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THE GROWTH BEHAVIOUR OF THE LOCAL ISOLATE OF LUMPY SKIN DISEASE VIRUS (LSD)

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SUMMARY

The aim of this study was to identify and evaluate the kinetics and growth characteristics of LSDV in MDBK cell culture.

The growth curve of the virus on MDBK cells denoted that the highest titre of cell associated virus was 4, 48 $TCID_{50}$/ml obtained 48 hours post inoculation.

The highest titre of cell free virus was 4.63 $TCID_{50}$/ml 48 hours post-inoculation. LSDV is more cell associated than cell free. It is observed that virus growth and release was usually correlated with progression of CPE. The form and sequential cytopathic changes in MDBK cell culture were described.

INTROUCTION

LSD is endemic in many countries in Africa, where regional outbreaks occur from time to another. The disease was introduced for the first time to Egypt most probably through cattle imported from Somalia in April 1988 (Fayed et al., 1988).

The virus of Lumpy Skin Disease (LSD) was primarily isolated in embryonated chicken eggs as well as primary cell culture like embryonic bovine lung (EBL), goat kidney (GM) and lamb testicle cells (LTC).

Further, identification steps have been done at the FADDL, Plum Island, USA.

The virus was successfully neutralized by specific LSDV serum. (House et al., 1990).

The aim of the present study is the detection of the growth behaviour of LSDV as a part in rapid and accurate diagnosis of the disease, as well as vaccine production.

MATERIALS AND METHODS

VIRUS STRAIN

Local Ismailia strain of LSD, isolated in FADDL, PLUM ISLAND, USA, was used throughout the experiment.

CELL CULTURE:

Madin-Darby Bovine Kidney (MDBK) cell were obtained from the virology laboratory of Department of Microbiology, Faculty of Veterinary Medicine,Cairo University, monolayer cell culture was grown in Eagle's MEM supplemented with 10% bovine serum.

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METHYL VIOLET STAIN: (MVS)

Methyl violet 0.5gm
Formaline (36%) 4.0ml
PBS (Phosphate buffer saline) 96.0ml

NVS used for staining of cell culture in the microtiter plates.

VIRUS PROPAGATION:

The LSD virus was propagated for 20 successive passages in MDBK cells. One ml of the virus diluted to 1:10 in Eagle's MEM was inoculated onto the confluent monolayer of cells in Roux bottle. After virus adsorption for 2 hours at 37°C, the culture was washed and fresh Eagle's MEM was added and incubated also at 37°C.

The incubated bottles were daily examined microscopically to follow up the appearance of Cytopathogenic Effect (CPE).

VIRUS TITRATION:

- A microtiter tissue culture, plate, 96 wells was used for the titration of LSDV.
- The suspension of MDBK cells were added in wells.
- Ten fold dilutions of the virus were prepared in sterile vials ranged from 10^{-1} to 10^{-8}.
- From each viral dilution, 50 ul were then transfered into individual wells (5 well per each dilution)
- 100 ul of Eagle's MEM supplemented with 5% horse serum containing MDBK cells were dispensed to each well containing the virus.
- The plates were incubated in CO_2 incubator at 37°C till the 5th day with daily microscopic examination to detect CPE.
- At the end of the test plates were stained with methyl violet and the TCID50 was calculated according to Reed and Muench (1938).

GROWTH KINETICS OF LSDV:

- Three tissue culture bottles were seeded with MDBK cells suspended in Eagle's MEM with 10% bovine serum, when monolayers of cells had formed, they were washed twice with MEM.
- Each bottle was then inoculated with 5ml of undiluted virus. Adsorption of the virus was caried out at 37°C for 2 hours after that inoculum was removed.
- Culture was washed with Eagle's MEM then fresh 80 ml of MEM + 2% horse serum was added into each bottle then inoculated at 37°C (20th passage).

  At various time intervals, 2, 8, 12, 16, 24, 48, 72, 96 and 120 hours post virus inoculation three tissue culture bottles per each time were removed from the incubator.
- Culture supernatants were pooled, then centrifuged at 3000 rpm for 10 minutes at 4°C.
- The harvested supernatants represent the extreacellular virus (free virus) were frozen at, -70°C until its use for virus infectivity.
- The culture bottles containing the cell, still adhering to the glass were resuspended with Eagle's MEM to its original volume and subjected to 3 cycles of freezing and thawing and clarified at 3000 rpm for 10 minutes.

- The supernatants represent the cell associated virus were pooled and kept at -70°C.

- Infectivity titration of extracellular (cell free) and intracellular (cell associated) virus in each time of harvest were assayed in cell culture plates.

-Ten fold dilutions were carried out for cell free and cell associated and 0.1 ml of each virus dilutions were dispensed into 5 individual wells containing 5 X 10^4 cell suspended in MDM+ 5% horse serum.

- The microplates were then incubated at 37°C and were observed for one week.

50% and point was calculated according to the method of Reed and Muench (1938).

RESULTS

SEQUENTIAL DETECTION OF CPE OF LSDV IN MDBK CELL LINE:

The local LSDV strain was serially propagated for 20 times in MDBK cells.

The microscopical examination revealed that no notable changes could be detected during the first 24 hours. Observation after which the infected cells began to show rounding (Fig. 1) was after 48 hours. There was obvious increase in the percent of rounded cells which appeared to be bulging above the monolayer with some cells showing elongation. The cells became detached leaving irregular spaces free from these detached cells (Fig 2).

On the third day about 50% of the sheet showed foci of clumped rounded cells and the areas of the detached cells progressively increased accompanied by appearance of cavitaton in between (Fig 3). On the fourth day most of the sheet cells and the remaining cells were either gathered in clumps or become elongated (Fig 4) also during the period of examination the normal control cells did not show any changes (Fig 5).

Growth Kinetics of LSDV in MDBK cell culture

This step was adopted to study the growth behaviour of LSDV by assessment the infectivity titre of the cell free and cell associated virus. Equal portions of virus harvests were obtained at different time intervals from the supernatant tissue culture medium and cell sediment of MDBK cells.

From the end titre indicated in table (1), one can notice that the titre of the cell free virus was increasing gradually till 16 hr post inoculation (P1) thereafter, the infectivity of the cell free virus particles found to give a constant plateau of gradually increasing virus titre till 96 hr. P. I., where the highest titre obtained was $10^{4.6}$, after which the virus infectivity found to be decreased gradually till 120 hr. P. I. with titre of $10^{3.82}$. Virus titer in the cell associated part increased rapidly till 12 hr. P. I. after which the level of virus titre found to decrease slightly at 15 hr. P. I. where the titre obtained was $10^{3.6}$ and then started again to elevate to $10^{4.43}$ to $10^{4.48}$ at 24 and 48 hr. P. I., respectivley. This rise in virus titre was followed by sharp decrease at 72 hr. P. I. with a titre of $10^{3.3}$ till it reached to $10^{1.8}$ at 120 hr. P. I. as clearly shown in table 1.

TABLE 1:

GROWTH KINETICS OF LSDV IN BDBK CELL CULTURE:

TIME P.I. (post inoculation) (hours)	TITRES OF VIRUS INFECTIVITY (Log 10/0.1 ml)	
	CELL FREE	CELL ASSOCIATED
2	1	1.83
4	1.83	1.7
8	2.74	2.62
12	3.14	3.81
16	3.4	3.6
24	3.4	4.43
48	4.63	4.48
72	4.3	3.3
96	4.6	2.3
120	3.82	1.8

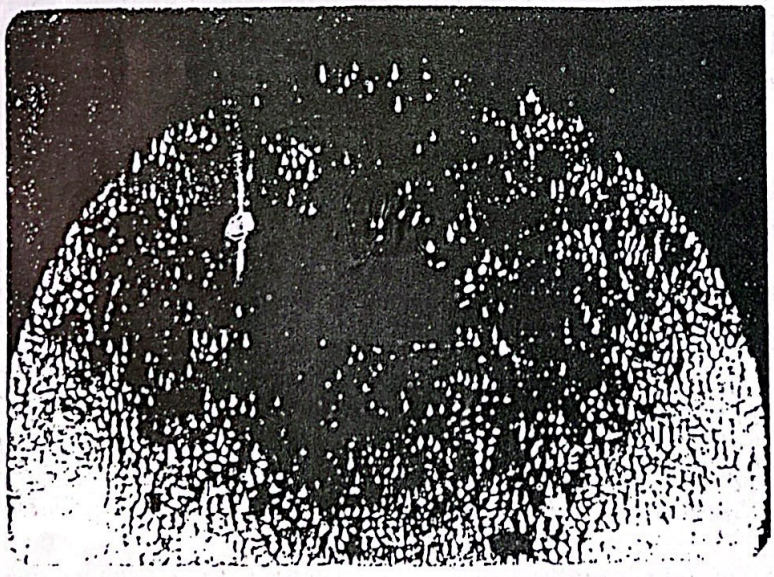


Fig. (1): Infected 11 DBK cells with local strain of I.S.D.V. 24 hours P. I. showing cell rounding. (100X)

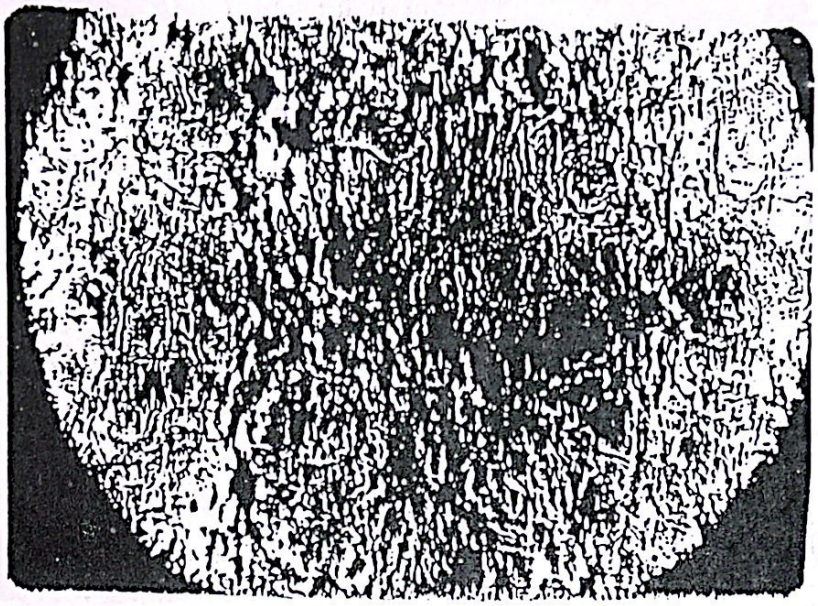


Fig. (2): Infected 11 DBK cells with local strain of I.S.D.V. 28 hours P. I. showing irregular spaces free from detached cells. (100X)

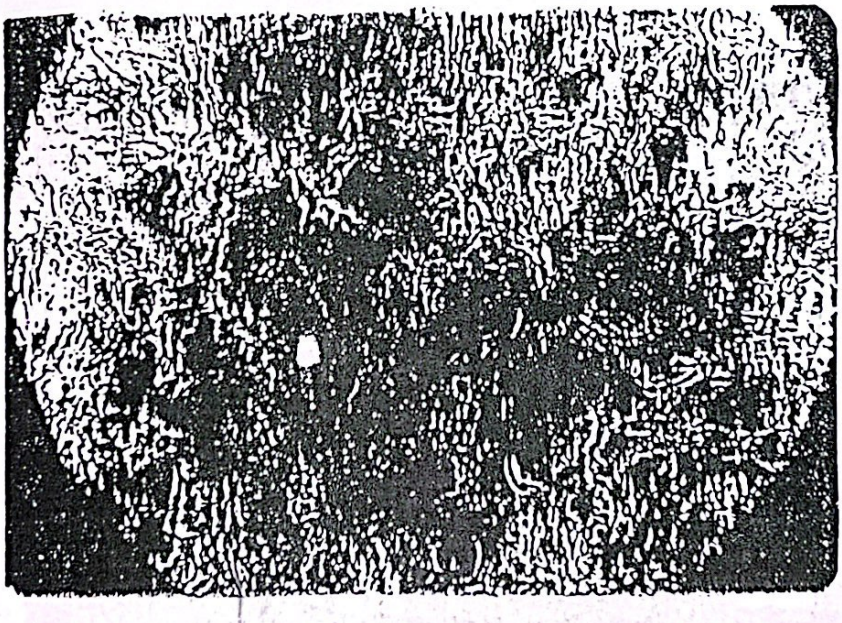


Fig. (3): Infected 11 DBK cells with local strain of I.S.D.V. 72 hours P. I showing cell aggregalion cluster of infected cells. Progressive detached cells and clongation of infected cells (100X)

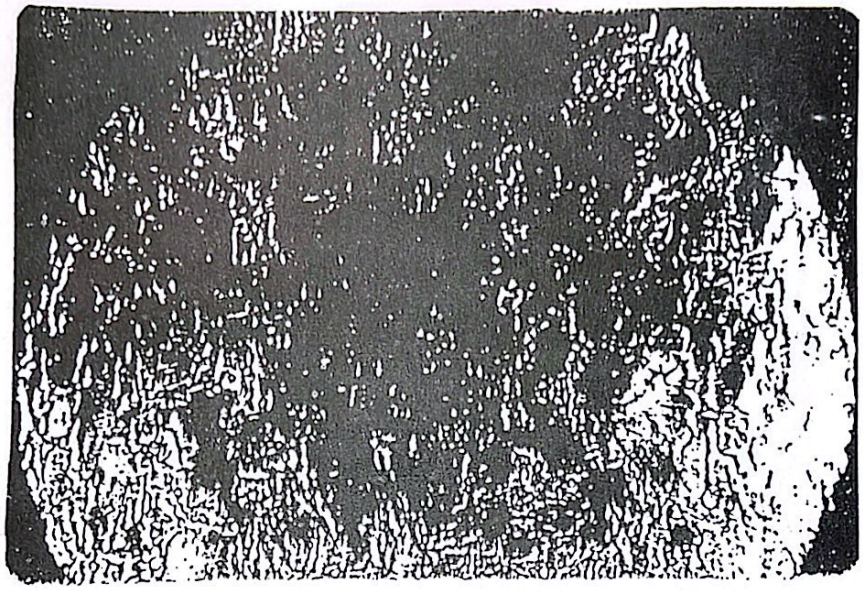


Fig. (4): Infected HDBKcells with local strain of I.S.D.V. 96 hours P.I. showing detachment of most sheet cells. (100X).

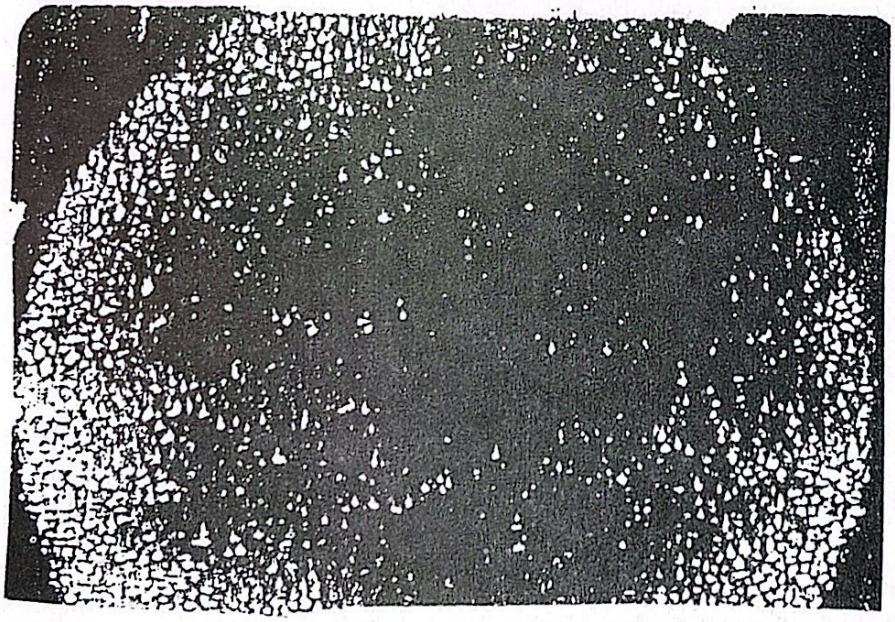
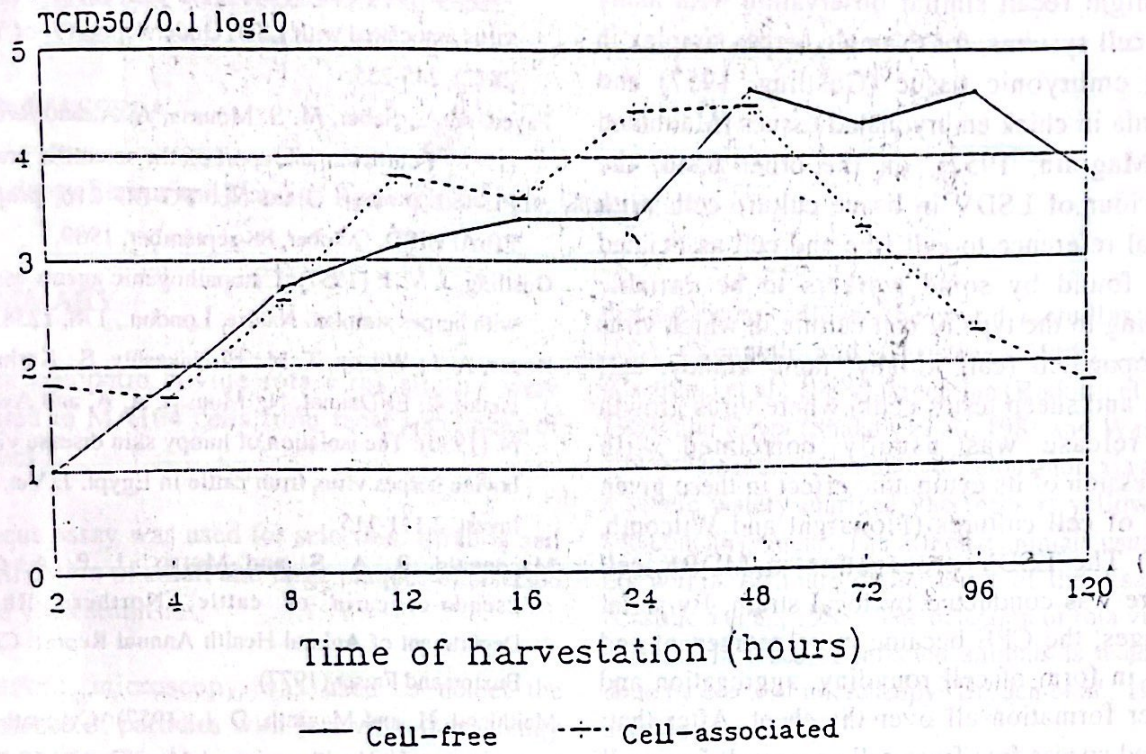


Fig. (5):Hormal unintected HDBK cels . (100X)

Fig.(6): Growth kinetics of L.S.D.V. in MDBK cell culture.



DISCUSSION

LSD is a viral disease which had been known since long time to affect cattle from which the virus was first described and isolated in Northern Rhodesia in 1929 (Macdonald, 1931 and Morris, 1931). The disease was first observed in Egypt in 1988 (Fayed et al., 1988) and was found to be associated with the great economic loss in milk and meat production as well as in hide industry.

The virus was identified at FADDL, Plum Island, USA.

(House et al., 1990). The aim of the present study was oriented to study the kinetic of characteristic growth behaviour as well as sequential follow up of the development of LSD viral protein in infected tissue culture cells (in progress).

From Table (1), it is recommended that the cell associated part was constantly increasing till it reached a titre of $10^{3.81}$ at 12 hr. P. I. with slight decrease in the titre 4 hours later to be $10^{3.6}$ at 16 hr. P. I. and then increased again to reach its highest peak at 24 hr. and 48 hr. with titers of $10^{4.43}$ and $10^{4.48}$ respectively. The same table indicated that LSDV is much more cell associated than cell free because of the clear observable difference in virus titre exhibited by cell free virus especially at 24 hr. and 48 hr. with lower titre of $10^{3.8}$, and $10^{4.63}$, respectively. An interesting observation that sharp drop in the cell associated virus part began 72 hr. to 120 hr. P. I. when compared with cell free part which was constantly increasng till 96 hr. This behaviour might be attributed to the destructive action of LSDV on MDBK cells and the detachment of infected cell from the underlying surface of tissue culture bottle. The constant demonstration of varying

quantities of firmly bound infected LSDV in the cell might recall similar observation with many virus cell systems, for example herpes simplex in chick embryonic tissue (Gostling, 1957) and vaccinia in chick embryonated tissues (Maithland and Magrath, 1957), on the other hand, the behaviour of LSDV in tissue culture cells with special reference to cell free and cell associated parts found by some workers to be variable acording to the type of cell culture in which virus is propagated (calf kidney, lamb kidney, calf testes and sheep testes cells) where virus growth and release was usually correlated with progression of its cytopathic effect in these given types of cell cultures (Plowright and Witcomb, 1969) The LSDV propagation in MDBK cell culture was conducted by local strain. By serial passages, the CPE became more promenant and fixed in form of cell rounding, aggregation and cluster formation all over the sheet. After that; marked spaces free from cell as a result from cell detachment became more increased allover the bottle surface and according to the obtained results the CPE started to appear after 24 hr post inoculation and reached to the maximum level after 48 hr, after which a complete destruction of the cell sheet (96 hr. P. I.) was noticed.

This is in agreement with Deland (1959), Nawathae et al., (1978). Woods (1988) and Abraham and Avivazissman (1991).

REFERENCES

Abraham, A. and Avivazissman, A. (1991). Isolation of lumpy skin disease virus from cattle in Israel. Israel J. of Vet. Med.46 (1), 20-23.

De-Lange, M. (1959): The histology of the cytopathogenic changes produced in monolayer epithelial culture by virus associated with LSD. Onderstepoort J. of Vet. Res. 28 (2), 245-255.

Fayed, A. A.; Saber, M. S. Moussa, A. A. and Reda, I. M. (1988): Fourth annual report on the scientific progress of PL-480. project, Grant No. TG-EG 216, project No. PB-APHISD, October, 88-september, 1989.

Gostling, J. V. J. (1957): Cytopathogenic agents associated with herpes simplex. Nature, London,, 178, 1238.

House, A. J.; Wilson, T. M.; El-Nakashly, S.; Karim, I. A.; Ismail, I.; El-Dannaf, N.; Moussa, A. A. and Ayoub, N. N. (1990): The isolation of lumpy skin disease virus and bovine herpes virus from cattle in Egypt. J. Vet. Diagn. Invest. 2:111-115.

Macdonald, R. A. S. and Morris, J. P. A. (1931). Pseudo-urticaria of cattle. Northern Rhodesia Department of Animal Health Annual Report. Cited by Buxton and Fraser (1977).

Maithland, H. and Magrath, D. I. (1957): Cytopathogenic agent associated with vacinia. J. Hyg. Camb., 55, 347.

Morris, J. P. A. (1931): Pseudo-urticaria-Northern Rhodesia Department of Animal Health Annual Report, 1930, 12.

Nawathae, D. R.; Gibbs, E. P. J.; Asagaba, M. O. and Lawman, M. J. P. (1978): Lumpy skin disease in Nigeria. Tropical Animal Health and Production 10 (1), 49-54.

Plowright, W. and Witcomb., A. M. (1969). The growth in tissue culture of a virus derived from lumpy skin disease of cattle. J. Path. Bact. 79, 397-406.

Reed, L. M. and Muench N. (1938): A simple method for estimating fifty percent end point. Am. J. Hyg. 27: 493-497.

Woods, J. A. (1988): Lumpy skin disease, a review. Tropi. Anim. Hlth. Prod. 20 (1), 11-17.