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Some Studies on Lumpy Skin Disease Virus

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Abstract

Lumpy skin disease virus (LSDV) of cattle has been present in Egypt since 1988 which causes severe economic loses. An effort to eradicate this virus which has been carried out by vaccination has showed no great success. This research was performed as a trial to improve LSDV situation in Egypt using data obtained from affected farms in Sharquia Governorate. Virological studies confirmed the presence of LSDV in farms under study, while pathological investigations give deep sight about the pathogenesis and pathological effect of the disease using recent pathological techniques. The advances in pathology make this branch able to diagnose all diseases by more accurate and non-doubtful manner, with lower costs compared to egg inoculation, Polymerase Chain Reaction (PCR) or serological approaches. The pathological examination showed diagnostic findings as vasculitis, necrosis which seen in all cell kinds in the affected area and all skin layers, cellular infiltration, thrombosis with necrosis of smooth muscles of blood vessels, endothelial cell damages, along with the presence of intracytoplasmic and intranuclear viral inclusion bodies, in addition to positive immunofluorescence. Agar Gel Precipitation as well showed strong line of precipitate. It is presumptive that LSDV present in Egypt is of high virulence, as it causes severe skin lesions.

Introduction

Lumpy skin disease (LSD) acquired its name from the skin lesions in cattle infected by LSDV. It is characterized by formations of hard nodules on skin and mucous membranes accompanied by fever, lesions on the internal organs, general deterioration of health with emaciation, enlarged lymph nodes, oedema of the skin, and sometimes death. LSD in cattle usually is complicated by secondary infection/infestation by bacteria, screw worm, and other contaminant viruses like ‘orphan viruses’ which affects the productivity of dairy animals and may cause deaths as a result of complications of secondary infections or from primary infections by more pathogens that endemic in the environment **(OIE, 2010; Von Backstrom, 1945; Haig, 1957; Diesel, 1949**). LSDV (genus *Capripoxvirus*, family *Poxviridae*) is stable and can survive at ambient temperature for long periods. It is highly resistant to inactivation, surviving in necrotic skin nodules for up to 33 days or longer, in desiccated crusts for up to 35 days, and at least 18 days in air-dried hides **(Fauquet et al., 2005)**. The virus is susceptible to sunlight and detergents containing lipid solvents, but in dark environmental conditions, such as contaminated animal sheds, it can persist for several months. The virus is susceptible to 55°C/2 hours, 65°C/30 minutes and can be recovered from skin nodules kept at –80C°C for 10 years and infected tissue culture

fluid stored at 4°C for 6 months. It is affected by high alkalinity or acidity and can be killed by ether (20%), chloroform, formalin (1%), and some detergents, e.g. sodium dodecyl sulphate **(OIE, 2010;Gershon and Black, 1987)**

There is no specific antiviral treatment available for LSD-infected animals except with Neethling and Kenya sheep and goat pox virus vaccines which have been used widely in Africa successfully for that purpose (**OIE, 2010).** Diagnosis of LSD is usually depending on its unique and characteristic clinical signs and its specific macroscopic and microscopic pathologic picture that never share resemblance with other diseases. Laboratory diagnosis of LSD virus could be achieved by different techniques as electron microscopy (EM), embryonated chicken eggs inoculation (ECE), cell cultures, immune-fluorescent antibody test (IF), and Polymerase Chain Reaction (PCR) **(Ali et al., 1990**; **Davies, 1991).** Effective control of LSD requires solutions focused on combating the LSDV by finding remedy and safe vaccine.

As Lumpy skin disease causes severe economic losses in the affected herd, efforts are directed towards discovering the efficient and economic diagnostic techniques. The continuity of old disease in affected area indicated the inefficiency and shortages in control programs. The research was directing to investigate recent infections and update the situation of LSDV in one of the Egyptian Governorate.

Materials and Methods

Lumpy Skin Disease virus (LSDV)

1- A field strain (Ismailia strain) of LSDV was isolated during the outbreak of the disease in Egypt in 1988. It was used for cell culture adaptation and propagation **(House et al., 1990)**.
2- Local isolate of LSDV from natural outbreak in Damenhour City, Al Behera Governorate 17-9-2014, tested by code RL/202 AHRI and confirmed by Immunoflurescent test and RT-PCR.

Tissue culture Cell lines

Maiden – Darby bovine kidney cells (MDBK) were prepared and provided by VACSERA Egypt. The MDBK cells were used for adaptation of LSDV, virus titration as well as serum neutralization test (SNT).

LSDV titration and determination of infectious dose (ID50)

MDBK cell lines were used for determination of virus' infectivity titer. Fifty percent of tissue culture infectious dose of a virus (TCID50), was carried out by traditional methods of virus quantification. Viral infectivity titer was evaluated according to the method adopted by **(Reed and Muench, 1938)**.

Serum samples

Sera samples were collected from infected (20), convalescent (5), and apparently healthy in contact cattles (10). The blood samples were collected in sterile tubes from jugular vein

and sera separated from clotted blood by centrifugation at 1000 rpm for 5 minutes. Then the sera were kept in refrigerator until use **(Payment and Trudel, 1993)**.

Skin samples from naturally infected animals

Skin biopsies, comprising epidermis and dermis of the nodular skin lesions, were collected from local cattle in Sharquia Governorate during 2014, 2015, 2016. Biopsy specimens were taken aseptically and the incisions were sutured. Samples were divided into two sets; one was collected in tubes filled with 10% formalin solution and other collected in 15 ml sterile tubes and stored at -20°C until use. The procedures were performed according to **(Payment and Trudel, 1993)**

Embryonated chicken Eggs inoculation

Three groups of 11 days old embryonated chicken eggs (ECE) were used to test the viability of LSDV. The inoculation was performed via the chorio-allantoic membrane (CAM) route. The dose of all samples was 0.1 ml/ egg. First group was infected with LSDV from natural samples; second ECE group infected with LSDV isolated in tissue culture (Egyptian strain) and the third group kept as control negative without injection. All groups were incubated at 36ºC in CO_2 incubators **(Payment and Trudel, 1993)**.

Virus Neutralization test

Infective tissue culture material containing disintegrated cells and maintenance medium was centrifuged at 1500 rpm for 10 minutes. Serial 10 fold dilutions of the supernatant were made using Hanks saline and were mixed with equal volumes of undiluted serum. Parallel dilutions were mixed using normal serum. Contact was allowed to take place for one hour at 37 °C after which the mixture was inoculated onto established monolayers in culture tubes (five tubes were used per dilution). All tubes were examined daily for cytopathologic effects (CPE) **(Payment and Trudel, 1993)**.

Agar Gel Precipitation Test (AGPT)

AGPT was performed for detecting the antigenic properties of LSDV under experiment; the original viruses used in the experiment were tested by AGPT. LSDV was tested against known reference antisera obtained from Institute of Sera and Vaccine, Agriculture Research Center, Ministry of Agriculture, Egypt **(Kitching et al., 1986)**.

Results and Discussion

Post mortem findings of CAM and embryo following LSD specimens inoculation of Embryonated Chicken Eggs (ECE):

The pathological changes in Chorio-Allantoic Membranes (CAM) were observed beginning from the third day PI as minute individualized whitish pock lesions and become more prominent at fifth day PI where whitish, necrosed, irregular opaque well circumscribed great number of plaques with spots of hemorrhages could be observed. Also the CAM become very turbid and thick [Fig. 16] .

Virus isolation, propagation, adaptation and titration of LSDV in MDBK cells

Table (1): Titration of different passages of LSDV on MDBK cells, showed highest virus titre at tenth passages with Log $_{6.4}$ TCID $_{50\%ml.}$

Virus passage	CPE Appearance (days	Virus titre Log_{10} TCID $_{50/ml}$
3	**3**	**5.2**
6	**2**	**5.4**
8	**3**	**6**
10	**3**	**6.4**

CPE: Cytopathic effect ; **$TCID_{50}$:** Tissue culture infective dose 50

The isolated virus was confirmed as LSDV by its characteristic cytopathic effect (CPE) and tissues culture subsequent cultivation and identifications. Pock lesions were seen by histopathological examination. Slides were stained by Fuchsin stain and were examined by immune fluorescent technique.

Agar Gel Precipitation Test (AGPT)

The test was performed for detecting the antigenic properties of LSDV under experiment, the isolated virus and the reference viruses used in the experiment were tested by AGPT. The test showed that, the antigenic properties was strong which indicated by forming well defined and strong line of precipitate (Fig.1).

Fig. (1): Showed the positive result of Agar Gel Precipitation Test (AGPT) as the strong line of precipitate.

Fluorescent antibody technique (IF) in diagnosis of LSDV:

Biopsy samples stained with the indirect method showed specific flurescence in the epidermis and the associated inflammatory tissue (Fig.17). our findings are in accordance with **(Davies et al, 1971)** who mentioned that by IF indirect method

applied on biopsy of skin nodules a very bright granular flurescence in the cytoplasm of epidermal cells and in some cells the granular material coalesced to give a uniform flurescence was observed.

Pathological findings:

Clinical symptoms: Off-food, rise of body temperature that was persist for weeks. Lacrimation, nasal discharges, and fatigue were seen in cattle showed skin lesions. Lumps were seen on skin with raised red coloration and swellings of surrounding tissues. Superficial lymph nodes were edematous and enlarged. Limbs and dewlaps showed edema. Some lumps lesions were necrotic and opened with exudates inside. Skin nodules were firm and hot. These findings are in accordance with **(Davies, 1981; Davies, 1982; Michael et al., 1990).**

Microscopic examination: By examination of skin biopsy from infected cattle; fibrin exudates were seen inside vesicles that seen in the dermis which were surrounded by severe interstitial edema and serous exudates, hemorrhage were seen inside the necrotic tissue of lumps. One could speculated that the proliferation of macrophages and the sub-epidermal edema and necrosis lead to formation of small and large vesicles which compress adjacent tissue. As well as the serous fluids accumulate in a tiny cysts, where some cyst filled and dilated by fibrin exudates. Skeletal muscles suffer necrosis and dissociation by exudates, inflammatory cells, hemorrhage, and suppuration (Figures 2-5) .

It was observed that, the massive number of empty and/ or serous filled vacuoles inside the affected skin by lumps which compress adjacent tissues is the prominent finding. Also, it could be emphasize that, these vacuoles may be filled by gases produced by secondary bacterial infections of lumps by anaerobic gas producing bacteria. However, the presence of gas producing bacteria may be another important factor in the pathogenesis of LSDV. LSDV affect skin of cattle causing formation of lumps, which starts by infection of the epithelium of prickle cell layer causing hyperplasia which lead to hyperkeratosis and parakeratosis. Then, the endothelial cells of blood vessels infected by the virus suffer necrosis which leads to thrombus formation with subsequent infarction. LSDV has the affinity to produce pox lesions in various organs besides skin nodules and mucous membranes. Pathogenesis of this disease showed its destructive behaviors towards affected cells, as LSDV cause necrosis to blood vessels' endothelium and smooth muscles surrounding blood vessels' wall followed by ischemia of tissues and direct necrotic effects on affected skin cells leading to the typical pathological figure of lumps. Internal organs are also affected by two or more factors; one by toxins of necrotic tissues and secondary infections of skin, another by infection of LSDV to mucous and endothelial linings of different organs. The present study was performed by using new techniques and

new staining methods. These findings are in accordance with **(Burdin, 1959; Bida, 1977; Jubb et al., 1991)**

Intra-cytoplasmic inclusions were seen inside areas of necrosis as eosinophilic oval or round homogenous mass surrounded by hallow zones. Also, intranuclear inclusions were seen inside nuclei of infected epidermal cells (figures 6-9). This study showing the pathogenesis and pathology of lumpy skin disease more clearly and comprehensive. Our findings are in accordance with **Plowright and Witcomb, (1959)** who described that the inclusion bodies resembling isolated inclusions towards the periphery of eosinophilic matrix and **Young, (1970)** who observed intracytoplasmic and intranuclear inclusions which were present in the epidermis within or adjacent to the necrosis areas. Moreover, **Haig, (1957)** was mentioned that intracytoplasmic inclusion bodies have been observed in histiocytes, epithelial cells and smooth muscle cells; these bodies are seen in sections stained with Haemalum - Phloxine stain when they appear as red to reddish purple round or oval bodies varying in size. Also, **Nawathe et al. (1982)** stated that LSDV skin nodules showed intracytoplasmic inclusion bodies which seen inside mononuclear cells and cells of sweet glands.

The necrotic muscle cells were apparently clear from adjacent tissues. Blood vessels showed multiple thrombi which infiltrated by white blood cells. However, necrosis of blood vessels were obvious and distinguished from other tissues by the special techniques that was first applied for lumps disease. Necrosis of tissues inside granuloma was apparent and fibrin exudates were seen inside the necrotic areas. Hemorrhages were seen inside inflammatory edema and exudates of affected dermis and epidermis. Blood vessels showed plasma cells which indicating body response towards the infection. Eosinophil was seen inside lumps and inside blood vessels which indicated allergic reactions and\or parasitic infestation of the necrotic areas (figures 10-15) .

The obtained results are in accordance with **Woods (1988), Michael et al. (1990), Jubb et al. (1991), Jones and Hunt (1983), Prozesky and Bernard (1982), and Davies (1981);** they described that the cellular infiltration, the micro vesicles which some coalesced to form large vesicles, lymphangitis, vasculitis and perivascular reactions, extensive edema with hemorrhages, necrosis, thrombosis and intracellular inclusions of lumpy skin disease virus make diagnosis on this basis fairly certain.

Chorio-allantoic membrane was stained by histological methods showed aggregations of cells with dark nuclei resemble lymphocytes. Moreover, inclusion bodies were seen in other types of primitive cells of CAM (figure 16) these findings are in accordance with **Prozesky and Bernard (1982); Clayden, (1971)**

Conclusion

Lumpy skin disease (LSD) causes severe sickness of cattle; however, its control without vaccination is extremely difficult in endemic areas. In Egypt protection of cattle against the disease was carried out using Kenyan sheep pox culture vaccine. So development of a specific vaccine prepared from the Egyptian local isolate of LSDV is still required. Accordingly, effective control of LSD requires not only rapid and accurate laboratory diagnosis supported by clinical findings but also real solution focused on combating the LSDV by effective vaccination. The present work was conducting for discovering new available and economic methods that could be used to diagnose LSDV. Embryonated chicken eggs inoculation is consider the best recommended method required for LSDV isolation from the tissue specimens, followed by several passages in the specific tissue cultures. There is no specific antiviral treatment available for LSD infected cattle. Sick animals may be removed from the herd and given supportive treatment consisting of local wound dressing to discourage fly worry and prevent secondary infections. Systemic antibiotics may be given for skin infections, cellulitis or pneumonia, and food and water should be made readily available these findings are in accordance with **Tuppurainen et al., (2005); Bhan and Mecksney (1988); Babiuk et al., (2007).**

List of figures

a-Figures showed interstitial edema, dermal empty cysts, vesicles, fibrin exudates:

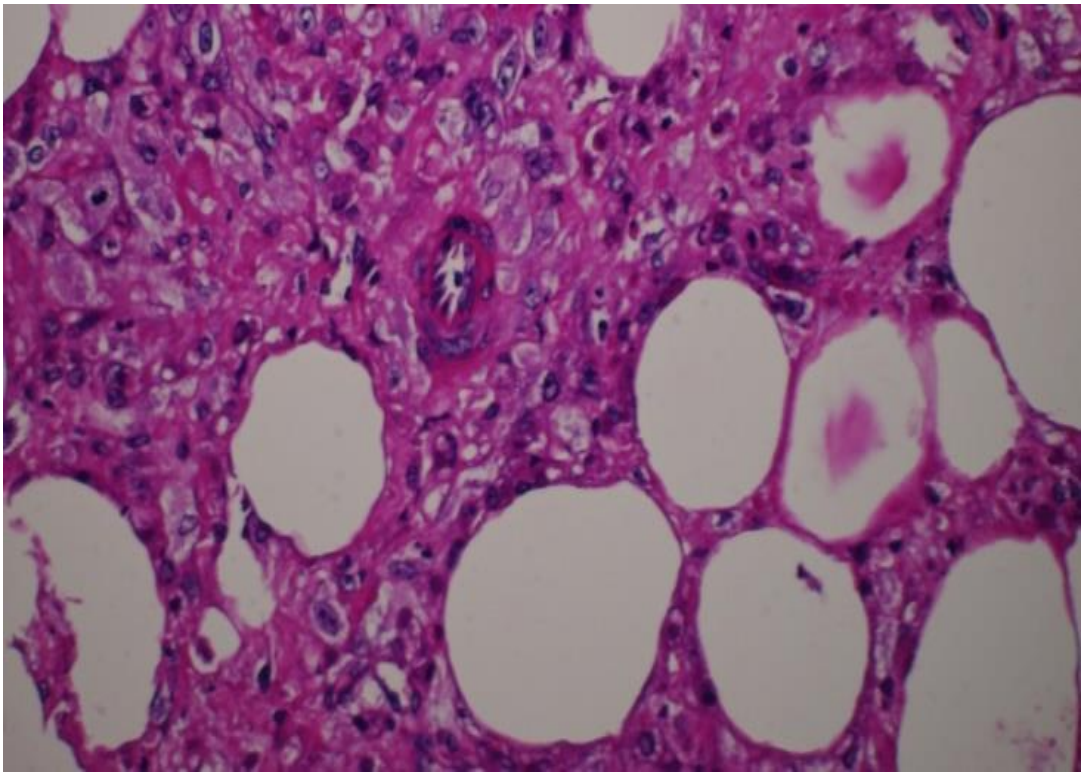

Fig (2): Cattle: Skin nodules showed dermal cysts, necrosis in small blood vessel (Fuchsin stain X400)

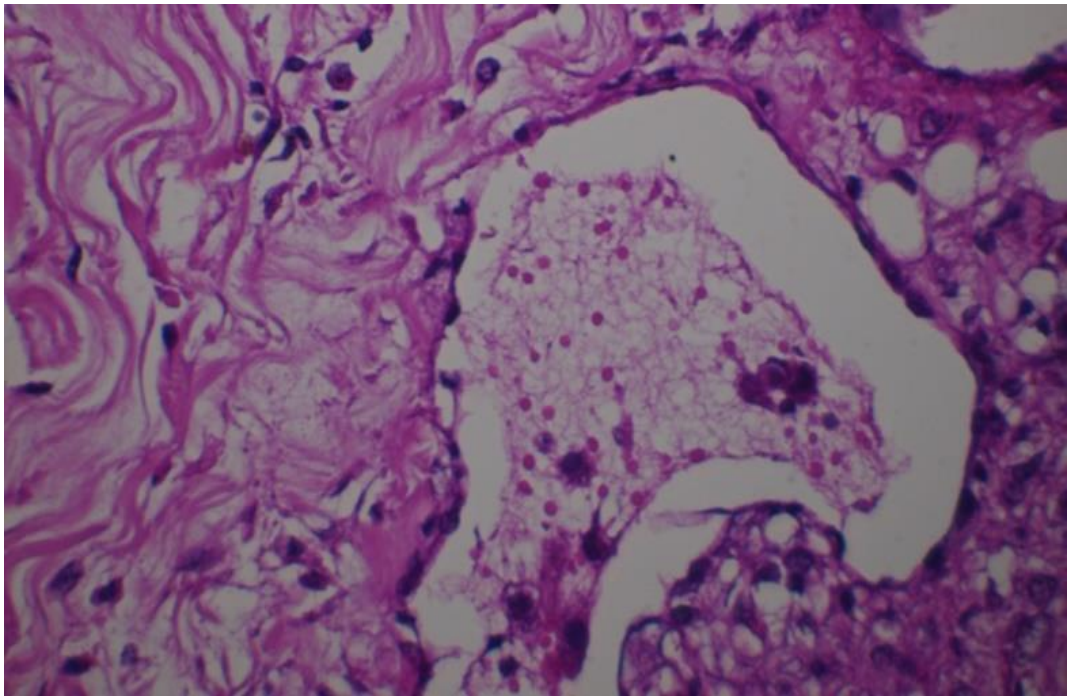

Fig (3): Cattle: Skin showed edema and dermal cyst filled with fibrin exudate (Fuchsin stain X400)

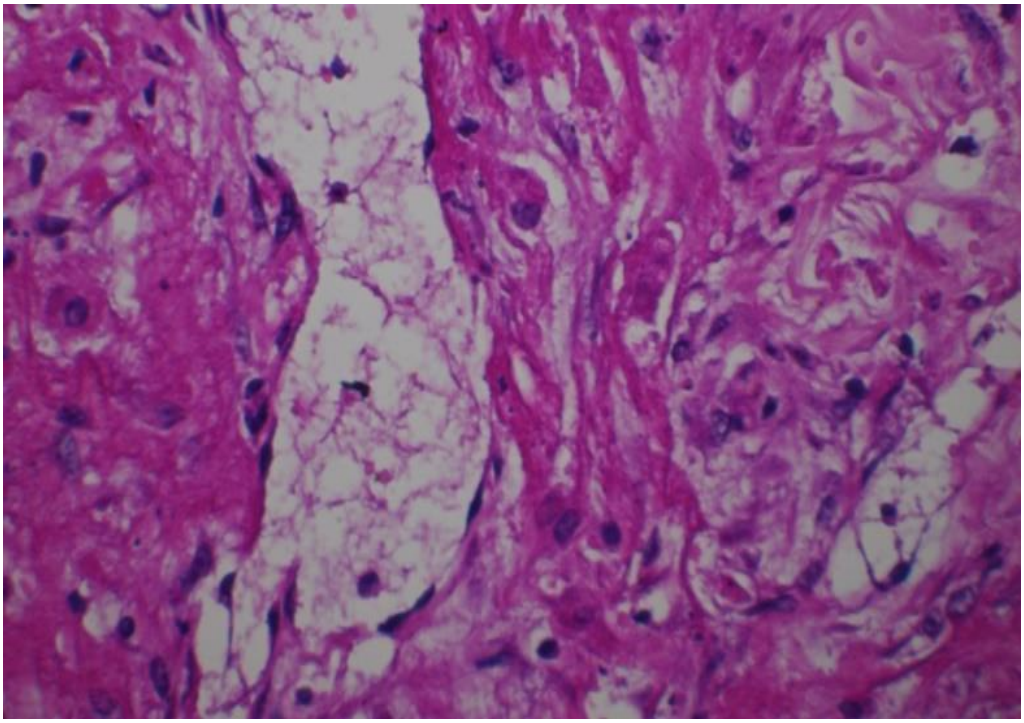

Fig (4): Cattle: Skin showed edema and dermal cyst filled with fibrin exudate (Fuchsin stain X400)

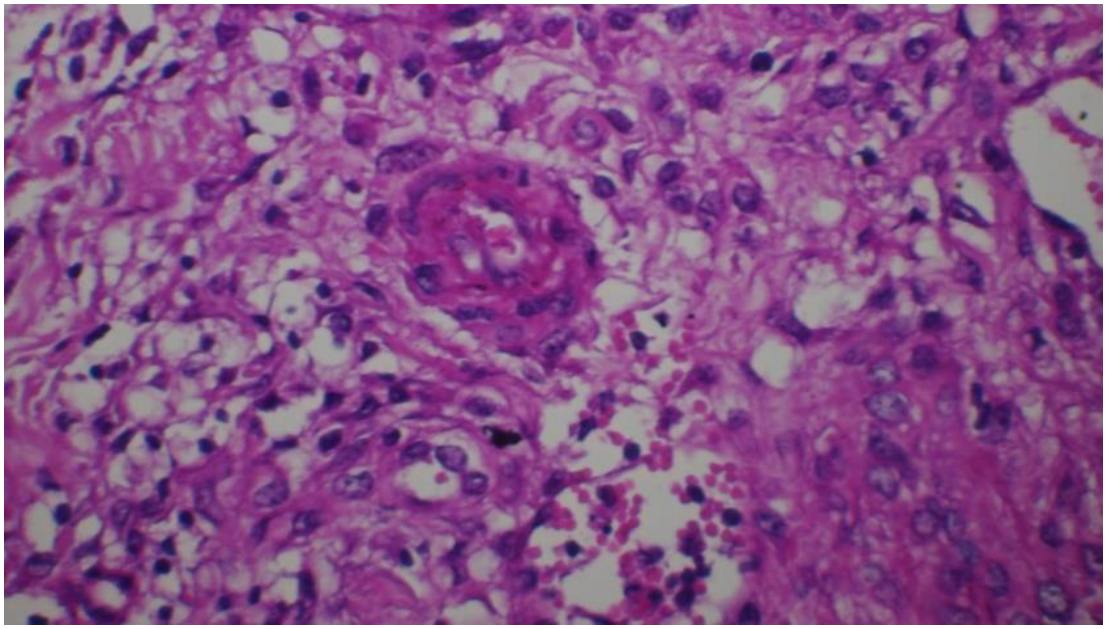

Fig (5): Cattle: Skin showed edema, necrosis in blood vessel and hemorrhage (Fuchsin stain X400)

b-Inclusion bodies:

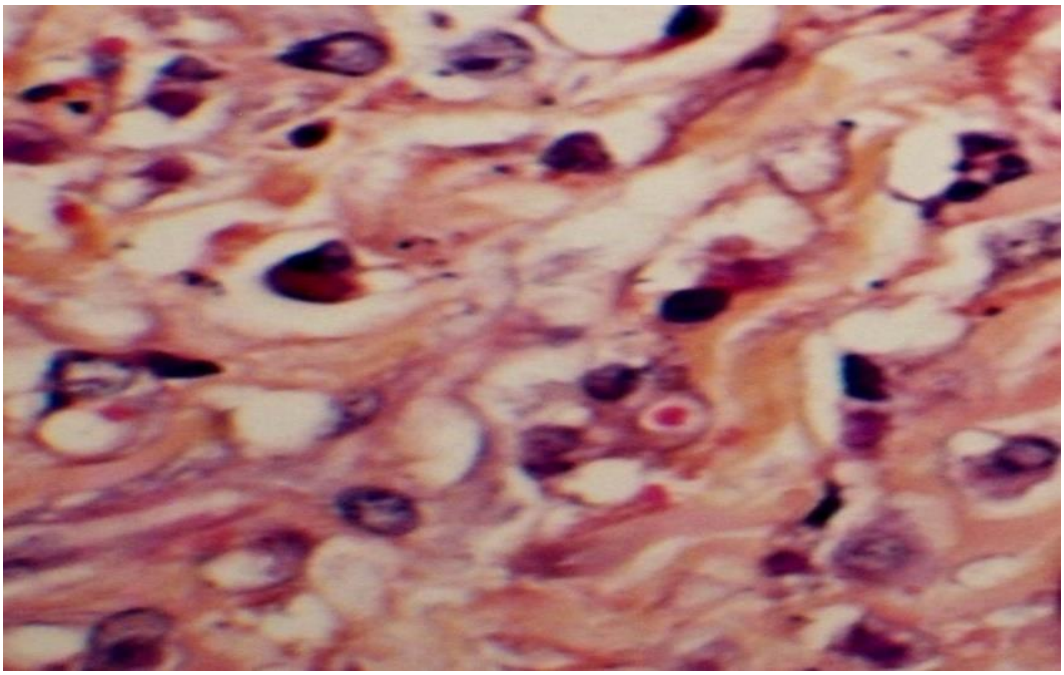

Fig (6): Cattle: Lumps in skin of cattle showed red intracytoplasmic inclusion bodies, surrounded by hallow zone (ploxine-tartrazine stain X1000)

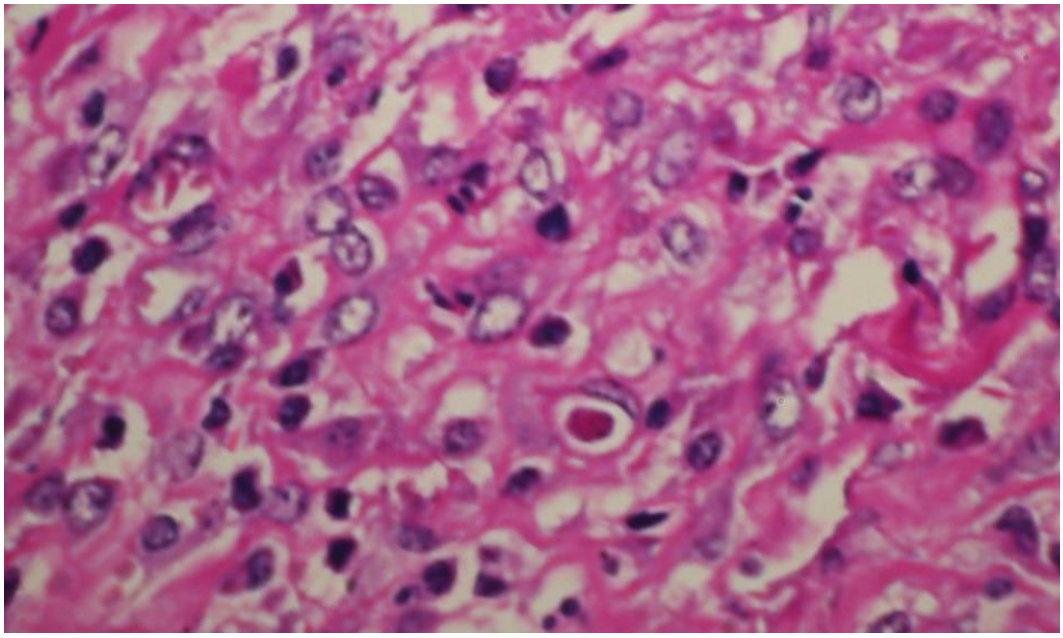

Fig (7): Cattle: Skin nodules of LSDV showed red intracytoplasmic inclusion bodies surrounded by hallow zone. (Fuchsin stain X 1000)

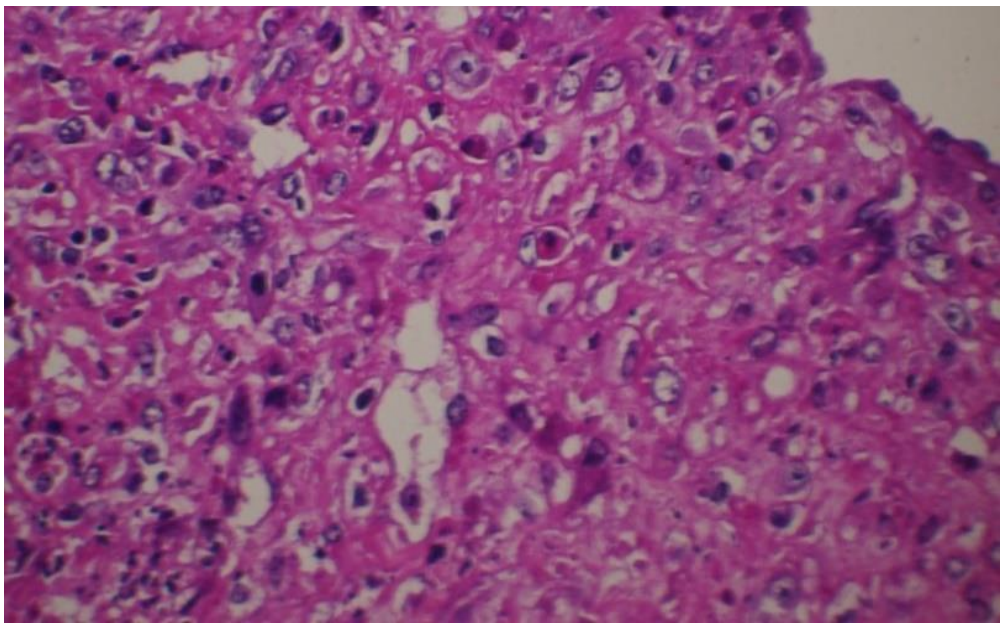

Fig (8): Cattle: Skin nodules of LSDV showed red intracytoplasmic inclusion bodies surrounded by hallow zone and intranuclear dark inclusion like bodies surrounded by hallow zone. (Fuchsin stain X 600)

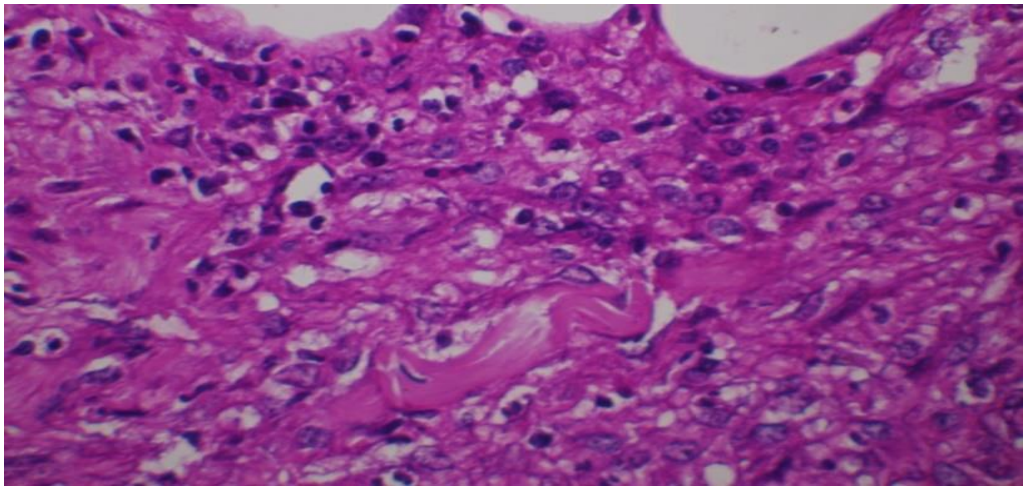

Fig (9): Cattle: Skin nodules of LSDV showed red intracytoplasmic inclusion bodies surrounded by hallow zone inside necrotic granulomatous tissue of the dermis. (Fuchsin stain X 600)

c-Blood vessels:

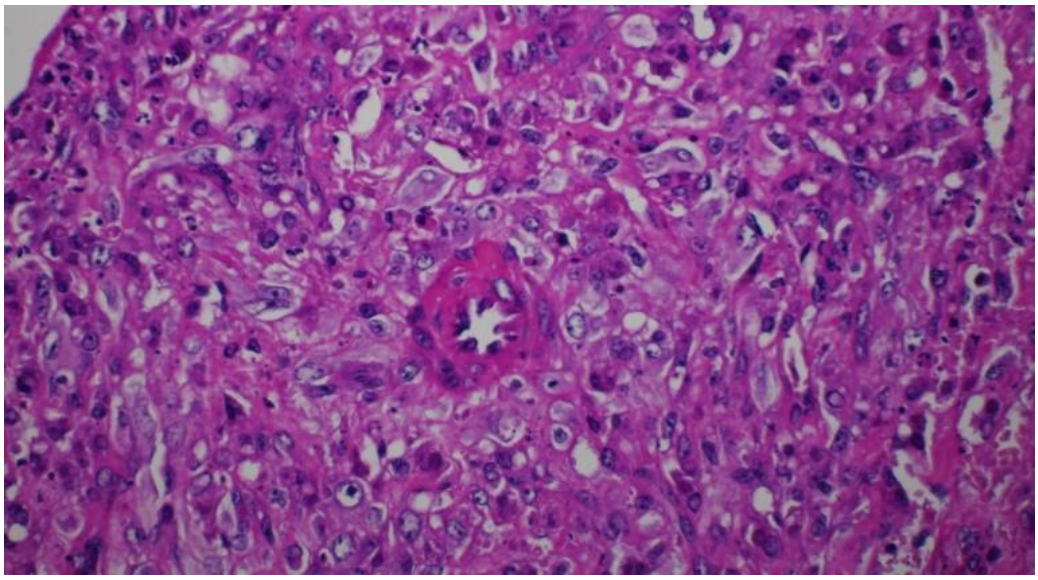

Fig (10): Cattle: Skin nodules of LSDV showed granulomatous necrotic focus and necrotic blood vessel. (Fuchsin stain X 600)

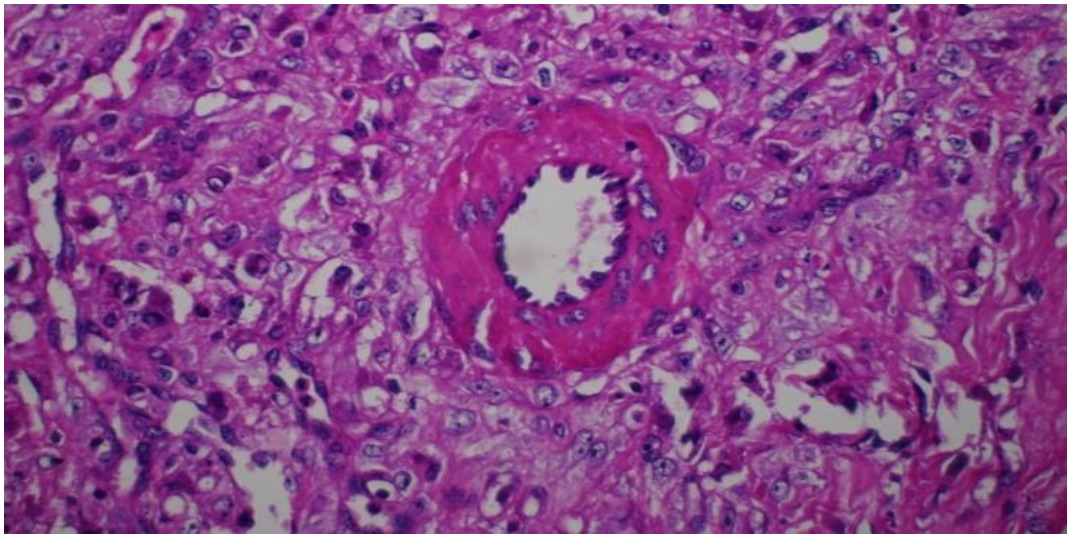

Fig (11): Cattle: Skin nodules of LSDV showed granulomatous necrotic focus and necrotic blood vessel. (Fuchsin stain X 600)

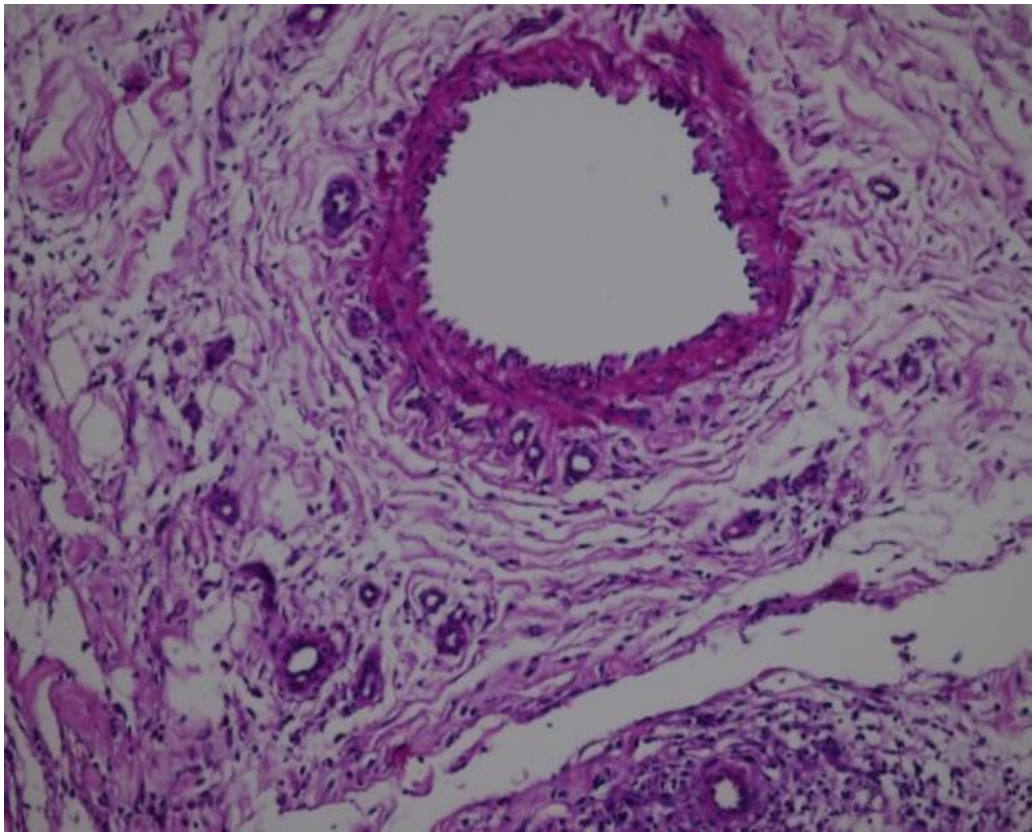

Fig (12): Cattle: Skin nodules of LSDV showed granulomatous necrotic focus and necrotic large blood vessel. (H&E stain X 200)

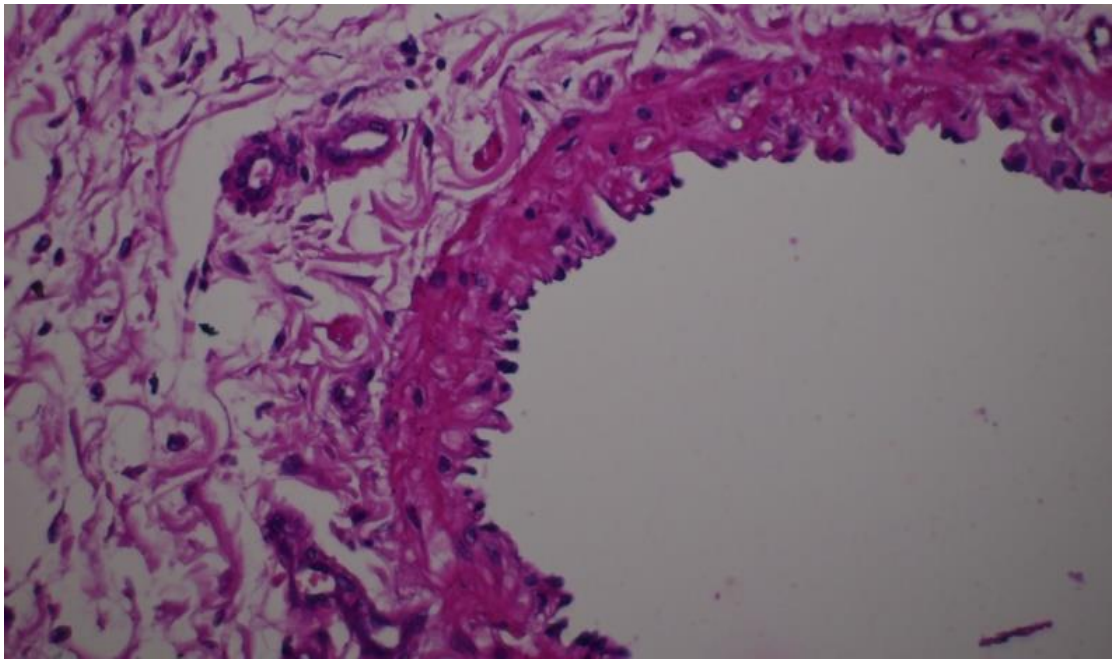

Fig (13): Cattle: Skin nodules of LSDV showed vasculitis with necrotic endothelium and wall of blood vessel. (Fuchsin stain X 600)

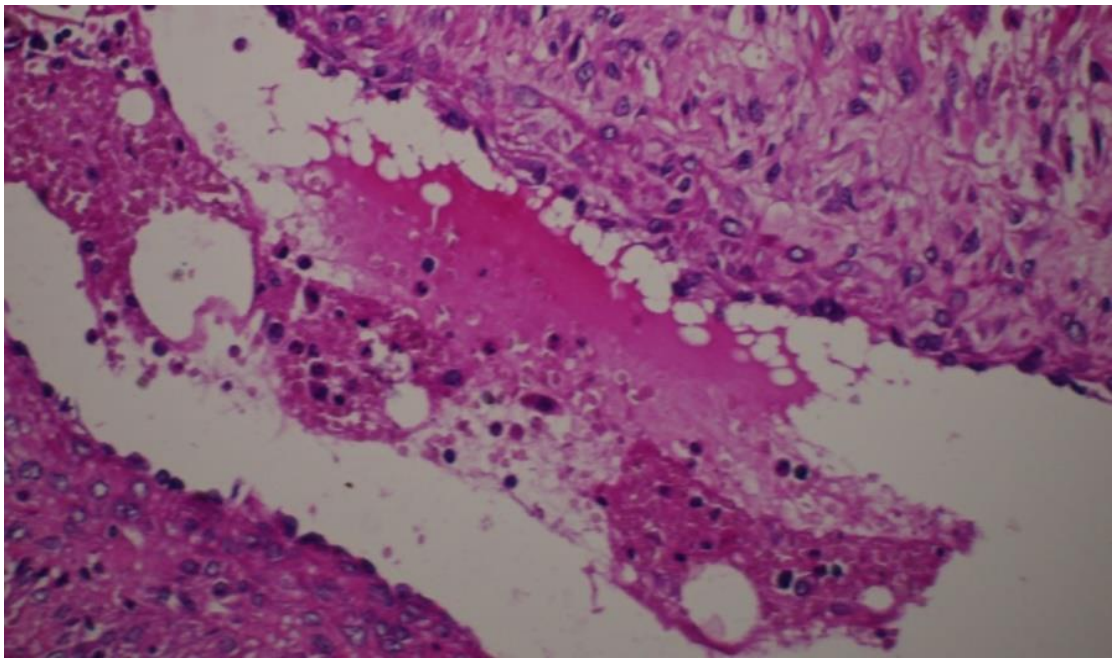

Fig (14): Cattle: Skin nodules of LSDV showed granulomatous necrotic focus and necrotic blood vessel with throumbus inside lumen which infiltrated by white blood cells. (Fuchsin stain X 600)

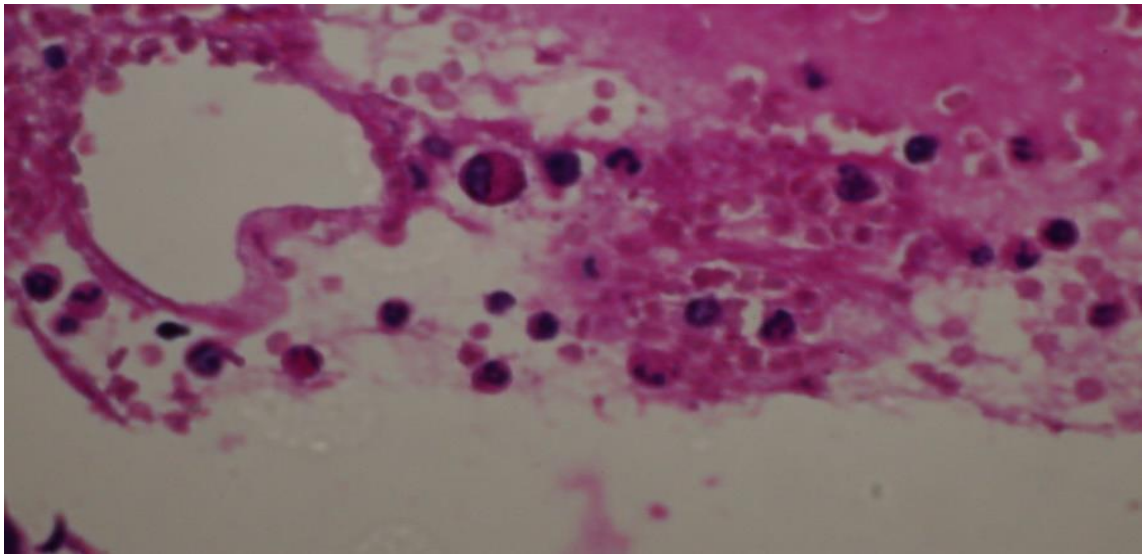

Fig (15): Cattle: Skin nodules of LSDV showed thrombus inside necrotic blood vessel which infiltrated by plasma cells, lymphocytes and esinophils. (Fuchsin stain X 1000)

d-Histopathology examination of Chorio- Allantois membrane of infected ECE:

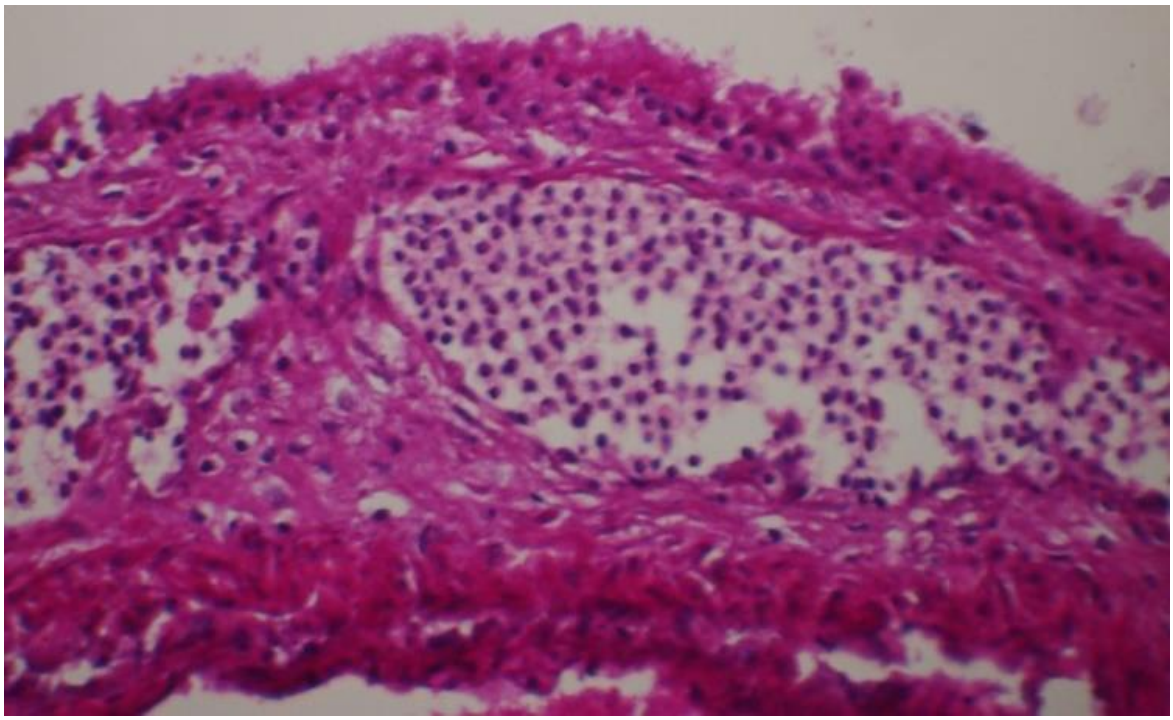

Fig (16): CAM showed congested blood vessel and proliferated endodermal, ectodermal and mesodermal layers (Fuchsin stain X400)

e-Immune fluorescence:

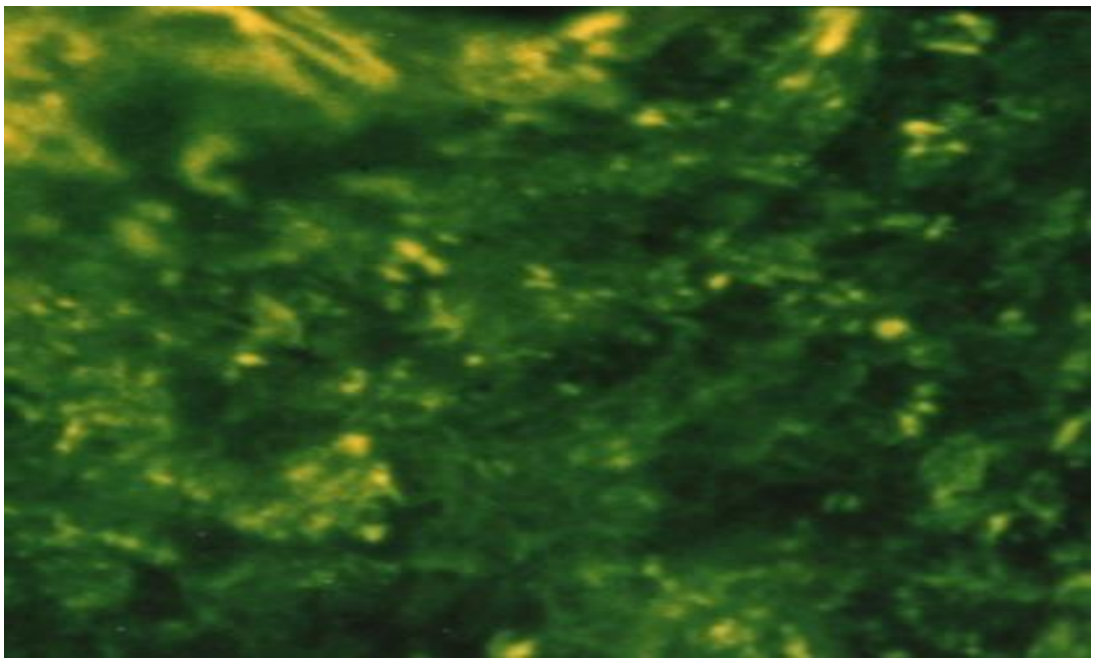

Fig (17): Cattle skin nodule of LSDV showed positive reactions inside necrotic dermis (X1000)

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