

## A study of Lumpy skin disease outbreak in Thi Qar Province

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### **Abstract:**

In this study the clinical diagnosis of lumpy skin disease was depended. A total 1606 serum samples and skin biopsies collected from infected cows clinically with skin nodule of different size which change to a necrotic nodule or form a deep scab, from wide different areas and cities of Thi Qar , extended from June to November at 2016.

ELISA were performed for identification of LSDV antibodies, LSDV isolation was carried out on CAM of ECE and Viral DNA was extracted from skin biopsy for PCR.

All serum samples was show seropositivity against LSD. Isolation of LSD virus from skin biopsy was conducted on CAM and the isolated virus was identified by PCR. collection and processing of clinical samples, viral isolation and PCR assay, for LSDV are much sensitive and rapid diagnostic of LSD their importance in controlling the rapid spread of disease in Iraq.

**Keywords:** Lumpy skin disease, ELISA, PCR

### **Introduction:**

Lumpy skin disease (LSD) is an economically important generalized skin disease ,( Tuppurainen *et al.*, 2015) caused by a virus of the genus *Capripoxvirus* and family Poxviridae, also known as Neethling virus. It mainly affects cattle and zebus, but has also been seen in giraffes, and impalas (Alkhamis and VanderWaal, 2016).

The World Organization of Animal Health (OIE) has classified LSDV as a notifiable disease due to its economic impacts on the global cattle industry (Alkhamis and VanderWaal, 2016). All *Capripoxvirus* DNA are double-stranded with lengths of around 150 kbp (Zhixun *et al.*, 2017). Usually strains from sheep pox and goat pox share 147 genes; LSDV have an extra 9 genes that are non-functional in SPPV and GTPV. Strains of SPPV, GTPV, and LSDV generally have genome identities similar by at least 96% ( Fevik *et al.*, 2016).

Successful control and eradication of LSDV depend heavily on early detection of outbreaks; swift laboratory confirmation of the clinical diagnosis; rapid application of stamping-out of all or only those animals presenting clinical signs of LSD; strict animal movement control; vaccination; quarantine; disinfection; vector control; and preventive measures at affected regions (Tuppurainen and Galon, 2016).

The transmission of LSDV is mainly via arthropod vectors and its outbreaks are associated with wet and warm seasons, Mosquitoes are efficient mechanical vector for the transmission and maintenance of LSDV . Direct and indirect contact between affected and susceptible animals is not reported to be a pathway for transmission (Alkhamis and Vander Waal, 2016 ; Abdulqa *et al.*, 2016 ).

Jarullah, (2015) was reported the Incidence of Lumpy skin disease among Iraqi cattle in Waset

governorate. Mansour *et al.*, (2015) was recorded the lumpy skin disease in cattle in Al.Qaddissyia province.

Lumpy skin disease has an incubation period of two to four weeks (Magori-Cohen *et al.*, 2012). The disease is characterized by fever which can sometimes exceed 41°C and may last up to 2 weeks, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, edema of the skin, also poor milk production, abortion, and sometimes death (Abera *et al.*, 2015)

Clinical lesions can be confused with bovine herpes virus infections, insect bites, dermatophilosis, and bovine besnoitiosis . Therefore, laboratory confirmation is needed. Laboratory diagnosis of LSD can be performed by using serological and molecular techniques and by virus isolation in cell cultures . Serum Biochemistry of Lumpy Skin Disease Virus-Infected Cattle (Aspden *et al.*, 2003).

The economic losses due to this disease is associated with decreased milk production, loss of traction power, weight loss, poor growth, abortion, infertility and skin damage (Aspden *et al.*, 2003). .

Poxviruses have been widely investigated as vaccine vectors as they activate both cellular and humeral mediated immunity (Tuppurainen *et al.*, 2014).

Vaccination is the only effective way to control LSD outbreaks in endemic countries. Unfortunately, currently no marker vaccines allowing the differentiation of infected from vaccinated animals are available. Cross-immunity is known to occur between the members of the genus Capripoxvirus (Tulman *et al.*, 2001).

**The objective** of this study to investigate an outbreak of LSD in the Thi-Qar province from clinical infected animals and to confirm the diagnosis with ELISA, CAM and PCR

### **Materials and Methods:**

The clinical diagnosis of lumpy skin disease was depended in the present study, The number of

samples that collected during the study was 1606 infected cows clinically were obtained from 34,440 cows of both sex, different age and from wide different areas and cities of Thi Qar . The study extended from June to November at 2016. The infected cows regions were recorded by Global positioning system (GPS).

The samples included:

A. Skin biopsies:

Skin biopsies nodules during the disease course at different stage for virus identification by PCR.

B. Serum samples:

Serum samples were collected from infected cows clinically obtained for antibody detection against LSDV by ELISA.

### **Serological Identification of LSDV:**

ELISA Bovine anti-Lumpy skin disease virus (LSDV) IgG ELISA kit (Biotrend, Germany) were performed for identification of LSDV antibodies according to the method described by OIE,(2010).

### **Virus Isolation:**

Embryonated chicken eggs 9-10 (SPF) Eight-day-old (ECE) were inoculated with the prepared samples (seropositivity) via the chorioallantoic membrane (CAM) route. LSDV isolation was carried out on CAM of ECE for PCR (House *et al.*, 1990).

### **Molecular Identification of Virus Isolates by PCR:**

Viral DNA was extracted from skin biopsy (Markoulatos *et al.*, 2000). and stored at -20°C till used for PCR.

The listed primer sets for *Capripoxvirus* manufactured by Tib – Mol Biol syntheselabor GmbH. H. Berlin, Germany were used in application of PCR assays (Ireland and Binpal, 1998). The PCR procedures were performed according Ireland and Binpal (Sambrook and Russell, 2000).

The specific primers set amplified a DNA fragment of 192 bp equal to the expected amplification product size from LSDV. the LSDV reference strain had the same size of attachment protein gene fragment 192 bp, Therefore it was certain that, these specimens contained DNA of LSDV.

Gene Primers	Sequence 5' - 3'	Tm	Size (bp)
Capripox	F 5'- TTTCTGATTTTC-TTACTAR 3'	60	192
	R 5' - AAATTATATACG-TAAATAAC 3'	48	

**Results:**

The clinical signs and symptoms of infected animals included : fever, skin nodule of different size which change to a necrotic nodule or form a deep scab. The skin nodule range ( 2 -5 ) cm seen mostly on the neck , head ,genitalia and premium as seen in (Figure1,2).



Figure (1):LSD lesions all over the body of infected Cow.



Figure(2):Skin nodules of different size in infected Cow by LSD.

Antibodies against LSDV were detected by ELISA test in all tested sera from infected cows clinically (1606), There was display the overall seropositivity.

The morbidity of LSD in Thi Qar province was (4.66%), while the mortality rate was (0.008%) and the case fatality rate was (0186%) (table 1).

Table (1):Morbidity , mortality and case fatality rates in Thi-Qar province of Lumpy skin disease

No. of susceptible cows in Thi Qar Province	No. of infected cows (%)	No. of deaths (%)	Case fatality rate
34,440	1606 (4.66)	3 (0.008)	0.186

There is clear seasonal variation in the number of infected cows, as the higher number of cases was recorded between September and November ,with a highest number in November(675) ; While the lowest number was reported in July (21).(figure3)

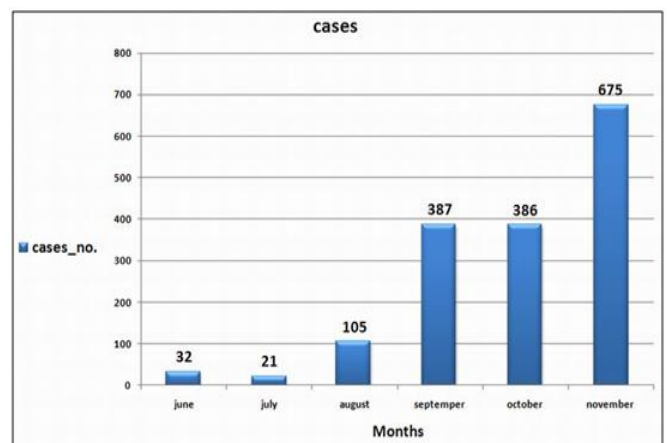


Figure (3): Occurrence of Lumpy skin disease in Thi Qar province according to the Months of the year 2016.

The disease was distribution according to rejoinis of Thi-Qar province of all infected cows, In this Figure the LSD was observed in most cities of Thi-Qar province (Figure4).

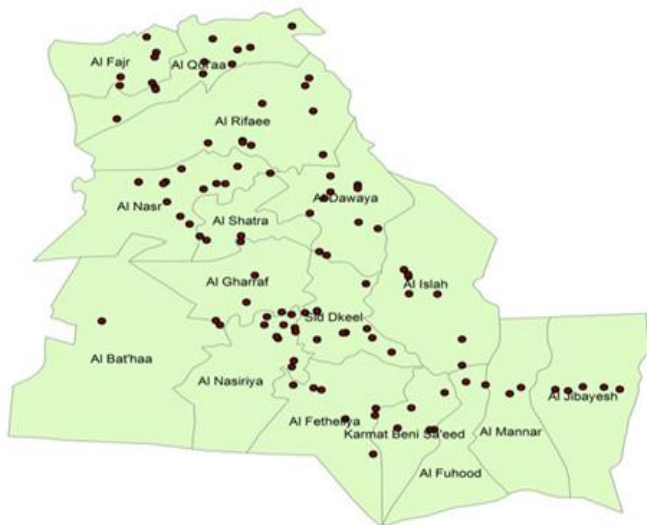


Figure (4): Map of Thi Qar province showing the distribution of Lumpy skin disease

Isolation of LSDV revealed the characteristic pock lesion on Chorioallantoic membrane of ECE, thickening of membrane numerous white foci. (Figure 5).



Figure (5): lesion of LSDV on CAM.

The specific primers set amplified a DNA fragment of 192 bp equal to the expected amplification product size from LSDV. The isolate from skin nodules, infected CAM had the same size of attachment protein gene fragment 192 bp, it was certain that these specimens contained DNA of LSDV (Figure 6).

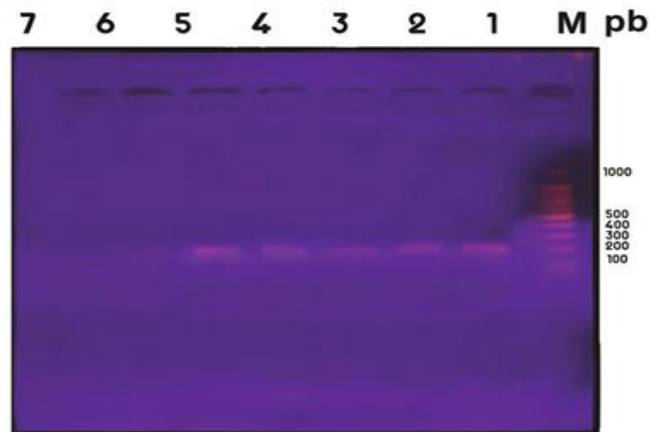


Figure (6) Shows the positive and negative result of PCR amplification of 192bp of LSD Lan (1,2,3,4,5) positive, Lan (6,7) negative and Lan (M) ladder marker.

### Discussion:

According to the clinical feature of LSD, the result indicate that (4.66%) of the cows were affected, as compared to the previous study conducted in Iraq which demonstrated that morbidity rate was (8.6%) among cattle in Waset (Jarulla, 2015) and (9.1%) among cattle in Nainawa and Baghdad (OIE, 2013).

The morbidity rate of LSD range from 5-45 % depending on several factors as health and nutritional status (OIE, 2013). the abundance and distribution of the insect vector and breed of the affected cattle (Wainwright *et al.*, 2013).

The higher prevalence rate of LSD was documented in Sudan, Southern, East, West of Africa and Oman (Wainwright *et al.*, 2013).

The LSD mortality usually ranged between 1-5%, but occasionally was reported between 10-40% (Wainwright *et al.*, 2013). The mortality rate in this study was (0.008) as compared to previous study in Iraq it was lower than the mortality rate in Waset (2.8%) (Jarulla, 2015) and Nainawa and Baghdad (0.5%) (OIE, 2013).

The severity of the disease depend on the route and the dose of the virus inoculation and health status of the animals; the young calves often affected by severe disease and *Bos*

*taurus* cattle are more susceptible (Wainwright *et al.*, 2013).

The result of the present study regarding the mortality and morbidity was in the range of the regional and local studies .

The clinical diagnosis was based upon the examination of the affected cows and the result demonstrated the presence of typical skin nodular lesion ( 2-5 )cm distributed all over the body , fever (40-41 c) enlargement of lymph node specially pre scapular and pre femoral. With time the skin nodules ulcerated ,necrotic and finally scab formation and these results in agreement with (Mansour *et al.*, 2015; body *et al.*, 2012; Stram *et al.*, 2008; Salib and Osman, 2011; Al-Habsy,2012).

Depending on the specific and characteristic skin lesion the tentative diagnosis was done .However ,in apparent and sub acute disease form need reliable and fast laboratory test to proved and confirm the diagnosis (Heine *et al.*, 1999; Stram *et al* 2008). For detecting the antibody and antigen of LSD, the ELISA technique have been discovered more recently (Heine *et al.*, 1999) which is a useful , quick and confirmatory diagnostic method of LSD .However ,there is a still need of virus isolation to demonstrate the virus during infectivity period (Tuppurainen *et al.*, 2005).

The incubation period of the LSD in experimental study was (4-14 days ) (Carn and Kitching, 1995). and( 4-5 ) days in other similar study (Tuppurainen *et al.*, 2005) and all the animals in the previous studies become infected and viremia was developed, the clinical sign and severity of LSD did not correlate with duration and length of viremic phase .By using virus isolation method ; the viremia can detected 1-12 days post infection , while PCR can detect the viremia at 4-11 days post infection (Carn and Kitching, 1995). So During the outbreak period, the diagnosing of the LSD by using PCR technique will facilitate the application of rapid control measure ( Sharawi *et al.*, 2011).

Because of the variability of the clinical feature of LSD (Davies, 1991)which range from

mild in apparent infection to severe disease , so severely affected animals with characteristic clinical feature can be diagnosed easily , however mild cases can be confused with other disease that have similar clinical sign as bovine popular stomatitis caused by *Parapoxvirus* , psudolumpy skin disease caused by Bovine herpes virus , insect bite, urticaria and photosensitization (OIE, 2009).

The disease was spread among the vaccinated cows during the outbreak regardless of the vaccination, The problem of vaccine failure and re infection of the vaccinated animal has been also reported by other author (Kumar, 2011).

The occurrence of LSD more in September and November due to wet and rainy weather which is the season of the development of insect population (Gari, 2010) . It is likely that during the LSD outbreak optimal condition for spread of LSD were created through the presence of high susceptible animals in a combination with high abundance of insect vector .

### **Conclusion:**

Lumpy Skin Disease is an important enzootic disease in Iraq. LSDV virus is circulating among Cows in Iraq. PCR is a simple, rapid, sensitive and accurate method for the detection of LSDV DNA in skin nodules and in the CAM of inoculated chicken embryos.

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