Investigation of Lumpy Skin Disease Virus in Baghdad City

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Abstract

In this study, the lumpy skin disease virus was isolated on the lamb testis cell culture for the first time in Iraq. Forty skin nodules were collected from cows suspected with lumpy skin disease (LSD) in Baghdad governorate. Real-time PCR detected LSD virus in 100% of these skin nodules. After that, the lamb testis cell culture was prepared for virus isolation. The prepared samples (0.5 ml) were inoculated into prepared lamb testis cell culture. Cytopathic effects (CPE) of the virus have appeared after 24hr post-inoculation and completed within 72hr. The noticed CPE were cell rounding, aggregation of cells, syncytia formation, and detached of the cells from the cell sheet. Fluids of lamb testis cell culture were examined by the real-time PCR to confirm that the observed CPE was due to the LSD virus. Cell culture fluids were positive by the real-time PCR (100%). Real-time PCR was sensitive for the detection of the LSD virus DNA in skin nodules, and it was a suitable confirmatory diagnosis tool of the virus in the tissue culture fluid. Finally, the locally isolated LSD virus in this study is valuable in future studies for making a vaccine to control the disease.

Keywords: *Cattle, Cell culture, Lumpy skin disease, Real-time PCR, Skin nodules*

Introduction

Lumpy skin disease (LSD) is a cattle pox illness caused by a virus called the Neethling virus. It mostly affects cattle and zebus but was also seen in giraffes and impalas[1]. The disease is characterized by fever, nodules on the skin, internal organs and mucous membranes, swollen lymph nodes, emaciation, skin edema, and occasionally death[2]. Abortion and pneumonia are the most usual sequels to LSD whereas the latter may be potentially fatal[3]. Lumpy skin disease virus (LSDV) has a double-stranded DNA genome that replicates in host cells cytoplasm[4]. This virus belongs to the *Poxviridae* family, of the *Chordopoxvirinae* subfamily, in the *Capri poxvirus* genus[5]. LSD is the main cattle health issue causing significant economic losses due to decreased milk production, a prolonged weakening of the clinical course, weight gain reduced, sterility in bulls, abortion of pregnant cows and permanent skin damage has a significant effect on the leather industry and this leads to a ban on international livestock trade[4]. Due to its economic effects on the global cattle industry, the World Organization of Animal Health (OIE) has listed the LSD virus as a notifiable disease[1]. OIE reported that the latest outbreaks of LSD when to occur in Iraq and Turkey, extending concerns that the disease will persist for spread into Asia and Europe[23].

In Iraq, LSD has occurred since 2013, and LSDV is circulating between Iraqi cows[6]. In autumn 2014, LSDV was detected by the polymerase chain reaction in Babil, Al-Qadysia, and Al-Muthana Governorate[7.23], was reported the occurrence of Lumpy skin disease among Iraqi cattle in Wasit province. [9], was recorded the LSD in Al-Qadysia province. There are many studies in Iraq were conducted about the detection of LSD virus[10], but the virus did not isolate so it was important to isolate LSD virus on cell culture to aid in the manufacture of a vaccine to control this disease in the future.

The aim of this study was the isolation of lumpy skin disease virus for the first time in Iraq on cell culture then the isolation will confirm by molecular technique.

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Materials and Methods

Samples collection

Throughout July to October 2019, forty (40) skin lesions were collected in sterile containers from animals that showed nodules or sitfasts on all the entire body, enlargement of lymph nodes, excessive salivation, mucous membrane ulceration, milk production decrease, anorexia, and pneumonia. These samples (sitfasts and nodules) were collected from five areas in Baghdad city (Abo-Ghraib, Al-Kadhimiya, Al-Wahda, Al-Ridhwania, and Al-Husua).

Extraction and amplification of DNA from collected samples

The collected samples divided into two parts, one part for detection of LSD virus by the real-time PCR, and the other for isolation of the virus on lamb testis cell culture. After sample preparation, the DNA was extracted by an extraction kit (Qiagen, Germany). Then, the extracted DNA was amplified by the real-time PCR kit (Genekam Biotechnology AG, Germany).

Preparation of primary lamb testis cell culture

Lamb testis cell culture Preparation was conducted in the virology laboratory of Baghdad University according to[11].

Isolation of LSD virus on lamb testis cell culture

After four days, the cell monolayer growth was completed, and growth media was removed from all flasks. LSD virus was propagated in lamb testis (LT) cell culture by inoculation 0.5 ml of the prepared sample into all flasks (except control flasks). Then, flasks were incubated at 37ºC for virus adsorption (1 hour). Thereafter, media (maintenance) was added to all flasks (infected and control), incubated at 37ºC, and cells were monitored daily by an inverted microscope for cytopathic effects. After that, flasks were frozen at - 20ºC (first passage). Flasks of the first passage were thawed to repeat the inoculation of undiluted fluid on new cells (second passage).

Detection of isolated LSD virus in cell culture by real-time PCR

After thawing of all infected flasks (passage 1 and 2), 0.5 ml was taken from each one of these flasks and were subjected to the extraction and amplification of DNA by real-time PCR for confirmation of the isolation of LSD virus in cell culture.

Results and Discussion

Clinical investigation of lumpy skin disease

Animals that used in this study were showed firm, circumscribed, raised and rounded nodules or sitfasts on all the entire body (figure 1), excessive salivation and nasal discharge, mucous membrane ulceration (figure 2), enlargement of lymph nodes, decrease in milk production, anorexia, and pneumonia. These results were compatible with[12, 13 and 14].

Figure 1: Lumpy skin disease sitfasts covered all the body of the infected cow.

Figure 2: Mucous membrane ulceration in the nose of a cow due to LSD ( ).

Detection of LSD virus in the collected samples by using real-time PCR technique

In the current study, skin nodules collected from naturally infected cattle detected by real-time PCR (Figure 3) and were positive (100%). These results revealed that the real-time PCR was sensitive in the detection of LSD virus in skin nodules of naturally infected cattle and indicated the incidence of an outbreak

of lumpy skin disease in Baghdad governorate in 2019. The findings of this study are consistent with those obtained by other researchers [15 and 16] who reported that the percentage of positive skin samples was 100%. However, these results are not compatible with[7], who pointed the detection of the LSD virus in 72% of skin lesions which is probably because of the collection of some samples were in the convalescent stage or due to presence of other diseases that clinically confused with LSD such as pseudo-lumpy skin disease.

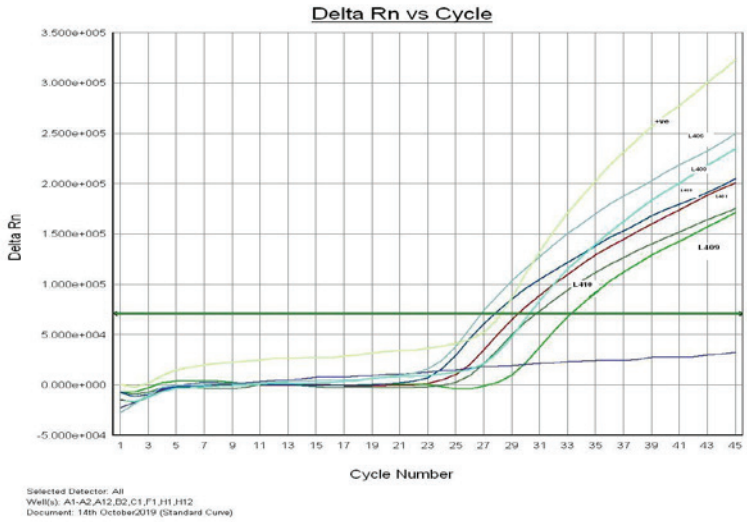

Figure 3: The plot of real-time PCR amplification of positive samples (L406, L403, L401, L408, L410, and L409) from Al-Husua, giving a Ct value of 27, 28, 29, 30, 31, and 33 respectively.

+ve: positive control give a Ct value of 28.-ve: negative control (did not give a Ct value).

Isolation of LSD virus in lamb testis cell culture for the first passage

The cytopathic effects of the LSD virus were rounding and aggregation of cells noticed after 24 hours post-inoculation. After 48 hours, the number of rounded cells increased, some of them separated from the flask surface, and the cells lost their cellular borders. Within 72 hours post-inoculation, the cytopathic effects were complete by observing the cell clusters detached from the cell sheet leaving empty spots and syncytia formation (Figures 4).

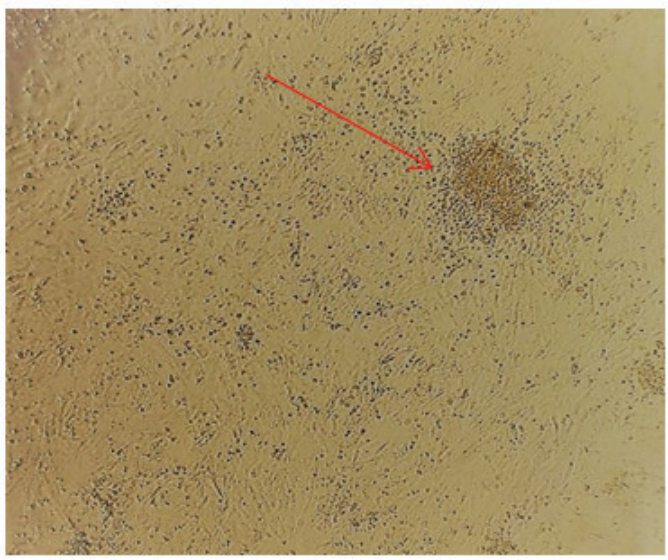

Figure 4: Cytopathic effects of LSD virus after 72hr post-inoculation (first passage), show syncytia ( ) (100x). Sample no. L379 from Al-Ridhwania.

Isolation of LSD virus in lamb testis cell culture for the second passage

The cytopathic effects of lumpy skin disease virus on lamb testis cell culture in the second passage (Figures 5 and 6) were fast, diffuse, and more evident than the first passage. The type of cytopathic effects was similar (as described in the first passage). In the present study, the primary lamb testis cell cultures were suitable for the cultivation of the virus, and the CPE appeared after 24hr post-inoculation in the first passage while in the second passage the CPE appeared in less than 24hr post-infection. This is similar to the studies of[18 and 22]. However, these findings are contrary to that published by[17 and 19], who pointed out that the CPE appeared within three days. The CPE of LSD was characterized by cell rounding, cell aggregation, syncytia formation, and detached of the cells from the cell sheet. These findings corresponded with the described results of other authors[17, 18, 19 and 21]. In this study, the cytopathic effects were completed within 72hr which found to coincided with[22] who recorded that the CPE completed in 48 to 72hr but it does not agree with[20] who reported that CPE completed in 9 days. These variations in the time of CPE appearance and completion were probably related to the type of cell culture, the dose of inoculated virus, strain of virus, media, and serum used.

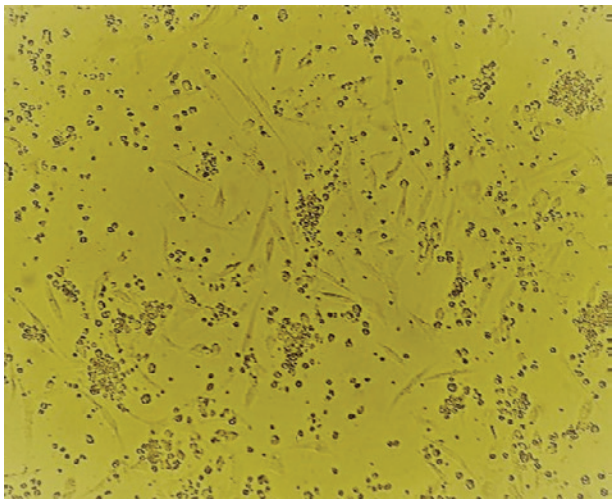

Figure 5: Cytopathic effects of LSD virus after 72hr post-inoculation (second passage), showed cell rounding, aggregation of cells, and empty spots (200x).Sample no. L379 from Al-Ridhwania.

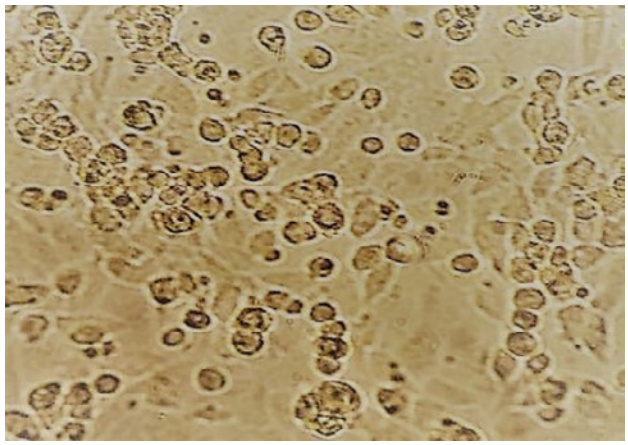

Figure 6: Cytopathic effects of LSD virus after 72hr post-inoculation (second passage), showed rounding of all cells (400x).Sample no. L399 from Al-Kadhimiya.

Detection of isolated LSD virus in cell culture by the real-time PCR technique

All fluids of cell culture (from both passages) were examined by the real-time PCR were positive (100%). This confirmed that the cytopathic effects were due to the LSD virus.

Conclusions

Incidence of infection with LSD virus in Baghdad governorate in 2019 was documented. The real-time PCR was sensitive for the detection of the LSD virus in skin nodules, and it was a suitable confirmatory diagnosis tool of the virus in the tissue culture fluid. It was favorable to collect skin nodules for diagnosis and isolation of the LSD virus. The primary lamb testis cell culture was suitable for lumpy skin disease virus isolation.

Ethical Clearance – This research was carried out under an agreement with the guidelines of the Canadian Council regarding using lab animals and animal care [Olfert] and approved by the office of the agricultural research ethics committee that recently took place in Iraq (approval number 5849/AGRO).

Source of Funding – Self

Conflict of Interest - Nil

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