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Clinical and Molecular Study of Lumpy Skin Disease in Cattle in Basrah

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Abstract:

This study was conducted in Basrah during the period from July 2019 till June 2020; 1022 cattle was concluded in this study; the tissue samples was collected from 80 clinically suspected cattle. This study revealed that the incidence of Lumpy skin disease (LSD) in Basrah according to clinical picture was 7.82% (80 out of 1022). All 80 samples of cows with clinical signs were positive to PCR examination. The study also revealed that, the incidence of LSD in calve was significantly higher than that reported in adult cows ($P < 0.05$). The study also revealed that there was no significant difference in the incidence of LSD between male and female ($P > 0.05$). The main clinical finding of LSD was fever, skin lumps, increase respiratory rate, nasal discharge, and limb edema, loss of weight, depression, diarrhea, lameness, enlarged lymph node, and dyspnea. The monthly distribution of disease revealed that the disease was higher incidence in spring months compared with other months ($p < 0.05$).

Keywords: Basrah, Cattle, Lumpy, skin, disease.

Introduction:

Lumpy skin disease (LSD) is a viral disease of cattle that caused by a capripoxvirus and also called Neethling vires. Lumpy skin disease virus (LSDV) of cattle, belong the genus of capripoxvirus in family Poxviridae, subfamily chordoxvirinae (13). The clinical manifestation of LSD is appearing as acute or sub-acute or in apparent depending on the health, breed, immunity status and strain of virus which cause the disease (10). LSD is characterized by fever, nodules on the skin, emaciation, enlargement of

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lymph nodes, and sometimes death (8, 12, 28, 15(. The disease is more severe in cows at peak of lactation and causes a sharp drop in milk yield, often leading to secondary bacterial mastitis. Temporary or permanent infertility may occur in cows and pregnant cows may abort (33, 7). The morbidity and mortality of the disease vary considerably, depending on the breed and immunological status of the cattle population and the insect vectors involved in transmission. In a few outbreaks morbidity has been reported as more than 50%, although the mortality rates were usually less than 10%. (29, 35).Diagnosis of LSDV is performed by observation of characteristic clinical signs, virus isolation, electron microscopy, histopathological examination, serological, and molecular techniques (28). Histopathological changes in the skin lesions of LSD are mainly characterized by vasculitis, necrotic epidermis, and eosinophilic intracytoplasmic inclusion bodies (14, 26).The effective control or eradication of LSD in endemic and non-endemic areas requires rapid and accurate diagnostic methods to confirm a presumptive diagnosis. Control strategies could not be implemented to control and eradicate the disease. Therefore, control measures through vaccination and restriction animal movement remain the most practical option in the country. (4, 16, 17) This study was aimed to detection of lumpy skin disease in cattle in Basrah Province.

Materials and Methods:

Ethical approval

The current study was approved by the College of Veterinary Medicine, University of Basra, Basra Iraq

This study was conducted during the period from July 2019 till June2020 in the Basra province. 1022 cattle were used in this study. The Samples collected from eighty (80) clinically suspected animals with a typical signs of lumpy skin disease**.**

Nodule skin biopsies samples were collected aseptically from cattle of different ages showing LSD-like lesions The nodules with surrounding area were shaved using a sterile scalpel; a small punch was made deeply into the skin to include all skin layers and the sample put in the phosphate buffered saline solution and store below -20 C for later testing**.** Genomic DNA was extracted from skin nodular tissues of cattle by using gSYNC^{TM} DNA Extraction kit quick protocol (Geneaid, Korea), and was done according to company

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instructions. The PCR assay was performed to amplify the V*P32* gene used in detection of lumpy skin disease virus from skin lesions. This assay was carried out according to DNA extraction and PCR assay.

DNA amplification was performed using primers developed from the viral attachment gene. The primers have the following sequences: forward primer 5′-TTTCCTGATTTTTCTTACTAT-3′ and reverse primer, 5′-AAATTATATA C GTAAATAAC-3′. PCR products were analysed on 1.5%agarose gel electrophoresis at 100 V for 30 min. The positive samples gave products of the expected; the specific primers set amplified a DNA fragment of 192 bp equal to the expected amplification product size from LSDV

Result:

Incidence of LSD according to clinical manufactures and PCR technique:

According to the clinical obstructions; the incidence of lumpy skin disease in Basra was 7, 82 %(80 out of 1022). And all 80 samples of cows with clinical signs was positive to PCR examination

Table 4-1: Percentage of infection of lumpy skin disease

Number	Positive clinical signs	Percentage %	Positive PCR	Percentage %
1022	80	7.82	80	7.82

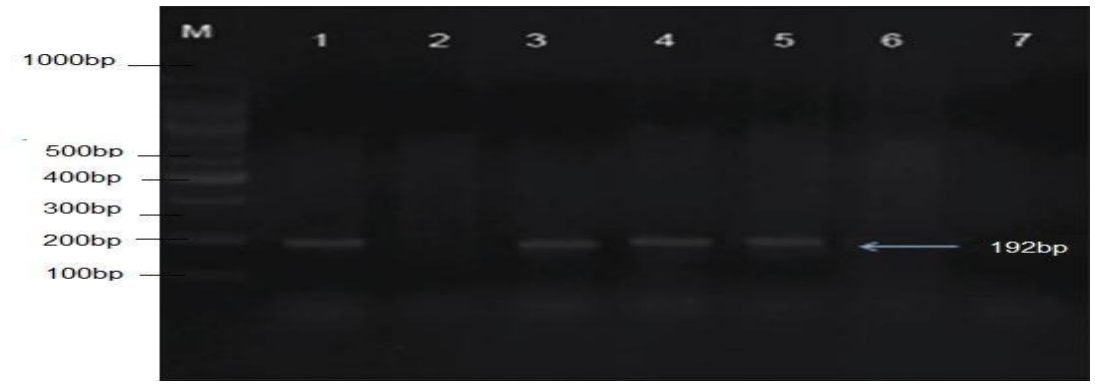

Figure 4.2: PCR result

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Incidence of LSD according to age group:

The study was also reported that; the incidence of LSD in calves was 12.6 %(47 out of 373 calf) which was significantly (P>0.05) higher than that reported in adult cow which was 5.08%(33 out of 649).

Table 4-2: Prevalence of LSD according to age group

Age group	Number	Positive	Percentage %
Adult cows	649	33	5.08
Calves	373	47	12.6
Total	1022	80	7.82

x^2= **17.8** ➡ **P**< 0.05

Incidence of LSD according to Gender:

The incidence of LSD in female was 7.7% (73 out of 948) and 9.4% (7 out of 74) in male. There are no significant differences in the incidence of LSD between male and female P< 0.05

Table 4-3: Prevalence of LSD according to gender

Gender	Number	Positive	Percentage %
Male	74	7	9.4
Female	948	73	7.7
Total	1022	80	7.82

x^2= **0.063** ➡ **P**< 0.05

Clinical signs of LSD:

The study was observed may clinical signs which appear on infected animal such as fever , skin lumps , increase respiratory rate , nasal discharge , Limp edema , Breast edema , Loss of weight , Depression , Diarrhea, Lameness , Enlarged lymph nodes , Dyspnea .

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Table 4-4: Clinical signs of lumpy skin disease

Sign	Number	Percentage%
Fever	80	100
Skin lumps	80	100
Increase respiratory rate	68	85
Nasal discharge	40	50
Limp edema	9	11.25
Breast edema	2	2.5
Loss of weight	58	72
Depression	68	85
Diarrhea	14	17.5
Lameness	9	11.25
Enlarged lymph nodes	54	67.5
Dyspnea	38	47.5

Effect LSD on milk production:

This study reveled that, the LSD was seriously cause decrease milk production in lactating cows 82, 35 %(14 out of 17).

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Table 4-5: Decrease of milk production in LSD

Lactating cow	Decrease milk production	Percentage%
17	14	82.35

The effect of LSD according to seasons:

The monthly distribution of the infected animals is shown that the infection in spring months was higher than those submitted in summer, winter and autumn.

Table 4-6: Distribution of infection according the month

Month	Number	Percentage%
July 2019	4	5
August 2019	6	7.5
September 2019	5	6.25
October 2019	6	7.5
November 2019	4	5
December 2019	2	2.5
January 2020	1	1.25
February 2020	3	3.75
March 2020	17	21.25
April 2020	14	17.5
May 2020	15	18.75
June 2020	3	3.75

Discussion

Lumpy skin disease virus is a highly contagious viral infection of cattle leading to economic impact on cattle industry (11).In this study the LSD was diagnosed by clinically according to typical signs of LSD this is the first study on molecular characterization LSD in Basrah governorate. This study revealed the incidence of LSD in Basrah according to clinical observation was 7.82% (80 out of 1022) this result was lower than reported by(30) in Iran who was reported the incidence 0 of LSD in north-western Iran was 17.9% this difference was attributed to the different climatic condition and vector distribution between Basrah and north of Iran .

This study was in agreement with the study of (5) who reported the

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morbidity of LSD in Jorden was 26% (124 out of 624).

The difference of result with our study related to the different climatic condition and vector distribution between Basrah and Jordan

In Turkey ; (31) reported that the incidence of LSD was 73% which was very higher than the result of our study this can be explained by the Turkish others made his study in the peak of outbreak of disease during the pandemic distribution of disease

The incidence reported in our study was lower than the prevalence of LSD was reported by (1) in Basrah the difference of result attributed to the type of examination use since the other researcher user ELISA rather than PCR.

The incidence reported in our study which agreement with previous studies in Iraq that reported by (20) in waste which reported 8,6% and (36) in Samawah which reported 8.9 % .The use of the PCR for the detection of LSD virus nucleic acid from skin biopsies and tissue culture supernatant has been published (19, 25) But no studies have been undertaken on the persistence of the virus in blood and skin using the PCR.

The incidence of LSD according to PCR was 7.82% (out of 1022) this result was different from these reported by(30) in Iran ; (5) in Jordan and (31) in Turkey due to the difference of climatic condition and vector distribution . The incidence reported in our study according to PCR was lower than the incidence of LSD was reported by (6) in sultanate of Oman which is 27.9% these values can fluctuate according to geography, climate, management conditions, immune status of status of animal, breed and strain of virus involved .

The result of our study agreement with that reported by (22) in Saudi Arabia which is 6%. This study was revealed that the incidence of LSD in calves was higher than reported in adult cows this result was in agreement with (18) this finding can be explained by the reported exposure of adult cows result in some protective immunity against disease (1)

The age distribution of disease revealed that there was significant difference in the incidence of disease between calves and adult cows this

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result was not in agreement with the previous study reported by (30) which reported that the LSD occurred more frequently in the older cattle than in calves

There was on statistical difference in the incidence of disease between male and female this result was in agreement with (24, 5). This result can be explained by the similarity of environmental condition for male and female in the same herds included this study (1)

Typical LSD clinical symptoms include fever that may exceed 41°C, marked reduction in milk yield in lactating cattle, depression, anorexia, emaciation, rhinitis, conjunctivitis and excessive salivation; enlarged superficial lymph nodes, cutaneous nodules of 2–5 cm in diameter, particularly on the head, neck, limbs, udder, genitalia and perineum occur within 48 hr of onset of the febrile reaction. These nodules are circumscribed, firm, round and raised, and involve the skin, subcutaneous tissue and sometimes even the underlying muscles (27)

The clinical signs of LSD reported in this study were fever , nasal discharge , skin lumps , increase respiratory rate ,limp edema , breast edema, loss of weight, depression , diarrhea , lameness , enlarged lymph node , dyspnea , this result were in agreement with (34,32)

In this study the LSD effect on infected dairy cattle and causing significant decrease in milk production this result were in agreement with (15; 9)

The Iraqi outbreaks of LSD occurred between February and October 2014 (3). These periods were characterized by moderate to hot weather that encouraged the growing and distribution of insects. This observation was compatible with the previous well-known fact that mentioned the mechanical transmission of LSD via different types of biting and blood-feeding arthropods (including mosquitoes, flies and ticks) (9). These results agreed with our finding where there is a significant increase of the LDS in the spring months.

Conflict of interest

There is no conflict of interest for the current study.

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