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Clinical and hematological studies of Local buffalo breeds infected with lumpy skin disease

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Abstract: *Lumpy skin disease of local Buffalo breeds at Basrah governorate, Iraq, has been investigated and diagnosed clinically. The present study was conducted to examine (88) local buffalo breeds 2-4 years old and of both sexes. Diseased animals show acute clinical manifestations and Cutaneous nodules of 2–5 cm in diameter develop at different body regions, These nodules are circumscribed, firm, round and raised, and involve the skin, subcutaneous tissue and might also involve the underlying muscles. Thirteen (13), clinically normal local buffalo breeds served as controls. A statistically significant increase has been indicated in body temperature, respiratory rate, heart rate and capillary refill time of diseased buffalo than in controls, However, the rate of ruminal contractions was significantly decreased. Results of hematological examinations revealed no change indicated in the values of total erythrocytes count and hemoglobin concentration, However, a significant increase was encountered in the packed cell volume, in diseased buffalo compared with controls. Moreover, a significant decrease has been found in total leukocytes count, which was due to a significant decrease lymphocytes. Examination of clotting factor indices indicated a significant decrease of the total thrombocytes count of diseased buffalo compared with controls, Whereas, a significant increase was encountered in thrombocytes volume, thrombocytes distribution width, fibrinogen time, clotting time, prothrombin time and activated partial thromboplastin time. It has been concluded that, lumpy skin disease could infect buffalo, reflecting poor health and productivity, resulting sometimes in high morbidity rate and mortalities and terminated with economic losses, Therefore, prophylactic and control measures should always be followed .*

Key words: *LSD, Buffalo, Iraq*

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I. Introduction

Lumpy skin disease (LSD) or what it calls, the Neethling virus disease, Pseudo-urticaria, , exanthema nodularis bovis, and knopvelsiekte) is an infectious disease caused by a virus (LSDV) in the family Poxviridae, genus Capripoxvirus. It is closely genetically related to sheep and goat pox virus. However, these viruses cannot be differentiated using routine serological test (1). LSD is a disease of cattle and water buffalo. It is a vector-borne disease transmitted by different biting blood-feeding arthropods. The disease causes considerable economic losses due to emaciation, damage to the hides, temporary infertility, mastitis, decrease milk production, and mortalities which could reach to 20% or more. The severity of clinical signs of LSD depends on the strain of capripoxvirus and the host cattle breed (2,3).

Lumpy skin disease is among the major health problems affecting the livestock industry of most developing countries, Moreover, It has been also revealed that the role of husbandry practices such as commingling of animals at communal grazing and watering points in the transmission of LSDV (4,5).

The disease is manifested by distinguishing, firm, circumscribed, few to multiple skin nodules which might spread all over body parts, as well as, sometimes involve mucous membranes of respiratory system, uro-genital system and other internal organs, However, In severe cases continuous high fever (40-41.5°C), depression and anorexia may ensue (1,6).

Until 1989, Lumpy skin disease is limited to the African continent. Nevertheless, the disease is moved outside Africa to Madagascar and the Middle East and causes serious economic loss to the livestock industry (7).

Lumpy skin disease was spread in all parts of Iraq (8) in a very noticeable way, and the rate of animal infection and its death in cows and buffalo was increased. Therefore, the disease was considered as one of the diseases prevalent in the north, middle and south part of Iraq, Nevertheless, The clinical significance of LSD began with clarification and the importance was detected at Basrah Governorate and its spread, especially in buffalo. By that, the study was conducted to achieve, study and recognized the common clinical manifestations showed by diseased Buffalo, with study the hematological changes as well as evaluation of clotting factor indices of diseased buffalo

II. Materials And Methods

Study design:-

The present study was conducted to examine (88) local buffalo breeds ***(Bubalus bubalis)*** 2-4 years old and of both sexes, representing different herds, raised indigenously in various parts of Basrah governorate, Iraq. Diseased animals show signs of high systemic body reactions, anorexia, and presence of nodules of different sizes distributed at most body regions. Thirteen, (13) clinically normal local buffalo breeds served as controls. Complete clinical examinations had been carried out in all infected and control buffalo, Moreover the fecal samples are screened for parasitic loud using the usual standard methods.

Blood samples and hematology :-

Five milliliters of blood were drained from each animal via jugular and /or milk vein-puncture. From these (2.5) milliliters of blood mixed in EDTA used to determine the Total erythrocytes count (TRBc), Hemoglobin concentration (Hb), packed cell volume (PCV), Thrombocytes count , mean thrombocytes volume , thrombocytes distribution width, and total leukocytes count, (Hematology analyzer, Genex, USA). Furthermore, differential leukocytes count was also examine according to Weiss and Wardrop (9). Other (2.5) milliliters of blood mixed with Trisodium citrate (used plasma) was used to determine, Fibrinogen time, prothrombin time and activated partial thromboplastin time (Biolabo / France). Clotting time was also estimated according to Bush (10).

Statistical analysis :-

The significance of variations between diseased buffalo and healthy animals were statistically analyzed using (SPSS) student *t*-test (11)

III. Results

Diseased buffalo show different clinical manifestations, Depression, anorexia and emaciation (85.2%), Lacrimation, nasal discharge (77.27%), Frothy Salivation(73.8%), Lameness (61.3%), Enlargement of superficial lymph nodes (50%), Cutaneous nodules presence in different body regions (100%).(Fig:1,2,3),The Cutaneous nodules are about 2–5 cm in diameter develop, in different parts of body regions, These nodules are circumscribed, firm, round and raised, and involve the skin, subcutaneous tissue and might also involve the underlying muscles. Table 1.

Table 1: Clinical manifestations of diseased buffalo infected LSD

Clinical manifestations	**% (n=88)**
Depression, anorexia and emaciation	85.2
Lacrimation and nasal discharge	77.27
Frothy salivation	73.8
Lameness	61.3
Enlargement of superficial lymph nodes	50
Cutaneous nodules presence at different body regions	100
Head and neck nodules	23
Trunk nodules	43
Limb nodules	8
Udder nodules	12
Genitalia and perineum nodules	11
Nostril nodules	3

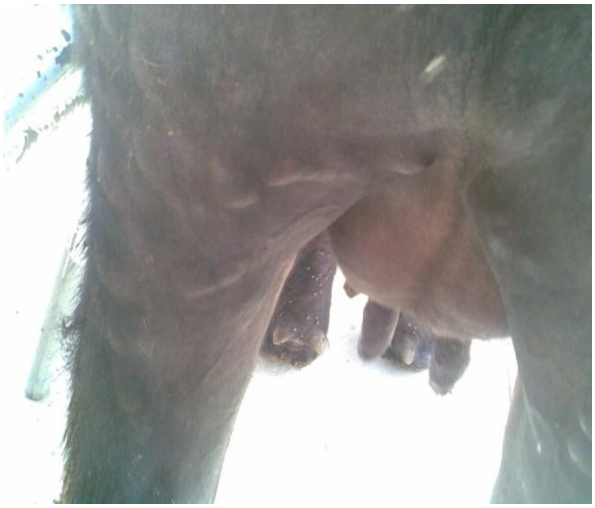

Fig 2: The Cutaneous nodules on hind limbs

Fig 1: The Cutaneous nodules on the animal's trunk

Fig 3: The Cutaneous nodules on perineum and udder

A statistically significant increase ($p<0.05$) has been indicated in body temperature, respiratory and heart rate, capillary refill time of diseased buffalo than in controls, However, the rate of ruminal contractions was significantly decreased in diseased buffalo compared with control animals. (Table 2).

Table 2: Body temperature, respiratory & heart rate, capillary refill time and ruminal contractions of diseased Buffalo and controls

Clinical parameters	**Controls n=13**	**Diseased n=88**
Body temperature C °	38.56 ± 0.76	41.5 ±0.8 **
Respiratory rate/ min	23.32 ±5.54	93.7 ±10.3 **
Heart rate/ min	58.3 ±4.2	125.4 ±12.2 **
Capillary refill time / min	1.22 ± 0.47	5.32 ± 0.72 **
Ruminal contractions / 5 mints	3.54± 0.62	1.66± 1.23 **

Values are mean ± standard error of mean. ** (P<0.05).

With respect to the hematological examinations, Results, revealed no change indicated in the values of total erythrocytes count (TRBc) and hemoglobin concentration (Hb), However, a significant increase ($p<0.05$) was encountered in the packed cell volume (PCV), in diseased buffalo compared with controls. Moreover, a significant decrease ($p<0.05$) has been found in total leukocyte count
(Leucopenia) which was due to a significant decrease ($p<0.05$) lymphocytes
(Lymphopenia) (Table 3).

Table 3: Blood parameters of diseased Buffalo and controls

Blood parameters	Controls n=13	Diseased n=88
RBC $\times 10^6$	7.43± 1.56	7.52 ± 1.45
Hb g/dl	13.3 ± 1.72	13.33 ± 1.41
PCV %	33.4 ± 4.32	42.21±5.72 **
TLC $\times 10^3$	10.83 ± 3.83	8.89 ± 9.33 **
Lymphocytes / Absolute	4887 ± 234.13	3578 ± 532.43 **
Nutrophiles / Absolute	4381 ± 522.22	4224.264 ± 535.13
Monocytes / Absolute	550 ± 362	552 ± 314
Eosinophiles / Absolute	391 ± 22	393 ± 21
Basophiles / Absolute	82 ± 61	82 ± 64

Values are mean ± standard error of mean. ** (P<0.05).

Examination of clotting factor indices indicated a significant decrease ($p<0.05$) of Total thrombocytes count of diseased buffalo compared with controls, Whereas, a significant increase ($P<0.05$) was encountered in Thrombocytes volume, Thrombocytes distribution width, Fibrinogen time, Clotting time, prothrombin time and activated partial thromboplastin time. Table (4).

Table 4: Indices of clotting factors in diseased Buffalo and controls

Parameters	Controls n=13	Diseased n=88
Total Thrombocytes count $\times 10^3$	567.431 ± 56.432	325.211 ± 72.63**
Thrombocytes volume /fl	11.521 ± 4.243	14.189 ± 2.725 **
Thrombocytes distribution width / %	13.551 ± 1.661	20.291 ± 7.582 **
Fibrinogen time / Sec	18.32± 2.16	22.14 ± 8.11**
Clotting time / min	3.422 ± 1.534	4.814 ± 2.878 **
Prothrombin time /Sec	13.175 ± 2.352	20.531 ± 5.121 **
Activated partial thromboplastin time /Sec	51.431±6.347	62.351± 14.767 **

Values are mean ± standard error of mean ,** (P<0.05).

IV. Discussion

It has been shown that, because of the widespread of lumpy skin disease , and it is noticeable in cows and buffalo in different regions of Iraq, especially at Basrah Governorate. The disease was diagnosed in the current study, depending on the accreditation and observation of clinical manifestations of infected animals.

Diseased buffalo show different clinical manifestations which agreed with those mentioned by (3,6,12).

The usual manifestations of LSD are multiple firm circumscribed nodules developed in the skin of the animals in which head, neck, perineum, genitalia, udder, and the limbs are principally involved. Moreover, The regional lymph nodes are easily palpable and enlarged which might reach to 3-5 times their normal size. However, Most cases may complicate or extend to other underlying tissues or internal organs and may sequel in economically significant disorders(13,14).

The so called higher body systemic reactions indicated in diseased buffalo was observed to change, As, high fever detected, might suggest the liberation of especial chemicals calls the endogenous pyrogens due to body cell lysis which commonly followed by the stimulation of the thermoregulatory center of the hypothalamus for fever crises(1). Furthermore, Increase respiratory and heart rate was also mentioned by others which reflected the acute phase of the disease (4,7). However, decrease ruminal contractions reflected the atony of ruminal smooth muscles which mostly reflected by the lack of ruminal fibers followed by anorexia (1,15).

Results were also indicated a significant increase in capillary refill time in diseased buffalo compared with controls. It has been documented that the capillary refilling time is a quick test done used to monitor some disease problems such as dehydration, shock, and peripheral vascular disease, thereby, prolong time of the test might reflect the less amount of blood flow reaches to tissues which were indicated in infected animals of the current study (14,16).

LSD has a narrow vertebrate host range. Cattle and Buffalo are the common species, which widely become infected naturally during field outbreaks. Five occurrences of clinical cases of LSD in Bubalus bubalis, the Asian water buffalo have been reported (7). No other domestic ruminant species become infected naturally during field outbreaks. All cattle and buffalo breeds appear to be equally susceptible to the disease. However,

some other workers found that imported breeds with thin skins, such as Bos taurus, Friesland cattle and the Channel Island breeds, were far more susceptible than indigenous breeds with thicker skins, such as the Afrikaner and Afrikaner cross- breeds, Moreover, Young calves are more susceptible to the disease and may develop the characteristic lesion within 24 to 48 hours, although all ages groups of animals are susceptible(17).

Enlargement of superficial lymph node was registered in the current study as one of the clinical manifestations encountered by diseased buffalo. It have been reported that some plasma and cells in the interstitial space, along with certain cellular material, antigens, and foreign particles enter lymphatic vessels, becoming lymphatic fluid, However, Lymph nodes filter the lymphatic fluid on its way to the central venous circulation, removing cells and other material, Moreover, The filtering process also presents antigens to the lymphocytes contained within the nodes, Furthermore, the immune response from these lymphocytes involves cellular proliferation, which can cause the nodes to enlarge(lymphadenopathy)(18).

In addition Smith (2004), added that pathogenic microorganisms carried in the lymphatic fluid can directly infect the nodes, causing lymphadenitis.

The hematological parameters of the current study show significant difference, As, no significant changes was encountered in TRBc. and Hb. concentration which was consistent with (2,19). On the other hand, The results revealed a significant increase in PCV of buffalo infected with LSD which may attributed to haemoconcentration, excessive loss of body fluid and dehydration, which lead to decrease plasma volume (20).

Hematological feature of the current study indicated a significant leucopenia due to an a significant Lymphopenia. Actually, this result could be indicative of the lesions in lymphoid tissue, which result in destruction and decrease of the lymphocytes and it is usually seen in the acute stage of most viral infection. Furthermore, previously studies shown that the virus localize in different tissues and cells like, pericytes, endothelial cells and probably other cells in blood vessel and lymph vessel walls causes vasculitis and lymphagitis in some vessels in affected areas (21,22).

Little documents had been mention the relation of LSD infection and the effect on clotting factors indices, Nevertheless, in infected buffalo thrombocytopenia might occurs regularly in acute stages of the disease although the reduction of platelets count does not always result in marked hemorrhages, even though the cause of decrease platelets count is not completely clear , However, The megakaryocytes lysis and reduced production of thrombocytes by megakaryocytes, with increased consumption of platelets in the periphery, and defects of its functions might all been suggested as factors predispose to decline its levels (15). Moreover, hemorrhagic diathesis was only indicated when platelets count are too low (23).

In the present study clotting factors indices indicated a clear disturbances in clotting system of diseased animals with imbalanced regulation which might lead to hyper coagulation and / or hypo coagulation which may reflects the initiation of disseminated intravascular coagulation (2).

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