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Overview on sheep pox disease and its vaccination under field circumstances

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Abstract

Sheep pox and goat pox are caused by a genus of *Capripox virus* causing a severe problem and great economic loss in sheep and goat in many parts of the world. The objective of this study is to give insight on hematobiochemical parameters, immunological and histopathological aspects in naturally infected sheep with *poxvirus.* For this purpose, 10 apparently healthy unvaccinated sheep, ١٠ sheep naturally infected with sheep pox and 10 sheep healthy vaccinated sheep were used to conduct the study (randomly selection).Crusted scabs were collected from infected sheep for virus isolation, immune-florescent antibody technique (IFAT) and PCR test. Blood samples were collected for hematological, biochemical and immunological tests. Organ samples were collected for pathological study. The virus was isolated on CAM of ECE which showed characteristic pock lesion. Isolated virus was identified by Immune-Florescent antibody technique (IFAT) revealed specific intracytoplasmic yellowish green fluorescence emission. Further investigations were carried out using Polymerase Chain Reaction (PCR).Sheep infected with pox virus showed insignificant decrease in haematological parameters beside significant decrease in total protein, albumin, globulin, Superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase coupled with significant increase in WBCs, lymphocytes, neutrophils, AST, ALT, ALP, urea, creatinine and melanodialdehyde coupled with non-significant increase in monocyte, serum nitric oxide and lysozyme activity.Histopathological examination revealed epidermal cell ulceration on the tongues, lips and skin which contained eosinophilic intracytoplasmic inclusion bodies in degenerated cells. The lymph nodes and spleen were depleted and the heart revealed focal myocarditis.

Vaccinated sheep with sheep pox vaccine showed non-significant increase in RBC, HB, PCV, MCV, AST, ALT, ALP, monocyte, eosinophils and basophils albumin, urea and creatinine, melanodialdehyde beside insignificant decrease in MCH, MCHC associated with significant increase in WBCs, total protein, globulin, serum nitric oxide, lysozyme, immunoglobulins (IgG & IgM), Superoxide dismutase, catalase, reduced glutathione and Glutathione peroxidase. No pathological change was recorded.

Results of this study indicated that sheep pox virus infection induce adverse effects on the blood picture, liver and kidney function tests, antioxidant enzymes and immunological status of sheep. In addition to adverse effects on the skin and tissues in the *sheep pox virus* infected group compared to the control group. Vaccination by SERVAC CAPRI-C has effective immunological status on vaccinated sheep.

Keywords: *Sheep pox virus; CAM; IFAT; PCR. Hemato-biochemical, immunological assay; histopathological study; pox vaccine.*

Introduction

Sheep seem to be one of most significant economic animal sources in Egypt (**Khameis *et al.,* 2018**). Sheep pox has worldwide distribution throughout southwest, Asia, Africa and Middle East (**OIE, 2017**). Sheep pox virus is a member of *Capripox virus genus*, which belongs to *Pox viridae* family Sheep *pox virion* is enveloped, brick-shaped particle of 270–290nm in size and contains a linear double-stranded DNA. The size of genome is approximately 150 kbp and it includes at least 147 putative genes **(Oguzoglu *et al.,* 2006).** Sheep pox disease is characterized by fever, anorexia, depression, inflammation of mucous membranes of eyes and nose, respiratory distress with appearance of pox lesions on areas devoid of wool as checks, lips, nostrils, inner aspect of thigh (**Mona *et al.,* 2019**). The virus transmitted by aerosols and close contact with infected animals (**Kitching and Carn, 2004**). *Sheep pox virus* is antigenically and genetically closely related to *goat pox virus* and *lumpy skin virus* recent studies showed that viruses are phylogenetically distinct and differentiated by molecular tools (**Bhanuprakash *et al.,* 2006).** Diagnosis of sheep pox is based on clinical signs and laboratory confirmation. The laboratory confirmation techniques are based on virus isolation recorded that *capripoxvirus* specific PCR assay was developed that differentiated between *Sheep pox virus* and *lumpy skin virus* (**Heine *et al.,* 1999).**

Vaccines are considered among the most valuable and cost-effective tools for control of infectious diseases. Vaccination is the only effective method to control disease in endemic areas. Vaccination has been cheapest and suitable means in control of *Capripox virus* by stimulating both humeral and cell mediated immune response (**Deshmukh and Gujar, 1992**). Active mass vaccination to SGP may induce strong herd immunity that can effectively control the disease. Single vaccination is considered as enough for providing life-long strong immunity (**Bhanuprakash *et al.,* 2011**).

In this paper, we describe viral isolation and identification of *sheep pox virus* and investigate hematobiochemical parameters, immunological and histopathological aspects in naturally infected sheep with *poxvirus* compared to vaccinated and non-vaccinated control group.

Materials and Methods

Animals

Survey study was done on 657 sheep animals during period of September 2018 to March 2019 in El-Asher Men Ramadan city, Sharkia Governorate, Arab Republic of Egypt. Different ages and sexes from same locality and reared in the same postural region were observed during the periodical protocol of vaccination. 76 sheep seemed to be infected with sheep pox (11.5%) mainly in lambs. Clinical signs, postmortem changes were reported. Sheep were showing signs of fever, generalized skin nodules (especially on lips, nostril, tongue and lower aspect of tail). Mild to moderate lesions were reported in respiratory and gastrointestinal tracts and lymph node enlargement lead to suspect infection with sheep pox disease.

Animals were divided into 3 groups as the following**:**

1. 10 apparently healthy non-vaccinated sheep from the same localities of rearing pastures (NV group). This group considered as control group.
2. 10 diseased sheep showing characteristic clinical signs (D group).
3. 10 apparently healthy vaccinated sheep by SERVAC CAPRI-C (live attenuated vaccine) from the same localities and rearing in the same pastures (V group).

Sampling: The samples were collected randomly from the same locality and postural areas**.**

Tissue Specimens:

A total 10 crusted scabs were aseptically collected from infected non-vaccinated sheep with typical clinical signs. Each sample was prepared for virus isolation and identification by IFAT and PCR **(OIE, 2017 and Santhamani *et al.,* (2013)** and stored at -70°C till used.

Serum samples:

Thirty serum samples were collected from diseased animal, vaccinated animals and unvaccinated animals apparently healthy animals in clean dry tubes without anticoagulant for biochemical and immunological tests.

Whole blood:
Thirty whole blood samples were collected from diseased, vaccinated and unvaccinated apparently healthy animals in clean dry tubes with anticoagulant for heamatological tests (RBCs, HB, PCV MCV, MCH, MCHC and differential leucocytic count).

Organs
Internal organs were used for histopathological investigation which include (oral mucosa, tongue, skin, lymph node, spleen, intestine, liver and heart).

Methods
1. Clinical signs and postmortem examination:
The main clinical signs and gross lesions among diseased and emergency slaughtered animals were recorded**.**

2. Viral identification: Reference Virus
The Egyptian strain of *sheep pox virus* was obtained from Virology department, Animal Health Research Institute, Egypt. It was used as positive control for IFAT, and PCR.
Virus isolation:
Specific Pathogen Free (SPF) 9-11 days old embryonated chicken eggs (ECE) were inoculated with the prepared samples via the chorioallantoic membrane (CAM) route for three passages according to **Rovozzo and Burke (1973)**. It was applied in Virology department, Animal Health Research Institute, Egypt.

Serological identification using IFAT:
It was applied in Virology department, Animal Health Research Institute, Egypt, according to **OIE (2017)**.

Polymerase chain reaction:
It was applied in Biotechnology unit-Reference laboratory for veterinary quality control on poultry production. Animal health research institute, Dokki, Giza, Egypt according to **Santhamani *et al.* (2013).**

DNA extraction:
DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56˚C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from Metabion (Germany)
Forward primer
5'-TCTATGTCTTGATATGTGGTGGTAG -3'
Reverse primer 5'- AGTGATTAGGTGGTG-TATTATTTTCC -3'

PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 5.5 µl of water, and 5 µl of DNA template.
Analysis of the PCR Products:
The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software **(Santhamani *et al.,* 2013).**

Table (1). Target genes, amplicon sizes and cycling conditions.

Target gene	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension
			Secondary denaturation	Annealing	Extension	
RPO30	151	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.

4. Hematological, biochemical and immunological identifications:

First sample was taken on tube contain EDTA for estimation the blood picture (**Jain, 2000**) and 2nd sample was taken for obtaining clear serum for estimation AST & ALT **(Reitman and Frankel, 1957**), ALP (**Kind and King, 1954**), total protein (**Doumas *et al.,* 1981**), albumin (**Bauer, 1982**), globulin (**Kapale *et al.,* 2008**), urea (**Fawcet and Scott, 1960**), creatinine (**Husdan and Rapoport 1968**), nitric oxide (**Rajarman *et al.,* 1998),** lysozyme activity **(Schltz, 1987).** Serum immunoglobulins (IgG and IgM) were performed using SANDWICH ELISA **(Erhard *et al.,* 1992),** total antioxidant (**Karecevic, 2001**) and melanodialdehyde (**Nielsen *et al.,* 1997**). Superoxide dismutase (**Nishikimi *et al.,* 1972**), catalase **(Aebi, 1974**), reduced glutathione **(Owens and Belcher, 1965)** and Glutathione peroxidase **(Paglia and Valentine, 1967**).

5. Histopathological identification:

Tissue specimens from different organs (skin, oral cavity mucosa, tongue, intestines, liver, heart, sublingual lymph nodes and spleen) from different groups were fixed on neutral buffered formalin then they were processed by routine methods according to **Bancroft and Gamble (2002**) and embedded in paraffin wax. Sections (5 um) were mounted on glass microscopic slides, stained with Haemotoxylin and Eosin (H & E) and examined microscopically

6. Statistical analysis:

The obtained data was analyzed firstly for the means and standard errors according to **Tamhane and Dunlop (2000).** Then, the significance of the results was evaluated using analysis of variance (ANOVA) using computerized SPSS program version 16.

Results

1. Clinical signs and post mortem results:

Diseased sheep (D group): Affected sheep were febrile, depressed, recumbent and off feed. Respiratory signs included catarrhal nasal discharge and mucopurulent conjunctivitis were seen. Mortalities were more pronounced among lamps. These lesions comprised slightly raised round plagues of 0.5- 3 cm in diameter with dark center. Ulcerative lesions and erosions were also found on the gums, tongue and inner lips (Plate.1A & B). Scab formation were prominent during late stages in lips, nostrils, eye lids and skin. The conjunctivae were congested and the lymph nodes were enlarged. Pustular and scabby lesions on the head and buccal cavity were noticed. Erythematous nodular elevations were seen on mammary glands, inner aspects of tail (1C) and other wool devoid regions (maculae).

Post mortem results: Vesicles covered by hemorrhage and edema with serofibrenous exudate on the affected skin lesions mainly (buccalmucosa, nostrils, eye lids and devoid wool skin). Ulcerative lesions in the nasal cavity and erosions in the mucosa of the oesophagus were observed. The intestinal and rumenal mucosa showed multifocal areas of pale thick foci, while both kidneys , lungs and the heart had multiple slightly raised grayish to whitish foci on its surfaces (pock lesion) (.1 D). The mesenteric and regional lymph nodes were enlarged.

Vaccinated and apparently health animal (V group & UV group):

Clinical signs:

All sheep were apparently healthy and free from any clinical signs.

Gross Findings:

Post vaccination sheep did not have any characteristic gross lesions.

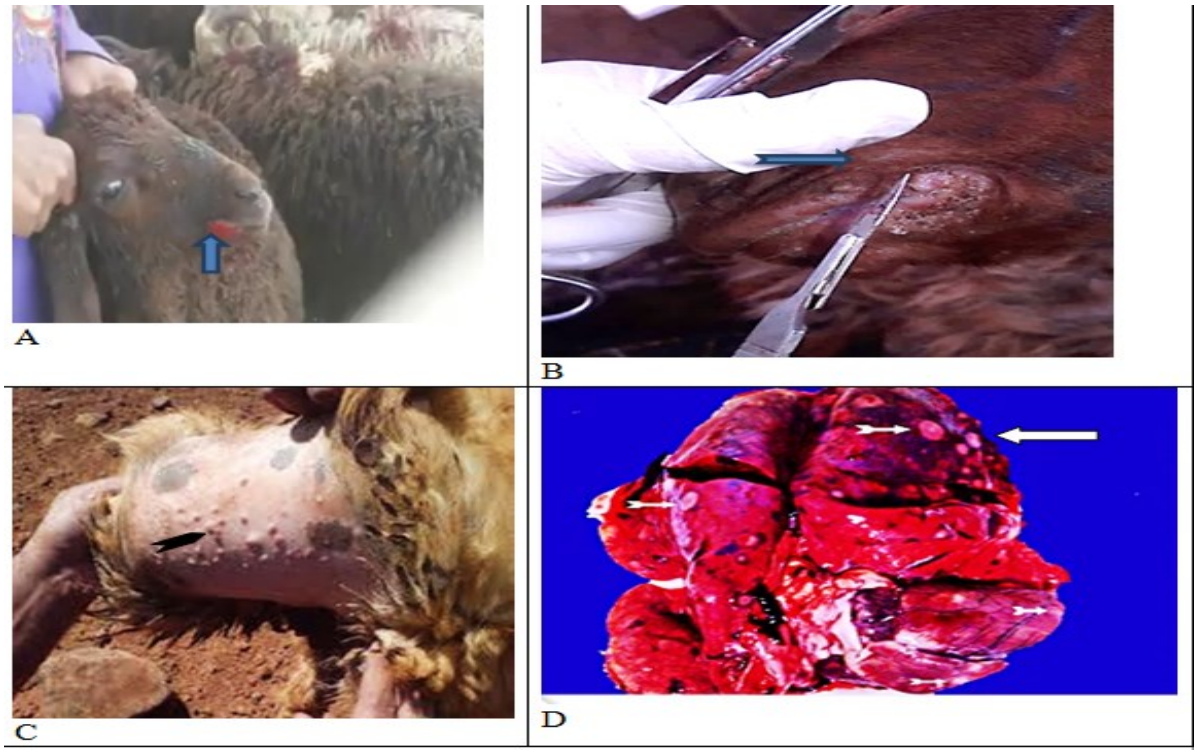

Plate (1). Gross lesions of sheep pox (A) showing hemorrhage and erosions on the lips. (B) The same as A showing that the ulceration includes deep oral mucosa (arrows) (C) Erythematous nodular elevations were seen on the inner aspects of the tail. (D) pock lesions on the surface in the kidney capsule and on the surface of the mesentry (Arrows).

Viral identification results:

Isolation on chorioallantoic membrane:

Isolation of SPV revealed the characteristic typical lesions (pock lesion), death of embryo, thickening, edema and hemorrhage of the membrane **(Fig. 2).**

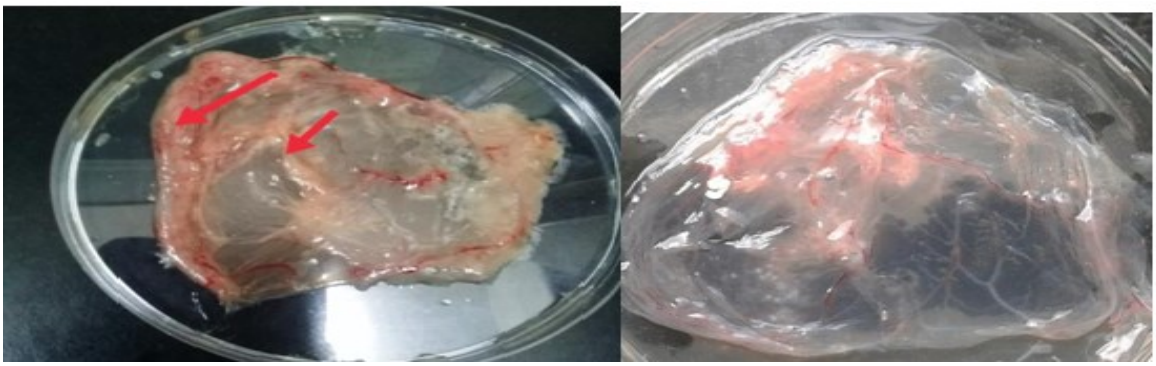

Fig. (2): Characteristic pock lesions of suspected skin scabs samples for SPV on CAM of SPF ECE after 3rd passages

Serological identification using IFAT:
Identification using IFAT demonstrated the specific intracytoplasmic yellowish green fluorescent granules **(Fig. 3).**

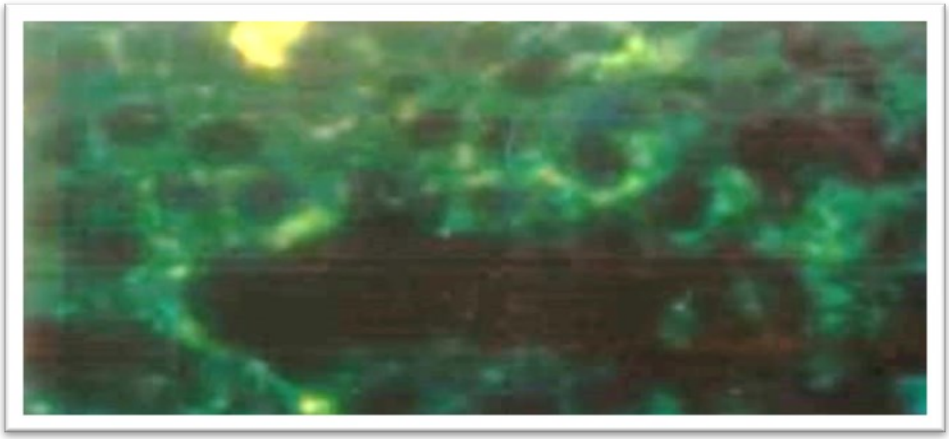

Fig. (3): Stained infected CAM suspension showing Intracytoplasmic yellowish green fluorescent granules.

2.3. Polymerase chain reaction:
Four samples were subjected to PCR using specific Primer targeted the PRO30 for detection of the local SPV strain. The primer was succeeded to amplify the specific SPV products (151bp) from the extracted DNA products **(Fig. 4).**

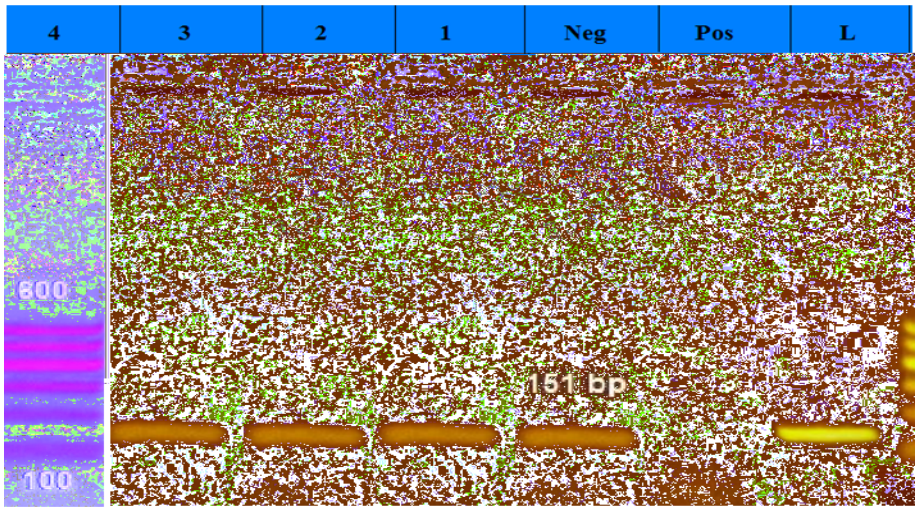

Fig. (4): Detection of DNA of SPV by PCR (agarose gel electrophoresis of the polymerase chain reaction products) The specific primers set amplified a DNA fragment of 151bp equivalent to the expected amplification product (amplicon) size from LSDV. Lanes: (L) 100 bp DNA ladder.
(Pos): positive PCR products from sheep pox virus reference strain.
(Neg): negative control (no primers).Lanes (1, 2, 3, 4) +ve samples

3. Clinicopathological results:

A) Hematological results: Sheep of (D group) showed insignificant decrease in RBCs, HB, PCV, MCV, MCH, MCHC and significant increase in WBCs, lymphocytes, neutrophils beside insignificant increase in monocyte and insignificant decrease in basophils, eosinophils. Vaccinated sheep (V group) sheep pox vaccine showed insignificant decrease in MCH, MCHC beside insignificant increase in RBC, HB, PCV, and MCV and insignificant in monocytes, eosinophils, and basophils and significant increase in WBCs, lymphocytes, neutrophils (table 2).

B) Biochemical results: Sheep of (D group) displayed significant decrease in total protein, albumin, globulin, SOD, CAT, GSH, GSH-Px and significant increase in AST, ALT, ALP, urea, creatinine and MDA. Vaccinated sheep (V group) showed insignificant increase in AST, ALT, ALP, albumin, urea, creatinine, MDA beside significant increase in total protein, globulin, SOD, CAT, GSH and GSH-Px (Table 3 & 4).

Table (2). Effect of sheep pox and pox vaccine on blood picture in sheep (n= 10).

Groups			NV sheep	D sheep	V sheep
R.B.Cs 10^6/cmm			8.11±0.15a	7.57±0.17a	8.13±0.14a
HB (gm/dl)			11.06±0.15a	11.16±0.58a	11.90±1.41a
PVC %			36.32±0.25a	35.99±0.49a	36.42±0.46a
Blood indices	MCV (fl)		36.30±0.36a	36.21±0.41a	36.41±0.35a
	MCH (pg)		11.83±0.32a	11.70±0.31a	11.76±0.31a
	MCHC (g/dl)		30.71±0.51a	28.71±0.49a	30.70±0.53a
Leukocytic count ($10/cmm^3$)	Total WBCs		9.97±0.11c	13.52±0.28b	14.88±0.27a
	Differential count	Lymphocyte	3.91±0.12c	4.98±0.69b	5.91±0.30a
		Neutrophils	2.27±0.11c	4.33±0.11b	5.07±0.20a
		Monocyte	1.58±0.09b	2.05±0.7a	1.65±0.09b
		Eosinophils	1.11±0.9a	1.11±0.9a	1.21±0.07a
		Basophils	1.15±0.06a	1.13±0.06a	1.13±0.11a

Means with different superscripts of the same raw indicate significant difference at $P < 0.05$
Mean value ±SE

Table (3). Effect of sheep pox and pox vaccine on liver and kidney function in sheep (n=10).

Groups			NV sheep	D sheep	V sheep
Liver function	liver enzymes	AST (U/ml)	74.13±1.32b	81.21±0.72a	76.95±1.33b
		ALT (U/ml)	63.28±0.20b	69.55±1.33a	65.48±1.13b
		ALP(U100ml	59.21±1.32b	65.08±1.69a	61.71±3.14b
	protein profile (gm/dl)	T. protein	8.67±0.23a	5.88±0.34b	9.97±0.7b
		Albumin	4.06±0.5a	2.81±0.15b	4.48±0.03a
		Globulin	4.63±0.06a	3.06±0.19b	5.67±0.13b
		A/G ratio	0.91±0.02a	0.90±0.20a	0.87±0.34a
Kidney function (mg/ml)		Urea	20.89±0.79b	26.32±0.88a	21.97±0.86b
		Creatinine	1.87±0.24b	3.32±0.49a	1.95±0.04b

Means with different superscripts of the same raw indicate significant difference at $P < 0.05$
Mean value ±SE

Table (4). Effect of sheep pox and pox vaccine on MDA and antioxidant enzyme in sheep (n= 10).

Groups		NV sheep	D sheep	V sheep
Melanodialdehyde (µmol/l)		5.59±1.93b	13.05±1.15a	5.88±0.67b
Total antioxidant capacity (mmol/l)		4.75±0.84a	2.53±0.44b	11.60±1.02b
Antioxidant enzymes	Cat (u/l)	61.89±1.94a	54.47±2.05b	71.87±1.94b
	SOD (mmol/ml)	39.66±2.36a	32.81±2.29b	48.99± 2.11b
	GSH (µmol/L)	7.18±0.71a	5.19±0.72b	13.06±1.60b
	GSH-Px (uml)	40.38.24±1.15a	31.36±2.30b	46.78±1.44b

Means with different superscripts of the same raw indicate significant difference at P < 0.05
Mean value ±SE

Immunological results: Infected Sheep showed insignificant decrease in IgG, IgM and non-significant increase in serum nitric oxide and lysozyme activity. Vaccinated sheep with sheep pox vaccine showed significant increase in IgG, IgM, nitric oxide and lysozyme. (Table 5).

Table (5). Effect of sheep pox and pox vaccine in some immunological parameters in sheep (n= 10).

Groups	NV sheep	D sheep	V sheep
IgG (pg/mL)	660.67±4.76a	652.56±22.01a	836.78±26.15b
IgM (pg/mL)	46.53±2.65a	45.89±2.96a	57.21±2.01b
Nitric oxide umol/L	5.77±0.72a	7.49±0.79a	15.69±1.25b
Lysozyme (µg/mL)	22.56±0.51a	23.94±0.47a	47.89±0.88b

Means with different superscripts of the same raw indicate significant difference at P < 0.05
Mean value ±SE

5. Histopathological Results:

Diseased sheep in Group (D): The early histopathlogical alternation of the majority of the **buccal mucosa** showed areas of congestion and or ulceration with leucocyte infiltration. The lips showed that most of the glands are necrosed and invaded with inflammatory round cells associated with necrotic materials filling the glandular lumen (Plate 5A). Mononucleaclear cells on the subepithilial layer and intracytoplasmic inclusions (Bollinger bodies) were observed (Plate.5B).Examination of tongue showed complete necrosis and dystrophic calcification of the covering epithelium beside complete destruction of the dermal, sub mucosal layer and leucocytic infiltration (Plate 5C). Epithelial vaculation accompanied with presence of intracytoplasmic inclusion (Bollinger bodies) were seen in the cytoplasm of the degenerated cells (Plate 5D). Ulceration of most of the covering epithelium replacing with necrotic material and round cells aggregations forming large circumscribed depression filled with mixture of necrotic epithelium and leucocytes mainly neutrophils (pustules) were seen (Plate. 6A). Muscle of the tongue showing edema, Zenker necrosis and aggregation of mononuclear cells (Myositis) (Plate.6B).

Skin crust lesion (scab formation): Dermis was hyperemic edematous and infiltrated by inflammatory cell aggregations (Plate 6 C). Necrosis with leucocytic infiltration in the epidermal layer and vacuolated cells contain one or more intracytoplasmic eosinophilic inclusion bodies were reported (Plate 6 D). At the late stage of the disease, areas of keratosis were seen on the epidermal layers in addition to necrotic debris and inflammatory cells in the stratum corneum.

Concerning the lymph nodes and spleen, the histopathlogical examination showed cortical

necrosis and lymphocytic depletion. These results were associated with sub capsular edema, congestion and /or thrombus in the blood vessels (Plate 7A&B).

The microscopic examination of the intestine showed submucosal round cells aggregations and necrosis of the intestinal villi and glands (Plate 7 C).

Liver revealed focal mononuclear cell infiltration and portal cirrhosis. Hyperplasia of the epithelial cells of the bile ducts, hepatocellular degenerative changes and congestion of central vein were seen (Plate 7D).

Examination of heart revealed congestion of blood vessels and myocardial necrosis in addition to edema associated with lymphocytes, few round cells and macrophages infiltration (Plate 7 E).

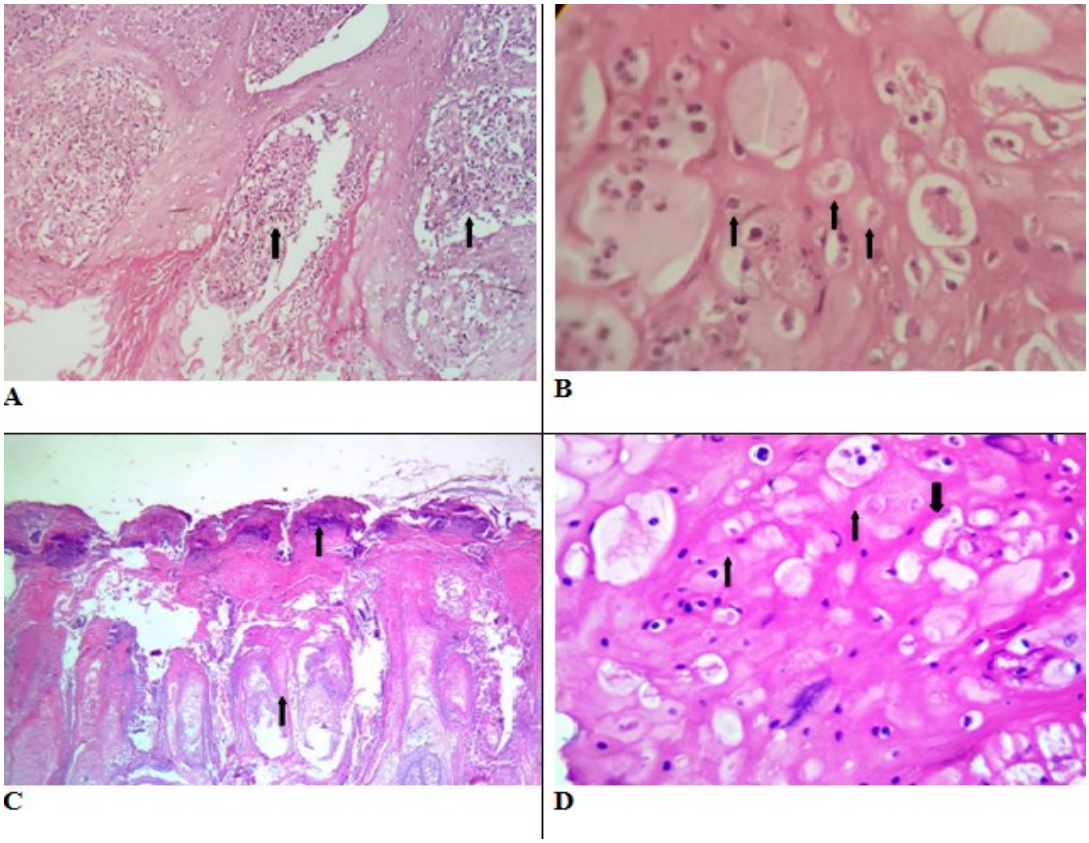

Plate (5). (5A) Lips showing that most of the glands are necrosed, invaded with inflammatory round cells associated with necrotic materials filling the glandular lumen. **(5B)** Lips showing invasion with polymorph nuclear cells on the subepithilial layer containing intracytoplasmic inclusions (Bollinger bodies) (black arrows). (**5C**) Tongue showing complete necrosis and dystrophic calcification of the covering epitheium beside complete destruction of the dermal structure. **(5D)**Tongue showing epithelial vaculation accompanied with presence of intracytoplasmic inclusion bodies (black arrows). H&E .X Powers 100 um, 50 um.

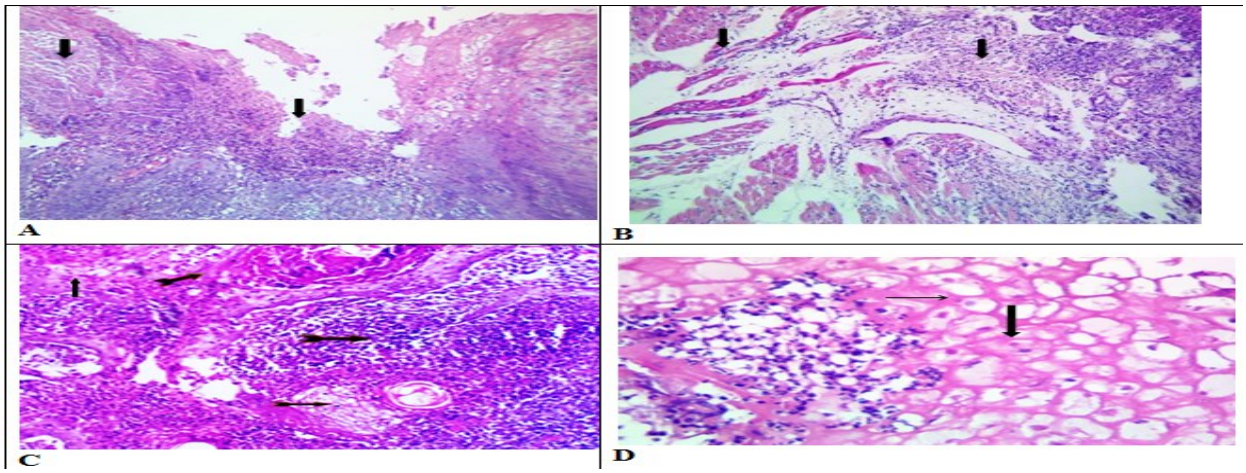

Plate (6). (6A)Tongue showing necrosis and ulceration of most of the covering epithelium replacing with necrotic material and round cells aggregations forming circumscribed depression (pustules)(black arrow). Inflammtory cells appeare invading the subepithelial layer. **(6B)** Muscle of tongue showing edema, Zenker's necrosis and aggregation of mononuclear cells (Myoscitis). **(6C**) Skin dermis showing cellular vacculation and infiltrated with inflammatory calls (black arrow). **(6D)** necrosis and leucocytic infiltration in the epidermal layer and vacuolated cells contain one or more intracytoplasmic eosinophilic inclusion bodies (black arrows). H&E, X Powers 100 um, 50 um.

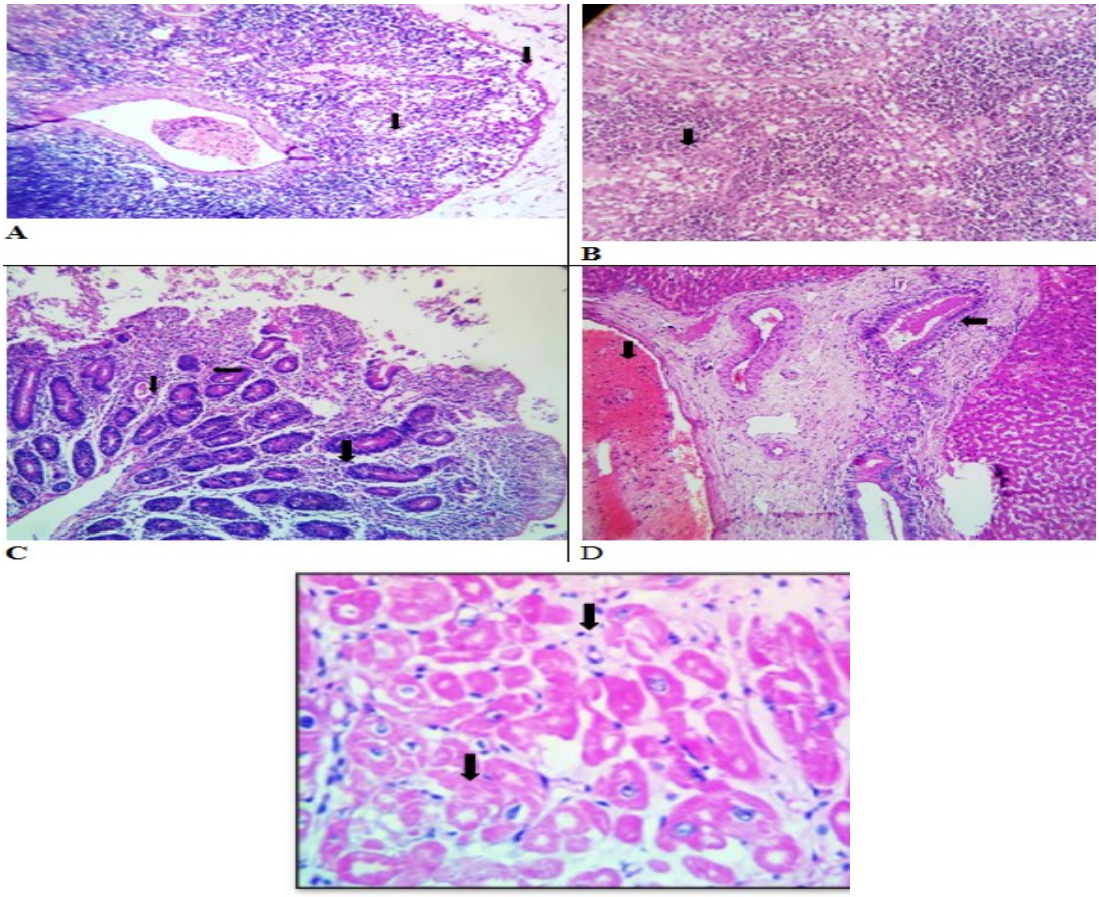

Plate (7). (**7A**) Lymph node showing sub capsular and interstitial edema associated with lymphocytic depletion and thrombus in the blood vessels. **(7 B)** Spleen showing marked lymphoid depletion and edema. . (**7 C**) Intestine showing and necrosis of the intestinal villi, glands and sub mucosal mononuclear leucocytic infiltration. (**7 D**) Liver showing mononuclear cell infiltration. The central vein appearing highly dilated and congested. (**7 E**) Heart showing edema, necrosis of some cardiac muscles and few round cell infiltration. (Black arrows). H&E, X Powers 100 um, 50 um

Vaccinated (V group) and apparently health sheep (NV group): Microscopic Findings:
Examined tissue sections from skin, oral cavity mucosa, internal organs and external lymph nodes of sheep, 30 days post-vaccination, revealed apparently normal cutaneous, subcutaneous and mucosal tissues free from the characteristic pox lesions and or the associated inflammatory reactions . Examined sections from heart, lungs and kidney showed apparently normal histomorphological structures with minor cardiac interstitial edema and mild renal nephrosis. Subcutaneous muscles, liver, intestines and lymph nodes of vaccinated sheep demonstrated apparently normal histological structures with immune reactive in the intestinal mucosa and Lymph nodes. Mild residual biliary proliferative reaction was seen in some cases.

Discussion
Sheep pox virus is a highly contagious viral infectious disease of sheep. The causative agent belongs to genus *Capripox virus* (**Garner *et al.,* 2000**). Sheep pox is endemic in Mediterranean region. It causes economic loss due to morbidity, mortality, reduced meat and milk production and abortion (**Dadousis, 2003**). Control and eradication sheep pox in endemic countries depends on diagnosis vaccination so, a rapid and sensitive diagnostic tool by screening affected sheep flocks is essential (**Bhanuprakash *et al.,* 2011)**.

In the present study, Trials for isolation of *sheep pox virus* from skin lesions of crusted scab samples which clinically suspected sheep pox by three blind passages through CAM of 9 -11 days old emberyonted chicken egg (SPF) which showed characteristic pock lesions, death of embryo, thickening, edema and hemorrhage of the membrane. These findings are in agreement with **Mahmoud and Khafagi (2016).**

Serological identification by IFAT for detection of SPV in infected CAM using specific hyperimmune serum against SPV. It showed specific Intracytoplasmic yellowish green fluorescent granules characteristic for SPV. Our results came in agreement with those of **Rao and Bandyopadhyay, (2000**).

The specific primers set amplified a DNA fragment of 151bp equivalent to expected amplification product (amplicon) size from SPV. SPV reference strain and local isolate from skin nodules had the same size of attachment protein gene fragment 151bp. Subsequently, it certain that these specimens contained DNA of LSDV.

In the present investigation, it has been shown that natural infected sheep with pox virus showed insignificant decrease in RBCs HB, PCV, MCV, MCH, MCHC, these may be due to depression anorexia that lead to decrease element forming RBCs and Hb. Our studies showed significant increase in total leucocytic count which was related to increase the number of lymphocytes and neutrophils due to viral infection and inflammation reported in pox virus infection. Healthy sheep vaccinated with sheep pox vaccine showed insignificant increase in RBC, HB, PCV, MCV, and WBCs beside non-significant decrease in MCH, MCHC but significant increase in lymphocyte and neutrophil. Increase in oxidative stress and lipid peroxidation in infected sheep lead to increase production of free radicals and caused bone marrow depression leading to hypofunction of erythropoietin and reduction in erythrogram (**Schalm *et al.,* 2003**). The infection with bovine pox showed leukocytosis, neutrophilia and lymphocytosis (**Rehfeld *et al.,* 2013**). *Pox virus* induces insignificant decrease in RBC, HB and PCV beside significant increase in lymphocytes lead to increase in total leucocytic count**.** These results had credibility with the result of **Numan *et al.,* (2016)** in buffaloes infected with *bovinepox virus* and **Shirish *et al.,* (2018)** in camels infected with *poxvirus*. Similar results were reported by (**De *et al.,* 2020**) who stated that camels infected with *poxvirus* induce significant increase in lymphocytes lead to increase in total leucocytic count**.** *Capripox virus* vaccine induce stimulate cellular immune response (**Deshmukh and Gujar, 1992**).

Our results were supported by the results obtained by **Zaghawa and Khalil (1997)** who noticed that sheep vaccinated with sheep pox vaccine induced decrease in RBC, HB, PCV and increase in MCHC WBCs lymphocyte and

neutrophil. Our results were agreed with results recorded by **Oloufat *et al.,* (2002)** who reported goats vaccinated with goat pox vaccine showed increase in WBCs and lymphocyte. Sheep pox vaccine induced insignificant decrease in RBC, HB PCV and increase in MCV MCH and MCHC **(Magda *et al.,* 2004)**.

The Same results were reported by **Ahmed *et al.,* (2007)** who stated that *Capripox virus* vaccine induces improved in WBCs mainly lymphocyte and neutrophil in sheep. Also, **Farag and Elnaker (2017)** stated that sheep pox vaccines increased WBCs mainly lymphocyte and neutrophil in cattle. Animals vaccinated with trivalent *capripoxvirus* vaccine improved cellular immunity (**Abd-Elfatah *et al.,* 2019**).

Activity of AST and ALT indicates the severity of liver damage of naturally infected sheep **(Tennant and Center, 2008)**. Our study showed sheep infected with *sheep pox virus* significant increase in liver enzymes but vaccinated sheep with sheep pox vaccine revealed non-significant increase in liver enzymes. In our opinion elevation in liver enzymes in diseased sheep in our study may be attributed to hepatotoxicity due to *sheep pox virus* and this agrees with **Altınel, (1993)**.

In addition, **Jemai *et al.,* (2007)** and **Bozukluhan *et al.,* (2018)** who stated that *Sheep pox virus* induced increase production of free radicals and caused liver cell damage which lead to increase in liver enzymes. Our data coincide with results of **Radostits *et al.,* (1995)** who stated that sheep pox vaccine induced insignificant increase in AST, ALT and ALP. These results are in agreement with those of **Manal *et al.,* (2001)** and **Magda *et al.,* (2004).** They stated that liver enzymes were insignificant increased in sheep vaccinated with sheep pox vaccine.

Our study revealed that, infected sheep with sheep pox virus showed significant reduction in total protein, albumin and globulin may be due to liver damage which is the main site for synthesis of albumen (hypoalbumenamia and hypoproteinemia) may be due to anorexia of sheep, increase protein catabolism due to stress of viral infection and also may be due to impaired kidney filtration capacity. The healthy vaccinated sheep showed significant increase in total protein, globulin but insignificant increase in albumin assisted by **Jemai *et al.,* (2007).** *Sheep pox virus* induced increase production of the free radicals and caused damage liver which lead to decrease albumin synthesis inducing hypoproteinemia. Another explanation for reduction of albumin was reported by **Issi *et al.,* (2008)** who mentioned that poxvirus infection induced liver disorders and anorexia leading to reduction in albumin and total protein. Also, **Bozukluhan *et al.,* (2018)** stated that sheep pox virus induced reduction in total protein, albumin and globulin in sheep **(Shirish *et al.,* 2018)**. In studies carried out by **De *et al.,* (2020)** and **Ujjwal *et al.,* (2020)** incamels naturally infected with pox virus showed marked reduction in total protein, albumin and globulin Increased serum total protein post vaccination may be due to absolute antibody producing cells in spleen and lymph nodes. The obtained results are in agreement with that of **Ahmed *et al.,* (2007)** who reported that vaccination leads to insignificant increase in serum albumin and globulin fractions where the later lead to increase in serum total proteins. Our results were supported by **Wassel *et al.,* (1996)** who reported that *caperipox virus* vaccines is the most effective immunogenic vaccine and provide increase in total protein and globulin**.** In addition, **Manal *et al.,* (2001)** and **Magda *et al.,* (2004)** stated that sheep vaccinated with sheep pox vaccine showed significant increase in serum total protein, albumin and globulin. Animals that vaccinated with trivalent *capripoxvirus* vaccine induced increase in total globulin (**Abd-Elfatah *et al.,* 2019**).

Our findings revealed that sheep infected with *sheep pox virus* showed significant elevation in urea and creatinine these may be due to kidney damage and increase in protein catabolism so considered an end product of protein catabolism .But healthy sheep vaccinated with sheep pox vaccine showed non-significant elevation in urea and creatinine. Elevation in urea and creatinine in sheep infected with *sheep pox virus* may be due to damage of kidney cells and tissues by viral infections via increased free radicals in host cells **(Akaike, 2010)** and (**Issi *et al.,* 2008**)**.** In addition, **Aral, (2015), Bo-**

zukluhan *et al.,* (2018) and Tennant and Center, (**2008**) who stated that elevations in urea and creatinine in sheep infected with *pox virus* may be due to systemic inflammation leading to impaired glomerular filtration. *Sheep poxvirus* induced increase in urea and creatinine. Our results were previously recorded by **Magda *et al.,* (2004)** who recorded that vaccination induced non-significant elevation in urea and creatinine. Same change in urea and creatinine were reported previously by **Manal *et al.* (2001)** in sheep vaccinated with sheep pox vaccine.

Significant increase in nitric oxide in our study may be due to increase lipid per oxidation in cells that lead to increase oxidative stress. Nitric oxide elevation is contributes to mammalian host defense against intracellular pathogens (**MacMicking *et al.,* 1997**). Significant increase in lysosome activity may be due to pox viral infection induced decrease cellular immunity against infection.

Infected sheep with *pox virus* showed insignificant elevation in serum nitric oxide and lysozyme activity. Meanwhile, Healthy sheep vaccinated with (V group) showed significant increase in nitric oxide and lysozyme activity. Lysozyme is a common constituent of biological tissues and secretions; it has been found in egg whites, tears, sweat and the digestive tract of ruminants. Lysosomes are multifunction cellular organelles that have important role in intracellular digestion in phagocytic cells due to its enzymatic contents. Lysozymes was known to be one of lysosomal enzymes attacks mucopeptide in cell walls of various bacteria **(Moore *et al.,* 2006)** and have bactericidal activity during phagocytosis **(Tizard, 1996).** Viral infections enhance nitric oxide productions and lysozyme activity **(Elanie *et al.,* 2015).** It is known that lysozyme seems to be effective against some viruses **(Lee Huang *et al.,* 1999)** and Gram-positive bacteria **(Blotskyi, 1976).** The antiviral activity of lysozyme is ascribed to its direct interaction with the virus (the blockade of viral fusion protein gp41) or with the cell (the blockade of CD4 receptor on lymphocytes) (**Behbahani *et al.,* 2018).** Therefore, the increase in lysozyme may be due to its leakage from lysosomal granules. **(Bussolaro, *et al.,* 2008).**

Our results were agreed with earlier reports of **Kirmizigul *et al.,* (2016)** who reported that sheep infected with *poxvirus* showed increase in serum nitric oxide. Similar findings were recorded by **Ujjwal *et al.,* (2020)** who stated that serum nitric oxide level and lysozyme activity was increased in camels infected with pox virus. The results depicted in the present study were similar to finding **Sorci and Faivre (2009)** they stated that the vaccinated sheep pox induced significant increase in production of nitric oxide and increase in lysozyme activity in serum of sheep **(Mohamed and Kamel, 2016)**.

In the present studies, infected sheep with *pox virus* showed insignificant reduction in serumIgG and IgM may be due to decrease humeral and cellular immunity as reduction in B lymphocytes lead to decrease in plasma cells producing antibodies and so decrease immunoglobulines .But healthy sheep vaccinated with sheep pox vaccine revealed significant elevation in IgG and IgM in comparison with control sheep .Our findings are harmonized with that of **Eman *et al.,* (2019) and Erhard *et al.,* (1992)** who stated that vaccinated animals empathize increase in the levels of IgG and IgM compared to non-vaccinated healthy sheep. In addition, the obtained results in the current study were in agreement with that of **Barmana *et al.,* (2010)** they reported that sheep vaccinated with sheep pox vaccine showed increase in IgG and IgM. Furthermore, the same finding in immunoglobulin IgG and IgM were reported by **Atkinson *et al.,* (2000)** in pregnant ewes.

In the current work, it has been noticed that infected sheep with *pox virus* revealed significant elevation in serum melanodialdehyde levels beside significant reductions in superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase. These results may be due to viral infection cause damage to the cells and tissues via increasing free radicals lead to oxidative stress. Healthy sheep vaccinated with sheep virus vaccine showed insignificant decrease in melanodialdehyde beside significant increase in superoxide dismutase, catalase, reduced glutathione**,** glutathione peroxidase) .Our result was supported by the results

obtained by A**bdel-Aziz and Ahmed (2005)**, **Issi *et al.,* (2008)** and **Mustafa *et al.,* (2008)** they reported that natural infection with *pox virus* exacerbated free radical formation and elevated of plasma melanodialdehyde beside significant reductions in activities of antioxidants enzymes (super oxide dismutase, catalase and reduced glutathione). Our result is supported by **Birben *et al.,* (2012)** who recorded that sheep pox infection in sheep induced impaired antioxidant status resulting in oxidative stress and increased plasma melanodialdehydebeside decrease in antioxidant enzymes activity: Our result was supported by the result of **Kirmızıgul *et al.,* (2016)** and **Bozukluhan *et al.,* (2018)** who stated that sheep pox virus increased melanodialdehyde level beside significant decrease in reduced glutathione. Our data are fit with those reported by **Ujjwal *et al.,* (2020)** and **De *et al.,* (2020)** who stated that melanodialdehyde concentrations were increased in serum of camels naturally infected with *pox virus*

In the present study, the histopathological examination of diseased sheep pox showed variable degrees of erosion and /or ulceration of the buccal mucosa, lips and tongue containing intracytoplasmic eosinophilic inclusion bodies (Bollinger bodies). Submucosal leucocytic infiltration mainly (lymphocytes) was reported. Crust lesions (scab formation) were prominent during late stages of disease in lips, nostrils, and eye lids and skin. Lips showed keratinocytes, necrotic debris. Also our studies showed lymphoid depletion of lymphoid organs (lymph nodes and spleen) . Submucosal leucocytic infiltration and necrotic villi and glands were noticed in the intestine. Our results revealed also portal cirrhosis, bile ducts epithelial hyperplasia in some cases. Myocardial necrosis and inter muscular edema was observed. No pathological changes in the different tissues of vaccinated and healthy non vaccinated sheep were noticed. These results agreed with **Abd El ghaffar and Khaled (2009)** who observed that sheep pox induced hyperkeratosis, acanthosis, hydropic degeneration and necrosis in epithelial layers. Mononuclear cells, neutrophils, giant cells infiltration were noticed in affected tissue. **Numan *et al.,* (2016)** and **Hamouda *et al.,* (2017)** who stated that sheep pox induced marked hyperkeratosis ballooning degeneration and intracytoplasmic eosinophilic inclusion bodies in keratinocytes

From our study, it could be concluded that sheep pox induced many adverse effect in blood picture, liver function, kidney function, some antioxidant enzymes, immunological status and pathological lesions. Prophylaxis by using attenuated vaccine (SERVACCAPRI-C) is considered among the most valuable effective tools for controlling the diseases. Vaccination has been considered to be suitable means in control of *sheep pox virus* stimulating both humeral and cell mediated immune response.

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