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VIROLOGICAL AND PATHOLOGICAL STUDIES ON BUFFALO POX VIRUS ISOLATED FROM DAIRY BUFFALOES' UDDER

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SUMMARY

Pox like lesions were frequently recorded in 43.75% dairy buffaloes recently introduced into small and moderate dairy barns at Kafr EL-Gabal and Zawet Abo Mesalem districts at Giza Governrate. These affections led to reduction of milk yield. This work was done to isolate and identify the causative agent to overcome the problem and its economic losses.

After inoculation of the prepared collected samples on the chorioallantoic membrane of embryonated chicken eggs, pock lesions were observed on the 4th or 5th day-post inoculation. Impression smears and samples were prepared from each harvested chorioallantoic membrane for identification of the pox virus using Agar gel precipitation and indirect immunofluorescent techniques. Both tests showed positive results in all examined samples in the form of precipitin lines or specific cytoplasmic fluorescent reaction, respectively.

Gross examination of the infected udders primarily revealed, tenderness followed by embedded or superficial papule with eroded or ulcerated surfaces on the teats and lower part of the udder. Histopathological study revealed focal infiltration of mononuclear and polymorphnuclear leucocytes in the dermal papillae with exocytosis of the inflammatory cells. Mild oedema and perivascular cuffing were also seen in the dermal layer. The cells of the stratum spinosum showed ballooning in addition to necrobiotc changes particularly near the ulcerative areas that was highly infiltrated by polymorphnuclear leucocytes.

INTRODUCTION

Pox viruses are DNA epitheliotropic viruses that infect most domestic, wild, laboratory animals and birds. The severity of pox viral infection is variable, in part due to the localized and systemic nature of the infection and sometimes to secondary infections.

Pox like lesions were frequently recorded in dairy buffaloes recently introduced into small and moderate dairy barns at Kafr EL-Gabal and Zawet Abo Mesalem districts at Giza Governorate. The lesions were developed two to four weeks after the newly purchased animals inter the farm. The lesions start as papules intra dermal in the teats that hinder easily milking of the animal. Moreover, the lesions progress to pustules, erosions and ulcers due to twice daily hand milking of the animals .In some cases mastitis and dryness of the affected quarters occurred. Tantawi (1974) isolated Pox virus from buffaloes in Upper Egypt. Tantawi and Sokaar (1975) also described tissue reaction of the buffalo Pox virus on chorioallantoic membrane and cell culture in 1975.The present work aims to isolate and identify the causative agent to overcome the problem and its economic losses.

MATERIAL AND METHODS

Animals:

Four moderate and small dairy barns at Kafr EL-Gabal and Zawet Abo Mesalem districts at Giza Governorate containing 130 dairy buffaloes, with continuous complain from development of papules and ulcers on the teat and lower parts of the udder in about 50% of newly introduced animals were examined. The lesions mostly developed within 15-30 days from interance of the animals. Seven out of 16 newly introduced buffaloes in these farms were infected. Clinical signs(temperature, appetite and lesions) of the infected were recorded.

Samples:

For virological examination, skin scrapings from the udder and teat lesions from all infected animals were collected. On the other hand, tissue samples were collected from teats of another three slaughtered infected buffaloes for pathological study,.

Isolation and identification of the pox virus:

Samples preparation was done according to the methods described by Van Rooyen and Weiss (1959). Briefly, 10% suspension of each grind skin scraping was prepared in phosphate buffer saline (PBS) containing 100 IU penicillin, 100 U streptomycin and 50 IU mycostatin per ml. Each suspension was centrifuged at 3000 rpm for 10 minutes and the supernatant was used for virus isolation.

One ml of each sample suspension was inoculated in 5-11 days-incubated fertile chicken eggs (0.2

ml /egg) via the chorioallantoic membrane (CAM). Five passages were done. The inoculated eggs were incubated for 5 to 7 days at 37° C and the CAMs were grossly examined for presence of pock lesions (Tantawi et al., 1973).

Three Impression smears were prepared from each harvested chorioallantoic membrane. Pox virus was detected on each prepared smear using indirect immunofluorescent technique (IIFT) according to the method described previously by Goldman (1968). The rabbit anti pox hyper immune serum was kindly supplied from veterinary Serum and Vaccine Research Institute, while the fluorescein conjugated anti rabbit serum was kindly offered by Prof. Dr. Nabil El Danaf, Professor of Microbiology, Animal Reproduction Research Institute. Agar gel precipitation test (AGPT) was done according to the method previously described by Manis (1958) for identification of the virus using 10% of the collected CAM. The used cattle pox hyper-immune serum was kindly supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt.

Histopathological study:

The tissue samples were fixed in formol saline, dehydrated in ethyl alcohol, cleared in xyline and embedded in paraffin. Four µm sections were prepared, stained with Heamatoxline & Eosin (H & E) staining technique and examined under light microscope.

RESULTS

Clinical signs:

Seven out of the 16 recently introduced buffaloes showed udder lesions. No lesions appeared on the remaining buffaloes. The affected animals showed hyperthermia (39.5 - 40°C) and transient decreased feed intake. Gross examination of the infected udders revealed, firstly tenderness followed by formation of embedded or superficial papule. Pustules were formed with eroded or ulcerated surface within one day and covered by dark brown crusts mainly on the teats and lower parts of the udder (Fig 1) Ulcers remained for two months on the teats of two animals while in the others healing occur within one month after the appearance of the lesions.

Virus isolation and identification:

Five days post inoculation of embryonated chicken eggs via chorioallantoic membrane (CAM), thickness and inflammatory oedematous spots (pock lesions) were observed at the site of inoculation (Fig. 2). The pock lesions regularly appeared on the 4th or 5th day post inoculation on the second, third and fourth passages of all inoculated embryonated chicken eggs.

The AGPT showed positive results (precipitin lines) in all tested samples (Fig. 3).

The results of indirect immunofluorescent tech-

nique for identification of the virus in CAM smears indicated positive reactions in all tested smears (specific intracytoplasmic fluorescent reaction in many cells) (Fig. 4).

Histopathological study:

Microscopic Examination of the skin sections of the affected animals revealed moderate focal infiltration of mononuclear inflammatory cells, lymphocytes in the dermal papillae with exocytosis of the inflammatory cells into the interdigitating lower epidermal layer (Fig. 5). The superficial dermal layer also showed mild oedema and congestion with heavy infiltrations of neutrophils and mononuclear cells particularly at the perivascular areas (Fig. 6&7). The epidermal layer usually showed hyperplasia of the stratum spinosum that was also infiltrated by polymorphnuclear leucocytes (Fig. 7) The cells of the stratum spinosum showed ballooning, particularly near the ulcerative areas, and were infiltrated by neutrophils (Fig. 8&9). At the area of ulceration, the central part showed liquifactive necrosis that was infiltrated by neutrophils and macrophages (Fig. 8). Certain epidermal areas showed atrophy of the interdigitating epithelium between the dermal papillae and its covering was abnormal stratified non keratinised epithelium (Fig. 10).

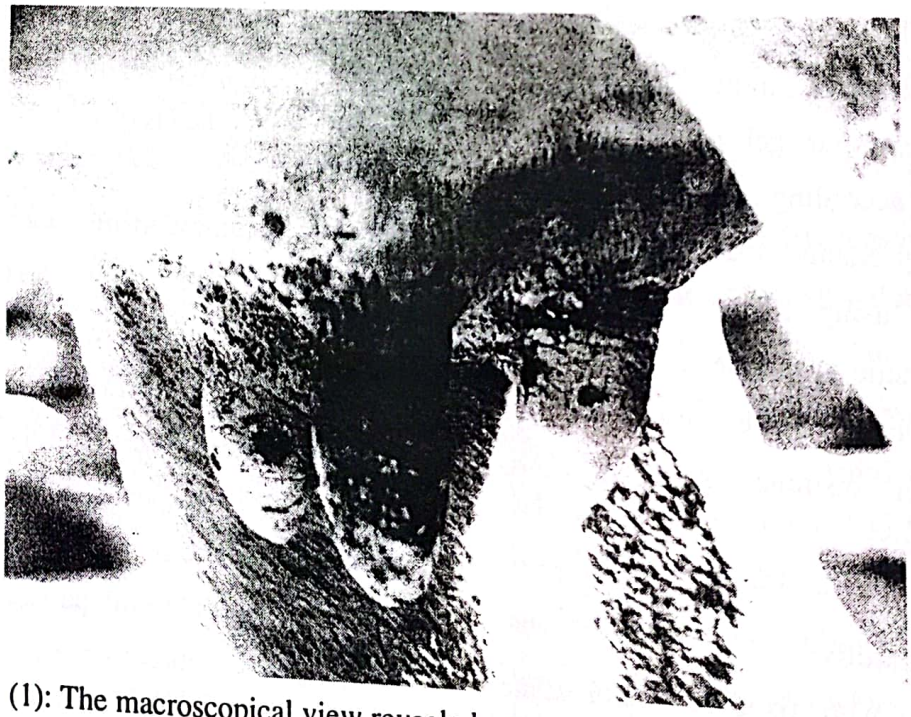

Fig. (1): The macroscopical view revealed presence of scape stage of pox virus infection on the lower parts of the udder and on the teats (arrows) in addition to ulceration of the anterior left teat.

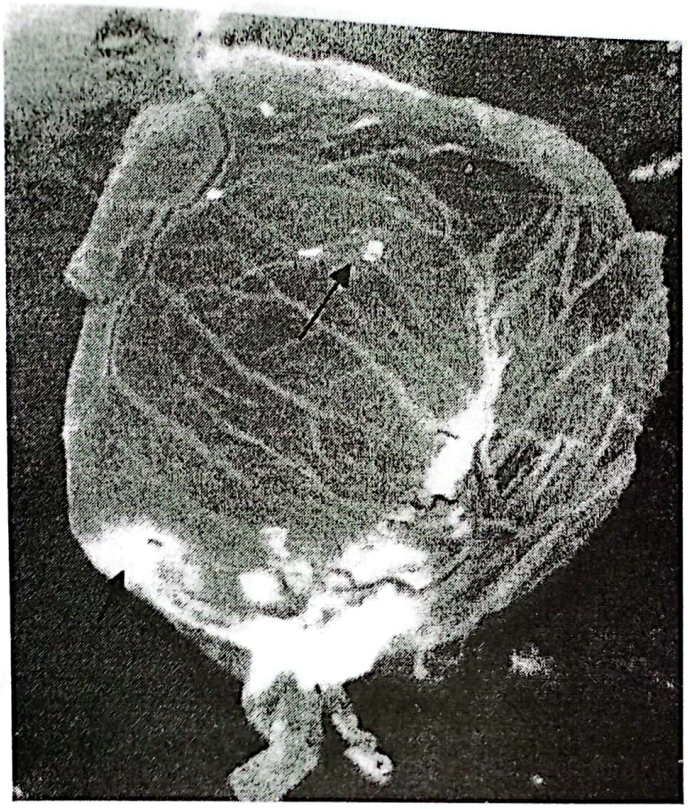

Fig. (2): Chorioallantoic membrance of embryonated chicken egg inoculated with tissue extract showing pock lesions.

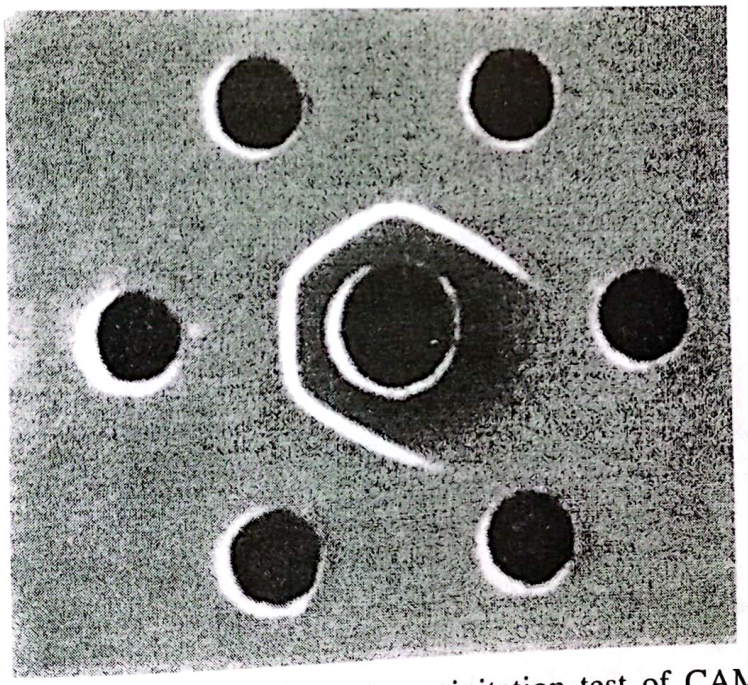

Fig. (3): Positive agar gel precipitation test of CAM harvested virus against rabbit anti buffalo pox hyper-immune serum.

Fig. (4): Positive agar gel precipitation test of CAM showing intracytoplasmic positive immunofluorescent reaction of Pox virus (arrows).

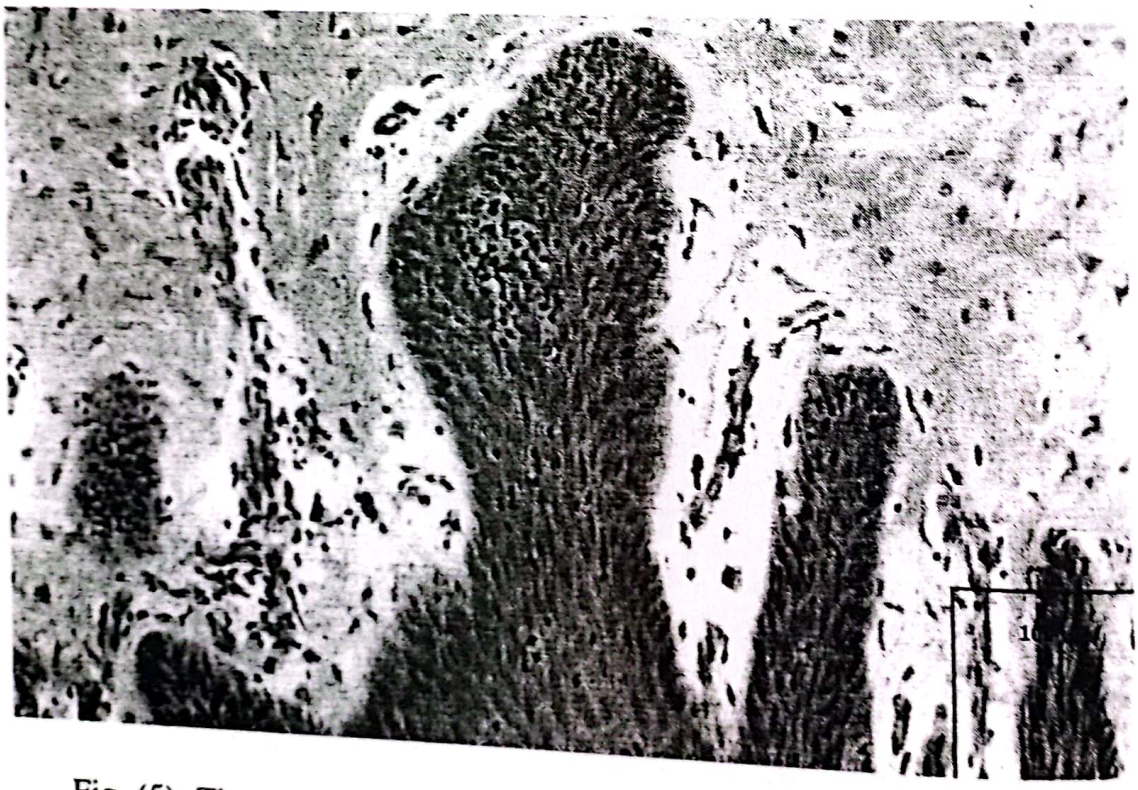

Fig. (5): The superficial dermal layer is infiltrated with mononuclear inflammatory cells and the epidermis showing exocytosis. (H & E 100 X).

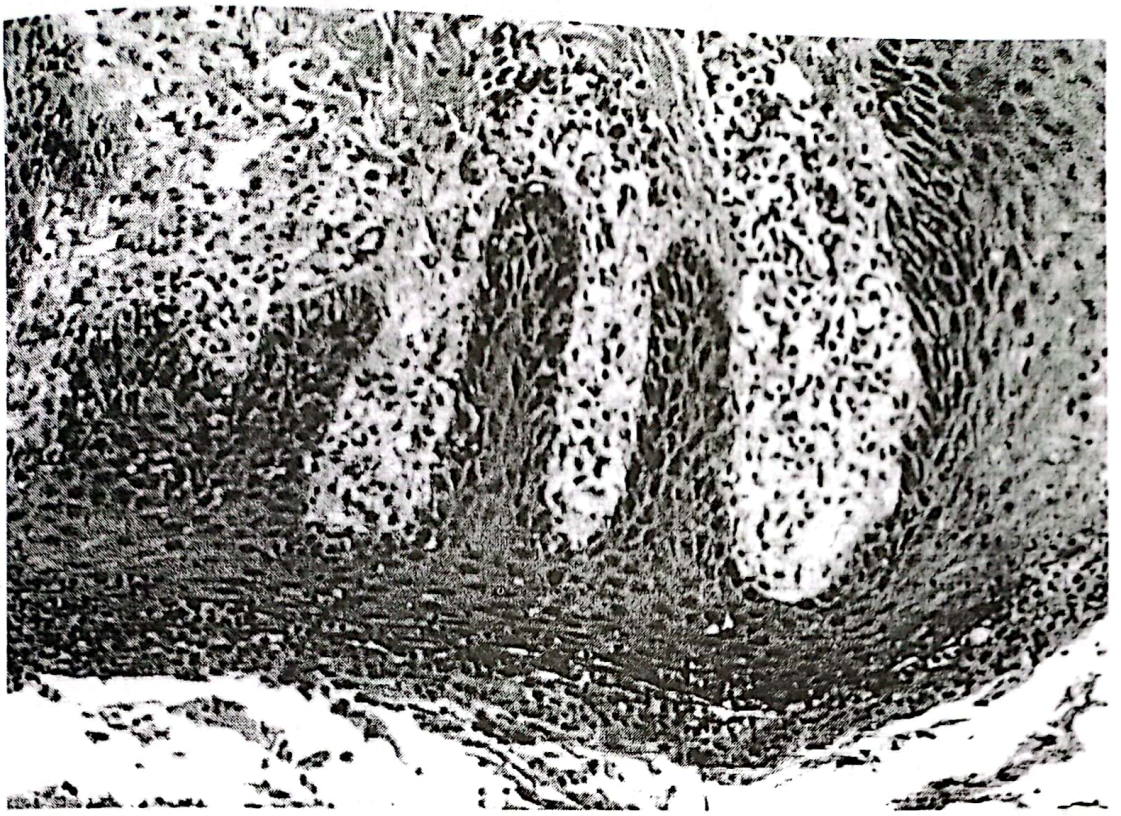

Fig. (6): The superficial dermal and epidermal layers are highly infiltrated with neutrophils with mild oedema in the superficial dermal layer (H & E 100 X).

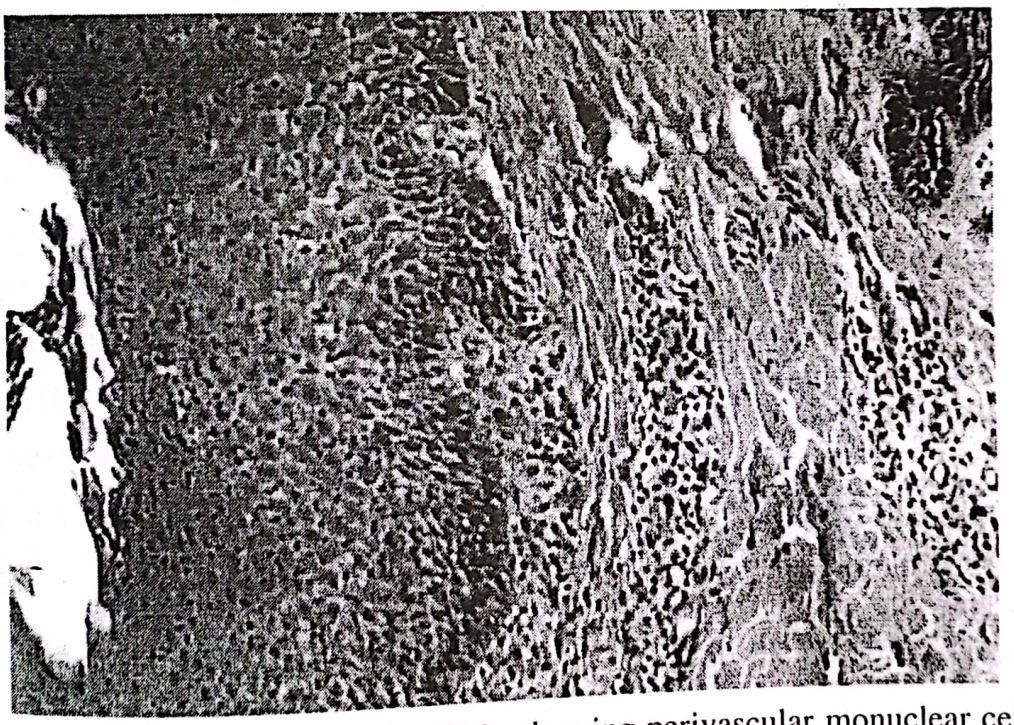

Fig. (7): Affected teat skin of buffalo showing perivascular monuclear cells infiltration in the dermal layer while the epidermal layer showing exocytosis and hyperplasia of the brickle cells (H & E 100 X).

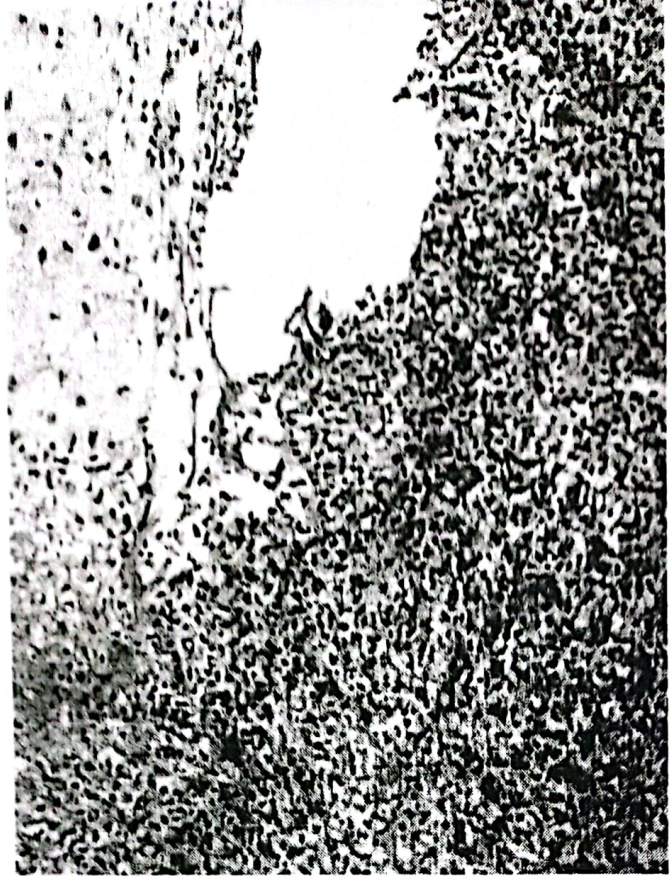

Fig. (8): Chronic ulcer in a teat of infected buffalo showing necrotic area highly infiltrated with neutrophils and the dermal epithelium beside it showing vacuolar degeneration (H & E 100 X).

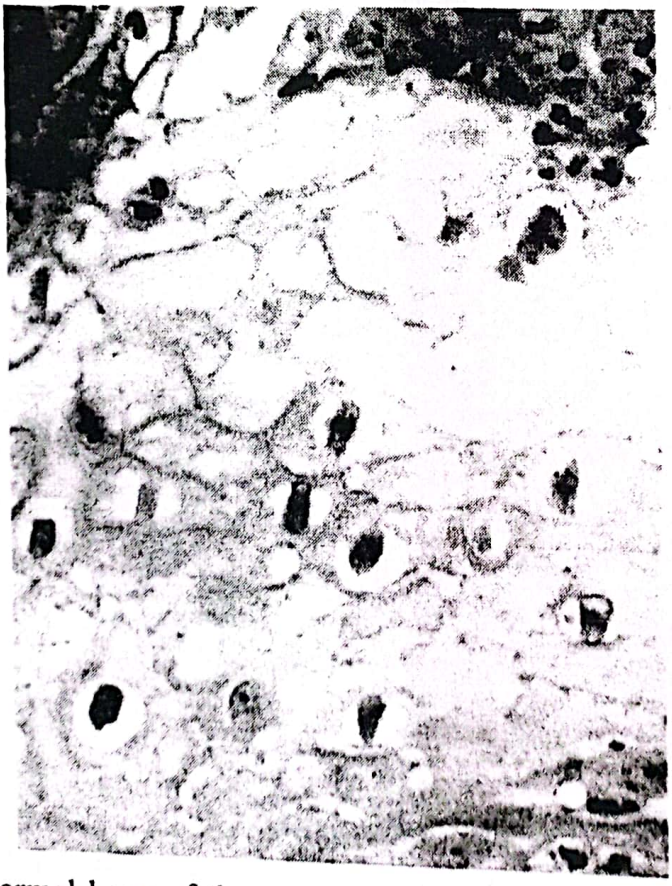

Fig. (9): Epidermal layer of the teat skin revealed ballooning of the stratum spinosum cells in addition to bursting of some cells accompanied with polymorphnuclear cells infiltration (H & E 100 X).

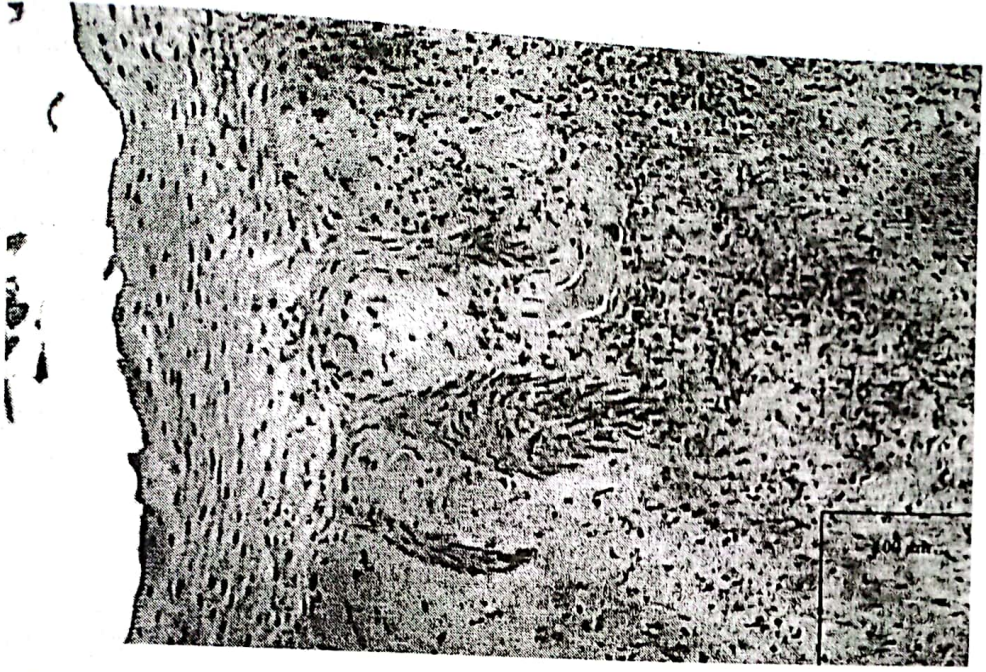

Fig. (10): Teat skin of infected animal showed hyalinised dermal collagen fibres around atrophied interdigitating epidermal epithelium, and abnormal stratified non-keratinised covvering epithelium (H & E 100 X).

DISCUSSION

In this study, the effected buffaloes showed inappitance and rise of body temperature (39.5 - 40°C). Similar signs were recorded by Rana et al. (1985) and Ali et al. (2003). Rana et al. (1985) also found that the disease run a course of 13 to 15 days in experimentally infected calves. However, in the present study, the lesions persisted up to two months. This may be attributed either to the milking process which done twice daily and hinder healing of ulcerated lesions or to the secondary infection.

In the present study, pox virus infection appear to be endemic at the localities where the virus was isolated since the lesion appeared regularly in newly introduced buffaloes with incidence of 43.75%. Mathew and Mathew (1986) demonstrated on epidemic of buffalo pox infection not only in buffaloes but also in cattle, horses, goats and human in India. Kuroda et al. (1999) also isolated parapox virus from epidemic disease among cattle in Japan with positive rate more than 50%. In Egypt, Ali et al. (2003) isolated buffalo pox from outbreak among buffalo calves within Dakahlia Governorate.

In this study, the prepared samples were inoculated in embryonated chicken eggs via chorioallantoic membrane (CAM) for pox virus isolation. After 5 days post-inoculation pock lesions were observed at the site of inoculation. The pox virus was also previously propagated on CAM by Ali

et al. (2003). The pox virus was detected in the impression smears which prepared from each harvested chorioallantoic membrane from inoculated eggs using indirect immunofluorescent technique (IIFT) and agar gel precipitation test when the isolates tested against cattle pox hyperimmune serum. The results indicated positive results in all tested smears. Also, the results indicated that buffalo pox virus antigenically related to cow pox and the IIFT and AGPT techniques are suitable for pox virus identification. The same conclusion previously mentioned by Tantawi (1974) and Ali et al. (2003). Also, Keen and Yeats (1999) stated that true pox virus are antigenically rather similar.

Although Ali et al. (2003) stated that pox lesion in naturally infected buffalo calves passed through the typical stages of pox viral disease (Macule, papular lesion, vesicle, pustule, scape and/or ulceration). The finding in the present study showed that papules and ulceration followed by dark scape were the only demonstrated gross lesions. On the other hand, Jones et al. (1996) and Ali et al. (2003) stated that these skin lesions of pox virus in experimentally infected rabbits were also lacking the vesicular character. The recorded lesions appeared on the teats and lower parts the udder. Kolhapure et al. (1997) supported this finding as they stated that the lesions of affected buffaloes were noticed mainly on the teats and udder, in outbreak of buffalo pox infection. Also, Rana et al. (1985) and Ali et al. (2003) demonstrated presence of lesion on breast and medial aspect the thigh in addition to generalized lesions in buffalo calves. Kuroda et al. (1999) and Buttner and Rziha (2002) also manifest udder lesions in cows and bovine papular lesions in calves caused by parapox virus infection. The last author added that the infection could be transmitted to man in hands and fingers causing pseudo-cow pox or milker nodules. In the present study the presence of the lesions on the teat and lower parts of the udder may indicate mechanical transmission of infection through hands of milkers during milking process.

Microscopical examination of the infected skin showed ballooning degeneration of some cells of stratum spinosium with destruction of others that were infiltrated by lymphocytes and neutrophiles. Hargis (1995) stated that when pox virus invade the epidermis, the virus replicate in the stratum spinosum causing ballooning degeneration and rupture of some cells of the epidermal layers (reticulate degeneration). Ali et al. (2003) also demonstrated the presence of polymorphnuclear leucocytes and round cells aggregations in dermal layer of experimentally infected rabbits but the author stated the epidermal changes were not seem until 20-35 days post infection.

Examination of the dermal layer revealed congestion, mononuclear and polymorphnuclear leucocytic cells infiltration accompanied by mild particularly around blood vessels and in the in the

dermal papillae. Hargis (1995) stated the cellular constituents of the ruptured epithelial epidermal cells release chemotactic factor for leucocytes and increase blood flow that cause dermal oedema due to increase vascular permeability. The author added that, initially lymphocytes migrate with variable numbers of neutrophiles around blood vessels in the dermis followed by exocytosis and development of pustules in the epidermis. Ammar et al. (1999) stated that heavy round cells infiltrations were constant finding in dermis of sheep infected with poxvirus. Ali et al. (2003) also supported the presence of congestion, oedema and mononuclear cells infiltration in the dermal layer of infected buffalo calves. Jubb et al. (1997) also reported presence of vasculitis, oedema and perivascular cuffing in pox virus infections.

The heavy neutrophilic infiltration, in some cases in both dermis and epidermis may be attributed to ulcerative changes and/or secondary invadors. Ali et al. (2003) reported that erosions and ulcer on skin of experimentally infected rabbits 28-35 day were accompanied by heavy infiltration of leucocytes, mainly lymphocytes, macrophages, plasma cells and fibroblasts.

In the present study, although the causative agent was identified as pox virus further molecular genomic and antigenic characterization still required for proper identification to prepare vaccine for problem control in animals and to hinder its transmission to human. Dumbell and Richardson (1993) supported this view as the author stated that 13 strains of buffalo pox virus isolated from scapes of pox lesions on buffaloes were indistinguishable from vaccinia virus in their biological properties but the restriction profile of their DNA differed them from vaccinia strain and buffalo parapox isolates. Moreover Kolhapure et al. (1997) also advised monitoring buffalo pox carefully to avoid emerge of a serious zoonotic disease as the author not only detected neutralizing antibodies in some affected human and their contacts but also demonstrated lesions in few children.

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