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ISOLATION AND IDENTIFICATION OF LUMPY SKIN DISEASE VIRUS FROM UPPER EGYPT

(With 1 Table and 8 Figures)

By

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عزل وتصنيف الفيروس المسبب لمرض التهاب الجلد العقدى في مصـــر العليـــا

طه العزاوى ؛ مختار الطرابيلى ؛ مراد اسماعيل ؛ صديق رشوان

لقد تم عزل ٢٠ عترة من فيروس التهاب الجلد العقدى من بين ثلاث أماكن مختلفة من مصر العليا وهي : نجع حمادى، الوادى الجديد، (موشا والبدارى) محافظة أسيوط . وقد تم التعرف عليها بتأثيرها على الغشاء السلي السجقي وعلى خلايا خصية الأغنام وخلايا كلية المواشى . وتم التعرف عليها أيضا باستخدام الاختبارات السيرولوجيه مثل اختبار الاليزا واختبار المصل التعادلي.

SUMMARY

Twenty local isolates had been obtained from skin nodules and skin scapes from 3 different localities Nag-Hamadi, New Vally and Mousha, El-Badary (Assiut in Upper Egypt). These isolates were identified by their cytopathogenic effect on CAM, MDBK and LT. As well as serologically by solid phase Elisa Technique and surm neutralization test.

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INTRODUCTION

Lumpy skin disease (LSD) is a highly infectious disease of cattle caused by a member of pox virida and exsists in various areas in Africa (MAC-DONALD, 1931; VON BAKSTROM, 1945; HAIG, 1957; MaCOWEN, 1959; AWAD, 1966 and EL-KANAWATY, 1989).

VANDENENDE et al. (1949) used a filtrate from an emulsion of skin nodes and milk duct nodules from a dead calf by bovine LSD to isolate the agent by serial passage in chick embryo. The agent appears to be filterable virus approximately 10–25 mu in diameter.

ALEXANDER et al. (1957) in the union of South Africa isolated 3 cytopathic virus from skin nodule of cattle suffering from LSD virus type one was Orpha the second Allerton and the 3rd was the Neethling type virus. The last one was the true causative agent of LSD. They added that the true causative agent (Neethling type virus) can be propagated in cell culture of calf and lamb kidney, calf and lamb testis with clear CPE change and on chorioallantoic membrane of chicken embryo (9–11) days old with production of macroscopic pock lesion.

LOSOS (1986) proved that LSD virus can be propagated in a number of mammalian cell culture as tissue from lamb, calves, rabbits, hamster and embryonated chicken egg (9–11) day old and incubated at 33–35.5 C for 6 days.

EL-KANAWATY (1989) isolated LSD virus on CAM from skin nodules and internal organs obtained from 5 cattle in El-Tal-Kabeer and 2 cattle from El-Noberia dairy farm.

WOODS (1988) reported that isolated virus of LSD from infected cattle could be confirmed by SNT with specific antisera. VERSTEEG (1985) mentioned that the Elisa was a widened scope for detection of viruses and their antigen. This work was carried out as a trial to isolate and identify the causative virus of lumpy skin disease in cattle in Upper Egypt.

MATERIAL and METHODS

1) Skin samples:

Skin nodules or scales were taken aseptically from 58 clinically infected cattle ageing between 4 months to 8 years for viral isolation. These

samples were homogenised then kept in tubes containing 4 ml phosphate buffer saline containing 100 Iu of pencillin, 100 ug of streptomycine and 250 Iu of mycostatin. These emulsions, either left at room temperature or incubated at 37 C for 1 hour, were centrifuged at 3.000 r.p.m. for 30 minutes in a cold centrifuge to remove any dehris. The collected supernatant fluid were stored in deep freeze at-70 C until used (ALI and OBIED, 1977).

2) Embryonated hens eggs:

9-11 day-old embryonated hens egg were obtained from agriculture college of Assiut University and examined by candling before used for chorioallantoic (CAM) inoculation to GRIST et al. (1977).

3) Tissue culture:

MDBK as well as primany and secondary (LT) were obtained as cell line from the Institute of Veterinary Serum and Vaccine Research and Production Abbasia. These cell lines were propagated in Eagles MEM suplemented with 10% horse serum or new born calf serum and used for virus isolation according to PLOWRIGHT and FERRIS (1958) and for SNT according to DARCEL (1975).

4) Reference sera:

Specific antisera for LSD virus were obtained from Faculty of Veterinary Medicine, Cairo University, Department of Microbiology.

5) Solid phase Eliza:

Indirect microplate Eliza according to KENDAL et al. (1983).

RESULTS

Table (1) indicates that twenty viral isolates were obtained from skin nodules and skin scales using CAM inoculation and then on MDBK and LT cell cultures, 15 isolates were from the aluminum farm, Nagh-Hamadi, 2 isolates from Mousha and EL-Badary (Assiut) as well as 3 isolates from New Valley.

Table (1) and Fig. (1,2) showed that most of the twenty isolates produce pin point pock lesion in the form of streks or strips. In some cases thickneas and congestion of membrane without any macroscopic lesion were observed.

Fig. (3, 4 & 5) demonsterate the cytopathic effect of the twenty isolates on MDBK, cell culture, which start from the third day post inoculation untile complete destruction of the cell sheet on the eight day.

Fig. (6, 7 & 8) showed the CPE of these isolates on primary and secondary lamb testis (LT) cell culture which start from the third day post inoculation till detachment and complete destruction of cell sheet after about 6–8 days post inoculation.

The identification and confirmatory test to performed on the twenty local isolates using SNT and Elisa in which specific LSD antisera with a titre (1/64) was used. The results revealed that these twenty local isolates were positive LSD virus.

DISCUSSION

Lumpy skin disease is a highly infectious skin disease of cattle caused by a virus, characterized by fever, skin nodules all over the body, oedema especially in hind limb and abortion in some cases (BLOOD and RADOSTITS, 1989).

The results, as shown in table (1) indicated the isolation of LSD virus on CAM, MDBK cell culture and LT cells from skin nodules and skin scales obtained from infected animals from 3 different localities in Upper Egypt. The same methods of isolation were applied by (VANDENENDE, 1949; ALEXANDER et al., 1957; MADIN & DARBY; 1959 and ALI & OBEID, 1977); WAADS, 1988 and EL-KANAWATY, 1989). In addition PROZESKY and BARNARD (1982) and EL-KANAWATY isolated LSD virus from lymph node, internal organs as liver, lung and kidney.

Table (1), on the otherhand, demonstrated that identification of LSD virus in this investigation depends on the cytopathic effect of thevirus on CAM and tissue culture (MDBK and LT). These CPE were also used by VAN-ROOYEN et al. (1949), ALEXANDER et al. (1957); PLOWRIGHT and WITCOMB (1959); VAN-ROOYEN et al. (1969); ALI & OBEID (1977); NAWATHE et al. (1980); WOODS (1988) and EL-KANEWATY (1989). Table (1) revealed also, that the 20 local isolates of LSD were identified and confirmed by S.N.T. AND Elisa using specific LSD antisera. These tests were previously used by (MARTIN & MAUREEN, 1968; VAN-ROOYEN et al., 1965; ALI 7 OBEICL, 1977; HEDGER 7 HAMBLIN, 1983; WOODS, 1988; EL-KANAWATY, 1989; CHO & BAHAC, 1985; LITTLE et al., 1985 and VERSTEEG, 1985). They also stated that Elisa was superior in viriological diagnosis than other methods.

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LEGENDS

Fig. 1: Chorioallantoic allantoic membrane showed pook lesions (6–7 days post inoculation).

Fig. 2: Chorioallantoic allantoic membrane showed pock lesions after (6–7 days post inoculation).

Fig. 3: Normal non infected MDBK showed a complete confluent sheet (5 days post inoculation) (10x40).

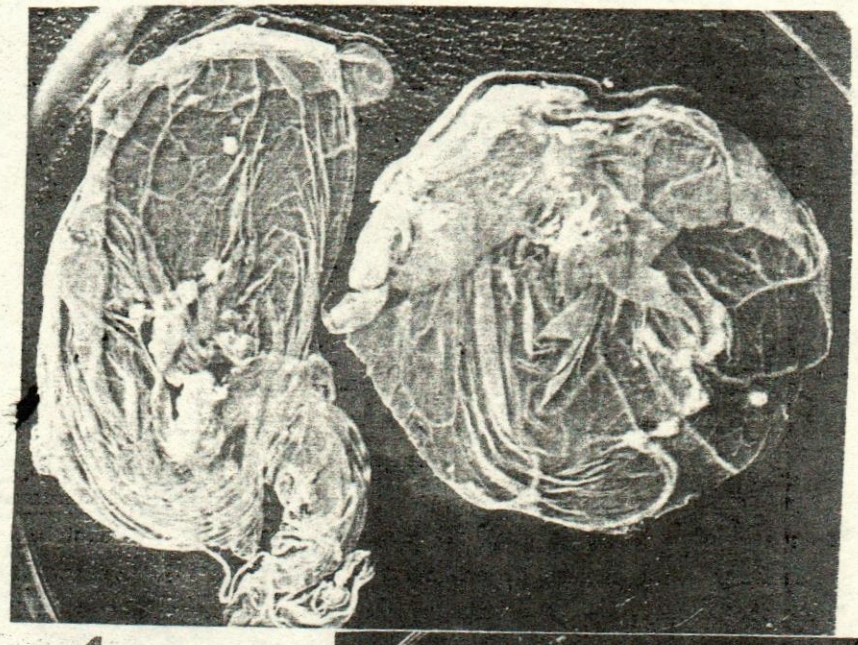
Fig. 4: Infected MDBK showed rounding shrinking anastomoss and destruction of cells (5 days post inoculation) (10x40).

Fig. 5: Infected MDBK showed complete destruction of cells sheets (7 days post inoculation) (10x40).

Fig. 6: Normal non infected LT 4–day–old showed complete confluent sheet.

Fig. 7: Infected LT alls showed rounding, shrinkage, clumping in grapes like formation (4 days post inoculation).

Fig. 8: Infected LT cells 6 days post inoculation showed complete destruction of the cell sheet (10x40).



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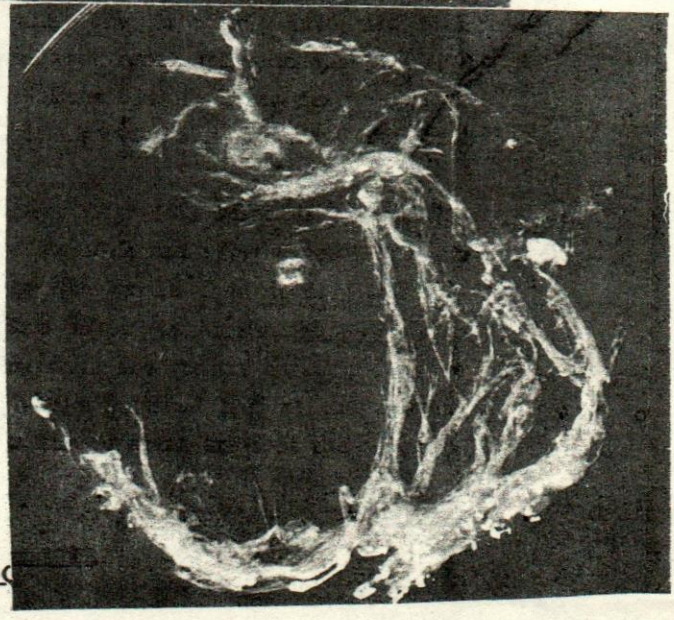

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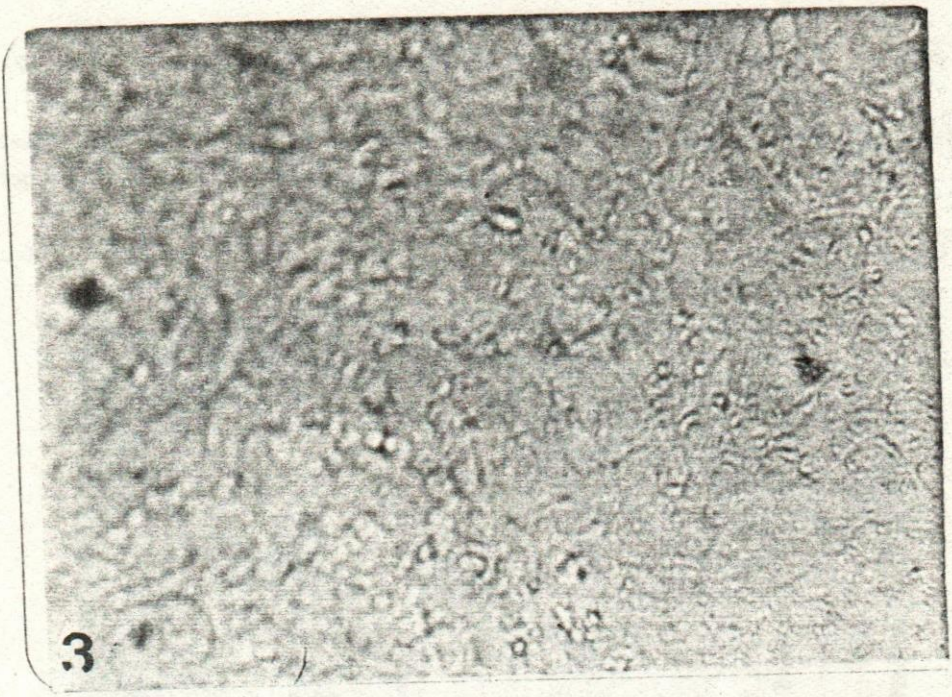
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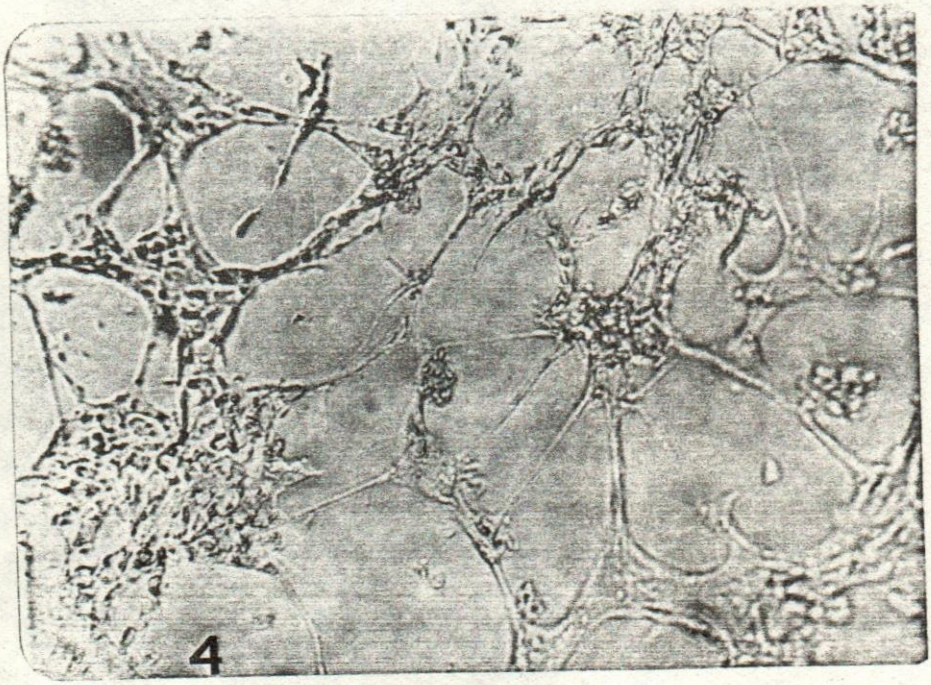
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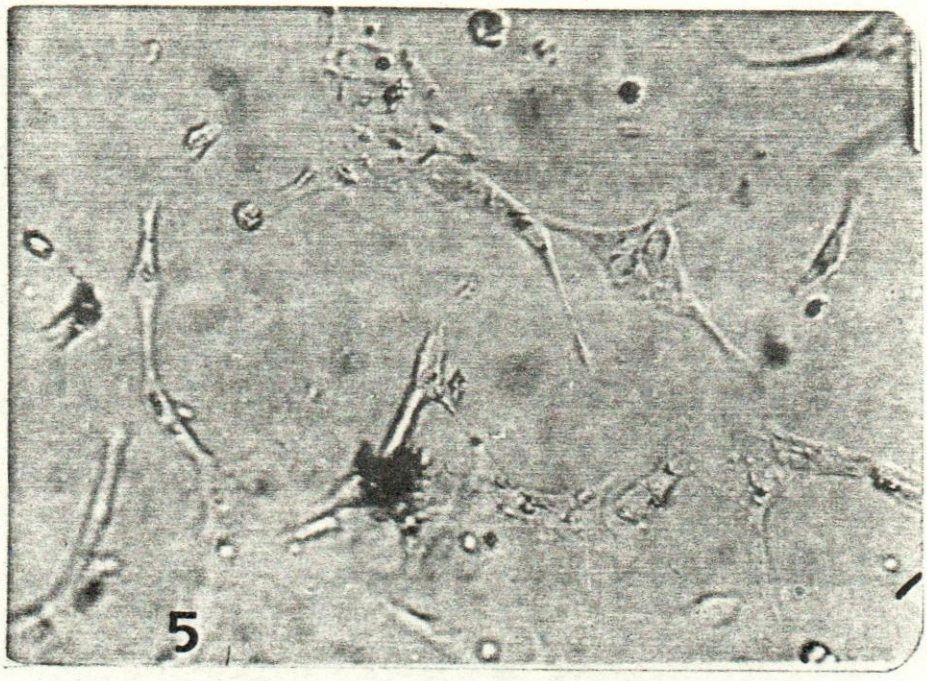
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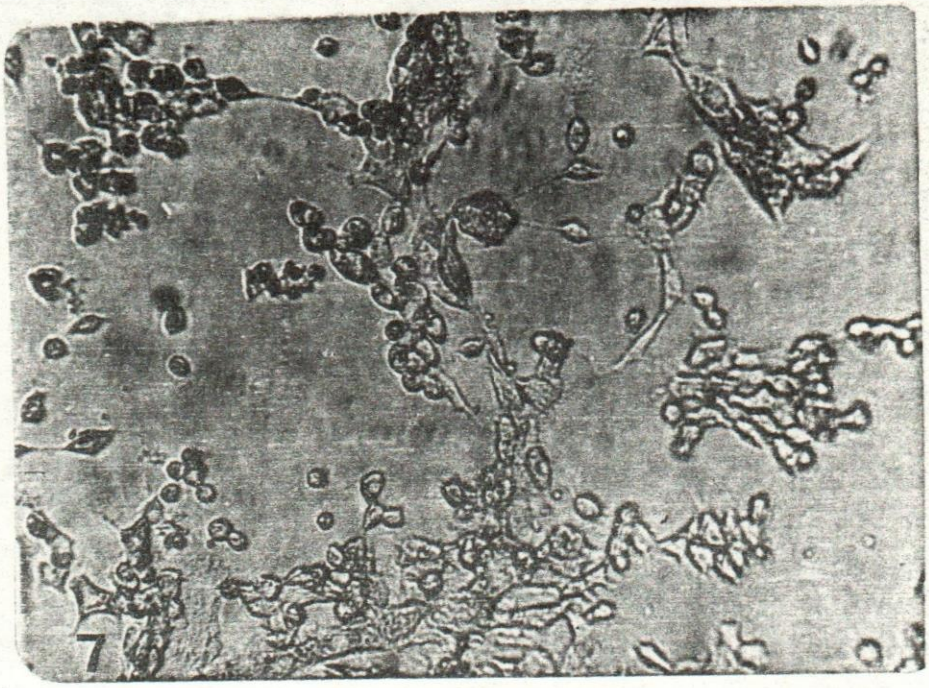

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Table,(1):Isolation and identification, of 20 local isolates of LSD virus.

No.	Locality	Breed	Type of samples	Primary isolate CAM	MDBK	LT	Description of cytopathogenic effect CAM,9-11 Days	MDBK,4 days	LT,4 days	Identification local isolates SNT	ELISA	Agar gel PT	Isolate identification
15	Nagh-Hamadi	Freizian cattle	Skin scrabs	+	+	+	Pock lesion 6-7 days one case gave congestion & thicking of CAM.	Round cell, shrinkage, anastomosis, granulation, cell detachment,3-8 day	Round cell, granulation, shrinkage, clumping in grapes like formation, cell detachment,2-6 day	+	+	-	LSD virus
3	New Vally	Freizian cattle	Skin nodules	+	+	+	Pock lesion 6-7 days	Round cell, shrinkage, anastomosis, granulation, cell detachment,4-8 day	Round cell, granulation, shrinkage, clumping in grapes like formation, cell detachment,3-6 day	+	+	-	LSD virus
2	Mousha Badary (Assiut Governorate)	Freizian Native	Skin nodules	+	+	+	Pock lesion 6-7 days	Round cell, shrinkage, anastomosis, granulation, cell detachment,4-7 day	Angular separation, round cell, granulation clumping in grapes like formation shrinking and cell detachment,3-5 day	+	+		LSD virus