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Original article

MOLECULAR DETECTION AND PHYLOGENETIC ANALYSIS OF OVINE HERPESVIRUS-2 IN SHEEP AND GOATS OF AL-QADISIYAH PROVINCE, IRAQ

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Summary

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This study aimed to identify ovine herpesvirus 2 (OHV-2) infections in sheep and goats in Al-Qadisiyah Province of Iraq, using molecular and phylogenetic methods. Nasal discharge swabs were collected from 60 sheep and 60 goats from 3 different animal sale bars. The samples were subjected to semi-nested-polymerase chain reaction (PCR), sequencing, and phylogenetic tests involving OHV-2 tegument protein gene (OHV-2T). The results of the semi-nested PCR showed the presence of OHV-2 in all 60 (100%) sheep and 52 (86.6%) goats. The samples from both sheep and goats were sent for partial-gene-based sequencing to confirm the PCR results. Phylogenetic analysis was conducted and 6 PCR amplicons (10%) of positive samples from each goat and sheep were submitted for sequencing. The sequence results were reassembled and deposited in the NCBI-GenBank database under the accession numbers of MF004402.1 for sheep and MG875327.1 and MG875328.1 for goats. Multiple alignments of sequences showed close identities with some global isolates of this virus. This study not only reports new sequences from the local OHV-2 isolates that have been deposited in the NCBI GenBank, but also provides important data about the presence and shedding of OHV-2 in the nasal discharge of healthy sheep and goats, and suggests OHV-2 as the major cause of malignant catarrhal fever in cattle.

Key words: goat, ovine herpesvirus 2, semi nested-PCR, sheep, tegument gene

INTRODUCTION

The ovine herpesvirus (OHV-2) is a gamma-herpesvirus that belongs to the genus *Macavirus*. This virus is present mostly in an asymptomatic status in sheep as the carrier animal. Due to poor adaptation of the virus in other animals, such as cattle, bison, and cervids, malignant catarrhal fever (MCF) in cattle is considered to be a sheep-associated infection. The infection causes a life-threatening disease that encourages lymphoproliferation and vasculitis (Davison *et al*., 2009; O'Toole &

Li, 2014), with no effective cure or vaccination yet (AlHajri *et al.,* 2017).

OHV-2 is one of the widely distributed viruses worldwide that causes MCF. It is a member of the gamma-herpesvirus family of herpesviridae. It causes severe to fatal disease in ruminants (Khudhair *et al.,* 2019). The tropism of the virus *in vivo* includes CD8+ T cells and epithelial cells. In sheep lungs, this tropism is mostly shown in alveolar type II epithelial cells (Simon *et al.,* 2003; Taus *et al*., 2010). The lack of proper cell-culture system limits a detailed understanding of the molecular properties of the virus, such as its entry pathway into host cells (AlHajri *et al.,* 2017). Perinatal infection usually does not occur in sheep; therefore, carrier animals (sheep and goat) play an important role in transmitting the virus across sheep and goats. This implies that separating the virus-free animals from the carrier animals may enable the prevention and control procedures (Li *et al.,* 1998).

In Iraq, especially Al-Qadisiyah province, the lack of molecular studies, such as phylogenetic analyses, delays the understanding of evolution of the virus and thus the prevention and control of the disease in susceptible animals. This study was focused on exploring ovine herpesvirus 2 (OHV-2) infections in sheep and goats in Al-Qadisiyah Province, Iraq.

MATERIALS AND METHODS

Ethics approval

The Animal Ethical Committee of the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq had approved the present study under R.N. 00100.

Animals and sample collection

Samples were collected from February 2017 to May 2017. One hundred and twenty animals (60 sheep and 60 goats) were selected randomly from 3 different animal sale bars (20 sheep and 20 goats from each sale bar). Animals were submitted for typical clinical examination of respiratory parameters such as nasal discharge, respiratory rate, respiratory type and rhythm, abnormal vascular sounds, body temperature, and mucus membrane of mouth and buccal cavity.

Nasal swabs were collected, and transported to a lab under cool conditions (2–8 °C), and the PCR assay was performed immediately in the College of Veterinary Medicine, University of Al-Qadisiyah, under sterile conditions.

DNA extraction

Extraction of DNA from nasal swab samples was performed using a kit (Geneaid, New Taipei City, Taiwan), based on the manufacturer's instructions. DNA yield was measured by NanoDrop (Bioneer, Korea) for quantity and quality.

Semi-nested-PCR technique

The OHV-2 tegument protein gene was used as a marker gene. Pan-primers (that can detect group of viruses associated with MCF in domestic and wild ruminants and classify as either OvHV-2, CpHV-2, or classical MCF in white-tailed deer virus) were used for the experiment. The primers used in semi-nested-PCR were: forward 556: AGT CTG GGT ATA TGA ATC CAG ATG GCT CTC (121692–121722) and reverse 755: AAG ATA AGC ACC AGT TAT GCA TCT GAT AAA (21312–121340). They were used in the first amplification of 442-bp DNA fragments. The internal reverse primer 555: 5′-TTCTGGGGTAGTGGC GAGCGAAGGCTTC-3′, with the same forward primer, was used for the second amplification (Baxter *et al*., 1993; Li *et*

al., 1995; Khudhair *et al.,* 2019). The PCR reaction mix for both amplifications consisted of 20 mM Tris HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 10 pM of each primer, 1 U Taq polymerase (IDT, Belgium), and 4 μL DNA (35–200 ng/μL). PCR was performed in a thermocycler under 95 °C/ 5 min for initial denaturation, followed by 40 cycles of 94 °C/2 min for denaturation, 52 °C/45 s for annealing, and 72 °C/60 s for extension, and 72 °C/7 min for final extension. The PCR products were tested using gel electrophoresis technique on 1.25% agarose gel and visualised by a Bio-Rad Gel Doc XR image analysis system.

Sequencing

Amplicons from sheep (6) and goats (6) were used in both-direction-based sequencing, using a sequencing kit applied in an automated sequencer (Macrogen, Korea), involving the above-mentioned external primers.

Phylogenetic analysis

The local strains were compared with global isolates of OHV-2. The phylogenetic tree was constructed using MEGA X (Version 10.0.5) software, following a rooted neighbour-joining tree (Saitou & Nei, 1987).

RESULTS

Clinical examination showed all animals to be healthy. The PCR results revealed high circulation of OHV-2 in sheep and goats of the study area; its presence being identified in all 60 sheep samples (100%) and 52 (86.6%) goat samples, as shown in Fig. 1. Semi-nested-PCR data, used for confirming the study results, have been depicted in Fig. 2. Since OHV-2 was detected at all ages in the animals, it might indicate hyperendemicity of the virus in the study area.

Sequences of amplicons were aligned using the MEGA X program; the ones with close identities (100%) were reassembled and deposited in the NCBI-GenBank database under the accession numbers of MF004402.1 for sheep and MG875327.1 and MG875328.1 for goats. The current isolates, when compared with global sequences, matched closely with those from Japan (LC203437.1), Germany

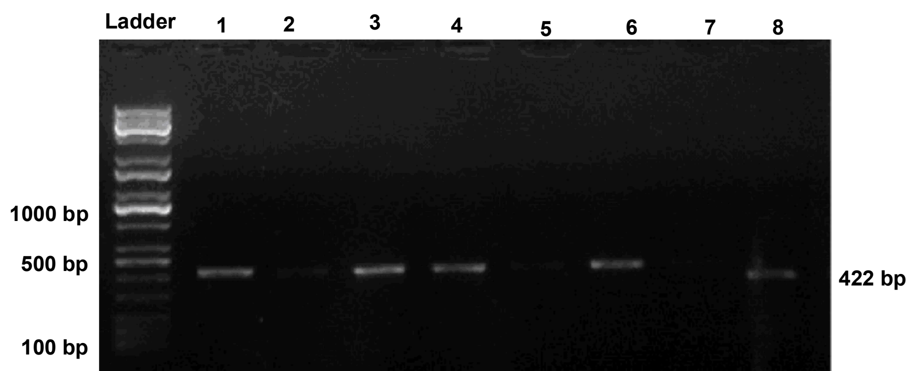

Fig. 1. Ethidium bromide-stained agarose gel electrophoresis of PCR products of OHV-2, using specific primers, from the nasal discharges of sheep and goats. Left to right: lane ladder, external primer reaction PCR of 8 samples of OHV-2 tegument protein gene, and the 422-bp fragment corresponding to the ladder.

Table 1. Comparison of the sequences of partial fragments of tegument protein gene of OHV-2 with published global sequences; those in bold refer to short distances among the sequences

Iraq_(sheep)_MF004402.1													
United_kingdom_HG813096.1	0.00												
Iraq_(Goat)_G-q2_MG875328.1	**0.00**	0.00											
South_Africa_EU851178.1	1.35	1.40	1.36										
Japan_LC203437.1	0.00	0.00	0.00	1.41									
Germany_HM216481.1	0.00	0.00	0.00	1.42	0.00								
Brazil_KC123170.1	0.01	0.02	0.01	1.38	0.02	0.01							
Iraq_(Goat)_G-q1_MG875327.1	**0.00**	0.00	**0.00**	1.36	0.00	0.00	0.01						
Italy_KJ420947.1	0.01	0.00	0.01	1.40	0.01	0.01	0.02	0.01					
Canada_KX060582.1	0.07	0.08	0.07	1.61	0.06	0.08	0.07	0.07	0.07				
Iraq_khiq2_KY635410.1	0.02	0.03	0.02	1.44	0.02	0.02	0.03	0.02	0.03	0.08			
Egypt_KT725443.1	0.00	0.00	0.00	1.34	0.00	0.00	0.01	0.00	0.01	0.07	0.02		
India_KJ020269.1	0.00	0.00	0.00	1.42	0.00	0.00	0.01	0.00	0.01	0.07	0.02	0.00	
Iraq_khiq1_KY635409.1	0.02	0.05	0.02	1.41	0.04	0.02	0.03	0.02	0.04	0.09	0.03	0.02	0.02

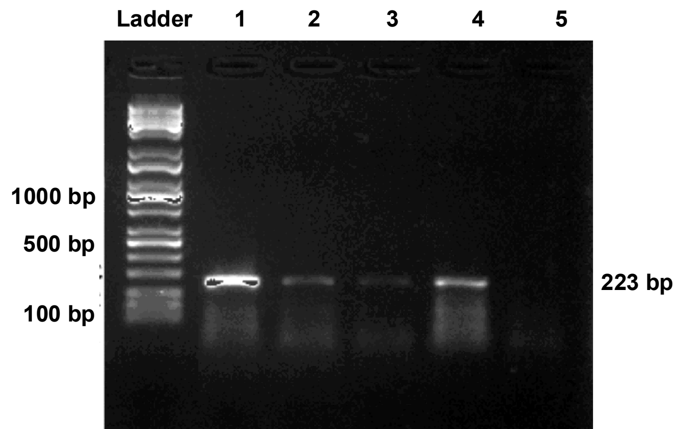

Fig. 2. Ethidium bromide-stained agarose gel electrophoresis of PCR products of OHV-2, using specific primers, from the nasal discharges of sheep and goat, internal primer-related semi-nested PCR of the four samples (1–4) found positive for OHV-2 in the same gene with 223 bp fragment length; only the 5th sample was negative.

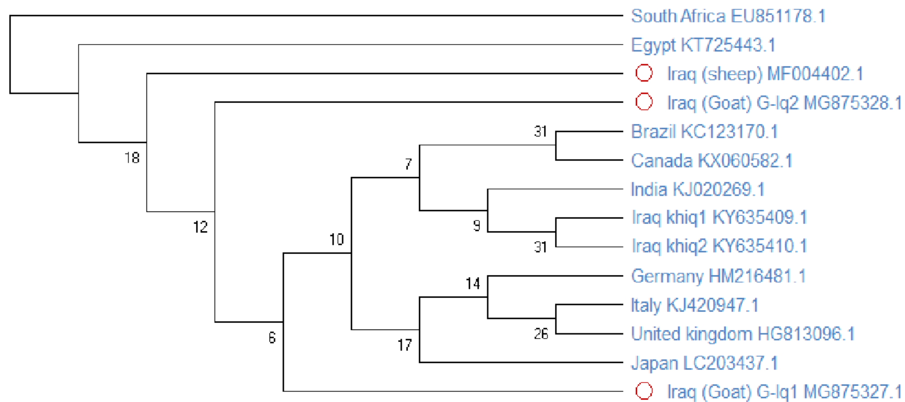

Fig. 3. Phylogenetic tree constructed using MEGA X software following a rooted neighbour-joining method. The local strains obtained in the current study were compared to some global isolates shown in the figure, with local and excision numbers of OHV-2.

(HM216481.1), Egypt (KT725443.1), and India (KJ020269.1), whereas they were highly distant from those of South Africa (EU851178.1) (Table 1).

Phylogenetic analysis, using 2 variant sequences from goats and 1 sequence after reassembly of sequences from sheep, showed close identities with those of some global isolates; the locations and excision numbers of the compared isolates are mentioned in Fig. 3.

DISCUSSION

Sheep and goats play crucial role in the origination and disseminated of the ovine herpesvirus (OHV-2) (Li *et al.,* 2014;

O'Toole & Li, 2014). The current study showed the presence of OHV-2 in the nasal discharge samples of sheep and goats. Using PCR, the percentage of OHV-2 was determined to be present in 60 (100%) sheep and 52 (86.6%) goats. The results agreed with those reported by Wiyono *et al.* (1994) using PCR, showing the presence of OHV-2 in Indonesian ruminants, normal sheep, and MCF clinical cases; the authors had suggested that an identical virus affecting the ovine might have affected goats, as per the PCR results. Despite goats being considered the natural hosts of CpHV-2, there is evidence of asymptomatically infected goats with OvHV-2 that transmitted the virus to a susceptible host (Chmielewicz *et al.,* 2001; Li *et al.,* 2001). A high percentage of goat infections with OvHV-2 suggested the goat as an important OvHV-2 reservoir and source of infection for susceptible animals. This disagrees with the previous observation that an MCF-like disease in goats presented with generalised arthritis and detectable OvHV-2 (Foster *et al.,* 2005).

PCR was considered to be an important tool in detecting OHV-2. The results also agreed with those by (Wiyono *et al.,* 1994), which showed OHV-2 in both normal sheep and in MCF clinical cases, using a PCR technique. Moreover, OHV-2 was detected in cows and sheep of Iran using PCR (Momtaz *et al.,* 2009). Experimental studies in susceptible species revealed two peaks of viral gene expression, the first being a pre-clinical peak, involving the respiratory system only, and the second being a clinical disease phase, involving multiple organ systems (O'Toole & Li, 2014). The first phase only occurs in small ruminants without clinical signs. Infectious virus gets transmitted from oral and nasal secretions of sheep and goats, during shedding episodes, to infect susceptible species (Li *et al.,* 2011; 2012).

The phylogenetic analysis results, using sequences of tegument protein gene of OvHV-2 from nasal swabs of sheep and goats, showed close identities with those of global isolates of this virus. These results showed close relationships between the current isolates and global isolates as demonstrated here. This might even suggest slight evolution of the Iraqi isolates studied here. The results of the phylogenetic analysis showed close identity (100%) between a sheep isolate and a goat isolate, indicating shared OHV-2 that may affect both sheep and goats. However, these two isolates showed far branching on the tree, from other goat isolates, suggesting that other different isolates may also affect goats (Doboro *et al*., 2019).

CONCLUSIONS

This study is possibly the first report of OHV-2 infection in sheep and goats in Iraq. Three new sequences of local OHV-2 isolates were identified in sheep and goats, and deposited in the NCBI GenBank. This study provides important data about the presence of OHV-2 in sheep and goats of the city studied, and the evolution status of this virus.

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