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RESEARCH ARTICLE

Molecular, Epidemiology and Phylogenetic analysis of Camel Contagious Ecthyma in Iraq (CCE)

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Abstract

Some epidemiological features of camel contagious ecthyma (CCE) were studied , with infection rate of 35.25% in four Iraqi governovates; The results showed variability in regarding age groups and months of the year.Genomic DNA of major envelop protein B2L gene 1088bp from Iraqi isolates of the virus from the clinical specimens of this study were amplified by endpoint polymerasechain reaction (conventional PCR) using specific primers , then sequenced and analyzed by phylogeneiticcomparison with world reference strains of other *parapoxviruses* . The Iraqi isolates were clustered together with the Indian camel contagious ecthyma virus strain accession number GQ39365-1 *B2L* (camel India) and Indian camel Orf virus strain accession number GU460370.1 *B2L* (camel ,India) indicating high genetic homology with them .

INTRODUCTION

Camel are slow producers , calving is usually (24) months or may be more . High calf mortality appears to be one of the major limitants to higher productivity in camels . Many factors play a role in calf mortality among which are infectious diseases (Wilson ,1978) . Camel contagious ecthyma (CCE) consider as an important cause of loss of calves (Mohamed, 1997) . The disease is consider of young calves less than one year old caused by *parapoxvirus* characterize by appearance of vesicles and pustules mainly occur on lips and around the month and rarely on the eyes and feet , The lesions are painful which usually seen on muzzle that can cause anorexia and starvation (Wilson *et al* 1982) .Molecular techniques that have generallybeen used in parapoxvirus diagnostic include (RT – PCR) and endpoint PCR which nowadays one of the most powerful and applied methods in virus diagnosis (Mackay *et al* 2002) . The identity of the amplified target sequence can be verified using direct sequencing (Chan *et al* ., 2009). The B2L gene has been used for demonstrate molecular characterization and phylogenetic analyses of CCE virus (Hosamani *et al*., 2007) . This study aimed to investigate the predominate strains of the virus and their relation with world reference strains of the virus sequences available in the Gen Bank. .

Materials and Methods:

Clinical examination of 20 camel flocks in four Iraqi governorates (Waist , Smawa , Diwaniya and Najaf) with the total number 3750 in different age groups . Typical camel contagious ecthyma lesions was recorded .A total 96 dried scabs were collected from infected animals and transported in cooled box to the laboratory as soon as possible . Viral genomic DNA was extracted from scabs samples by using genomic DNA extraction kit (Geneaid – USA) .

Primers of major envelope protein gene (*B2L* gene) that used in endpoint PCR has been designed and provided by (Bioneer - Korea)

PCR master mix was prepared by using (Accu Power ^{R}PCR premix kit) .PCR was performed for amplification of 1088 bp*B2L* gene of CCE . The PCR products were used in gene sequencing and phylogenetic analysis of local CCE virus isolates, after visualization of PCR products of CCE virus B2L gene by agrose gel electrophoresis the product was extracted and purified by (EZ . EZ – spin column DNA gel extraction kit Biobasic – Canada) .

The purified B2L gene products were used for DNA sequencing system by using Dye – terminator sequence method .Nucleotide sets were used to demonstrate identity score of our isolates with selected world reference strains by using NCBL- Blast program and alignment by using NCBL – clustle w2 program soft ware .Phylogentic tree was constructed by using nighbour joining method with MEGA -6 software according to (Tamura *et al* 2007) . Phylogenetic tree were inferred with distance parsimony and maximum likelihood method and reliability of the trees were determined by 1000 data set bootstrap resembling.

Results and Discussion

Primer	Sequence		Size
B2L-PCR Primer	F	**AAATGTGGCCGTTCTCCTCC**	**1088bp**
	R	**GGACAGGTCCTTGACGATGT**	

The our results showed that the total number of camels with characteristic skin lesions around the mouth and nostril and other part of the body (Fig. 1) and confirmative molecular assay were 1332 out of 3750 in percentage 35.25% with no significant importance among different governorates (Table 1) . The same environmental conditions of study areas was gave almost same infection rate while the epidemiological features of CCE in various areas of the world differ according to many factors including etiological strains and many other environmental influences , despite the effect of CCE varied according to ecological conditions (Bncnenev *et al* 1987) in Asia , (Mollin and Zessin 1988) in Africa . Similar results of different geographical areas of this study may due to similarity of climate and grazing area in agreement with (Gitao 1994) . Who found same results when he studied CCE in four areas of Turkana in Kenya . The results according to age groups , less than one year , 1-3 years and more than 3 years were 79.89% , 47.41% and 8.29% respectively which was significantly differ at $P<0.05$ (Table -2) . There were high infection rate in camel calves less than one year in agreement with (Kalafallah, 2000) and may refered to the susceptibility of young camel due to absence of control measures and there were some degree of resistant of adult animals from previous natural infection . The results of infection rate according to the months July , August , September and October were (52.38% , 42.2% , 20 % and 21.42%) respectively (Table -3) , which were significantly differ recording high infection rate in July and August that may due to seasonal pattern of occurrence of the disease as observed by (Kalafallah, 1994).CCE occurred more common in young calves born in the same season and the hot weather increase the chance of skin damage by dried prickle plants that enhance the entrance of the virus and establishment of infection . The results of PCR for detection of CCE virus by *B2L* gene (1088) amplification and electrophoresis in ethidium bromide stained gel were gave positive results (Fig. 2) when visualized by UV ray in lane,1,2,3,4,5,6,7,8,9, and 10) as compare with lane M ladder size 2000bp. The PCR technique was sensitive enough to identify a low DNA concentration of 5 ng of genomic DNA of the virus in suspected samples . PCR using B2L gene primers system for diagnosis of CCE in the field without the need to use cell culture or electromcroscope EM as these biotechnology consider as time consuming and expensive assay . PCR was more reliable method to identify viral gene B2L (1088 bp) regardless the disease stage (Scaliarini*et al* 2004) . Sequence analysis of CCEV isolates and its comparison with selected world reference strains published in GenBank give an idea on epidemiology of CCEV and pociepalemerges of novel strains in the envivoment . In the present study 12 positive PCR isolates of B2L gene were sequenced showing 95 – 98 % identity with other selected foreign *parapox*nucleolide by using NCBL – Blast sequenceof GenBankstrains (Fig. 3) . The results were in accordance with (Nagarajan *et. al*.,2008) who was sequenced partially B2L gene of CCEV isolated from camel in India . The results of sequencing gave good forwards nucleotide sets as wave like sequence by dye terminator

sequencing method (Fig. 4) .The results of multiple sequence alignment is very important step for phylogenetic study , which drive the evolutionary relationships among different sequences sets . This obtained by application of clustal w2 program which allow to align many sequences sets together in a computationally efficient manner according to (Goujon *et al* 2010) which showed high identity in specific location sequence , the results depended on the Basic local Alignment Search Tool (BLAST) (Fig. 5) . Phylogenetic analysis of 12 isolates of this study and four selected world reference strains of other poxviruses which were Indian CCEV strain accession number GQ39365-1 B2L camel India,camelorf virus accession number GU460370.1.CO B2L camel India , Bovine papular stomatitis virus accession number AY42973.1 BPSV B2L and Bovine pseudo cowpox virus accession number KF 554010.1 BPCPV B2L Turkey . The isolates of the present study were clustered together with CCE GQ390365.1 and camel orfs traind GU460370.1 CO isolated from camel in India (Fig.6).

(Fig. 1)Scabby lesions around the lips , and nostrils

Table (1) Total percent of infection rate and disruption of infection according study areas Similar letters refer to the non – significant differences (P<o.o5)

Governorats	Flock ON.	NO. animals	Infected Camel	Morbidity Rate %
AL- Diwaniya	5	1036	372	35.9% a
Al- Samawa	5	993	320	32.22% a
AL-Najaf	4	824	290	35.19% a
Waist	6	897	340	37.9% a
Total	20	3750	1322	35.25%

**Similar letters refers to the non-significant differences , while different letters refers to the significant differences at ($p < 0.05$).

Table (2) Infection rates according to the age groups of camel .

Age of groups	NO. Camels	Infected,NO.Camels	Morbidity Rate
Less than one year	756	604	79.89% a
1 – 3 year	1006	477	47.41% b
More than (3 years)	1832	152	8.29 % c
Total	3750	1322	35.25%

**Similar letters refers to the non-significant differences , while different letters refers to the significant differences at ($p < 0.05$).

Table (3) Infection rates according to months of the year.

Months	camels No.	Infected NO .	Morbidity
July	1050	550	52.38% a
August	1000	422	42.2% a
September	1000	200	20% b
October	700	150	21.42% b
Total	3750	1322	35.25%

**Similar letters refer to the non-significant differences , while different letters refer to the significant differences at ($p < 0.05$).

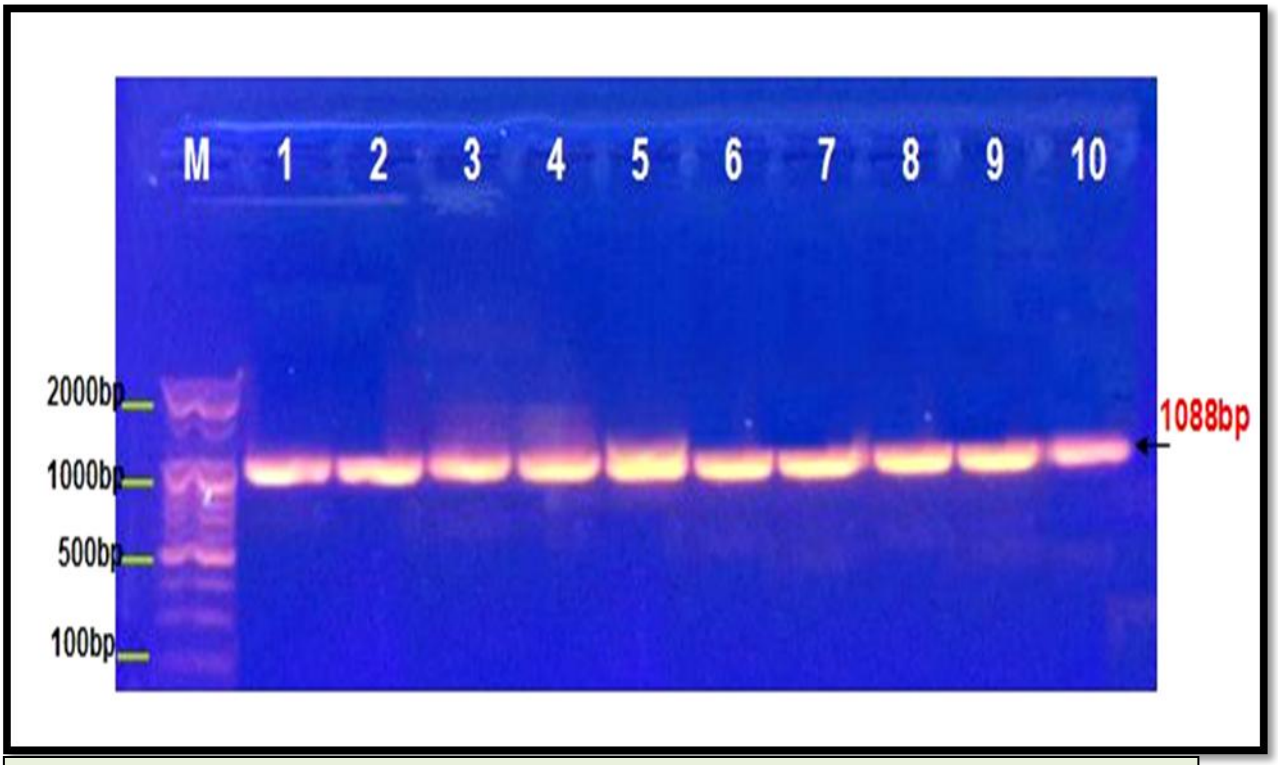

Figure (2)positive results of endpoint conventional PCR of B2L gene in CCEV samples .

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TGTCCACCATCAAGAACCTCGGGCTCTACTCCACCAACAAGCACCTGGCCGCGGACCTCATGAACCGCTACAACACCTTCT
ACTCCATGATCGTGGAGCCGAAGGTACCGTTCACTCGGCTCTGTTGCGCCATCGTCACGCCCACGGCCACGGACTTCCAC
CTGAACCACTCAGGCGGCGGCGTCTTCTTCTCGGACTCGCCGGAGCGCTTCCTGGGCTTCTACCGCACACTCGACGAGGA
CCTCGTGCTGCACCGCATCGATAACGCGAAGAACAACATCGACCTCTCGCTGCTCTCGATGGTGCCGGTGATCAAGCACG
CCAGCGCCGTGCAGTACTGGCCGCAGATCATCGACGCACTGCTGCGTGCGGCCATCAACCGCGGCGTGCGCGTGCGCAT
CATCACCACCGAATGGAAGAACGCGGACCCGCTATCGCTCTCGGGCGCGCGCAGCCTCAATGACTTCGGCGTGGGCAGC
GTGGACATGTCCGTTCCCAAGTTCGTGATAACGGGCCGAGACGACACCACCTACAACACCAAGCTGCTCATCGGGGACAA
CACCTTCACGCACGTGACGGTGGCCAACCTCGACGGGGCGAACTACCGGTACCACGCCTTCCTGAACGGGAAC

Fig. (3) Forward nucleotides sets sequencing B2L of CCEV isolates

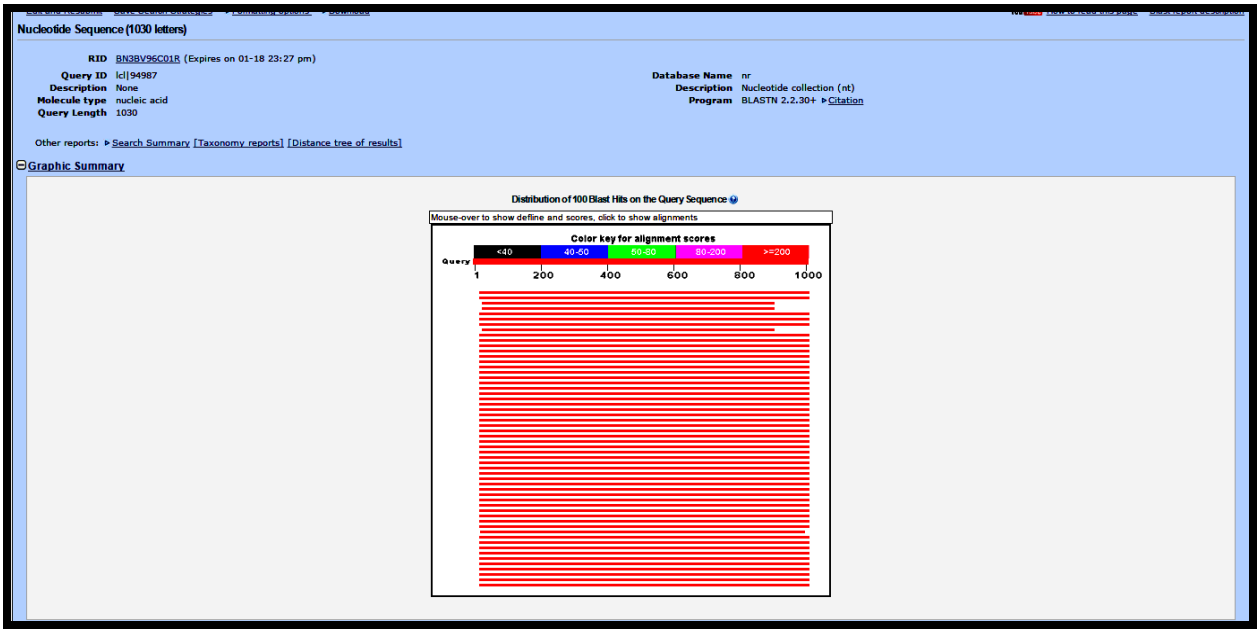

Fig. (4) Examination of forward nucleotide sets of any sample in this study compared with world strains of B2L gene CCEV.

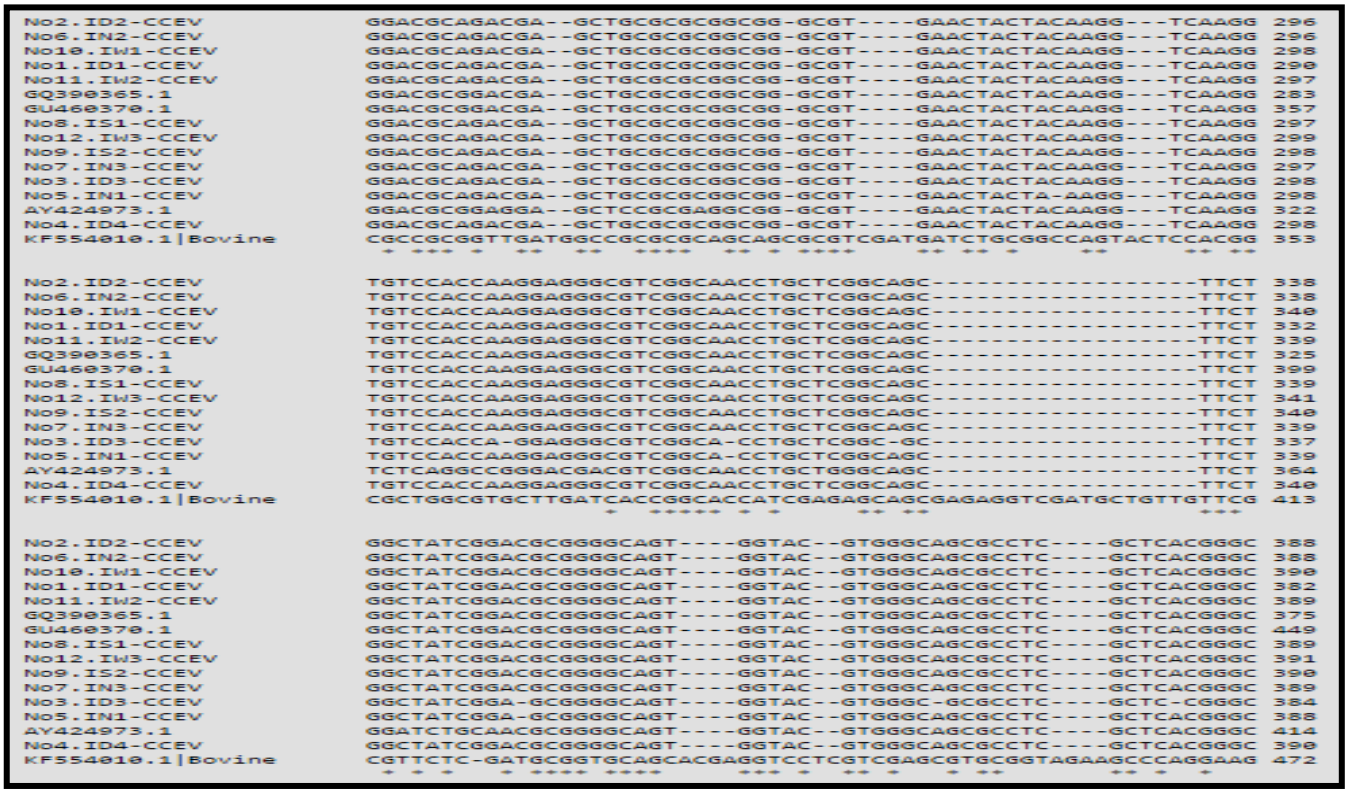

Fig. (5): Multiple sequence alignment analysis of the *(B2L)* gene of Camel Contagious Ecthymavirus(CCEV) and selected world reference strains .

(*) Alignment sequence site of DNA .(High Identity locations)
(space) Variation of the multiple alignment sequence.

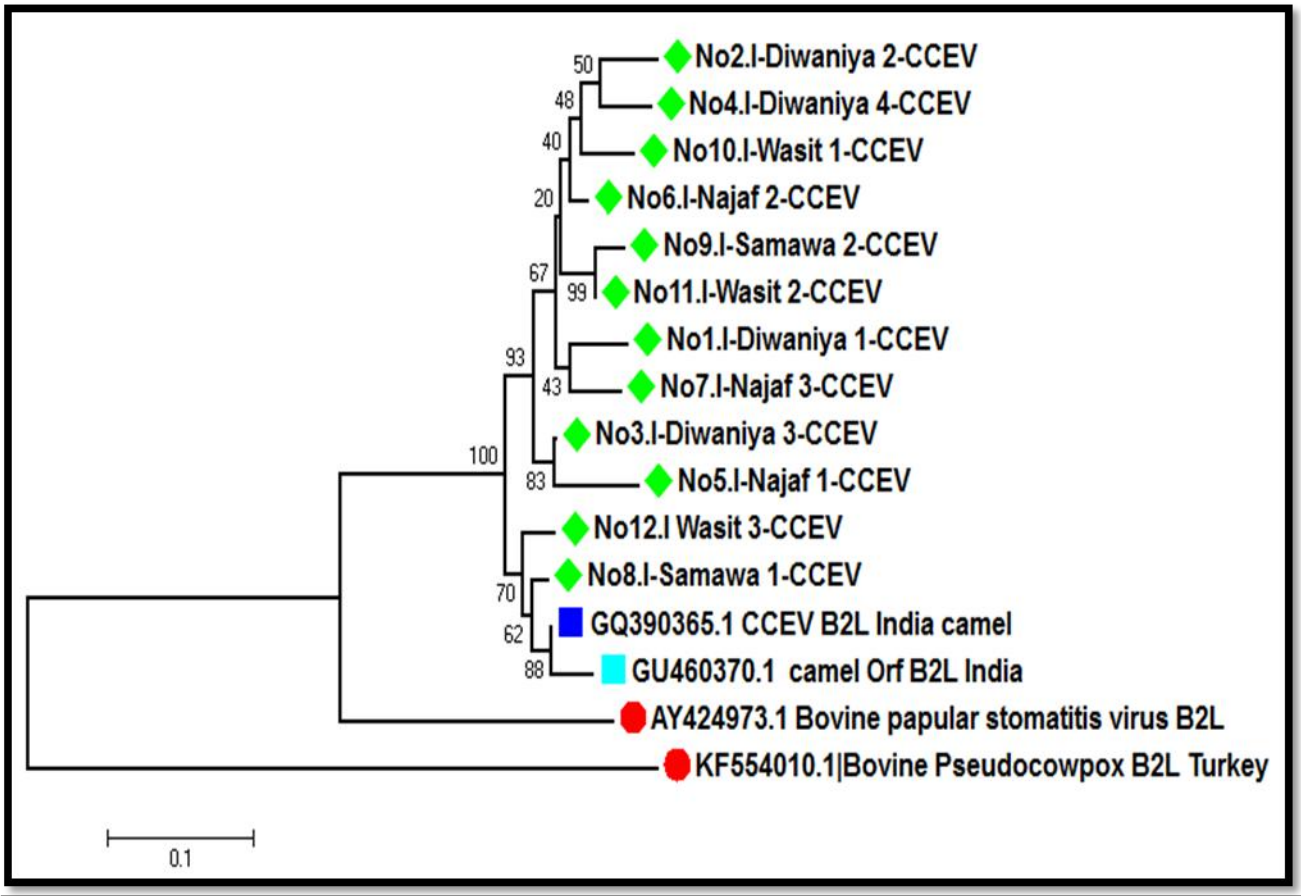

Fig. (6): Traditional (Rectangular) phylogeny tree using a neighbour – joining method based on (1088 bp) *B2L* gene of (CCEV) .

**(Numbers on the tree) units of the number of base substitution per site .*

** Green spots (Iraqi CCEV isolates).*

** Blue spot (CCEV B2L India) .*

References:

1- Wilson R.T., 1978. Studies on the livestock of Southern Darfur, Sudan. *Trop. Anim. Health Prod.*, 10: 19-25.

2- Mackay, I.M., Arden, K.E., Nitsche, A., 2002. Real-time PCR in virology. Nucleic Acids Res. 30, 1292-1305.

3- Hosamani, M.,Yadav, S., Kallesh, D.J., Mondal, B., Bhanuprakash, V., Singh, R.K., 2007. Isolation and characterization of an Indian ORF virus from goats. Zoonoses Public. Health. 54, 204-208.

4- Chan, K.W., Yang, C.H., Lin, J.W., Wang, H.C., Lin, F.Y., Kuo, S.T., Wong, M.L., Hsu, W.L., 2009. Phylogenetic analysis of parapoxviruses and the C-terminal heterogeneity of viral ATPase proteins. Gene 432, 44-53.

5-Buchnev K.N., Tulepbaev S.Z., Sansyzbev A.R., 1987. Infectious diseases of camels in the USSR. *Rev. Sci. Tech. Off. Int. Epizoot*., 6: 487-495.

6- Khalafalla A.I., Mohamed M.E.M., 1997. Epizootiology of camel contagious ecthyma in Eastern Sudan. *Revue Elev. Méd. vét. Paystrop.*, 50: 99-103.

7- Khalafalla, A. I. (2000): Camel contagious ecthyma: Risks in young calves. Rev. Elev. Med. Pays. Trop. 53, 173-176.

8- Moallin A.S.M.,Zessin K.H., 1988. Outbreak of camel contagious ecthyma in Central Somalia. *Trop. Anim. Health Prod.*, 20: 185-186

9- Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 6. Mol. Biol. Evol. 24, 1596-1599.

10- Goujon ,M.;McWilliam, H.; Li, W.; Valentin, F.; Squizzato, S.; Paern, J.and Lopez, R. *(2010).*Clustal W2 *A new bioinformatics analysis* tools *framework at EMBL-EBI .Nucleic acids research, W695-9doi:10.1093/nar/gkq313*.

11-Wilson A. j., H. J. Schwartz , R. Dolan C. R. Field and D. Roettcher 1982 Epidemiology is che Aspekt ebedeutent end erkamel krankheiten in ausge wahltenGebieten Kenias . Der praktischeTierarzt 11: 974-987.

12- Nagarajan,G.,Ghorui,S.K., Kumar,S. and Pathak,K.M.L.,Lucinda,N., Inoue Nagata,A.K., Kitajima,E.W. and Nagata,T. Camel contagious ecthyma virus isolate Jodhpur envelope protein (B2L) gene, complete,cds. India. 2008