Research Article

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Genetic Phylocomparative Analysis of B2L, F1L Genes in Orf Virus Isolated from Felid Infected Sheep

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Abstract

Contagious ecthyma virus Large, oval, double-stranded DNA viruses from the family Poxviridae they are distinct from other poxviruses due to their unusual spiral coat. Orf virus encoded highly conserved F1L gene, B2L gene, which codes for highly immunogenic envelope protein. Orf lesions were observed on the upper and lower lips, upper and/or lower eyelids, and around the mouth and nose of sheep. This study evaluated the histology and genetics of Orf virus in AL-Qadisyah sheep infected with infectious ecthyma. Positive histology is defined by the presence of subcorneal pustules, hydropic degeneration of necrotic keratinocytes, and epidermal hyperplasia with pronounced rete ridges. Samples were fixed in paraffin and sectioned into 5m slices. PCR on DNA-extracted samples was positive for both the B2L and F1L genes. Four positive samples were sequenced and recorded in GeneBank, and phylogenetic analysis was performed. Histopathology and clinical symptoms can aid in the diagnosis of infectious ecthyma rapidly and affordably, whereas PCR distinguishes between identical diseases in endemic regions. Analyzing the divergence between the inferred amino acid sequences of the incomplete B2L gene in different strains from Iraq OK336711.1, OK336710.1, and other Indians, we found that two locations contain different amino acid changes, resulting in a nucleotide and amino acid identity of 97.8% and 97.6%, respectively. The F1L envelope protein of the Iraqi strain OK330734.1 was comparable to those of China and India, while the envelope protein of the Italian strain OK330733.1 was identical to that of Italy.

Key words: Orf virus, B2L gene, F1L gene, DNA viruses

Introduction:

Contagious ecthyma is widespread in the wild populations of small ruminants across Asia, Africa, and other continents, and is contagious to these animals (1). Although adult sheep mortality is rare, secondary infection can kill up to 15% of lambs (2). Crusty sores, papules, and pustules appear on the lips, gums, and nose of infected animals (3), this contagiousness, zoonotic potential economic impact of ORFV infection can be seen in the signs of decreased food intake, slowed growth, and a drop in the market value of the animals (4, 5). In most cases, CE recovers spontaneously, however severe cases due to infections or delayed nursing care cause mortality and wasting, most human infections are minor and clear up without complications, but those with impaired immune systems might develop painful, slow-to-heal wounds (6). ORFV is a parapoxvirus, having 134 genes. ORFs 009-111, the virus's central core region, is highly conserved and essential for viral, maturation, and virion structural development and shape (7). Immunogenic ORFV B2L gene-encoded 42 kDa protein produces high antibody response (8). In late viral infection, ORFV F1L gene transcriptional coding can express an immunological protein on the mature virion envelope, full-length F1L protein (9) have a good immunogenicity.

Material and Methods

Sample collection, genomic extraction, and pcr approach: The research was conducted in Al-Qadisyiah province in November 2020 using a scabs were homogenized in phosphate-buffered saline and sterilized sand to create a 10% virus solution (PBS), 0.45-m syringe filter was used to filter the mixture after it was centrifuged at 1000 RPM for 10 minutes. Finally, after filtration, penicillin, streptomycin, and nystatin were added, and the solution was kept at $^{-}80$ C° DNA was extracted using a G-spin kit (iNtRON Biotechnology, Seongnam-Si, South Korea), and the resulting DNA was sequenced. Primer designated table 1 based on NCBI database strains under excision KX951407.1 (B2L) gene/ORFV 011, and KX951408.1 F1L (F1L) gene/ORFV 059 and supplied.

Table 1: designated primers.

Genes	Sequences	Product size
B2L	F(ATGTGGCCGTTCTCCTCCATCC) R(ATTTATTGGTTTGCAGAACTCCAGCGCC)	950bp
F1L	F(ATGGATCCACCCGAAATCACGGCC) R(CACGATGGCCGTGACCAGCAG)	840bp

A 2x SuperHot PCR Master Mix (Jena Bioscience, Jena, Germany) was denatured at 95°C for 3 minutes, then exposed to 35 cycles of 1 minute at 55°C, 1 minute at 72°C, 1.5 minutes at 72°C, and 10 minutes at 72°C as an extension temperature. A 1.5% agarose gel was made by immersing 1.5 g of agarose into 100 cc of TBE solution and exposing the mixture to an 80V at 100A electrical field for 45 minutes to sort the amplified DNA pieces. Ethidium bromide (Bioshop Canada) was used to color and scan the gel. Macrogene in Korea sequenced the PCR result. The genetic study, editing, and genome matching were compared to gene bank and NCBI public database data.

Histopathological examination

Biopsies of infected sheep mouth skin were taken using sterile scissors and forceps, and then fixed in 10% formalin according to conventional protocols for histological diagnosis. The pathology lab at veterinary medicine college of Al-Qadiysih university processed and examined the slides. Olympus optical microscope slides stained with (HE) as described by (10).

Results

Gross examination and histopathological changes.

uneasy excruciating pustules erythema, vesicles, pustules, and scabs on lips, nose, infective lesions are typically limited to regions surrounding viral entry sites. A microscopic examination of skin lesions stained with hematoxylin and eosin. The superficial dermis is enlarged by the infiltration of inflammatory cells. Keratinocytes displayed intraepithelial ballooning degeneration, eosinophilic intracytoplasmic inclusion bodies, intracellular edema, and dispersed hemorrhage.

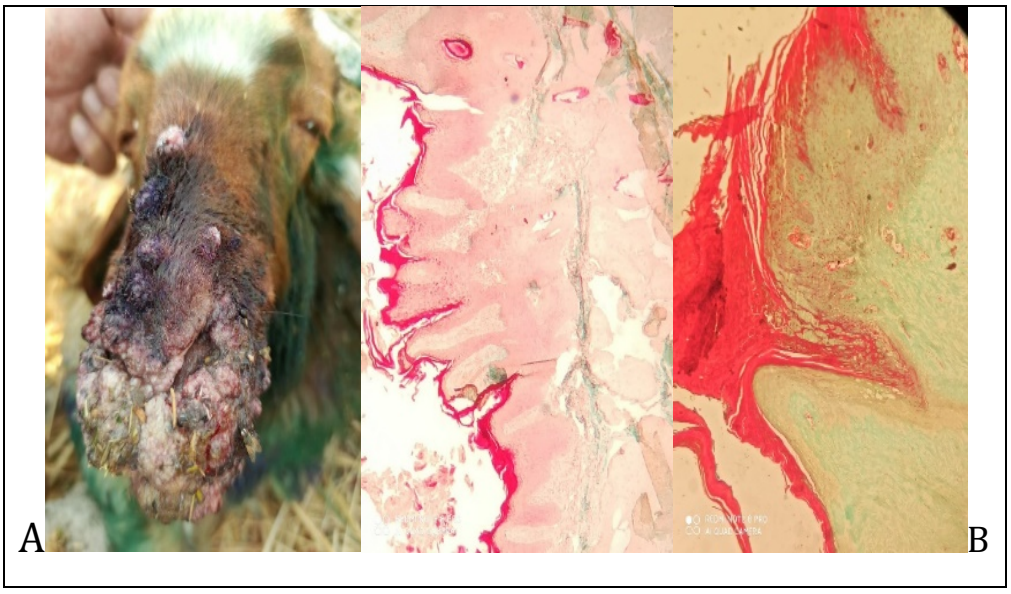

Figure (1) A: signs Sheep have skin lesions around the mouth. B: Histopathology changes shows vascular degeneration, keratinocyte swelling.

Molecular detection and PCR amplification: The amplified genomic DNA showed positive results for 2 different genes (F1L, B2L) at different product size when electrophoresed (figure 2).

Sequencing and phylogenetic analysis: On the basis of an alignment of the deduced amino acid sequences of the incomplete B2L gene from Iraqi strains OK336711.1, OK336710.1, and the other Indians, we discovered that two locations contain distinct amino acid alterations, resulting in a nucleotide and amino acid similarity of 97.8% and 97.6%, respectively, in a phylogenetic study using only the partial B2L gene. The F1L envelope protein of the Iraqi strain OK330734.1 was akin to that of China and India, whereas the envelope protein of the OK330733.1 strain was identical to that of Italy.

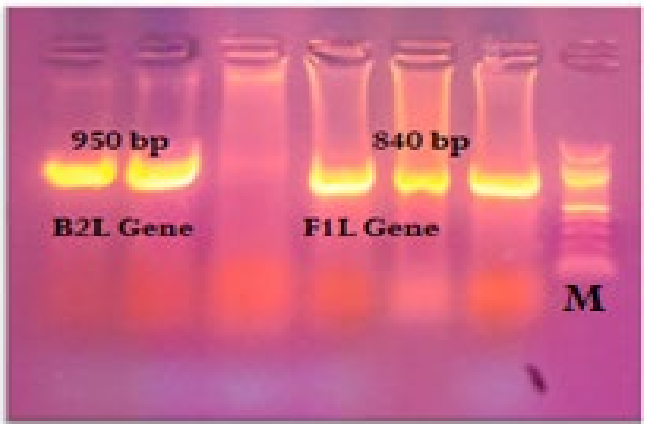

Figure (2) Image of electrophoresed 1.5%-agarose gel, B2L gene PCR products of CEV from sheep. M: DNA ladder (100-1000bp). Lanes 1,2 B2L gene: Positive amplified samples at 950bp. Lanes 4,5,6 F1L gene. Positive amplified samples at 840bp.

Figure 3: Phylogenetic tree of the complete sequencing of the B2L and F1L gene belongs to Orf virus. The analysis was based on the use of Maximum Likelihood method, Neighbor-Joining, and Maximum Composite Likelihood (MCL). The Iraqi strains labeled with blue triangular.

Discussion:

Orf virus is a global threat because it can cause repeated infection in the same animal, severe sickness in adults, death in young lambs and kids, and zoonotic nature (11). The clinical indications of infected sheep and lesions were consistent (12): painful, highly vascular, fragile, and quickly bleeding scabs; a progression from redness, rash, pustule, to crust and scab (13). Typically, the disease lasts three to four weeks, and lesions recover in one to two months without scarring (14). Lesions are typically confined to the epithelium of the oral mucosa, the skin of the lips, and the region surrounding the nostrils. Lesions can also be noticed on the teats of nursing animals and, on occasion, on the tongue and gums of diseased animals (15). The virus replicates in keratinocytes of the epidermis. ORFV evades the protecting keratinocytes of the host (16). Previous investigations have described the genetic characterization of Iraqi isolates and already a multitude of ORFV isolate sequences accessible in GenBank, from AL-Diwaniyah Al-Najaf,

Al-Smawa governorates targeted GM_CSF/IL-2 inhibition factor (GIF) gene (17) and in Al-Basrah (18)(19) targeted orf virus including some partial genomes data that have been the subject of substantial prior study none of these investigations characterized B2L, a significant and immunogenic envelope protein, genetically in sheep. On a phylogenetic scale, two significant clusters were identified, including both Iraqi and foreign orf isolates. ORFV F1L structurally mirrored the topology of its homologue, vaccinia virus. F1L immunodominant envelope protein showed similarity between Iraq strain OK330734.1 with China, India whereas OK330733.1 showed exact identity with Italy. Amino acid sequences derived from F1L expressing genes were determined and compared across all strains, demonstrating a wide variety of alterations. Structural analysis was carried out to ascertain the impact of amino acid substitutions on protein structure, and the results showed that the secondary structure was maintained. The results showed that despite differences in host cell selectivity and pathogenicity, parapoxvirus proteins, especially ORFV F1L protein and its homologues, are highly conserved globally and across species. Despite having a highly conserved C-terminal domain, the study confirmed that ORFV strains can be distinguished based on N-terminal variability (20). Based on an alignment of the deduced amino acid sequences of the incomplete B2L gene from Iraqi strains OK336711.1, OK336710.1, and the other Indians, we noticed that two places contain distinct amino acid alterations, yielding a nucleotide and amino acid similarity of 97.8% and 97.6%, respectively, in a phylogenetic study using only the partial B2L gene (Fig3).

Conclusions: PCR differentiates between endemic illnesses. Analyzing the divergence between inferred amino acid sequences of the incomplete B2L gene in different strains from Iraq OK336711.1, OK336710.1, and other Indians, we found two locations with different amino acid changes, resulting in nucleotide and amino acid identities of 97.8% and 97.6%, respectively. Iraq's F1L envelope protein was similar to China and India's, while Italy's was identical.

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التحليل الوراثي مقارنة النشوء والتطور لجينات B2L و F1Lفي فيروس Orf المعزول من الأغنام المصابة بالعدوى

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الخلاصة

يشمل جنس الفيروس parapoxvirus فيروسات DNA كبيرة، بيضاوية، مزدوجة الشريطة تنتمي إلى عائلة Poxviridae معطفهم اللولبي الفريد يميزهم عن فيروسات الجدري الأخرى. يقوم جين B2L الفيروسي بتشفير بروتين مغلف كبير وعالي المناعة بالإضافة إلى جين F1L محفوظ للغاية، وقد لوحظت آفات Orf على الشفتين العلوية والسفلية والجفون العلوية أو السفلية وحول فم وأنف الأغنام. قيمت هذه الدراسة الأنسجة والوراثة لفيروس أورف في الأغنام القادسية المصابة بالإكزيما

المعدية. تتميز الأنسجة الإيجابية بتضخم البشرة مع تلال شبكية بارزة، وتنكس مائي للخلايا الكيراتينية النخرية، وبثور تحت القرنية. تم تثبيت العينات في البارافين وتقسيمها إلى شرائح بطول 5 سم. كان تفاعل البوليميراز المتسلسل على العينات المستخرجة من الحمض النووي موجبًا لكل من جينات B2L و F1Lتم تسلسل أربع عينات إيجابية وتسجيلها في بنك الجينات، وتم إجراء تحليل النشوء والتطور. يمكن أن يساعد علم التشريح المرضي والأعراض السريرية في تشخيص الإكزيما المعدية بسرعة وبتكلفة معقولة، بينما يميز تفاعل البوليميراز المتسلسل (PCR) بين الأمراض المتماثلة في المناطق الموبوءة, بمقارنة تسلسل الأحماض الأمينية المستخلصة من جين B2L غير المكتمل من السلالات العراقيةOK336711.1 OK336710.1، وغيرهم من العزلات الهندية، وجدنا أن موقعين يحتويان على تغيرات مختلفة في الأحماض الأمينية، مما أدى إلى هوية نيوكليوتيد وأحماض أمينية بنسبة 97.8٪ و97.6٪ على التوالي كان بروتين المغلف F1L للسلالة العراقية OK330734.1 مشابهًا لبروتين الصين والهند، بينما كان البروتين المغلف للسلالة الإيطالية OK330733.1 مطابقًا لبروتينات السلالة الإيطالية.

الكلمات المفتاحية: فايروس الاورف, جينB2L ,جينF1L , فيروسات الدنا.