

List of Contents

1.	INTRODUCTION	1
2.]	REVIEW OF LITERATURE	4
3.	MATERIAL AND METHODS	35
4.	RESULTS	56
5.	DISCUSSION	97
6.	SUMMARY	108
7.	REFERENCES	111
8.	ARABIC SUMMARY	



List of Abbreviations

BK : Bovine Kidney

CAM : Chorio – Allantoic Membrane
 CFT : Complement Fixation Test
 CMI : Cell Mediated Immunity

CPD : Contagious Pustular Dermatitis

CPE : Cytopathic Effect

DDW : Double Distilled WaterECE : Embryonated Chicken Egg

EDTA : Ethylene Diamine Tetra-Acetic Acid

EM
 Electron Microscope
 FCF
 Foetal Calf Serum
 G+C
 Gunine + Cytosine

H&E : Haematoxylin and Eiosin
HI : Haemagglutination Inhibition
HBSS : Hank's Balanced Salt Solution
FAT : Fluorescent Antibody Technique

IFAT : Indirect Fluorescent Antibody Technique

IFAN : Interferon

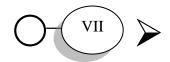
Ig : Immunoglobulin

IGDT : Immue-Gel Diffusion TestITR : Inverted Terminal RepeatIUDR : Iodo-deoxyuridine

MDBK
 Madin Darby Bovine Kidney cell
 Madin Darby Ovine Kidney cell
 MEM
 Minimum Essential Medium

MLV : Modified Living VaccinesmRNA : messenger Ribonucleic Acid

NCF : Newborn Calf Serum





NZ : New Zealand

OK : Ovine Kidney Cell Culture

Orf v-cc : Orf virus strain

ORF
 PBS
 Phosphate Buffer Saline
 PFU
 Pock Forming units

PCR : Polymerase Chain Reaction

PM : Post Mortum

RE : Restriction Endonuclease

RNA : Ribonucleic Acid Rpm : rotation per minute

SDS : Sodium Dodycyl SulphateSNT : Serum Neutralization Test

TC : Tissue Culture

TCID₅₀ : Tissue Culture Infective Dose fifty
 VEGF : Viral Vascular Endothelial Growth Factor

VNT : Virus Neutralization Test

VP : Viral Protein

 γ : Gamma Irradiation



6. Summary

- Twenty five (25) samples from the affected sheep and goats (15 from sheep and 10 from goats) from different governorates show clinical signs like nodules, pustules, and crusts which are obviously appeared on lips, gums, mouth commissures and eyelid were collected in order to isolate and identified the CPD virus by conventional and molecular means.
- The prepared samples from skin lesions were inoculated in embryonated chicken eggs (SPF) for three blind passages by the CAM route.
- By the 3rd passage the number of positive samples increased to 10 (66%) and 6 (60%) respectively for sheep and goats clinical specimens. These positive pathological changes were characterized by odema, thickening, hemorrhages and small grayish white foci and pock lesions.
- Eight samples from both negative and positive results on CAM Were inoculated on confluent sheet of MDBK cell culture and examined daily for developing the characteristic CPE.
- The developed CPE 5-7 days post inoculation appeared in the form of cell rounding, multinucleated cells, then progressing of the CPE till distortion of the monolayer and cell detachment.
- All the eight inoculated suspected samples giving positive results with inoculation on ECE were gave positive results with inoculation on MDBK cell culture after the third passage.



- On the other hand, only six suspected samples out of eight that giving negative results with inoculation on ECE with the ratio of 75% were gave cytopathic effect on MDBK cell line after the third passage.
- The MDBK cell line was more sensitive than ECE for isolation and propagation of CPD virus.
- The histopathological examination of CAM revealed that the ectodermal cells of inoculated CAM appeared with several protrusion to the underlying mesoderm. Some ectodermal cells become fused together forming multinucleated large cells known as syncytia with or without formation of intracytoplasmic inclusions.
- Identification and confirmation of isolated CPD virus was done either by serological and non serological tools. The indirect fluorescent antibody technique (IFAT) was done and the stained MDBK cells inoculated with the positive isolates showed clear specific yellowish green fluorescence.
- The second confirmatory tool applied for identification of viral isolates was done on the harvested CAM by using the electron microscope. The examined samples revealed that the isolated virus was ovoid in shape and characteristic ball of wool appearance.
- The physical and chemical character of the isolates described in this study confirmed that it is a CPD virus. Treatment with IUDR inhibited its replication, indicating its DNA virus. The viability of the isolates was destroyed at 37°C after 10 days, at 56°C within 25 minutes and It is sensitive to the action of diethyl ether, reflecting the presence of an essential lipids in the envelop.



- Sera collected from 225 sheep and goats were used to detect the presence of CPD antibodies by SNT. The test revealed that the titre of antibody (1/8) against CPDV was obtained with 31 samples (12.15%%), titre 1/16 with 36 samples (14.12%), titre 1/32 with 24 samples (9.41%), titre 1/64 with 11 samples (4.31%), titre 1/128 with 3 (1.17%) and finally, no antibodies detected with titre 1/256 in all tested samples.
- Our results concluded that the CPD virus is endemic among small ruminants in Egypt, and further investigations should be followed up.
- PCR test was done using known primer on tissue samples. Amplification and running of characteristic fragments of CPD viral DNA on Ethidium Bromide stained Agarose Gel gave typical results in many segments on DNA fragment as reference virus which indicate that the given sample contain CPD viral antigen.
- The PCR technique proved to be more rapid and efficient for diagnosis of CPD virus infection than other tests.
- Egyptian isolates have high homology % with some Orf isolate in gene bank and the most diversion isolate was Ismailia 1 where it had (92, 9-91, 9) identity with reference strains.