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Original Article

Assessment of Contagious Ecthyma Virus in Camels of Wasit Province, Iraq

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Abstract

Camel contagious ecthyma (CCE) is an infectious disease caused by the Paravox virus (PPV) of the family *Poxviridae*. Due to the importance of the camel breeding industry in tropical and subtropical regions, the present study aimed to isolate the causative agent of camel contagious ecthyma (CCE) using cell culture and molecular confirmation of virus isolate. A total of 210 camels aged 6 months to 4 years were selected from different districts in Wasit province (Iraq) from August 2017 to April 2019. These animals, which included 117 females and 93 males, displayed signs of papules, blisters, pustules, and scabs on the skin. To isolate the CCE virus, primary and secondary cell cultivation was performed using the lamb testis (LT) cells. The findings pointed out that there were cytopathic effects during the second passage of the virus, characterized by rounding and cells aggregation after 72 h. Furthermore, there were dramatic changes, including sloughing off and detachment from the surface of the monolayer, in monolayer cells after 48-72 h. The titration values of the isolated Orf virus in LT cells were obtained at 10^{-5} TCI50 /0.05 and 10^{-6} TCID 50 / 0.05 ml in the third and fourth passages, respectively. As expected, the *B2L gene* of affected camels was amplified from a skin biopsy DNA sample to produce nearly 594 base pairs. In conclusion, the results of the current study focused on epidemiological and virological characteristics of CCE in Wasit province; moreover, the virus was confirmed by a specific gene called the *B2L gene*.

Keywords: *B2L* gene, Camel contagious ecthyma, Orf, PCR, LT cells

1. Introduction

In tropical climate conditions, the breeding of camels is more important than other livestock species living in the same environment since they are the most adapted species to climate changes and drought (1, 2). Moreover, metabolic processes in camels use less water, as compared to that in other ruminants, and the consumption of a wide range of food sources, such as herbaceous and woody species, has been also considered in the livestock industry (3). Camels have thick and rubbery lips that make food prehension easier during the consumption of prickly and thorny desert plants (4). They also have a strong digestion system that breaks down indigestible materials, such as plastic plates; consequently, camels can convert low-quality pasture into milk and meat (5). Camel is probably an exceptional animal that can be used worldwide as a natural breeding tool and works well in food insecurity (6). Viral skin disease in farm and wild animals is a worldwide problem caused by direct contact with infected animals (7). The epitheliotropic ecthyma virus infects and replicates in epidermal keratinocytes in damaged skin (8).

Camel pox (Orthopoxvirus) and camel contagious ecthyma (CCE), also known as a *Parapoxvirus*, are two major poxvirus diseases that can affect camels. They

can be differentiated using the technique of polymerase chain reaction (PCR) (9, 10). The CCE, also called the Auzdik disease or Orf, was first detected in Kazakhstan in 1968 and has been reported in countries with camel breeding systems in the Middle East, Asia, and Africa Africa (8, 11). The clinical symptoms of this disease, which is caused by a virus from the *Chordopoxvirinae* subfamily of *Poxviridae* family, usually include nodular skin lesions, papules, and vesicles, which are most commonly observed around the mouth, lips, and eyes.

In addition, young camels have been reported to have a high rate of morbidity and mortality due to CCE (11-13). This virus is extremely contagious causing severe dermatological lesions, particularly in small ruminants, such as goats and sheep, which are highly susceptible. Moreover, the virus can transmit to humans, resulting in solitary or multiple skin lesions commonly observed on fingers, hands, or forearms (14-17).

In camels, contiguous ecthyma is genetically characterized by two CCE strains. The first strain which has been obtained from Bahrain and Saudi Arabian camels has sequence homologies and phylogenetic relationships with the pseudocowpox virus (PCPV) species of the PPV genus of the family *poxviridae* (18). The second strain of CCE has been detected in the Indian dromedary camels due to infection with the PCPV (19). There is a dearth of information on virus characteristics and their effects on the camel breeding industry in Wasit province in Iraq. Therefore, the present study aimed to focus on some viral aspects in camels of this province through the isolation of the virus and confirm some isolates using molecular PCR techniques.

2. Materials and Methods

2.1. Specimen Collection and Study Areas

This study was conducted on 10 camel flocks in different districts in Wasit province, including Al-Numaniyah, Badra, Sheik Saad, and Al-Hay, from August 2017 to April 2019. The research sample consisted of 210 camels, 117 females and 93 males, within the age range of 6 months to 4 years. All study animals showed the signs of papules, blisters, pustules, and scabs on the skin. Skin scrapings (cutaneous scabs) were collected under aseptic conditions in sterile plastic containers that were saved cooled in the icebox and transported to the laboratory as quickly as possible.

2.2. Isolation of the Virus

2.2.1. Primary and Secondary Sheep Testes Cell Culture

To prepare the cell culture, testis of embryos of sheep were placed in a sterilized petri dish that contained the transport media and antibiotics and remained for 30 min at room temperature to prepare these cells. The testicular tissues were subsequently extracted using sterile scissors and forceps, placed in a sterile graduated cylinder containing penicillin-streptomycin antibiotic, and then washed with sterile phosphate buffer saline (PBS). To remove the blood remnants, the tissues were placed in a sterile trypsinizing flask containing a magnetic bar with the addition of 25% of sterile proteolytic trypsin solution.

The flask was placed on a magnetic stirring for 15 min to obtain a cell suspension. Following that, the cell suspension was filtered throughout different layers of gauze into a sterile flask, and the suspension was centrifuged at 4°C for 15 min at 1500 rpm. Thereafter, the solution was filtered and the cell precipitate was added to the growth media 150 ml/1 ml of cells (MEM (GIBCO ®), mixed well with 10% fetal calf serum, and distributed in falcons. The falcon was incubated at 37°C for 24 h, and the cells were then checked using an inverted microscope to detect if they had formed a confluent monolayer after 48-72 h (20). Just after the monolayer was fully functional, the growth media was removed, and trypsin 37°C with antibiotic was added for 1-2 min until the cells were rounded. Subsequently, the part of the trypsin which was added to growth media was removed, and part of the suspension was transferred to another falcon flask and incubated at 37°C for 2-3 days and examined daily.

2.2.2. Isolation of Virus on Tissue Culture

Orf virus strains were propagated in primary lamb testis (LT) cells by inoculating 0.3 ml of the viral entire monolayer. After removing the growth media, the virus was incubated at 37°C for 1 h to ensure virus adsorption. When infected cells are prepared at the same time, control cells inoculated with 0.3ml of PBS are prepared using the same method. The cytopathic effect (CPE) of infected and control cells were monitored using an inverted microscope on a daily basis, and the flask was then frozen at -20°C. Frozen and thawed methods were used to repeat inoculation on new cell monolayers, resulting in strong CPE in infected cells. The media used here are called maintenance media [MEM (GIBCO ®)] and mixed with 2% bovine fetal serum.

2.2.3. Titration of Viruses

To determine the titration, the Orf virus was serially diluted from 10^{-1} to 10^{-12}. The LT fibroblast cells were cultured in a microtiter cell culture plate. In each well, 0.05 ml of the virus was added, and the wells were incubated at 37°C for 1 h to allow the virus to adhere to the cells. Thereafter, one milliliter of maintenance media and 0.05 milliliters of PBS were added to each well, the wells were covered with adhesive paper, and incubated at 37°C for 5 days. The titration was calculated following the method previously described by Reed and Muench (21).

2.3. Molecular Examination

Following the manufacturers' instructions on the use of the High Pure Viral Nucleic Acid Kit (*Geneaid. Korea*), DNAs were extracted and examined by the Nanodrop (BioRad, USA) to estimate the concentration and purity. Targeting *B2L* gene, specific primers [(F: 5' GTCGTCCACGATGAGCAGCT-3') and (R: 5' TACGTGGGAAGCGCCTCGCT-3') were designed (9), Mastermix tubes were prepared at a final volume of 25 µl using a PreMix Kit (Bioneer, Korea), and PCR reaction was performed using the thermal cycler (Applied Biosystem, Singapore). The steps of DNA amplification involved 1 cycle initial denaturation (94ºC / 3 min); 35 cycles for denaturation (94ºC / 1 min), annealing (55ºC / 1 min), and extension (72ºC / 1 min); and 1 cycle final extension (72ºC / 10 min). Finally, agarose-gel electrophoresis was carried out at 90 volt for 1.5 h, and the detected bands using the ultraviolet transilluminator were considered positive at ~594 bp.

2.4. Statistical Analysis

All obtained data were documented using Microsoft Office Excel and analyzed in SPSS software (version 20). The Chi-square test was applied to detect significant differences at $P<0.05$.

3. Results

3.1. Clinical Findings

The outbreaks of CCE in camel herds have occurred in different parts of Wasit province. All 210 examined animals (aged 6 months to 4 years) were severely injured. The clinical findings in infected camels included severe papules on their lips and legs, increased body temperature, copious salivation, foulmouthed odor, and facial edema (Figure 1).

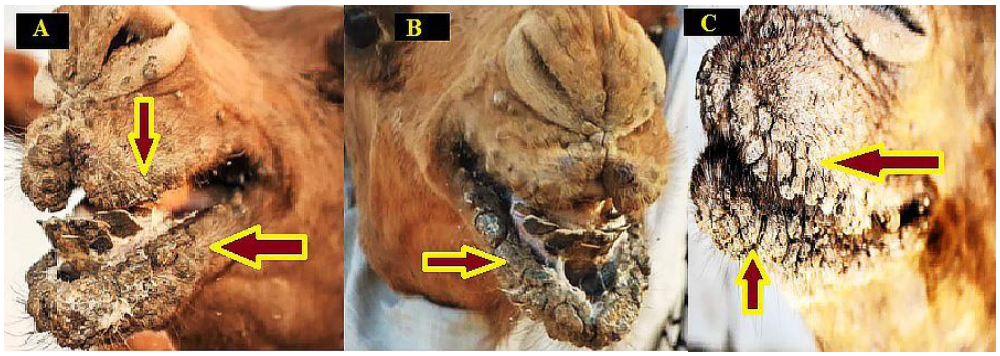

Figure 1. Camel Orf clinical presentations on lips

The obtained results pointed out that the morbidity rate at sheik saad city was higher than that in other districts (Table 1). Moreover, the age group of less than one year demonstrated a higher morbidity rate, as compared to other age groups (Table 2). It was determined that the 10 youngest camels died due to suspicious complications and starvation caused by oral lesions. The incidence rate was higher in summer and spring, as compared to that in other seasons.

Table 1. Distribution of ORF in infected camels according to study areas

District	No. of flocks	No. of camels	No. of infected	Morbidity rate
Sheik saad	4	76	48	63.1% A
Badra	2	40	23	57.5% A
AnNumaniyah	2	48	21	43.7% B
Hay	2	46	18	39.1% C
Total	10	210	110	50.8%

Variation in large letters vertically refer to significant differences (*P<0.05*)

Table 2. Infection rates of ORF according to the age of camels

Age of camels (Year)	No. of tested	No. of infected	Infection rate
0.5- 1	82	63	76.8% A
1-3	67	30	44.7% B
3-6	61	17	27.8% C
Total	210	110	49.7%

Variation in large letters vertically refer to significant differences (*P<0.05*)

3.2. Virus Isolation Findings

The findings of this study indicated that monolayer cells of the lamb testes cells illustrated the cytopathic effects of the second passage of orf virus, characterized by rounding and cell aggregation after 72 h. There were dramatic changes, including sloughing off and detachment from the surface of the monolayer, in monolayer cells after 48-72 h, as compared to the control group (Figure 2).

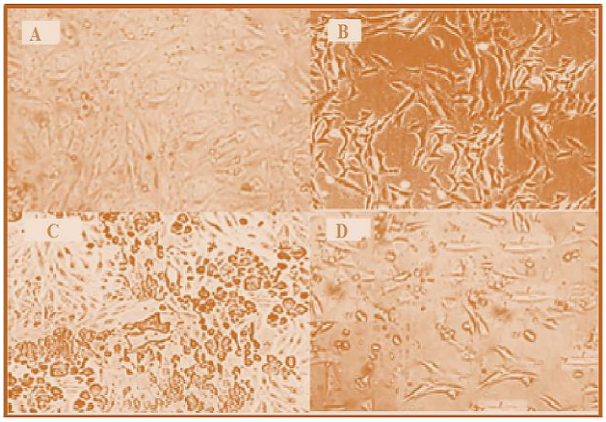

Figure 2. Results of ORF virus isolation on tissue culture. **(A):** Non-infected cells, **(B):** 3rd passage after 72 hours of infection, **(C):** 2nd passage after 72 hours of infection, **(D):** 4th passage after 48 hours for infecting the cells of sheep testes with orf virus.
CPE was appeared sloughing and detachment of the cells (B and D) with rounding and clumping in C (40×)

3.3. Titration of the Virus

The results of the titration experiment indicated that the titrations of the isolated ORF virus in infected sheep testes cells was 10^{-5} TCID50 /0.05 ml in the third passage and 10^{-6} TCID50 /0.05 ml in the fourth passage.

3.4. Molecular detec#tion

Virus isolation was performed using a combination of *B2L* gene analysis from scab lesions and cell culture PCR amplification. The *B2L gene* of affected camels was amplified from a skin biopsy to produce nearly 594bp (Figure 3).

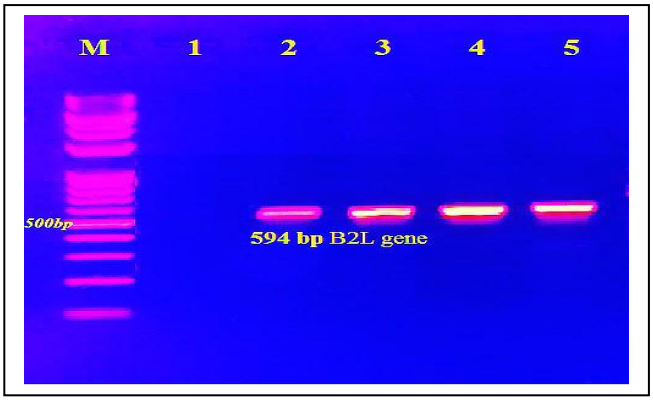

Figure 3. Agarose-gel electrophoresis targeting *B2L* gene, Lane **(M):** Ladder Marker (100-1500bp), **(1):** Negative control, **Lane (2):** Positive control, **Lane (3):** DNA from cell culture, Lanes **(4 and 5):** DNA from scab lesions

4. Discussion

Contagious ecthyma caused by the *Parapoxvirus* has been ignored for a long time since it was believed to be a mildly self-limiting disease (22). For the first time, ruminants, as well as camels, were examined to detect PPV in the field and laboratory. As a result, affected animals develop a proliferative exanthematous dermatitis which is marked by pustules and crusts mostly observed in their mouths (23, 24). Camels have edema in the neck and head, while in sheep and goats, edema is observed in heads and legs (13, 25) *Parapoxvirus* ovis or Orf virus (ORFV) is known to cause such lesions; nonetheless, none of them was detected on the teats of lactating animals (26, 27). The results of the current research are consistent with the observations of some other studies (5, 24, 28, 29).

The infected animals by the *Parapoxvirus* had proliferative crusted gross lesions covered by a dark coarse fissured crust that was extended to the mouth, nose, and ears. These clinical manifestations were similar to those described for CE in sheep, goats (30, 31), camels (26), and other species of animals (32).

In Wasit province, camels were examined in order to collect the main epidemiological data on the CE infection, and the findings demonstrated that clinical signs and symptoms are of great help in the differential diagnosis of the disease from other similar infections. Nevertheless, most owners believed that the disease does not require medical attention or notification of veterinary state officials on a routine basis. The virus was isolated using lamb testes cell culture which also showed a high tendency for its growth. Consistent with the findings of the studies conducted by Sheikh, Mansour (33), (34), the results of virus replication suggested that these types of cells are more suitable for culture, easily proliferated, and more susceptible to viral infection, as compared to other types of virus. The examination of infected sheep and goats has shown that the disease mainly affects young animals and all infected camels were under one year of age; however, the infection has been also reported in some adults (35). In accordance with the results of the studies by Gharib Mombeni, Gharib Mombeini (28), (34), as well as Abdelbasset, Rabia (5), the use of the *B2L* gene is considered to be more specific and confirmative for the diagnosis of the CCE.

Clinical manifestations, virus isolation, and PCR results were used to diagnose CCE in Wasit province. To investigate the epidemiological details, interactions, and genetic variations of the circulating ORFV in all districts, further studies should be performed in the future.

Authors' Contribution

Study concept and design: H. A. M. A.
Acquisition of data: H. A. M. A.
Analysis and interpretation of data: H. A. M. A.
Drafting of the manuscript: H. A. M. A.
Statistical analysis: H. A. M. A.
Administrative, technical, and material support: H. A. M. A.

Ethics

This study was approved by and performed under the supervision of the Scientific Committee in College of Veterinary Medicine, Wasit University, Wasit province, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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