BIOCHEMICAL AND MOLECULAR GENETICS DISTINCTION AMONG SHEEP POX VIRUS STRAINS

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Sheep pox (SP) is a highly contagious viral disease of sheep (Butox and Frazzer, 1977).It is caused by one of the Capri pox virus. The mortality rate of disease is usually low but it causes very important economic losses. It reduces yield of milk, meat and depreciation of wool, abortion and mastitis in ewes and deaths in unweand lambs (Sabban, 1955; Fenner, 1996; Oguzoglu *et al.,* 2006). The Sheep pox viruses (SPV) are large double stranded DNA (dsDNA) virus brick-shaped, SPV genome range size from approximately 130 to300 kbp. The disease is characterized by local ridding (Macula), the popular stage, the vascular stage and final pustule (Sabban, 1960; OIE, 2004).

Vaccination is the only and major weapon of controlling these maladies (Jadhav *et al*., 1989). In Egypt, SPV vaccine was prepared by attenuated live SPV (Michael *et al*., 1997). The first vaccine was prepared from Romanian strain of SPV by the subcutaneous inoculation of susceptible lambs. Another vaccine was prepared from Kenyan strain of SPV by successive propagation in Vero cells (Samir, 1994). The virulent Egyptian strain of SPV (local strain) was adapted first in lamb testicle cells then in Vero cells for 20 successive passages (Aboul-Soud *et al.,* 1998). The present work aimed to differentiate between the Egyptian, Romanian and Kenyan strains of SPV by estimating the total viral protein using spectrophotometer protein profile by Sodium Dodecyle Sulphate Polyacrylamid Gel Electrophoreses (SDS-PAGE), and polymerase chain reaction (PCR) based strategy using primers designed from the sequence of SPV DNA (ITRS). The results obtained may help in choosing the most suitable strain for vaccine production that may give a good immune response for controlling the circulating virus in Egypt.

MATERIALS AND METHODS

A- Materials

1. Virus Source

Egyptian strain of SPV was isolated by Sabban (1960) at Pox Dept., Veterinary Serum And Vaccine Research Institute, Abbasia, Cairo, Egypt. The virus was adapted in Vero cells by Aboul Soud *et al*. (1998). Romanian strain of SPV was used for vaccine production in lambs in

Egypt till 1994, and then adapted in lamb testicle cells and Vero cells by Samir (1994). Kenyan strain of SPV is a vaccinal strain adapted in lamb kidney cells and obtained by Dr. House from the Foreign Animal Disease Diagnostic Laboratory (FADDL), USA.

2. Animals

Three susceptible lambs about 6 months old were purchased from El-Wade El-Gedeed Governorate, Egypt.

3. Cell culture and medium

African green monkey kidney cell line (VERO) was obtained from FADDL, plum island, USA. It is maintained using Earl's minimum essential medium (EMEM) with 10% new born calf serum. This medium was used for virus propagation and titration.

4. Titration and propagation

Titration and propagation were prepared according to Dulbeco and Voget (1954) and Daoud *et al.* (2003). The chemical reagent and buffer include 0.25% trypsin, phosphate buffer saline (PBS), pH (7.2), sucrose; lactalbumin hydrolyzate was obtained from Difco laboratories (Michigen, USA), methyl violet stain and bovine serum albumin (BSA)

5. Sodium dodecyle sulphate-poly-acrylamide gel electrophoresis (SDS-PAGE)

This was performed according to Laemmli (1970) and Sambrook *et al.* (1989) and includes 30% acryl amide mixture, 10% separating gel, 5% stacking gel, running buffer, SDS loading buffer, coomassie blue staining and destaining solution and prestained standard protein marker (Gibco-BRL).

6. Western blot

A nitrocellulose membrane (NCM), T-PBS, blocking buffer, hyper immune serum which prepared in rabbits, anti ovine horseradish peroxides conjugate and 3-amino -9ethyl carbazol (AEC) substrate

7. Polymerase chain reaction

DNA extraction was carried out according to Sambrook *et al*. (1989) the SPV (DNA) fragments was generated by PCR based strategy using Oligo Ins-1.1/Ins-1.1 primers F: 5'- AGA AAC GAG GTC TCG AAG CA-3, R: 5-GGA GGT TGC TGG AAA TGT GT-3 designed from the sequence of SPV DNA Inverted Terminal Repeats (ITRs) described by Gershon and black (1989) and Black *et al*. (1986).

B- Methods

1-Propagation and titration of SPV strains

The three strains of SPV used in this study were propagated individually in Vero cells according to the method described by Das and Malick (1986) and Singh and Rai (1991). The viruses was inoculated to confluent Vero cells, incubated for one hour at 37°C to allow

the virus to be absorbed on the cells with occasional tilting, covered with maintenance medium, then incubated at 37°C for 4 days with daily observation for the development of CPE. The inoculated cultures were exposed to three cycles of freezing and thawing then centrifuged at 3000 rpm for 10 min. The viral Supernatant fluid was harvested. Virus titration was carried out according to Mateva and Stoichev (1995). Ten fold dilution of each strain of SPV were prepared separately in PBS .tissue culture micro plate 96 well were seeded with Vero cell and incubated at 37°C in CO_2 incubator for 1 hour till obtaining the confluent monolayer of Vero cell. Then add 100 ul/well of each dilution of the virus. And reincubate for 7 days in CO_2 incubator then examine daily for CPE detection and record the result after staining with crystal violet, calculate the titer as log 10TCID50 and the virus titers were estimated according to Karber (1978).

2- Determination of protein concentration and SDS-PAGE

The protein concentration was estimated using modified lowery (Ohnishi and Barr, 1978). SDS was done on SPV strains according to Laemmli (1970) and Sambrook *et al.* (1989). The viral protein was boiled for 5 minutes with lysis buffer and loaded at the same gel with the prestained standard protein marker. The gel was run and read by computer programs through scanner. The molecular weight (M.W.) of protein bands were estimated by comparing their electrophoretic mobility's with these of known standard M.W marker loading in the same gel.

3- Preparation of hyper immune serum against sheep pox virus strains

Thirty five rabbits were divided to three groups, each 10 rabbits and 5 rabbits were used as a control. For the first group, ten rabbits were inoculated intradermally (ID) with (0.5 ml) containing $10^{5.2}$ $TCID_{50}$ /ml of Egyptian sheep pox virus (ESPV). For the second ten, rabbits were inoculated (ID) with (0.5 ml) containing $10^{5.2}$ $TCID_{50}$/ml of Romanian sheep pox virus (RSPV). For the third ten, rabbits were inoculated (ID) with 0.5 ml containing $10^{5.0}$ $TCID_{50}$/ml of KSPV each of them emulsified with 0.5 ml of complete Freund's adjuvant in the first week of injection.

Then four equal injections were given with a week interval between injections by 0.5 ml of incomplete Freund's adjuvant followed by an intravenous (IV) inoculation of 1 ml of the purified virus fluid in the ear vein in the last week.

Rabbits were bled 10 days after the last injection. Collected blood was left for 2 hours at room temperature then overnight at 4°C for clotting and serum separation. Collected sera were centrifuged at 2500 rpm for 10 minutes. The sera were dispensed in bottles.

4-Western blot

It was performed as described by Towbin *et al.* (1979) and Burnette (1981).

The separated viral proteins in SDS-PAGE were transferred electrophoretically to NCM rinsed thoroughly in T-PBS and blocked in blocking buffer with skimmed milk on a rotating shaker wash and incubated with diluted hyperimmune serum for two hours, washed again and incubated with anti ovine horseradish peroxides for 1 hour. The color reaction was developed by incubation the membrane in substrate for 30minutes, then stopped and read by scanning.

5- Polymerase chain reaction (PCR)

PCR reaction was carried out using 100 pmol of each oligonucleotide primer and two units of *Taq* DNA polymerase (Finzyme, Finland).Each genomic DNA preparation sample(1.5ul) that containing 1ug total DNA were placed in 50 ul of the final volume of 50 ul reaction mixture containing 50 mMKCl, 10 mM Tris-HCL (pH 8.5), 1.5 mM of each dNTP. The DNA was denaturized 95°C for followed by 35 cycles, annealing at 55°C for 1 min and extension at 72°C min for 1 min. The 35 cycles were followed by a final extension at 78°C for 8 min.

RESULTS AND DISCUSSION

1-Propagation of SPV strains

Each SPV strains was inoculated in a confluent sheet of Vero cell culture and examined daily for detection of the cytopathic effect (CPE). The results showed that, in case of Egyptian SPV strain in the 1st and 2nd passages, no CPE was observed at 1st day post inoculation (DPI) but 3rd DPI the CPE was inform of small aggregations of Vero cells and intra cytoplasmic inclusion bodies (Fig. 1C), the CPE was delayed till the 5th DPI after 18 passages (Fig. 1F). In case of Romanian SPV strain, the 1st and 2nd passages, no CPE was observed at 1st day post inoculation (DPI) but 3rd DPI the CPE was inform of small aggregations of Vero cells and intra cytoplasmic inclusion bodies (Fig. 1D), the CPE was delayed till the 5th DPI after 8 passages (Fig. 1G), whereas, CPE in case of Kenyan SPV strain in the 1st and 2nd passages, no CPE was observed at 1st day post inoculation (DPI) but 3rd DPI the CPE was inform of small aggregations of Vero cells and intra cytoplasmic inoculation bodies (Fig. 1E), the CPE was delayed till the 6th DPI after 13 passages (Fig. 1H).

The CPE of Egyptian, Romanian and Kenyan SPV strains characterized by the presence of intra cytoplasmic inclusion bodies in Vero cell culture begin to appear before the appearance of CPE by 24 hours it was small granular at the beginning, after that it became large dense and rounded masses which has a jute position to the nucleus At the 5th DPI, the CPE characterized by rounding and aggregation of cell forming grape like appearance then the cell membrane was fused forming multinucleated giant cell. The infected giant cells were detached leaving holes in the cell sheet and some cell aggregates were detached forming a focus of clear area by time a complete destruction of the cells was developed forming network.

The control non inoculated Vero cells appeared to be glistening cells arranged together and forming intact sheet with no abnormalities as shown in Fig. 1 (A and B). These results agreed with those previously obtained (Vegada and Sharma 1973; Mateva and Stoichev, 1975; Tozzini *et al.,* 1987).

2- Titration of SPV strains

The titration of propagated SPV strains on Vero cell revealed that the titers were 5.5 $\log_{10}$, 5.2 $\log_{10}$ and 6.3 $\log_{10}$ (TCID 50/ml) for Egyptian, Romanian and Kenyan SPV strains, respectively. These results are presented in Table (1) and Fig. (2A, B and C). Egyptian SPV strain has a titer of 5.5 TCI D 50/ml, which has obtained at 10^{th} passage after 5 days post inoculation (DPI). This result was illustrated in Fig. (2C). However, the result obtained in Fig. (2B) demonstrated that the Romanian SPV strain reaching the level of 5.2 TCI D 50/ml, at 5 DPI which obtained at 8^{th} passage. In case Kenyan SPV strain (Fig. 2A), the titer was 6.3 TCID50/ml, which obtained at 9^{th}, 10^{th} passage at 6 DPI. These results are in full agreement with Plowright and Ferris (1958).

3- SDS-PAGE

The total protein amount of (Egyptian, Romanian and Kenyan) SPV strains were 0.69 g/dl, 0.71 g/dl and 0.66 g/dl, respectively (Table 1).

The electro-photometric characterization of SPV strains by SDS-PAGE revealed that each of these viruses has a complex protein profile but although the over all protein profiles of SPV strains were visually similar. There were some substantial differences between individual strains. The electrophoretically separated based on Molecular weights as shown in Fig. (3). The Maximum bands number was 12 there are 4 Monomorphic bands in three strains. The MW of the electrophoretic products ranged from -~ 13 to -~172 kDa the bands exhibite the most common bands were located In the case of Egyptian and Romanian strains (172, 155, 128, 91, 72, 52, 40, 30, 29, 30, 17, 15 and 13 kDa). In The case of Kenyan strain the protein resolution in to (130, 50, 40 and 15 kDa). The result of protein separation of the (SPV) Strains in comparison with standard marker was presented in Fig. (4). The comparison between the obtained results and those obtained with Black *et al*. (1986), Kitching *et al*. (1986) and Rao *et al*. (1996) indicated there are complete agreements between them. They proved that by SDS-PAGE, the major structure polypeptides of Capri pox viruses (sheep, goat, and lumpy skin disease viruses) migrated on SDS-PAGE and the MW of these polypeptides were 72, 67, 40 kDa. Also, they added that the Capri pox viruses share a common soluble major antigen which consists of 210 kDa proteins contained 3 polypeptides of 100, 30 and 17 kDa.

4- Western blot

In which virus-specific antibody responds to structural proteins of the virus were analyzed using western blot assay

(Fig. 5). The results appeared the immunogenic protein fraction (67, 34, 32 and 26 kDa) in the case of Egyptian SPV and Romanian SPV strains. In the case of Kenyan strain (100, 75, 67 and 49 kDa). This result appeared some substantial difference between strains in there immunogenic protein fraction and their density by using the first polyclonal anti body (hyper immune serum) as anti species then with the second anti body (anti-ovine immunoglobulins conjugated with enzyme hors radish peroxides. Antigen-antibody complex is visualized with substrate 3-amino-9carbazole (AEC) by developing common perceptible color reaction.

These results agreed with those obtained by Chand *et al.* (1994) and Romano (2000), where they reported that the protein fractions of Capri pox viruses were 75, 67, 42, 32, 26, 19 and 17 kDa by using SDS-PAGE and the immunogenic proteins were 67, 32 and 26 kDa by using western blot. Also, Hanan *et al*. (2002) proved these results.

5- Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) of SPV strains for SPV inverted terminal repeat (ITRS) gene indicated that the three strains showed positive (+ve) amplification with primer ins-11/ins-1.1' which amplified to 289 kbp (Fig. 3). This result agreed with Gershon and Black (1989), who found that sheep pox virus genome consists of a dsDNA of 130-300 kbp. This differentiation revealed that SPV strains have a similar level of positive reaction. These results agreed with Ireland and Binepal (1998) and Mangana-Vougiouka *et al*. (2000).

From this study, it has been concluded that there was no differences between Egyptian, Romanian and Kenyan strains of SPV in tissue culture. A study of total protein and protein analysis by SDS-PAGE and western blotting showed some variations in the protein profile although the viral proteins were applied under the same conditions. These variations may be due to some differences in major immunogenic protein fractions (epitopes). These variations are very important for vaccine evaluation or production.

SUMMARY

This study was planned to investigate SPV strains in order to choose the most suitable strains for vaccine production which will give e a good immune response for controlling the circulating virus in Egypt. For this purpose, the following experiments were done: propagation of ESPV strain on Vero cell for 18 passages, RSPV strain for 8 passages and KSPV strain for 13 passages the CPE was clear and completed after (5, 5 and 6) DPI for Egyptian, Romanian and Kenyan strains respectively. Titrations of SPV strains were 5.5, 5.2 and 6.3 TCID 50/ml for Egyptian, Romanian and Kenyan SPV strains, respectively. Concentration of virus to 1/10 from its size to prepare antigen of SPV to each strain. Hyper immune serum was used in western blot as a polyclonal antibody

which reacts with nitrocellulose membrane. Sodium dodcyl sulphate-poly acrylamide gel electrophoresis revealed that there are similarity in molecular weight between Egyptian and Romanian strains were estimated by comparing their electrophoreses mobilities with those of known standard molecular weight marker after electrophoresis in the same gel following staining by coomassie blue stain the M.W of Egyptian and Romanian strains (172, 155, 128, 91, 72, 52, 40, 30, 29, 17, 15, and 13 kDa). But there are differentiating between them and Kenyan strain which M.W is (130, 50, 40, and15 kDa). Western blotting technique from this test concluded that there is relationship in immune response between Egyptian. Romanian which immunogenic protein fractions were ESPV and RSPV (67, 34, 32, 26) kDa KSPV (100, 75, 67, 49 kDa). From this result the RSPV is a best strain for vaccine production which gives high immune response for sheep pox. Polymerase chain reaction (PCR) revealed that no difference between three strains which have a similar positive reaction from these results we conclude that RSPV have close relation ship with ESPV

REFERENCES

Aboul Soud, E. A., S. S. Samir, A. M. Ishak, Soad M. Soliman and Wafaa A. Zaghloul (1998). Adaptation of Egyptian sheep pox virus in cell culture. 8thSci. Con. 15-17 Nov .1998, Fac. VET. Med., Assuit Univ., Egypt.

Butox, A. and G. Frazzer (1977). Animal microbiology. 1st Published 2: 745-747. Distributed in USA by J. E. Lippincott Company. Philadelphia and in Canada by J. El. Lippicott Company of Canada LTD Toronto.

Black, D. N., J. M. Hammond and R. P. Kitching (1986). Genomic relationship between Capri poxvirus. Virus Res., 5: 277-292.

Burnette, W. N. (1981). Western blotting electrophoretic transfer of protein from sodium dodecyl sulphate polyacrylamide gel to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Am. Biochem., 112: 195-203.

Chand, P., R. P. Kitching and D. N. Black (1994). Western blot analysis of virus specific antibody responses for Capri pox and contagious pustular dermatitis viral infections in sheep. Epidemiol. Infect., 113: 377.

Das, S. K. and B. B. Mallik (1986). Evolving of live modified sheep pox viral strains: modification of indigenous viral strains. Ind. J. Exper. Biol., 24: 282-286.

Daoud, A. M., F. F. Zaki, E. A. Aboul Soud, Elham A. El-Ebiary and Soad M. Soliman (2003). Comparative studies on the protein of the local sheep pox strain with standard Kenyan and Romanian

strains. Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo.7th Sci. Cong. Egyptian Society for Cattle Diseases, Assiut, Egypt.

Dulbeco, R. and M. Voget (1954). Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med., 99: 167

Fenner, F. (1996). Pox viruses. Field virology, 3rd Ed. pp. 2673-2720.

Gershon, P. D. and D. N. Black (1989). A Capri poxvirus pseudo gene whose only intact homologous are in other pox virus genomes. Virology, 172: 350-354.

Ireland, D. C. and Y. S. Binepal (1998). Improved detection of Capri poxvirus in biopsy samples by PCR. J. Virol. Methods, 74: 1-7.

Jadhav, K. M., A. K. Pandey and N. S. Radadia (1989).Field observations on Sheep Pox tissue culture vaccine. Indian veterinary Journal, 66: 908-912.

Kitching, R. P., J. M. Hammondand D. N. Black (1986). Studies on the major common precipitating antigen of Capri pox virus. J. Gen. Viral., 67: 139-148.

Karber, (1978). Manual of standardized method for veterinary microbiology. Bull. Int. Epizo., 63: 1607-1625, sheep pox. Ind. J. Vet. Sci., 31: 132-140.

Laemmli, U. K. (1970). Cleavage of structural protein during assembly of the head of bacteriophage T4. Nature (London), 227: 680-695.

Mangana-Vougiouka, O., P. Markoulatos, G. Koptopoulos, K.Nomikou, N. Bakandritsos and O. Papadopoulos (2000). Sheep pox virus identification from clinical specimens by PCR cell culture, immunofluorescence and agar gel immunoprecipitation assay. Molecular and Cellular Props, 14: 305-310.

Mateva, V. and S. Stoichev (1975). Adaptation and cultivation of sheep pox virus (strain Prego) in tissue culture and evaluation of its immunogenic properties. Veterinarno Meditsink, Nauki, 12: 18-23.

Michael, A., M. S. Saber, Soad M. S., A. A. Mousa, S. A. Salama, A. A. Fayed, I. N. Mounira and J. Hause (1997). Control of lumpy skin disease outbreak in Egypt with Romanian sheep pox vaccine. Assiut Vet. Med. J., 36: 173-180.

Office International des Epizooties (OIE) (2004). Sheep pox and goat pox. Manual of Diagnostic tests and vaccines for Terrestrial animals. Part 2, Section 2-1, Chapter 2.1.10: 1-16.

Oguzolu, T. C., F. Alkan, A. Ozkul, S. A. Vural, A. B. Gungor and I. burgu

(2006). A sheep pox virus outbreak in central turkey in 2002: Isolation and identification of Capri poxvirus ovis. Vet. Res. Commun., 30: 965-971.

Ohnishi, S. T. and J. K. Barr (1978). A simplified method of quantitating protein using the Biuret and phenol reagents. Anal. Biochem., 86: 193-200.

Plowright, W. and R. D. Ferris (1958). The growth and cytopathogenicity of sheep pox virus in tissue culture. Br. J. Exp. Pathol., 39: 424-435.

Ramano, M. (2000). Manual of standards Diagnostic Tests and Vaccines, Chapter 2: 1-7.

Sabban, M. S. (1955). Sheep pox and its control in Egypt using a desiccated live virus vaccine. Am. J. Vet. Res., 16: 204-213.

Sabban, M. S. (1960). Sheep pox and its control in Egypt. Bull. Off. Int. Epiz., 53: 1527-1539.

Sambrook, J. E. F., E. Fritsch and T. Maniatis (1989). Molecular cloning: A Laboratory Manual. 2nd Ed. Cold Spring Harbor., Laboratory Press, New York

Samir, S. S (1994). Further Studies on Sheep Pox Disease in Egypt PhD. Coll. Vet. Med., Cairo Univ.

Singh, B. and A. Rai (1991). Adaptation and growth of sheep pox virus in Vero cell culture. Ind. Vet. Med. J., 15: 245-250.

Towbin, H, T. Stochein and J. Gordon (1979). Electrophretic transfer of proteins from Polyacrylamide gels to nitrocellulose sheet. Procedure and some applications Proc. Natl. Acad. Sci., USA, 76: 4350-4554

Tozzini, F., H. A. Salim, H. D. Abdyabar and A. F. Abdulcadir, (1987). Growth characteristics of vaccine strains of Rinderpest virus and ovine pox virus on subcultures of bovine fetal lung fibroblasts. Arch. Vet. Italiano, 38: 9-12.

Vagada, J. L. and G. L. Charma (1973). Cutaneous and pulmonary lesions of sheep pox. Ind. J. Anim. Sci., 43: 1061.

Table (1): Cytopathic effect, titration and protein quantities of SPV strains.

SPV strains	Titre/$\log_{10}$ TCID 50/ml	CPE/DPI	Viral protein g/dl
Egyptian SPV	5.5	5	0.69
Romanian SPV	5.2	5	0.71
Kenyan SPV	6.3	6	0.66

SPV: Sheep pox virus, TCID 50: Tissue culture infected dose fifty, CPE: Cytopathic effect, DPI: Day post inoculation.

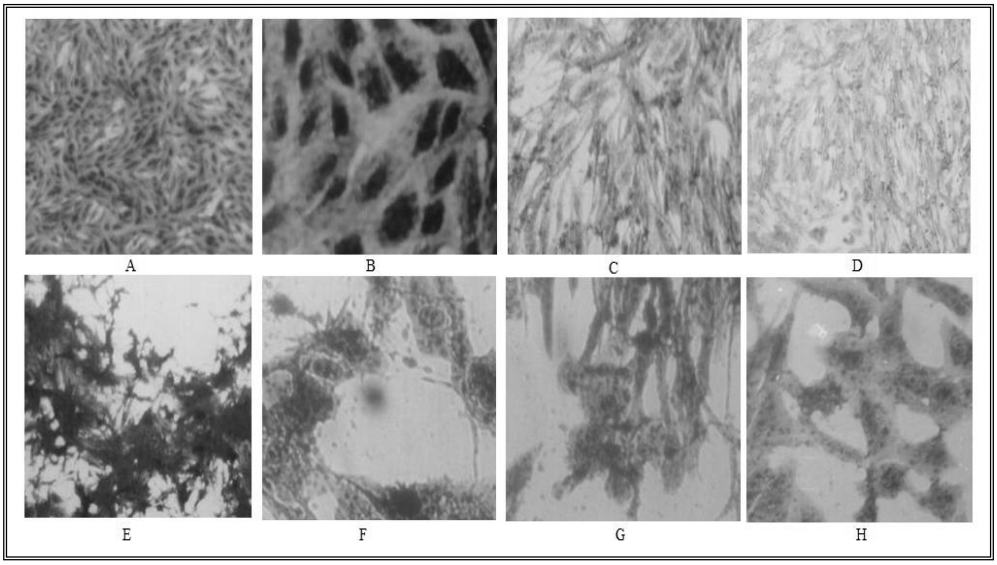

Fig. (1): Normal Vero cells stained with crystal violet (low power 10x) A, : B: high power 32x, C: cytopathic effect of ESPV at 3rd DPI 18 passage showing cell aggregation and foci of clear area (low power 10x), D: Cytopathic Effect of RSPV at 3rd DPI 8 passage showing cell aggregation and foci of clear area (low power 10x), E: Cytopathic Effect of KSPV at 3rd DPI 13 passage showing cell aggregation and foci of clear area (low power 10x), F: Cytopathic Effect of ESPV in Vero cell at 18 passages at 5th DPI showing giant cell formation and intracytoplasmic inoculation bodies (power 32x), G: Cytopathic effect of RSPV in Vero cell at 8 passages at 5th DPI showing giant cell formation and intra cytoplasmic inoculation bodies(high power 32x), H: Cytopathic effect of KSPV in Vero cell at 13 passages at 5th DPI showing giant cell formation and intra cytoplasmic inoculation bodies (high power 32x).

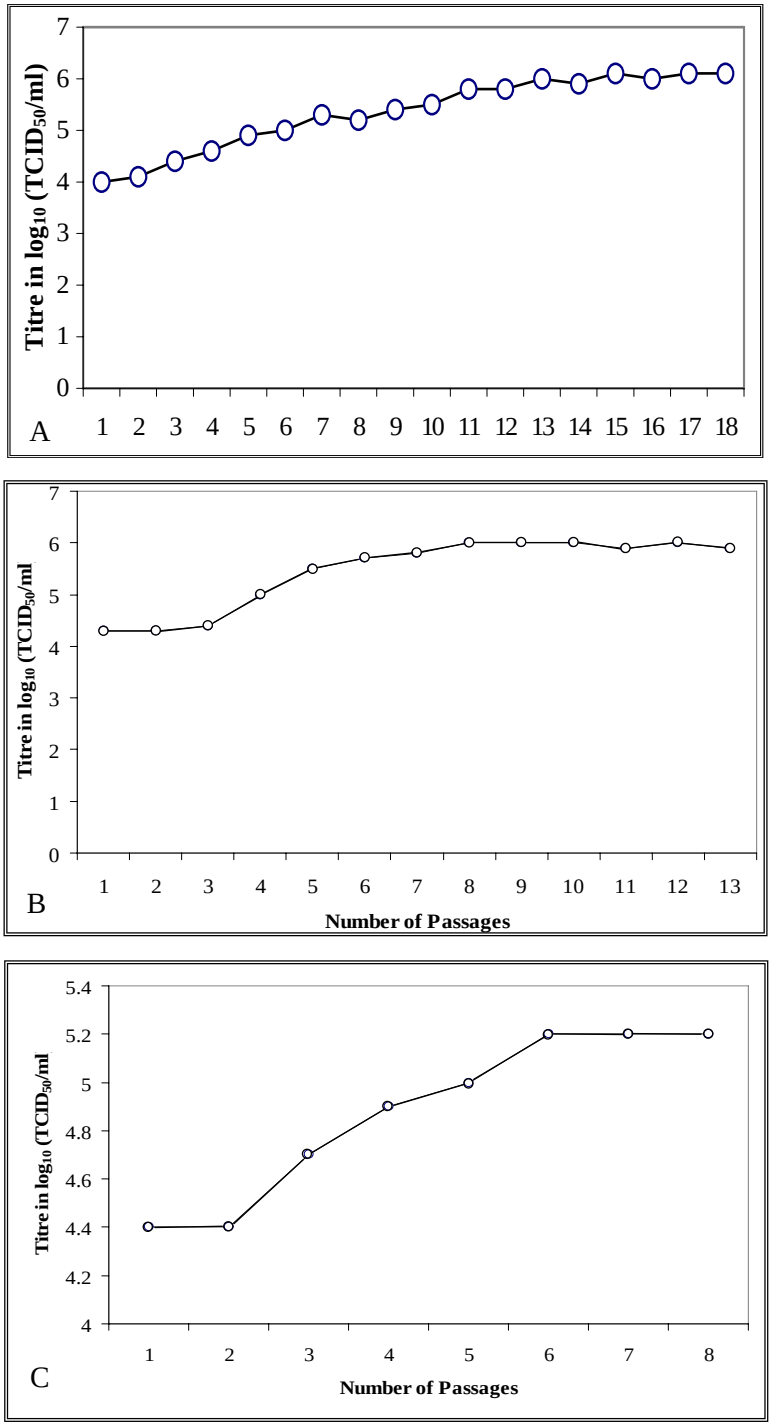

Fig. (2): Titration of Egyptian (A), Kenyan (B) and Romanian (C) sheep pox virus on Vero cell cultures.

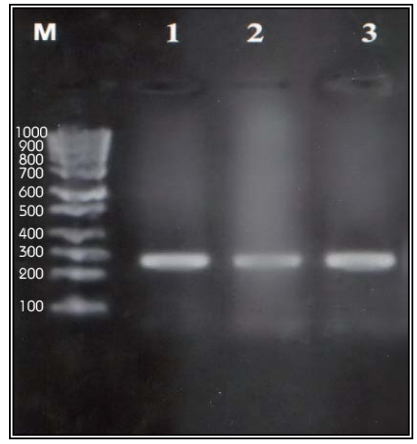

Fig. (3): Electrophoretic pattern of polymerase chain reaction (PCR) product, Lane (M): *HaeIII* DNA digest molecular marker, Lane (1): Egyptian sheep pox virus strain (ESPV), Lane (2): Kenyan sheep pox virus strain (KSPV), and Lane (3): Romanian sheep pox virus strain (RSPV).

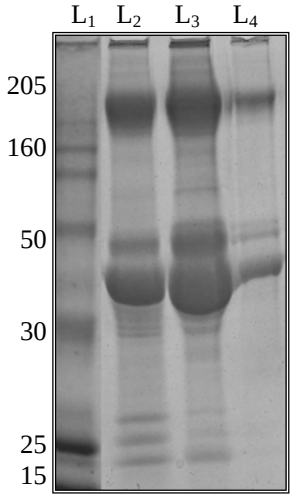

Fig. (4): SDS PAGE of whole viral protein of SPV strain in 10 % separating gel and stained with Commassie blue stain, Lane (1): Standard high range protein marker, Lane (2): Egyptian SPV strain, Lane (3): Romanian SPV strain and Lane (4): Kenyan SPV strain.

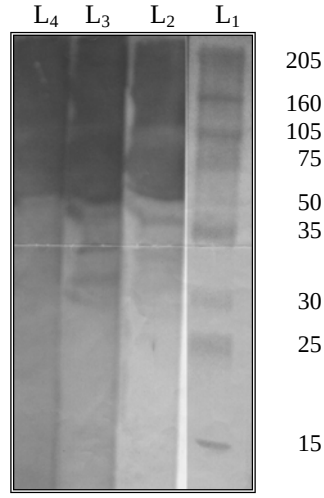

Fig. (5): Western blot of viral proteins of SPV strains, Lane (1): Immunogenic protein of Kenyan SPV strain, Lane (2): Immunogenic protein of Romanian SPV strain, Lane (3): Immunogenic protein of Egyptian SPV strain and Lane (4): Standard high range protein marker.