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Histopathological Changes in Skin and Lymph Nodes of Sheep Following Vaccination with Anthrax, Capripox and Combined and Anthrax and Capripox Vaccines

[1]Abbas Mohamed Ahmed, [1]A.M. Zakia, [2]M.M. Mukhtar and [3]A.M. El-Hussein
[1]Central Veterinary Research Laboratory (CVRL), Khartoum, Sudan
[2]Institute of Endemic Diseases (IENDs), University of Khartoum
[3]National Central Laboratory (NCL), Khartoum, Sudan

Abstract: Skin sections of sheep inoculated with live spores anthrax vaccine revealed edema, intense effusion of mononuclear cells and proliferation fibroblast in the dermis and subcutaneous tissues, whereas sections of skin of those inoculated with capripox vaccine showed hyperplasia and hydrophopic degeneration of epidermal epithelium and instance infilteration of cellular exudates in dermis and subcutaneous tissues. However, the lesions presented by the combined vaccine included the fore mentioned lesions.

Key words: Histopathological changes, anthrax, vaccines, mononuclear cells, subcutaneous tissues, skin

INTRODUCTION

Animal anthrax occurs in at least three different forms, peracute or apoplectic form, acute form and subacute to chronic form. Ruminants are most likely to manifest the peracute and acute form, horse the acute form and dogs cats and pigs a subacute to chronic or localized condition (OIE, 2004). The acute form is usually seen in horses and varies with the site of exposure. Fever and depression usually accompany enteritis and colic. Death usually occurs within 48-96 h. Pores introduced subcutaneously, for example by biting insects result in a hot oedematous swelling at the site that spread to the throat, thorax, abdomen, prepuce or mammary gland. Dyspnoea due to throat swelling with resulting compression of the trachea may also be apparent. The course of the disease is usually 1-3 days, with some animals surviving for one week or more.

The subacute to chronic form of anthrax occurs in domestic and wild pigs, dogs and cats. The infectious bacteria are usually ingested when the host feeds on contaminated source. The organisms tend to localize in the regional lymph nodes of the pharyngeal area, where severe swelling may occur, resulting in death by occlusion of airway. In case this does not occur, a fatal bactraemia may develop, although recovery after a few days of illness is not uncommon. An intestinal form with severe acute gastroenteritis is also seen in carnivores and omnivores.

Plowright and Ferris (1959) stated that sheep pox virus causes a severe systemic disease which had clinicopathological manifestation comparable to those observed in variola and ectrometra and myxamatosis. However, a number of workers (Vegad and Sharma, 1973; Davie, 1967; Katiyar, 1961) described a typical sheep pox infection in which the vesicular and pustular stages of lesion development were absent. Instead of the characteristic pustule formation,there was an accumulation of purulent material between the necrosed crust of the epidermis and underlying granulation tissues. (Vegard and Sharma, 1973). The main histological changes occurred in the connective tissues under the epithelium and subcutis. Microvesicles formation in the epidermis was reported by Murry. In the dermis there are the characteristic sheep pox cells or cellules calveleuses of Borrel which have a vacuolated nucleus and large intracytoplasmic inclusions. The histological and ultrastructures features of the cells demonstrated that they include monocytes, macrophages and fibrocytes.

Both intracytoplasmic and intranuclear inclusions are observed in the macrophages. Because the hydropic degeneration does not result in the rupture of cell, there is no vesicle formation. (Katiyar, 1961; Plowright and Ferris, 1959; Vegard and Sharma, 1973). The nodules are present throughout the lungs and the mucosa of the trachea, mouth, pharynx and abomasums. There are lesions that occasionally appeared on the mucosa of the duodenum and large intestines. Desquamation of the nodules lead to the formation of ulcer in the mucosa (Katiyar, 1961). Ramachandran reported that pulmonary lesions developed in only 30-46% of the animal experimentally infected with sheep pox or goat pox viruses where as pulmonary involvement was exhibited in 70-90% of the naturally infected sheep and goat.

Corresponding Author: Abbas Mohamed Ahmed, Central Veterinary Research Laboratory (CVRL), Khartoum, Sudan

We aimed from this study to see if there is any interference between *Bacillus anthracis* and the capripox virus at the site of inoculation and in the prescapular lymph node.

MATERIALS AND METHODS

Animals: Local breed sheep of no history of vaccination with anthrax vaccine, capripox vaccine or the combined anthrax and sheep pox vaccine were purchased from the market. These animlas were divided into 4 groups,group 1 vaccinated with anthrax vaccine, group 2 vaccinated with the capripox vaccine and group 3 vaccinated with the combined vaccine, the last group remained as non-vaccinated control. All the three vaccines were adminstred Subcutaneously (S/C).

Anthrax vaccine: The live spore anthrax vaccine was prepared according to the OIE (2004).

Capripox vaccine: This vaccine was also prepared as described by the OIE (2004).

Combined anthrax and sheep pox vaccine: Fifty mL of concentrate of anthrax vaccine (5×10 9) was mixed with 450 mL of capripox vaccine (5 TCID 50) then mechanically homogenized and lyophilized in small vials so that the dose of the combined vaccine is equal to the doses of the two individual vaccine.

Histopathological method: Specimens of the skin at the site of inoculationand prescapular lymph nodes were fixed in 10% formalin and processed for paraffin embedding sections using the conventional method. 0.5 micron sections were cut and stained with Hematoxylin and Eosin (H and E).

RESULTS

Histpathological changes following vaccination with anthrax vaccine

Skin: No evidence of histopathological changes in all section of the non-vaccinated control were observed (Fig. 1).

At the site of inoculation with anthrax vaccine the epidermal layers revealed local area of mild cell proliferation. The dermal reaction included edema, proliferation of fibroblast and intense effusion of cellular exudates which consisted of lymphoid, plasma, histocytes and few eosinophils (Fig. 2). Aggregates of these inflammatory cells were present between the degenerated hair follicle cells and around blood vessels in the subcutaneous connective tissues.

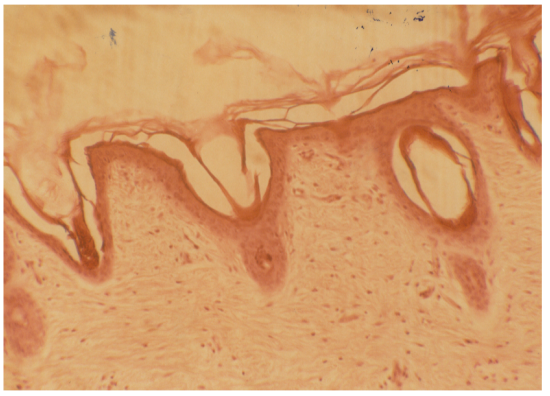

Fig. 1: Section of control animal. Note: Normal structure of epidermis and connective tissue dermis. H and EX100

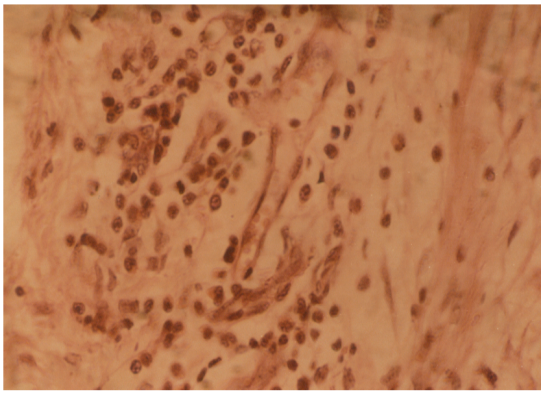

Fig. 2: Section of skin of sheep 4 days post inoculation with live spore anthrax vaccine. Note edema, effusion of mononuclear cells and proliferation of fobroblast t the site of inoculation. H and E 400

Lymph node: Section of lymph node from animal vaccinated with anthrax vaccine showed atrophic lymphoid follicles which formed cavities in the germinal center and subcapsular areas concomitant with numerous cavities of different sizes, scattered in both cortex and medulla (Fig. 3).

Histpathological changes following vaccination with capripox vaccine

Skin section: On the fourth day post inoculation with capripox vaccine, stained section revealed epidermal acanthosis with very long rete-ridge, hydrpoic degeneration and exfoliation of epidermal layer exposing the dermis (Fig. 4). The histological changes of the hair follicles epithelium were comparable to those of the epidermal layers with occasional formation of

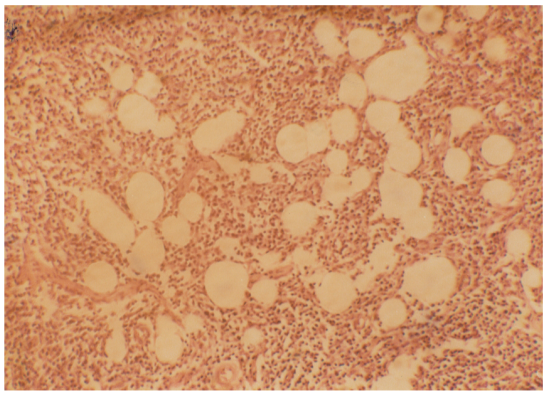

Fig. 3: Atrophic lymphoid follicles which formed cavities scattered in both cortex and medulla of prescapular lymph node due anthrax vaccine reaction (X10)

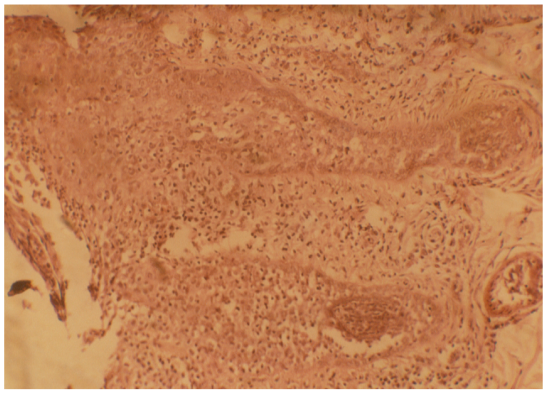

Fig. 4: Epidermal acanthosis with very long rete-ridge at the site of inoculation with capripox vaccine (X10)

microvesicles. The changes in the epidermal epithelium were accompanied with edema, proliferation of fibroblasts and intense accumulation of cellular exudation in the dermis subcutaneous connective tissue and underline muscles and fat. This cellular exudation was also observed between the degenerated disassociated epidermal and follicular epithilium and around some blood vessels. The inflammatory cells consisted of lymphoid cells, plasma cells and histocytes. In severely affected areas the dermis and subcutaneous tissues showed extravasated erythrocytes and coagulative necrosis which was lined with effusion of inflammatory cells intermixed with tissue debris.

Lymph node: The section of lymph node from animal vaccinated with capripox vaccine revealed edema and aggregation of mononucular cells around blood vessels (Fig. 5).

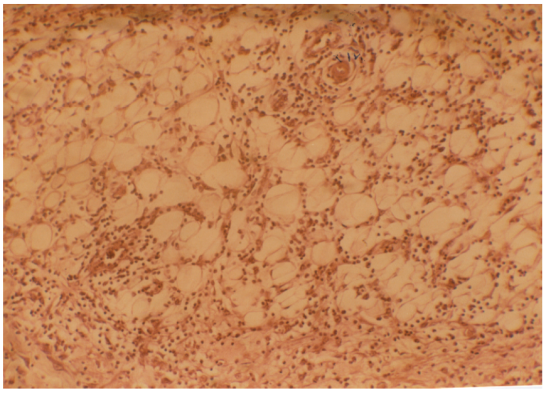

Fig. 5: Section of skinof sheep 4 days post inoculation with capripox vaccine. Note, edema, infilteration of mononuclear cells and proliferaion of fibroblast at subcutaneous tissue. H and EX10

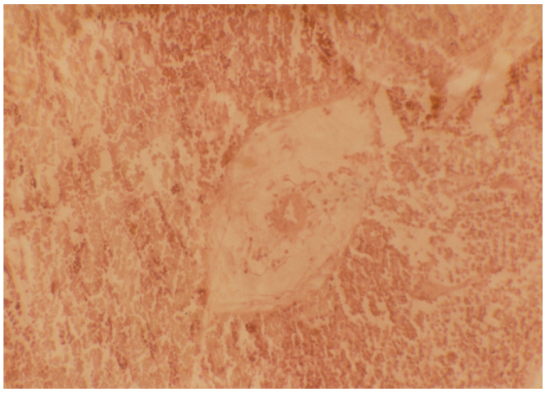

Fig. 6: Lymph node section showing edema and aggregation of mononuclear cells around blood vessels due capripox vaccine inoculation (X10)

Histpathological changes in skin and lymph node following vaccination with combined vaccine:

Skin section: Following subcutaneous inoculation with this vaccine, the skin displayed multiple area of epidermal and hair follicle epithelial cells, hyperplasia, ballooning and some vacculations. Some of these cells revealed marginal nuclear heterochromatin. Homogenous eosinophilic inclusion bodies were scarcely observed in the degenerated follicular epithelial cells (Fig. 6). The histological changes in the epithelial tissues was accompanied with edema, fibrotic reaction, hemorrhage and effusion of epithelial tissues. The cellular reaction consisted of plasma, lymphocytes and histocy.

Lymph node: The section showed necrosis with depletion of lymphoid cells in the germinal center of the lymphoid

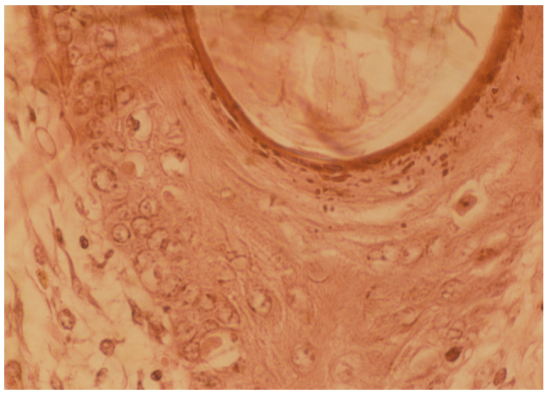

Fig. 7: Homogenous eosinophilic inclusion bodies in degenerated follicular epithelial cells due to combined vaccine inoculation (X40)

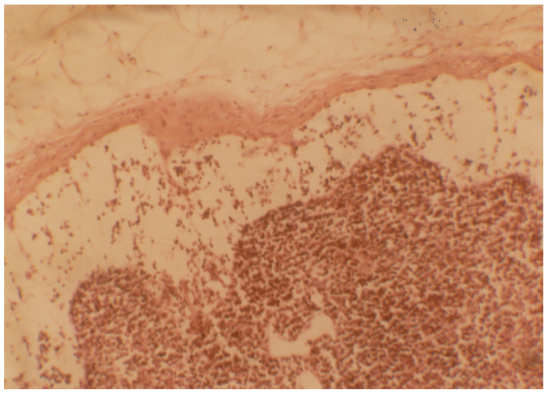

Fig. 8: Section of prescapular lymph node showing necrosis with depletion lymphoid cells in the germinal center of the lymphoid follicle and in the subcapsular area resulted from combined vaccine inoculation (X10)

follicles and in the subcapsular area (Fig. 7). Effusion of mononuclear cells, plasma, histocytes and lymphoid cells were seen in medullary sinuses.

DISCUSSION

Cutaneous anthrax accounts for 95% in human in countries like the United State (Taylor *et al*., 1993). Necrosis and massive edema with lymphocytic infilteration are the usual histological lesions (Mallon and McKee, 1997). In this study, at the site of in inoculation in sheep with anthrax vaccine (Fig. 2), dermal reaction that included edema, proliferation of fibroblasts and effusion of some cellular exudates were observed. This was almost similar to report published by Wun on human cutaneous anthrax. When endospores are introduced into the body by abrasion, inhalation, or ingestion, they are phagocytosed by macrophages and become vegetative bacteria (Ross, 1957; Guidi *et al*., 1999) then released from macrophages and multiply in the lymphatic system. Multiplication of the organism in the prescapular lymph node results in atrophic lymphoid follicles and cavities in both cortex and medulla (Fig. 3).

The histopathological changes of the capripox virus were proliferative changes typical of all pox lesions (Austvet plan, 1996). These changes of the skin section revealed epidermal acanthosis with the characteristic long rete-ridge, edema and cellular exudation in both dermis and epidermis (Fig. 4). Acanthosis, hyperkeratosis andhydropic degeneration are the cause of the increase of thickness of the skin. The prescapular lymph node showed edema and cells perivascular infiltration. The main histological changes occurred in the connective tissues under the epidermis and the subcutis were edema, proliferation of fibroblasts and intense accumulation of cellular exudation in the dermis subcutaneous connective tissue and underline muscles and fat.

There was proliferation of the endothelial cells lining the small subcutaneous blood vessels. The change was followed by development of severe vasculitis which is characterized by necrosis of the vessel cells, aggregation of polymorphonuclear leukocytes in the lumen, wall and surrounding adventitia and thrombosis. Severely affected area showed extravasation of erythrocytes and coagulative necrosis of the dermis and subcutaneous tissues. At the site of inoculation of the combined vaccine, the histopathological changes were almost similar to those demonstrated at the sites of inoculation of both sheep pox and anthrax vaccine. They were consisted of hemogenous eosinophilic inclusion bodies in the follicular epithelial cells (Fig. 7) and a necrosis with depletion lymphoid cells in the germinal center of the lymphoid follicle and in the subscapular area of the prescapular lymph node (Fig. 8). The intracytoplasmic inclusion bodies are one of the lesions of sheep pox present in the dermis and the columnar cells of the trachea (Kitching, 1994). They were more prominent at the site where combined vaccine was introduced (Fig. 7). These results indicated that no interference occurred between the inoculated virus and the bacteria.

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