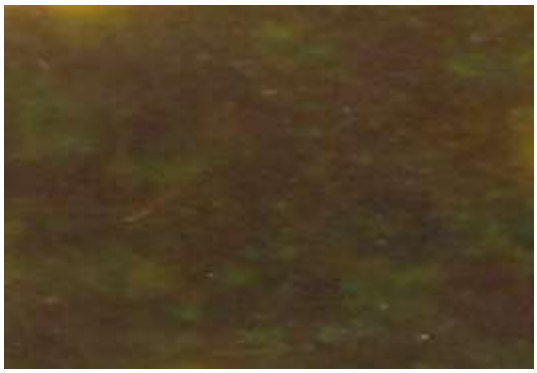

Fig. (4): Normal rounded cells infilterated between and under epithelial cells lining CAM impression smears without any emission.

3.4. Detection of nucleic acid of suspected CPDV isolates using Polymerase Chain Reaction (PCR):

Viral DNA was extracted from the samples collected from affected animals and also from the chorio-allantoic membranes were screened with PCR. The expected size of PCR product as 392 bp of the VLTF-1 gene of the CPDV was successfully detected in 30 /40 (75.00%) in total samples before isolation (23/30 of sheep samples with 76.66% and 7/10 goat samples with 70.00%) while the PCR detection of CPDV in positive samples upon isolation showed that 26 /36 (72.22%) in total samples after isolation (20/28 of sheep samples with 71.42% and 6/8 goat samples with 75.00%), (Table 3 and Fig. 5).

Table (3): Detection of CPDV using PCR on samples before and after isolation:

Species	Number of samples	Positive samples using PCR directly upon samples		Positive samples on isolation	Positive samples using PCR upon virus isolates	
		No.	%		No.	%
Sheep	30	23	76.66%	28	20	71.42%
Goats	10	7	70.00%	8	6	75.00%
Total	40	30	75.00%	36	26	72.22%

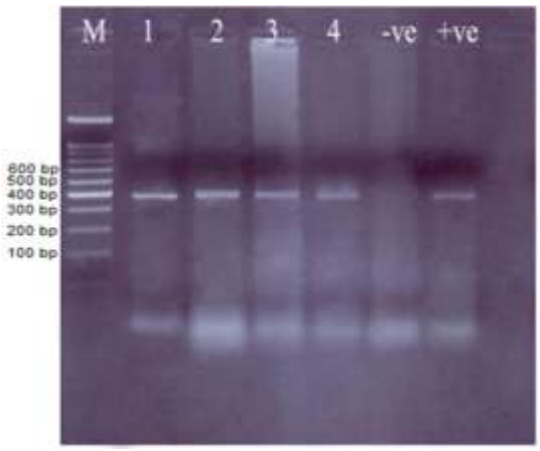

Fig. (5): Electrophoretic pattern of amplified product ofVLTF-1 gene of CPDV using PCR in agarose gel (1.5%).Lane (M): 100 bpDNAmarker.Lanes (1, 2) demonstrate the 392 bp amplification productfrom sheep samples.Lanes (3, 4) demonstrate the 392 bp amplification productfrom goat samples.Lane (-ve) refers to negative control sample of FMDV.Lane (+ve) refers to positive control sample of reference CPDV.

4. Discussion

CPDV also known as Orf, contagious pustular stomatitis and contagious ecthymavirusis an epitheliotropic DNA parapoxvirus with a worldwide distribution that induces acute pustular lesions in the skin of sheep, goats and man **(Haig and Mercer, 1998)**. CPDV belong to genus Parapoxvirus, family Poxviridae**(Robinson and Balassu, 1981)**. The disease is characterized by Papules, pustules and scabs covering ulceration at oral mucocutaneous junction, oral commissures and spread to muzzle, oral cavity**(Radostits et al. 2008).** Many serological tests were used for diagnosis of CPDV include virus neutralization, complement fixation, and agar gel immunodiffusion**(Zarnke et al., 1983;Tryland et al., 2005).**

This work aimed to isolate CPDV from the scabs and nodules samples collected from infected sheep and goats from Qaliubeya Governorate in specific pathogen free-emberyonated chicken eggs then its identification using agar gel immunodiffusion (AGID) test, immunofluorescence technique and PCR.

Virus isolation from prepared samples on CAM of ECE showed that 28/30 (93.33%) from sheep samples and 8/10 (80.00%) from goats samples were positive by the 3rd passage with the inoculated CAM showed thickening at 6 -7 days post-inoculation (Tables 1 and 2). These resulted agreed with **(Mangana et al., 1999, 2000)**whopreferred isolation on ECEforits ease of use, simplicity of procedures and equipments required and rapidity with the result appeared within 5 days only, than using cell culture which need more than 14 days to develop a CPE**.**

Using of AGIDT and indirect-IFAfor detection of CPDV antigen after being successfully isolated on ECE showed that 22/36 of the samples (61.11%) were positive for the presence of CPDV antigen, 17/28 (60.71%) for isolated sheep samples and 5/8 (62.50%) for isolated goat samples (Table 2 and Fig. 4 and 5). This result agreed with the results showed that the indirect IFA and AGIPT have been reported to be rapid confirmatory assays for the detection of CPDV inoculated on CAM of ECE that could be used routinely in diagnostic laboratories

(**Mazur and Machado, 1990 and Mangana et al., 2000))**.

Although virus isolation is considered a gold standard for CPDV detection and AGIDT isa useful method for confirmation of the virus, both are time consuming, laborious and sometimes show lack of sensitivity **(Mazur and Machado, 1990, Wittek et al., 1980 and Mangana et al., 2000)**. Also cross-reactionsof CPDV with sheep and goat poxviruses due to serological relationships between these viruses are considered a problem in diagnosis of CPDV **(Kitching et al. 1986 and Chand et al. 1994)**. So, PCR is considered specific, sensitive and rapid toolfordetection of CPDVthat preferred than the conventional serological techniques.

CPDV genome consists of linear double-stranded DNA that measures 138 kbp in length and contains 132 putative genes most of them are located in the central part of the genome, well conserved and homologous to other poxvirus genes that encode products involved in replication, structure and morphogenesis of the virus. Other genes are located in the terminal regions and encode genes with no homology with other poxvirus sequences, whose products are involved in determination of virus virulence, host range and evasion of the immune response (**Mercer et al., 2006).** The late transcription factor (VLTF-1) gene has been successfully used for the detection, molecular characterization and phylogenetic analysis of Egyptian CPDV isolates. Phylogenetic analysis of 392 bp fragment of VLTF-1 gene revealed 99% identity with other ORFV strains reported worldwide [**Mahmoud et al., 2010**].

Viral DNA extracted from prepared samples collected from affected animals and also from that isolates on CAM of ECE were subjected to PCR using specific primers for the late transcription factor (VLTF-1) gene of CPDV according to (**Kottaridi et al., 2006)**. The expected size of PCR product as 392 bp of the VLTF-1 gene of the CPDV was successfully detected in 23/30 (76.66%) of sheep samples and in 7/10 (70.00%) of goat samples with 30 /40 (75.00%) in total samples before isolation, while the PCR detection of CPDV in positive samples upon isolation showed that 26 /36 (72.22%) in total samples after isolation (20/28 of sheep samples with 71.42% and 6/8 goat samples with 75.00%), (Table 3 and Fig. 5).Theresults proved superior PCR sensitivity than that of egg inoculation method then detection with indirect-IFT and AGIPT as it detected four more cases which gave negative results with egg inoculation. These results agreed with conclusion that the sensitivity and specificity of the PCR assay is 100% and have considerable potential as a specific, sensitive and rapid diagnostic approach for CPDV detection and differentiation from sheep pox and goat pox viruses directly from skin biopsy samples (**Magnana et al., 2000, Kottaridi et al., 2006 and Mahmoud et al., 2010**).In conclusion CPDV is best isolated on CAM of emberyonated chicken eggandidentified using AGID and indirect-IFT while PCR is the most specific and sensitive diagnostic tool for rapid detection of CPDV in skin biopsy samples.

Reference:

1. **Abu Elzein, E.M.E. and Housawi, F.M.T.(2009):** Drastic cutaneous multi-focal orf infection in goats, causing severe dysfunctioning. Rev. sci. tech. Off. int. Epiz., 28 (3), 1025-1029.
2. **Ahmed, M.H., Mervat, M.M. and Eweis, M. (2001):** Isolation and identification of CPD virus from sheep

and goats. J. Egypt. Vet. Med. Assu. 61 (6): 137-141.

3. **Billinis, C.; Mavrogianni, V. S.; Spyrou, V. and Fthenakis, G. C. (2012):** Phylogenetic analysis of strains of Orf virus isolated from two outbreaks of the disease in sheep in Greece. Virology Journal, 9:24.
4. **Chand, P., Kitching, R.P. and Black, N. (1994):** Western blot analysis of virus-specific antibody responses for capripox and contagious pustular dermatitis viral infections in sheep. Epidemiol.Infect., 113, 377-385.
5. **Christine, K., Kyriako, N., Liana, T., Giovanni, S., Rossella, L., Panayotis, M. and Olga, M. (2006):** Phylogenetic correlation of Greek and Italian orf virus isolates based on VIR gene. Veterinary microbiology, 116: 310-316.
6. **Davis, A.G. and Otema, C. (1981):** Relationship of Capri pox viruses found in Kenya with two Middle Eastern strains and some orthopox viruses. Res. In Vet. Sci. 31(2), 253-255.
7. **Eisa, M.I., Hanan, A.F., Fatma, M.D. and Ahmed, M.H. (2003):** Clinical, virological and pathological studies on contagious pustular dermatitis in sheep and goats. 7th sci. cong. Egyptian society of cattle diseases, 7-9 Des, Assiut, Egypt.
8. **Gabry, G.H. (1987):**Ser-epidemiological studies on contagious pustular dermatitis in sheep and human contacts. M.V.sc. thesis, Fac. of Vet. Med., Cairo University.
9. **Haig, D.M. and Mercer, A.A. (1998):** Orf "Review Article". Veterinary Research, 29: 311-326.
10. **Hosamani, M., Yadav, S., Kallesh, D.J., Mondal, B., Bhanuprakash, V. and Singh, R.K. (2007):** Isolation and Characterization of an Indian Orf V irus from Goats.Zoonoses Public Health, 54:204-208.
11. **Kitching, R.P., Hammond, J.M. and Black, D.N. (1986):** Studies on the major common precipitating antigen of capripox. J Gen Virol 1986; 67: 139-48.
12. **Kottaridi, C., Nomikou, K., Lelli, R., Markoulatos, P., Mangana, O. (2006):** Laboratory diagnosis of contagious ecthyma: comparison of different PCR protocols with virus isolation in cell culture. J. Virol. Meth. 134, 119–124.
13. **Lojkic, I.; Cac, Z.; Beck, A.; Bedekovic, T.; Cvetni c, Z. and Sostaric, B. (2010):** Phylogenetic analysis of Croatian orf viruses isolated from sheep and goats. Virology Journal 2010, 7:314.
14. **Mahmoud, M., Abdelrahman, K. and Soliman, H. (2010):** Molecular and virological studies on contagious pustular dermatitis isolates from Egyptian sheep and goats. Res. Vet. Sci.; 89(2):290-294.
15. **Mangana-Vougiouka, O., Markoulatos, P., Koptopoulos, G., Nomikou, K., Bakandritsos, N. and Papadopoulos, O. (1999):** Sheep poxvirus identification by PCR in cell cultures. J. Virol. Meth. 77, 75–79.
16. **Mangana-Vougiouka, O., Markoulatos, P., Koptopoulos, G., Nomikou, K., Bakandritsos, N. and Papadopoulos, O. (2000):** Sheep poxvirus identification from clinical specimens by PCR, cell culture, immunofluorescence and agar gel immunoprecipitation assay. Mol. Cell. Probes 14, 305–310.
17. **Mazur, C. and Machado, R. D. (1990):** The isolation and identification of the contagious ecthyma virus of caprines in cell-cultures. Revista de Microbiologia, Vol. 21, No. 1, pp. 127-130.
18. **Mercer, A.A., Uedaa, N., Friederichs, S., Hofmann, K., Fraser, K.M., Bateman, T. and Fleming, S.B. (2006):** Comparative analysis of genome sequences of three isolates of Orf virus

reveals unexpected sequence variation. Virus R esearch 116 :146-158

19. **OIE Manual of Diagnostic (2008):** Chapter 2.7.14 Sheep pox and goat pox Terrestrial Animals under the heading "Diagnostic Techniques".
20. **Radostits, O.M., Gay, C.C., Hinchcliff, K. W. and Constable, P.D. (2008):** Veterinary medicine, A Textbook of The Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th Edn., W.B. Sounders, London, New York, Oxford.
21. **Robinson, A.J. and Balassu, T.C. (1981):** Contagious pustular dermatitis (orf). Vet Bull, 51:771-82.
22. **Sabban, N.M.S., El-Dahby, H. and Hussein, N. (1961):** Contagious pustular dermatitis in Egypt. Bull. Off. Int. Epiz., 55: 635-656.
23. **Shemies, O.A., (2006):** Isolation and identification of a locally isolated contagious pustular dermatitis virus in sheep. J. Egypt. Vet. Med. Asso. 66 (1):87-96.
24. **Soad, M., Wafaa, A.Z., Micheal, A., Fayed, A.A. and Taha, M.M. (1996):** Studies on sheep pox and goat pox viruses from normally infected animals. Assiut Vet. Med. J. 35(70), 29-38.
25. **Sullivan, J.T., Mercer, A.A., Fleming, S.B. and Robinson, A.J. (1994):** Identification and characterization of an orf virus homologue of the vaccinia virus gene encoding the major envelope antigen p37K. Virology, 202:968-973.
26. **Tantawi, H.H., Fayed, A.A., Shalaby, M.A. and Skalinsky, E.I. (1979):** Isolation, cultivation and characterization of pox viruses from Egyptian water buffaloes. Journal of the Egyptian Veterinary Medical Association 37: 15–23.
27. **Tryland, M., Klein, J., Nordoy, E.S. and Blix, A.S. (2005):** Isolation and partial characterization of parapoxvirus isolated from skin lesion of Weddell seal. Virus Res. 108, 83–87.
28. **Wittek, R., Herlyn, M., Schümperli, D., Bachmann, P.A., Mayr, A. and Wyler, R., (1980):** Genetic and antigenic heterogeneity of different parapoxvirus strains, Intervirology 13, 33–41.
29. **Zarnke, R.L., Dieterich, R.A., Neiland, K.A. and Ranglack, G. (1983):** Serologic and experimental investigations of contagious ecthyma in Alaska. J. Wildlife Dis. 19, 170–174.

الملخص العربى

أيمن سعيد الهباء
قسم الفيرولوجيا-كلية الطب البيطرىجامعة بنها

ينتمى فيروس مرض التهاب الجلد النفطىالمعدى(CPDV)الى جنس parapoxvirus، تحت عائلةChordopoxvirinae، عائلة الجدرىPoxviridae وهو يسبب مرض التهاب الجلد النفطىالمعدىفي الأغنام والماعز في جميع أنحاء العالم وفي مصر. تم جمع ٤٠ عينة من الاصابات الجلدية من أغنام وماعز مشتبهفى إصابتها بالمرض من مناطق مختلفة من محافظة القليوبيةوأخضعت العينات للعزل على بيض الدجاج المخصب (ECE)، والكشف عن أنتيجين الفيروس باستخدام إختبارى الانتشار المناعىفى الاجار (AGIDT) و الوميض الفلورسينتىالمناعى الغير مباشر وكذلك الكشف الجزيئى عن الحمض النووىالفيروسى (DNA) باستخدامإختبار البلمرة المتسلسل(PCR). وقد ظهرت نتائج إيجابية مع ٣٦ عينة (٣٠/٢٨ من عينات الأغنام و ١٠/٨ من عينات الماعز) للعزل على بيض الدجاج المخصببعد التمريرة الثالثة، و مع ٢٢ عينة (٢٨/١٧ من معزولات الأغنام و٨/٥ من معزولات الماعز) باستخدامإختبارى الانتشار المناعىفى الاجار (AGIDT) و الوميض الفلورسينتىالمناعى الغير مباشر(IFA). أظهر الكشف باستخدام إختبار البلمرة المتسلسل (PCR) نتائج إيجابية مع ٤٠/٣٠ منالعينات مباشرة قبل العزلة مع (٣٠/٢٣ من عينات الأغنام و ١٠/٧

من عينات الماعز)، في حين أنها أظهرت نتائج إيجابية مع ٣٦/٢٦ من العينات بعد العزل (٢٨/٢٠ من معزولات الاغنام و ٦/٨ من معزولات الماعز). وقد خلصت النتائج إلى أن إختبارى الانتشار المناعفى الاجار (AGIDT) و الوميض الفلورسينتالمناعى الغير مباشر(IFA) من الطرق المناسبة للكشف عن فيروس مرض التهاب الجلد النفطالمعدى (CPDV)ولكن كان إختبار البلمرة المتسلسل (PCR) الأداة الأكثر تخصصا وحساسية للكشف السريع عن فيروس مرض التهاب الجلد النفطالمعدى (CPDV).