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CLINICAL, EPIDEMIOLOGICAL AND HISTOPATHOLOGICAL STUDY OF LUMPY SKIN DISEASE IN CATTLE OF DIYALA PROVINCE-IRAQ

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ABSTRACT

This communication provides details on an outbreak of LSD virus in cattle of Diyala province 2014-2015 and describes the clinical, PCR, heamatological, epidemiological, histopathological aspects of the infection .The present study was conducted on 150 cattle of different breed. Their age ranged from 1- 12 months and 1-6 years of different sexes. All suspected animals were clinically examined. The infected animals were classified according to the severity of clinical status into mild and severe forms. Effect of breed, sex and age of infected animals, were studied. The study show the LSD is more sever in crossbreed and Frisian cows than native cows and more sever in small age groups than adult .On the other hand the LSD was severe in male than adult. The hematological examination of LSD revealed to slight decrease of Total WBCs and increase in lymphocytes and monocyte. The Histopathological examinations of lumpy skin disease revealed ballooning degeneration of squmous epithelial cells with occasional intra- cytoplasmic inclusions are seen .The epidermis is extensively necrotic with perivascular cuffing cells .On the other hand, there is vasculitic necrosis with cell debris and presence infiltration of inflammatory cells mainly neutrophiles. A specific PCR assay was developed by the laboratory of molecular virology General Company for Veterinary in Baghdad, Iraq. The main target of treatment trials was to save the animal life and to prevent LSD complications.

KEYWORDS: Lumpy skin, Epidemiology, Blood parameter, Histopathology

INTRODUCTION

Lumpy skin disease (LSD) is an infectious viral disease of cattle caused by a virus (LSDV) of the family *Poxviridae* and genus *Capripoxvirus* characterized by pyrexia and sudden eruption of skin nodules (Tuppurainen and Oura, 2012).

Transmission: LSDV is thought to be transmitted primarily by biting insects. The virus was detected in mosquitoes of the genera *Aedes* and *Culex* and *Ixodid* ticks during some outbreaks

(Chihota *et al*., 2003; Tuppurainen *et al*., 2011). Disease incidence is highest in wet and warm weather. Incidence decreases during the dry season, which is possibly linked to decrease in insect vector (occurrence/numbers). Minor sources of infection could include direct and indirect contact (e.g. through infective-saliva contaminated feed and water). Other potential transmission routes include the milk of lactating cows and the semen of infected bulls, since the LSD virus can persist for extended periods of time in both (Irons *et al.,*2005 and Osuagwuh *et al.,* 2007)

Epidemiology: LSD was first described in Northern Rhodesia in 1929 by Morris (1930). Since then disease has spread over most of Africa in a series of epizootics as previously recorded by Davies (1991) and House (1990).The most recently affected countries include Kuwait in 1986-88 as mentioned by Anonymous (1988), and Israel in 1989 as previously recorded by Shimshony (1989). The diseases reappeared in Egypt at the summer of 1989 and, in a period of five to six months, it had been spread to 22 out of 26 Egyptian governorates. The disease also spread to Asia and appeared in non-African countries (Yeruham *et al*., 1995; Brenner *et al*., 2006; Body *et al*., 2012). Since 2000, LSD outbreaks have been reported across the Middle East and it is highly likely the disease will become endemic at least in parts of the Region. In subsequent years Bahrain, Kuwait, Oman, Yemen and the West Bank also reported LSD incursion. Lebanon and Jordan joined LSD affected countries in 2012 and 2013, and most recently Turkey reported the disease in October 2013 .LSD appeared in Iraq after importation of cattle from other neighboring countries of Iraq.

Clinical signs: The incubation period in natural cases is thought to be between two to five weeks (Carn and Kitching, 1995; Tuppurainen *et al*., 2005).The disease appears clinically as acute, subacute or subclinical. The acute disease is characterized by pyrexia, lymphadenopathy, skin nodules with subsequent sit-fasts and occasional orchitis and mastitis (Brenner *et al*., 2006). Other lesions observed at post-mortem examination include necrotic plaques in the body mucosa, chiefly of the upper respiratory tract, the oral cavity and rumen.

The pathology of LSD: Viral replication in pericytes, endothelial cells and probably other cells in blood vessel and lymph vessel walls causes vasculitis and lymphagitis in some vessels in affected areas (Lindsay Thomas, 2013). In severe cases pannicular infarction that were seen recently in some LSD biopsies (Ali *et al*., 1990 and El-Neweshy, *et al.,* 2013).

Treatment and control of LSD

There is no specific treatment (antibiotics, anti-inflammatory drugs and vitamin injections) and any therapy should be supportive and aimed at alleviating clinical signs and controlling

all secondary complications. Isolation of affected animals may, for most practical purposes, be of little use as the virus may be insect transmitted.

Effective control of the disease depends on immunization of the animals and in South Africa effective vaccines are produced from the Neethling strain virus. In other countries effective vaccines are produced from sheep pox and goat pox viruses. However, the use of these latter vaccines would probably only be feasible in countries where goat and sheep pox is endemic. All susceptible animals should be vaccinated annually. Calves born from vaccinated cows should not be vaccinated before 6 months of age, but calves from unvaccinated cows should be vaccinated before 6 months of age. (Jaargang and Mapham, 2008 and Lindsay Thomas, 2013).

In summer of 2014, outbreak of LSD was recorded in several district areas of Diyala province. All age groups and both sex of Diyalian cattle were infected with severe and serious complications. Clinical signs, epidemiological characters, histopathological findings and features of LSD in this outbreak were recorded in this study. Several trials in treatment had been used to control the complications after infection

Diagnosis: A tentative diagnosis of LSD can be made on the basis of the typical morbidity, mortality and clinical patterns (Jaargang *et al*., 2008).

Materials and Methods

Area of study

The present study conducted in different district areas of Diyala province (East-northern of Iraq). The total number examined 150 cattle in different age, breed and sex.

Clinical Examination All suspected animals were clinically examined. The animal examination was concentrated on physical statutes, temperature, superficial lymph node and skin lesions according to (Radostits *et al*., 1995). Gross lesions and complicated cases of different ages and sex were recorded.

Epidemiological data of Lumpy Skin Disease

Effect of breed, sex and age of infected animals were studied. Morbidity, mortality and case fatality of the disease were also calculated among examined animals.

Table (1).Distribution of LSD infected cattle according to age, sex and breed.

Age of examined animals	Number of the examined	Number of the infected	Number of the dead	Morbidity rate (%)	Mortality rate (%)	Case fatality (%)
1-12 months	38	38	2	100	1.3	1.3
1-2.5 years	42	42		100	0	0
3- 4.5 years	46	46	1	100	0.6	0.6
5- 7 years	24	24		100	0	0
Breed of animals						
Native cattle	30	30	1	100	0.6	0.6
Crossbreed (Native-Holstein-Frezian cattle)	95	95	2	100	1.3	1.3
Frezian cattle	25	25		100	0	0
Sex of animals						
Male	87	87	2	100	1.3	1.3
Female	63	63	1	100	0.6	0.6
Total	150					

Hematological examination

Blood sample collected from jugular vein after disinfected of area by alcohol 70%. 10 ml of blood sample collected and divided into 5ml of blood put in without EDTA tube to separation of serum to evaluation of some kidney function and 5ml of blood put in a vials containing sodium ethylenediamine tetracetic acid (Na2 EDTA) sufficient for 5 mL of blood to prevent coagulation. The tubes were gently rotated to ensure proper mixing of the blood with the anticoagulant without damaging the integrity of the cells and were transported to the laboratory. White blood cells count (WBC) and differential white blood cells count were calculated according to (Radostits *et al.,* 2007).

Confirmatory diagnosis

All suspected cases was confirmed by The virology laboratory-state company of veterinary and animal health –ministry of agriculture using Techne. qPCR Kit for Capripoxvirus (CaPV) genomes (http://www.genesig.com).

Histopathological examination

Histopathological sections were carried out by fixing of skin biopsy of living animals in 10% neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 micron) and stained with hemato-xyline and eosin according to Bancroft *et. al.,*(1996).

Results

Epidemiological study.

Effect of breed, sex and age of infected animals, were studied. The study show the LSD is more sever in crossbreed and frezian cow than native cow and more sever in small age groups than adult .On the other hand the LSD was severe in male than female. The results are illustrated in tables (1).

Clinical examination

In the present study, lumpy skin disease virus causes in apparent mild to severe form in intensity of disease . All ages of cattle were affected, but young calves were more severely affected and the males were more severely affected than female. The severity of the disease as measured by number of lumps and occurrence of complications. The infected animals were classified according to the severity of clinical status into

Mild form: It was only observed in native cattle and some crossbreed which, appeared as 3 or 6 lumps. Some cases showed detached lumps leaving ulcer. Depression, anorexia, excessive salivation, lachrymation, nasal discharge and emaciation were also noticed. Nodular lesions were seen on the animal body especially in the skin the muzzle, nares, nasal and oral mucosa, back, legs, scrotum, perineum, eyelids, lower ear and sometime in the tail.

Severe form: The severe form was recorded in all ages and both sex in crossbreed, Frezian cattle and some native cattle which firstly suffered from fever (40.5C°-41.0C°) that persisted for 6-12 days. The number of lumps about 20-100 lumps or more than which was a variable in sized and covered whole animal body as in figure (3) . Swelling of brisket, face and one or four legs was observed figure (1,3) . This swelling ruptured oozing pus. Subcutaneous nodules and enlarged of prescapular and prefemoral lymph node enlargement were also observed as in figure (4,A&B) .The nostrils and muzzle were crusted with mucopurulent discharge and the mucous membranes were congested. Unfortunately the nares orifice of calf was closed with mucoupurulant and lumps as in figure (2). The nodules were painful and hyperemic, decrease in milk production also observed. Some cows infected by LSD were passing from mixed infection with blood parasite as Babesiosis and Theileriosis. On the other hand some of cow suffered from abortion.

Treatment The main target of treatment trials was to save the animal life and to prevent LSD complications .The Prednisolone (tablet orally 5mg/100kg .B.W. twice daily and recovery was in 7 days) was firstly use to treatment of vasculatis and cellulitis in this study and some complication of LSD . On the other hand used of Bubarvaquone (Butalixe)and Dimenazine

to treated blood parasite ,Antihistamine to treated pruritic and lameness and Oxyteteracycline and tylosine to reduce of respiratory infection and Calciform, Cafosal®, AD3E® and dextrose5%) as intravenous infusion for recumbency

The (2) :complications of LSD and treatment of complications

Complication of LSD	Examined number of animals	Percentage of infection %
Cutaneous edematous	40	26.66
Recumbancy	8	5.33
Pneumonia	48	32.0
Cellulitis and vasculitis	30	20.0
Abortion	15	10.0
Diarrhoea in calf	22	14.66
Lameness	20	13.33
Clogged nostrils slot in calf .	1	1.5

Fig.(1;A&B) A:Calf suffered from edematous swelling in face ,neck and present lumps around muzzle. before treatment . B:Calf healing from the edematous swelling and lupus after treated by prednisolone drug .Recover stage.

Fig.(2). Complication of LSD ,closed of nasal cavity of calf and mucopurulent

Fig.(3;A& B& C): Cow affected by LSD suffered edematous swelling in the brisket ,neck ,head, diffusion of lumps along body and enlarged of prescapular lymph node .

Hematology :

The present study show significant difference in blood parameter and decrease in total white blood cells count and significant difference (P<0.05) in differential WBC as in table (3).

Fig.(4,A,B,C): (A) Sever infection form and diffusion of lupus on all body and vasculitis .(B) Enlargement of prescapular lymph node .(C) Mild infection form subcutaneous vasculitis.

Table (3). Effect of LSD on leukocytes of animals .

Parameter	Normal value	Infected value M. + S.D
Urea nitrogen (mg/dL)	20.2 ± 0.75	63.2 +10.109*
Creatinine (mg/dL)	1.0 ± 0.33	1.32 +0.164*
Significant difference (P<0.05) M= Mean , S.D= Standard deviation		

Table (4). The effect of LSD on some serum biochemical of kidney function activity of animals

Blood parameter	Before treatment M ± SD	After treatment M ±SD
White blood cells count WBC Per/µL	3929 ± 544.04	4780 ± 779.0022*
Band neutrophils %	0.2 ± 0.421	0 ± 0
Segmented neutrophils %	15.9 ± 8.288	25.8 ± 7.330*
Basophils %	0± 0	0.2 ± 0.421
Eosinophils %	6.4 ± 2.270	6.2 ± 1.988*
Monocytes %	10.1 ± 1.908	9.5 ± 3.064*
Lymphocytes %	66.6 ± 5.518	58.5 ± 4.813*
Significant difference (P<0.05)	M= Mean , S.D= Standard deviation	

Macro and Histopathology:

The macroscopic pathology show skin nodules are usually uniform in size, firm round and raised, but some may fuse into large irregular and circumscribed plaques. On the other hand, the cut surface nodules are reddish-gray and the subcutis oedematous with sometimes reddish grey serous fluid as in figure (5: A,B&C)

Fig (5:A,B,C) A: skin nodules, firm round, raised and uniform in size. B: the cut surface nodules, reddish-gray and the subcutis oedematous C: Cut surface contain reddish grey serous fluid.

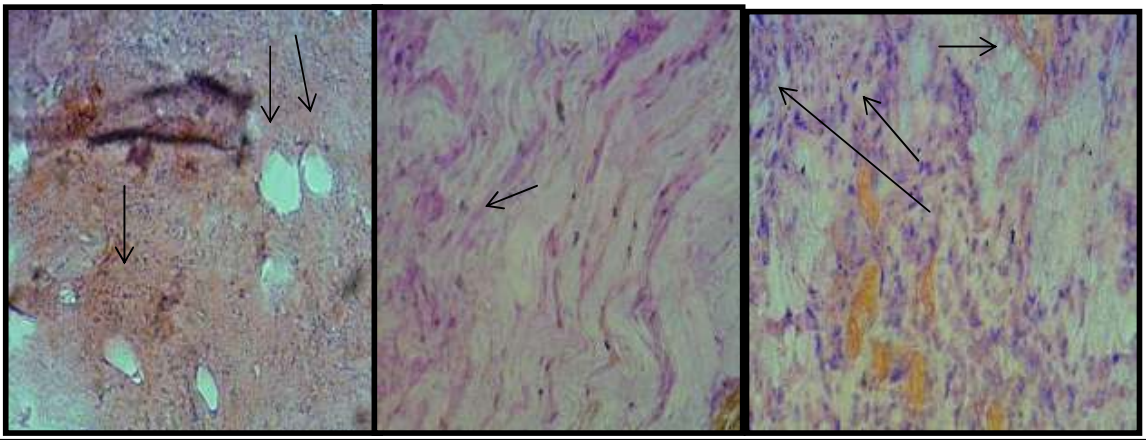

Fig. (6 A,B,C) A: Ballooning degeneration of squamous epithelial cells with occasional intra- cytoplasmic inclusions (arrows).
B: Epidermis is extensively necrotic with perivascular cuffing cells (arrows)
C:pannicular infraction and sever vasculitic necrosis with cell debris and presence infiltration of inflammatory cells mainly neutrophiles **(H&E x 1000).**

The histopathological examination of LSD revealed ballooning degeneration of squamous epithelial cells with occasional intra- cytoplasmic inclusions are seen .The epidermis is extensively necrotic with perivascular cuffing cells .On the other hand, there is vasculitic necrosis with cell debris and presence infiltration of inflammatory cells mainly neutrophiles.

Discussion

LSDV has spread from Africa to the Middle East and Asia and there is a major concern about further spreading into Europe and other parts of the world. LSD outbreak was recorded in Iraq 2013-2014 after importation of cattle from other Neighboring countries. A tentative diagnosis of LSD can be made on the basis of the typical morbidity, mortality and clinical patterns. Depending on epidemiological analysis the morbidity rate for LSD about 100% this attributed to distribution and abundance of insect vectors, breed of cattle affected and general health and nutritional status of the animals in question. This finding agreement with (Fayez and Ahmed 2011). The incidence of LSD in crossbreed and Frisian cows were found to be significantly higher than in native cows this attributed to the native breed may have stronger immune response which concentrated the virus lesions in one or two skin. This was in agreement with other studies, and may be due to breed susceptibility (Davies, 1991; OIE, 2008 ; Fayez and Ahmed 2011).

The males animals had higher cumulative incidence than females. This might be attributable to the stress factor of exhaustion, physiological hormone of androgen and fatigue rather than to a biological reason (Blood *et al.,* 1983, OIE, 2008).

The clinical signs of LSD agreement with other research (Tuppurainen *et al*., 2005, Fayez and Ahmed 2011).The nostrils and muzzle were crusted with mucopurulent discharge and the mucous membranes were congested this may be due to pneumonia complication of disease. Ulceration lesion of leaving lumps attributed to the nodular lesions penetrating through the subcutaneous fasciae into the deeper fasciae layers and even into the musculature of the hind quarters. (Vorster and Mapham, 2008). As shown in table (2) the complications of LSD resulted from damage of skin or mucous membranes that were followed by secondary bacterial invasion in addition to stress induced immunosuppression, anorexia, persistent fever and severe debilitation. This finding close agreement with (Davies 1991, Fayez and Ahmed 2011and El-Neweshy *et al*.,2013).

The hematological reveal to decrease in total number of WBC and increase in lymphocytes this attributed to causative agent virus seems to cause lesions is viral replication in cells such as the pericytes and endothelial cells in lymphatics and blood vessels walls, giving rise to vasculitis and lymphangitis and enlargement lymph node and proliferation of lymphocyte.

The pathological effect of LSD on macroscopic examination of present study agreement with (Jaargang *et al.,* 2008) in which the cut surface nodules are reddish-gray and the subcutis oedematous with sometimes reddish grey serous fluid as in figure (5: A,B&C).

Whenever, histopathological effect show the epidermis was extensively necrotic, while in the intact areas, some ballooning degeneration of squamous epithelial cells with occasional intra-cytoplasmic inclusions were seen. This agreement with **(**Brenner, *et al* 2006 ,Fayez and Ahmed 2011 and El-Neweshy et al.,2013).

Use of prednisolone in treatment of vasculatitis and edema due to the prednisolone inhibit the inflammatory response to a variety of inciting agents and, it is presumed, delay or slow healing. They inhibit the edema, fibrin deposition, capillary dilation, leukocyte migration, capillary proliferation, fibroblast proliferation, deposition of collagen, and scar formation with inflammation. This finding agreement with (Lambrou *et al.,*2009). A specific PCR assay was developed by the laboratory of molecular virology General Company for Veterinary in Baghdad, Iraq. (The PCR Max qPCR Kit for Capripoxvirus (CaPV) genomes) on the base of the African strain. Other treatment trials was to save the animal life and to prevent LSD complications this agreement with (Fayez and Ahmed 2011and El-Neweshy *et al*.,2013).

Conclusion

In the present work LSD had a high prevalence in cattle of Diyala province of Iraq. LSD is more sever in crossbreed and frezian cow than native cow and more sever in small age groups than adult. Use of prednisolone in treatment of vasculatitis and edema.

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