

Clinical and diagnostic study of the Contagious Ecthyma in Dromedary Camels of Basrah, Iraq

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Abstract

Contagious ecthyma is an important viral disease infect animals' skin mostly small ruminants, it could infect other ruminants with a zoonotic pattern. In dromedary camels of Basrah province, Contagious ecthyma was detected and diagnosed. Seventy-eight local dromedary breeds, 2-6 years old show clinical manifestations of Anorexia and difficulty grazing, lesions of Contagious ecthyma appear as papules, vesicles, and scabs located at the upper and lower parts of the lips, commisure and /or around the mouth, Furthermore, Lesions in the form of Fissures with lips edema was also detected on diseased camels. Twenty, clinically healthy dromedaries served as controls. The infection was confirmed using the PCR technique. Blood parameters with acute phase response have been also evaluated. Results indicated that diseased camels show different clinical manifestations belonging to contagious ecthyma with a significant increase in body temperature, heart and respiratory rate, Moreover, Leukocytosis due to lymphocytosis was detected in infected dromedaries. a significant increase in ESR was detected in infected dromedaries compared with controls. Results referred to an increase in haptoglobin and Fibrinogen in diseased dromedaries with contagious ecthyma compared to controls. The contagious ecthyma of camels was still circulating in Iraqi areas, where appropriate preventive measures must be taken to prevent the spread of disease among camel herds and reduce the resulting economic losses.

Keywords: Ecthyma, camels, Iraq.

Introduction

Contagious ecthyma (ORF) is a viral disease affecting animal skin mostly sheep and goats, However, it could infect other ruminants with a zoonotic pattern. Lesions are always located on the lips, around the mouth, and sometimes at unusual regions of the body such as eyelids and udder causing pain, and anorexia with difficulty grazing (1,2)

Contagious ecthyma results from infection by the virus, belonging to genus *Para Poxvirus*, family Poxviridae, As the causative agent can be cultivated in cell culture of ovine, caprine and bovine sources (3). The disease has a zoonotic pattern, Therefore, all persons who handled the diseased animals or their infected tissues could be infected by direct contact, On the other hand, the disease is distributed globally in most countries especially those that rise sheep goats and camels (4).

It has been shown that normal animals can carry the contagious ecthyma virus in their wool or hides for a short period of time after the lesions have healed, the important type of virus transmission occurs via direct contact or with fomites, Thereby, it is thought to enter via abrasions or skin cuts, Further, Saliva can play a good role in the transmission of the virus especially in these diseased animals with oral lesions (5).

Contagious ecthyma often appears in a benign form and the malignant type of this disease could also be registered at a few locations around the world and the morbidity and mortality rates are higher,

especially in younger ages suffering from the disease for the first time (1,2).

The lesions of Contagious ecthyma are primarily peri labial involving oral commeasures, lips, muzzle, and mouth including oral mucosa, while sometimes it could be detected on the tongue, gums and dental pad, and even on the palate (6,7).

In contagious ecthyma, coalescing and may be ulcerated erythematous lesions of the diseased animals were detected and seen on the dental pad, gingiva, tongue as well as hard palate, On the other hand, lesions could also be detected on the pharynx and esophagus, and even in ruminal tissues (2). Moreover, skin lesions of the head, face, neck, ears, chest, flank, legs, nose, and mucosa of the nostrils are also not uncommon (8).

It's known that, Camels have dense thick, and rubbery matrix-like lips which let the food pretension easier during picking up the spiky plants, The epithelia-tropic contagious ecthyma virus could infect and proliferate in the specific dermal as well as keratinized skin cells in the harmful infected skin which is very suitable for infection and growth (3,4, 9).

Camel contagious ecthyma also called the Auzdik disease was first detected in Kazakhstan in 1968 and has been reported in other countries with camel breeding systems in the Middle East, Asia, and Africa (10). The clinical symptoms of this disease, usually include skin nodule like lesions,

papules and vesicular lesions, located mostly at commensures, lower and upper lips (2).

Camel contagious ecthyma was diagnosed in some areas of Iraq (4), It was first detected in camels in Basrah province, Iraq, Therefore, the clinical and diagnostic study was applied.

Materials And Methods

Animals and Study Areas: Seventy-eight (78) local dromedary breeds (one-humped dromedary) (Local Iraqi breeds), 2-6 years old (males 30 and females 48), and their weight ranged from (200-450 kg), show signs of contagious ecthyma used in this study, twenty clinically healthy dromedaries served as controls. Animals from both groups have been examined clinically. Further, their fecal and blood smears were examined to exclude any gastrointestinal and/or blood parasitic infection using the standard laboratory methods. This present study started from June to December 2022 in Basrah, Iraq.

Samples collection: Ten milliliters of jugular vein blood were collected via vein puncture. The blood sample was divided into 2.5 milliliters mixed with EDTA to evaluate total red blood cells TRBCs, Hemoglobin (Hb) concentration, packed red blood cells (PCV), and Total white blood cell count (TWBC) using the Hematology analyzer (GENEX, USA).

Blood smears method, stained with Giemsa, Weiss, and Wardrop (11) has been applied for evaluation of differential leukocytes count. However, the remaining blood was

used for ESR evaluation and extraction of serum. The erythrocyte sedimentation rate (ESR) has been estimated according to (12).

DNA extraction and Polymerase chain reaction (PCR): By using tissues of lip scrape of diseased dromedaries with Contagious ecthyma, Viral DNA has been extracted. As lip tissue samples were collected in sterile containers under aseptic conditions, However, they rapidly transfer to the laboratory unit and were stored at deep freeze (-20°C) till used for the viral genomic DNA extraction.

The Viral genomic DNA extraction: The Viral genomic DNA has been extracted using tissues of lip scrape by a kit of Genomic DNA extraction tissue, from (Geneaid. USA). The Polymerase chain reaction test was used to detect the polymorphisms of the contagious ecthyma-specific gene via forward as well as reverse primers. The performed application was applied using pairs of primers named as

5`CGCAGACGTGGCTCAGTACGT-3` and 5`TGAGCTGGTTGCCGCTGTCCT-3`.

As, Amplification reaction was done using a DNA Thermocycler, 1.5% Agarose gel electrophoresis which was stained using Ethidium Bromide under Ultraviolet light (13,14).

Evaluation of Haptoglobin:

By using an Enzyme-linked immunosorbent assay to evaluate haptoglobin According to instructions of the manufacturing company (Biotechnology co –china). Serum from diseased dromedaries with contagions ecthyma was used.

Evaluation of Fibrinogen: It was assessed using a kit from (Biolabo / France) and according to their manufacturer instructions, A plasma mixed with trisodium citrate was used.

Statistical analysis: Statistical analysis was achieved by applying t-test(unpaired). Statistically significant data were determined at a P value ≤ 0.05 . (15).

Results

Diseased dromedaries show different clinical signs which are illustrated in Table 1. As, diseased dromedaries show signs of Losing appetite, and difficulty grazing However, Contagious ecthyma lesions in the form of papules, pustules, vesicles, and scabs were seen in diseased animals, Lesions located around the mouth and commeasure,

Lesions located at the upper and lower part of the lips, whereas, Lesions on upper and/or lower eyelids are also detected and Lesions in the form of Fissures with lips edema was encountered in diseased camels. Table.1. On the other hand, diseased dromedaries show high body temperature($p < 0.05$) with increase respiratory and heart rate than in the control group. Table 2. To confirm the clinical diagnosis of contagious ecthyma in dromedaries, The results of (PCR) indicated that the contagious ecthyma causative virus has (480 bp) base-pair specific Polymerase chain reaction amplicons detected (Fig.2). A significant ($p < 0.05$) Leucocytosis because of lymphocytosis is indicated in infected dromedaries than the controls, Moreover, a Significant rise of ESR was indicated in infected dromedaries with contagious ecthyma. Table. 3

Table 1: The signs of infected dromedaries with contagious ecthyma.

Signs	Infected dromedaries		%	
	n=78			
	M	F	M	F
Losing appetite, and difficulty grazing	45	30	57.6	38.4
Contagious ecthyma lesions in the form of papules, pustules, vesicles, and scabs	48	29	61.5	37.1
Lesions located around mouth and commeasure	43	27	55.1	34.6
Lesions located at the upper and lower part of the lips	40	27	51.2	34.6
Lesions on upper and/or lower eyelids	2	6	2.5	7.6
Lesions in the form of Fissures with lips edema	1	5	1.2	6.4

M: Males and F: Females.

Table 2: The Body temperature, respiratory and heart rate of infected dromedaries with contagious ecthyma and controls

Clinical Parameters	Controls n=20		Infected dromedaries n=78	
	M	F	M	F
	Body temperature C°	37.1± 1.12	37.3± 1.15	39.2± 0.57*
Respiratory rate/ min	12±1.33	12±1.28	27± 3.42*	26± 4.42*
Heart rate/ min	43.57± 2.54	46.67± 1.31	73.6± 6.12*	78.5± 8.21*

* (P<0.05). M: Males and F: Females.

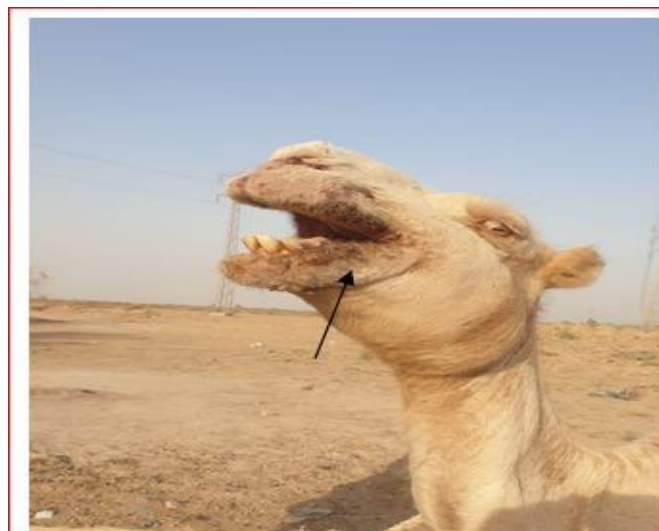
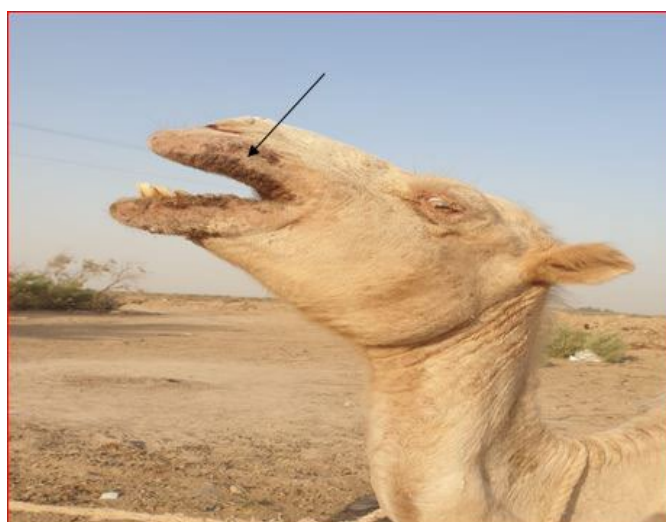


Fig.1&2: Contagious ecthyma lesions located at lips (upper & lower), commeasure, and with lips edema

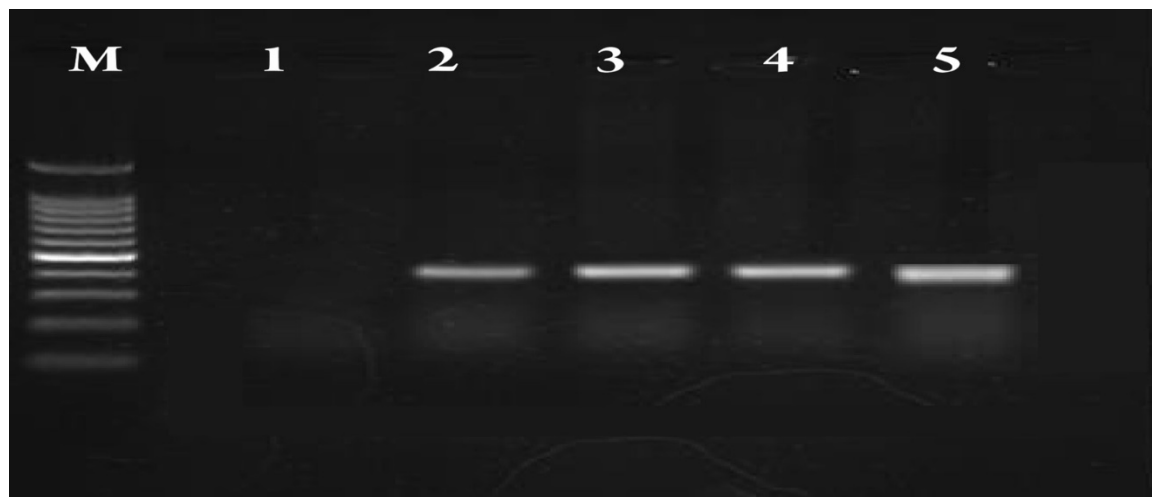


Figure.2: Lane (M): Marker ladder (100-1500bp), Lane (1): The controlling negative Lane (2): The control positive (480bp) Lane (3,4,5) positive samples (480 bp).

Table 3: The hematology of diseased dromedaries with contagious ecthyma and controls

Hematology	Controls n=20		Infected dromedaries n=78	
	M	F	M	F
RBC $\times 10^6$	7.56 \pm 1.11	7.16 \pm 1.91	7.13 \pm 1.32	7.24 \pm 1.12
Hb g/dl	12.41 \pm 1.81	12.62 \pm 1.58	12.71 \pm 3.65	12.41 \pm 1.85
PCV %	28.4 \pm 3.87	27.4 \pm 2.88	28.5 \pm 1.43	26.8 \pm 4.77
TLC $\times 10^3$	11.23 \pm 1.16	11.23 \pm 1.17	15.14 \pm 1.22*	15.12 \pm 1.12*
Neutrophiles $\times 10^3$	4.81 \pm 0.16	4.71 \pm 0.15	4.44 \pm 0.83	4.48 \pm 0.81
Lymphocytes $\times 10^3$	5.62 \pm 0.41	5.41 \pm 0.42	9.78 \pm 0.52 *	9.83 \pm 0.57 *
Monocytes $\times 10^3$	0.45 \pm 0.7	0.45 \pm 0.6	0.52 \pm 0.08	0.51 \pm 0.06
Esinophiles $\times 10^3$	0.51 \pm 0.31	0.53 \pm 0.21	0.52 \pm 0.14	0.52 \pm 0.17
Basophils $\times 10^3$	0.07 \pm 0.03	0.06 \pm 0.03	0.08 \pm 0.02	0.08 \pm 0.02
ESR mm/24hr	7.23 \pm 3.54	7.23 \pm 2.57	16.61 \pm 5.22 *	18.31 \pm 5.22 *

* (P<0.05). M: Males and F: Females.

On the other hand, results indicated a significant rise in values ($P<0.05$) of both Haptoglobin and Fibrinogen time of

diseased dromedaries with contagious ecthyma than in the control group. Table.4.

Table 4: The Haptoglobin and Fibrinogen of infected dromedaries with contagious ecthyma and controls

Parameters	Controls n = 20		Infected dromedaries n =78	
	M	F	M	F
	Haptoglobin g/dl	0.021 ± 0.011	0.023 ± 0.012	0.038± 0.008*
Fibrinogen time / Sec	25.12 ± 6.24	26.16 ± 8.11	33.15± 5.83*	34.12± 7.33*

* ($P<0.05$). M: Males and F: Females.

Discussion

The present disease of camels considered as a serious contagious viral disease distributed globally with its mainly benign form, However, the malignant type was also registered through scarce parts of the world caused by a poxvirus of the family Poxviridae (1,3). Contagious ecthyma is common in different regions throughout the globe and has been reported in Iraq (4), Further, direct contact with the causative virus is an important way for its transmission, As, The causative virus is resistant to external epidemiological condition, However, can remain active in the environment for a longer time, Moreover, Although the disease commonly infects younger's, However, it could also be detected in adult animals which grazing of

roughages which could predispose to infection with the scabby mouth(5,2). Different other names of the disease utilized in different outbreaks such as ecthyma contagiosum, sore mouth, infectious labial dermatitis, and ORF, However, Auzdik disease is commonly used in camels (1,7). Diseased dromedaries exhibited various clinical signs mentioned also by others,(1,5,6,7), Such as erythema, papules, pustules, vesicles, and scabs located around the animal mouth as well as lips and commeasure(4), However, under special environmental conditions as in younger ages the disease could appear as a chronic form, As, the lesions could stay longer time to resolve and heal completely, Therefore, those chronic lesions could transfer to another section of the skin parts (2, 16).On the other hand, It is important to mention

and note that there are infected sheep herds (of small numbers) were seen in the same area (intermittently and inconsistently) close to the grazing areas of the same diseased camels. Moreover, all diseased camels whom show clinical manifestations of contagious ecthyma signs are found positive with PCR test with no mortalities noted.

It was documented, that this infectious disease is distributed in the regions of the world that are famous for raising camels, with little epidemiological information's provided (1,4). On the other hand, the disease becomes more severe in younger's, nutritional deficient animals mostly grazing in rough pastures, and immuno-suppressed or overcrowded animals (17). Moreover, an increase in morbidity rate and mortalities might depend on the immune status of diseased animals besides the application of control measure (1,2).

Several diagnostic criteria have been applied to confirm the diagnosis of the disease; However, the Polymerase chain reaction test proved the request for the fit diagnosis of the causative agent (the contagious ecthyma causative virus) (3,8, 10).

Generally, upper and lower lips, mouth commensure and the surrounding skin are the common target areas of the virus infection, However, the udder of she-camels, face, and legs could also be targeted by the causative virus in which contagious ecthyma lesions will crust, scabbed and finally fall, Furthermore, lower eyelids could infect rarely (4, 18). Therefore, diseased camels show signs of Anorexia, and difficulty grazing with mouth pain and edematous lips sometimes, terminated with loss of animal condition (1,5).

It was documented that lesions located at the lips and around the mouth are more common because of the grazing nature of the diseased dromedary could lead to harmful mouth scraping and abrasions on the mouth as well as on the lips of the animal while grazing, Moreover, suckling baby-camel could be got the infection when sucking the infected teats (5).

A known scientific fact that the confirmative diagnosis of the contagious ecthyma virus could run via viral isolation and culturing, which is considered a specific technique for confirmation, However, the use of polymerase chain reaction test (PCR) through which a suitable identification of the causative virus via amplification of the gene through particular and specific primers, Since, Scientists, who work in the field of camel research confirmed that the polymerase chain reaction test is a fit diagnostic test for contagious ecthyma causative virus (13,19). Moreover, in current study, The primers used are specific targeting highly conserved nonstructural gene, So no need to compare the sequence with NCBI data base (4,13,14).

It has been indicated that diseased camels with contagious ecthyma has a Leucocytosis indicated because of lymphocytosis, was confirmed by (1, 20) whom mentioned that increase leukocyte count explained the reflex to various reactors, inflammatory causes, Since in those status, This response might arranged and stimulated through different molecules, which are liberated according to stimulators such as specific agents like white granulocytic cells, The stimulating factor of the colonies, The

macrophages, Furthermore, bone marrow stimulation due to the infection reflected increase in leukocytes release to an extent incomplete number of mature cells for delivery into the bloodstream, Where the disease will depress the number of immature cells, therefore, leukocytes number will significantly lowered and finally return back to natural values, over the time of maintenance, following an harmful inflammatory reaction, Moreover, Monocytosis might indicated, However, the lymphocytes become more abundant. Furthermore, it has been mentioned that the viral infection is mostly characterized by an increased number of small lymphocytes, However, without monocytosis, (11,21). On the other hand, (3) mention that several contagious viral diseases could be related to the presence of a specific type of large lymphocytes.

Results show high values of ESR in infected camels compared with the control group, As, this high values of sediment erythrocytes might prove the abundant inflammatory reactions which could trigger Hyper-fibrinogenemia and end with fast sedimentation of erythrocytes due to cells clumping (22- 24).

Results also show a significant increase in both Haptoglobin and Fibrinogen which indicated a positive stimulation of acute phase response.

It has been documented that any inflammatory response to damaged tissue will be reflected in the form of that the host could make a defense against any harmful states and soon will onset with the recovery and cure (25), The direct and quick reflexing is named acute phase response,

However, the prevalent use of acute phase response is the modifications of the density of different plasma proteins concerning the host reaction, These modifications might the final consequence of alterations in acute-phase protein structures in the liver (26). Furthermore, The acute phase response means a non-certain and unspecific reaction that could occur for short time after tissue damage, where, pro-inflammatory cytokines are liberated firstly at any location of an insult and are responsible for the induction of both local and systemic defenses (25). Haptoglobin was one of the acute phase proteins, believed to be a good marker of any inflammation in all animals which significantly increased in the present work (27).

On the other hand, Infected dromedaries exhibit a significant increase in fibrinogen time compared with the controls. It was shown that Fibrinogen is considered an important plasma protein that reflects the acute-phase response in animals (28), Therefore, this kind of protein was indicated as a good indicator for inflammatory responses, thus, it is used commonly as an active guide for the reflected inflammatory processes such as endocarditic, pneumonia, peritonitis, enteritis, and nephritis, (22,24).

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دراسة سريرية وتشخيصية لمرض الحميقاء الساري في الجمال وحيدة السنام في محافظة البصرة، العراق

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الخلاصة

يعد داء الحميقاء الساري من الامراض الفايروسية والتي تخمج جلد الحيوانات وبخاصة المجترات الصغيرة فضلا عن الحيوانات الأخرى ويعد من الامراض المشتركة بين الانسان والحيوان. تم الكشف وتشخيص المرض في الجمال وحيدة السنام في محافظة البصرة اذ لوحظ المرض في ثمان وسبعون من الجمال البالغة من العمر 2-6 سنوات وأظهرت هذه الحيوانات علامات سريرية تمثلت بفقدان الشهية وصعوبة الرعي كما لوحظت الافه المرضية وبشكل حطاطات وحوصلات فضلا عن ضهورها على شكل قشور متواجدة في الجزء العلوي والسفلي من الشفاه او حول الفم فضلا عن تواجد نفس الافه المرضية وبشكل شقوق مع توذم الشفاه. استخدمت عشرة من الجمال وحيدة السنام السويه سريريا كمجموعة سيطره، تم استخدام تقنية تفاعل البلمرة المتسلسل لغرض التشخيص النهائي للفايروس المسبب للمرض كما تم تقييم الفحوصات الدموية واستجابة الطور الحاد. اظهرت نتائج الدراسة ان جميع العلامات السريرية التي أظهرتها الجمال متوافقة مع مرض الحميقاء الساري فضلا عن حدوث ارتفاع معنوي في معدلات درجات الحرارة الجسم والتنفس وضربات القلب بالمقارنة مع مجموعة السيطرة، كما لوحظ وجود زيادة معنوية في العدد الكلي لكريات الدم البيض والناجمة من زيادة الخلايا اللمفاوية. استنتج من هذه الدراسة ان مرض الحميقاء الساري لا يزال متواجدا في البيئة العراقية حيث ان وضع البرامج الوقائية المناسبة يعد من الأمور الضرورية الواجب استخدامها لمنع انتشار المرض وتقليل خسائره الاقتصادية.

الكلمات المفتاحية: داء الحميقاء، الجمال، العراق.