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Risk factors and genetic diversity of border disease virus in small ruminants in Nineveh province, Iraq

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Abstract

Gathering information about the status of the Border disease virus (BDV) would limit its propagation and make monitoring efforts more effective. Numerous BDV genotypes are globally widespread, according to various reports. In Nineveh province- Iraq, the phylogenetic analysis and some associated risk factors of BDV virus in sheep were the subjects of this groundbreaking work. Blood samples from 264 sheep were collected in different regions of Nineveh province from the period between June till December 2022. The analysis for the sequences of BDV Ribosomal RNA (rRNA) was performed using the online GenomeNet multiple sequence alignment tool (CLUSTALW). Following that, the sequences were blasted against other available BDV virus strains in the GenBank using NCBI BLAST (BLASTn) of NCBI. Neighbor-joining (NJ) mode was used to create the phylogenetic trees. The result revealed that 15.9% (42/264) of sheep tested positive for BDV, and the associated epidemiological aspects, including herd size and interspecies management, had a significant impact of ($P<0.05$) on this rate. Forty-two 5' UTR sequences were subjected to individual sequence analysis, which identified the genotypes of BDV in Nineveh province for the first time. This finding could be potentially benefitting future studies and management of this disease status in the study zone.

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Introduction

The border disease virus (BDV) is classified within the family Flaviviridae, genus Pestivirus. BDV frequently spreads between small ruminants, large ruminants, and pigs, presumably making its diagnosis challenging. The majority of infections and viral maintenance occur mainly in sheep flocks. BDV infection can result in significant financial losses, such as prenatal and postnatal infections (1). Moreover, it causes congenital disorders, abortions, stillbirths, weak lambs, hairy fleece, immunosuppression, and the possibility of contracting other infections, all of which cause significant financial losses to the animal industry (2). There are four primary members of the family Flaviviridae, genus Pestivirus, including bovine viral diarrhea virus types 1 and 2, classical swine fever virus (CSFV), and border disease virus (BDV), in addition to several pestivirus species were detected in various domestic and wild animals. The original members were recently divided into eleven viral species annotated with letters from A to K (3,4). The first BDV infection in sheep was documented in 1959 in the border regions between England and Wales (5). In sheep, the seroprevalence of BD may range from 5% to 90% or more; this depends on the animal husbandry; however, the death rate relies on the time of infection and the agent's virulence, and the type of the host (6). Although the clinical signs in sheep with acute infection are often minor, they can also be asymptomatic or clinically severe (7). Persistent infections (PI) occur in fetuses exposed to the BDV in early pregnancy since their immune system is not well developed. These young animals may show symptoms known as "hairy shaker syndrome." Also, these persistently infected animals are usually seronegative and shed the virus lifelong. In addition, these animals are

considered a primary source for virus distribution in the flocks (8,9). The disease's epidemiology depends on vertical transmission via the placenta. Recently, in Xinjiang (China), *Melophagus ovinus*, one of the external parasites in small ruminants, was found to mechanically transmit BDV (10,11). Based on the current separation of BDV in ovine and caprine and the genetic classification of genotypes, BDV could be phylogenetically divided into at least 8 genotypes (BDV-1 to BDV-8) (12). The discovery of other ovine pestiviruses that cause BD-like disorders suggested a distinct evolutionary history that includes different genetic subgroups. In particular, the CSFV is more closely related to BDV according to their phylogenetic relationship (13). A recent work discovered that CSFV and a newly emerged ovine pestivirus (OVPV), which is remarkably different from other pestivirus types, are closely related genetically and antigenically (14,15). This suggests that OVPV is considered a novel species. BDV in animal populations has been documented in several regions worldwide. However, the majority of the available information originates from Europe. In fact, sheep are the most often affected animals among the domestic and wild ones (1). The BDV-2 genotype only contains the German isolates and BDV-1 (subgroups BDV-1a and BDV-1b), formerly known as BDV-A and BDV-B (16). In Europe, the common genotype is BDV-3, then BDV-1, which is widespread worldwide. BDV-4, formerly BDV-C, is Spain's most common genotype (17). The initial reports from France showed the presence of BDV-5 and BDV-6 genotypes. Later, the BDV-8 genotype was discovered in Italy (12,18) and sheep, sheep, and pigs in Switzerland (19,20).

Previously, no phylogenetic study of the ovine border disease virus in Nineveh province has been accomplished. The current work aims to validate the phylogenetic analysis and certain risk factors related to BDV in sheep.

Materials and methods

Ethical approval

This study was ethically permitted by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Mosul (UM.VET.2022.053) on 15 May, 201822.

Animals and sample collection

Twenty local sheep flocks (from 1.5 to 6 years old) were used as samples for this study, conducted between June 2022 and December 2022. A semi-intensive or intense breeding approach was employed by most of the included farms, and sheep in these farms were grown and reared close to farms with dairy and beef cattle. The herd size was ≤40 and >40 animals. Blood samples were obtained from 264 sheep, 20% randomly selected from each farm. The animals appeared to be in good condition or may have previously had reproductive, respiratory, or diarrheal disorders. It was also established that none of these farms had ever provided BDV vaccinations. The blood was drawn from the jugular vein with a disposable needle into sterile anti-coagulant vacutainers, which were transported on ice to the laboratory. Before further testing, samples were kept at -20°C (21).

RNA extraction and amplification using RT-PCR

The 264 whole blood samples were used to extract the RNA using the ultRNA Column Purification Kit (Abm, Canada). The process was done as directed by the manufacturer. The Nanophotometer was used to verify the RNA content and purity of the samples (BioDrop. Germany). The conserved region that included the 5' UTR sequence of BDV was amplified (n = 264) in the next step. Previous research used persistently infected (PI) animals as positive controls, and their cDNA was used as a positive control (22). Whereas the cDNA of healthy animals was used as a negative control. The primers were used in this study comprising forward primer BD-F (5'-TCGTGGTGAGATCCCTGAG-3') and revers primer BD-R (5'-GCAGAGATTTTTTATACTAGCCAGCCTATRC-3') with the amplification size 225 base pair (23).

One-Step RT-PCR Kit (V6V-2J5, Canada) and the thermocycler (Optimus 96G, United Kingdom) were used in this step. The PCR protocol included mixing the following contents: 25 µl of (2X) One-Step RT-PCR Buffer, 1 µl of OneScript®, 2 µl of BestaqTM DNA Polymerase, 2.5 µl of forward Primer (10 µM) and 2.5 µl of reverse Primer (10 µM), then dH_2O was added up to 50 µl for each reaction. The PCR amplification cycle setting was as follows: 42°C at 30 minutes for the cDNA synthesis step (1 cycle), 94°C at 3 minutes for initial denaturation (1 cycle), 94°C at 30 seconds for denaturation, 94°C at 45 seconds for annealing and 72°C at 45 seconds for extension (36 cycles), and 72°C at 5 minutes for Final Extension step (1 cycle), based on (24).

Sequencing of cDNA

For purification and sequencing, 42 PCR amplicons from sheep tested positive for PCR were shipped to Macrogen Company (South Korea). The sequences of 16S rRNA were analyzed using multiple sequence alignment with the online tool (CLUSTALW) GenomeNet and then compared to other available BDV sequences in GenBank by NCBI BLAST (BLASTn) from NCBI (http://www.ncbi.nlm.nih.gov). The Neighbor-joining (NJ) and CLUSTALW (GenomeNet) tools were used to create phylogenetic trees (25). To create phylogenetic trees, the 16S rRNA gene sequences of BVDV-2 (AY443026) in Argentina cattle were employed as an outgroup (100 replicates).

Statistical analysis

The two-sided Chi-square and Fischer's exact tests were used by the SPSS program to assess the difference in prevalence between the main risk variables for BDV. The statistical significance was determined for the data at the P value of (≤ 0.05).

Results

The BDV antigen was detected using RT-PCR in 264 samples of blood. 15.9% (42/264) of samples showed evidence of the virus in animals. The positive animals were re-examined after 21 days to check for potential PI cases. According to the findings, the prevalence of PI among animals showed 1/42(2.38%).

The findings also demonstrated a significant difference (P <0.05) of BDV based on the herd size; the animals >40 heads were highly susceptible to the risk of infection (odds ratio = 1.8819, Cl: 1.2283 - 2.8833), P = 0.043 (Table 1). The results also show a significant difference (P<0.05) in the prevalence of BDV in animals with closed and interspecies contact. The contacted animals showed a higher risk of infection (odds ratio = 3.03, cl: 1.1295-8.1697), P=0.02 (Table 1).

Out of 264 sheep blood samples, 42 sequences of the BDV were detected in Nineveh province for the first time using the individual sequence analysis (BLASTn). These sequences (n=42) shared 100% of their similarities, and One of these sequences was submitted to GenBank and assigned the accession number (MT823310) (Table 2).

By comparing the retrieved local sequence (MT823310) of the 5'UTR of BDV genotype to the available database in GenBank, it was possible to show that the local sequence was closely related to those of Japan (AB122085.1), Germany (AF144618.1), and China (89% identity) as well as other regions (Table 3).

Additionally, the analysis of the phylogenetic tree using the neighbor-joining program revealed that the local BDV sequence was closely related (99% identity) to the other available BDV genotypes in the GenBank database, including Germany and Japan genotypes (AB122085.1 and AF144618.1), respectively. The tree was rooted with BVDV-2 (AY443026) as an outgroup (Figure 1).

Table 1: The risk variables of BDV in sheep

Factors	No. case tested	No. of +ve (%)	OR	CI	P
Herd size					
≤40	58	4 (6.89%) [a]	1		
>40	206	38 (18.44%) [b]	1.88	1.2283- 2.8833	0.043
Management					
Close (non-contact)	144	6 (4.16%) [a]	1		
Interspecies (contact)	120	14 (11.66%) [b]	3.03	1.1295-8.1697	0.02

OR: Odds ratio, CL: Confidence of interval, P: P value.

Table 2: The nucleotide sequence of 5'UTR for the local border disease virus QSK10-BD (MT823310.1)

Local genotype	Gene	Sequence	Accession No.
QSK10-BD	5'UTR	TAGTAGGACTAGCAAACGGGAGGACTAGCTTACGTGGTGAGAT CCCTGAGTGGTCTAAGTCCCGAGTACGGGGCAGTCGTCAGTAGT TCTACGCAATGTGGAGTTGCCTTGAGATGCTACGTGGACGAGG GCATGCCCAAGACACACTTTAACCCTGGCGGGGGTCGCCAGGG TGAACTCACCTAATGGTGTTGGGATTACAGCCTGATAGGGTGCT GCAGAGGCCCACGCATAAGTTAGTATAAAAATCTCTGCTGTAC ATGGCACATGGA	MT823310.1

Table 3: Homology between the local sequence (MT823310) of BDV and other genotypes using BLASTn

Name of strains	NCBI No.	Query cover	Identity	Country
Border disease virus strain Casimir gene, 5'UTR	AB122085.1	100%	270/272 (99%)	Japan
Pestivirus reindeer-1 V60-Krefeld complete genome	AF144618.1	100%	269/272 (99%)	Germany
Border disease virus isolate LA1108 5' UTR	EU637000.1	92%	241/252 (96%)	Germany
Border disease virus isolate J1004 5' UTR	EU637001.1	86%	226/236 (96%)	Germany
Border disease virus isolate chemnitz 5' UTR	EU637006.1	79%	209/215 (97%)	Germany
Border disease virus isolate ST1507 5' UTR	EU637003.1	79%	208/215 (97%)	Germany
Border disease virus isolate ST1405 5' UTR	EU637002.1	79%	207/215 (96%)	Germany
Border disease virus isolate Stolpe 5' UTR	EU636998.1	78%	208/214 (97%)	Germany
Border disease virus strain AH12-01 polyprotein gene	JQ946320.1	100%	245/274 (89%)	China

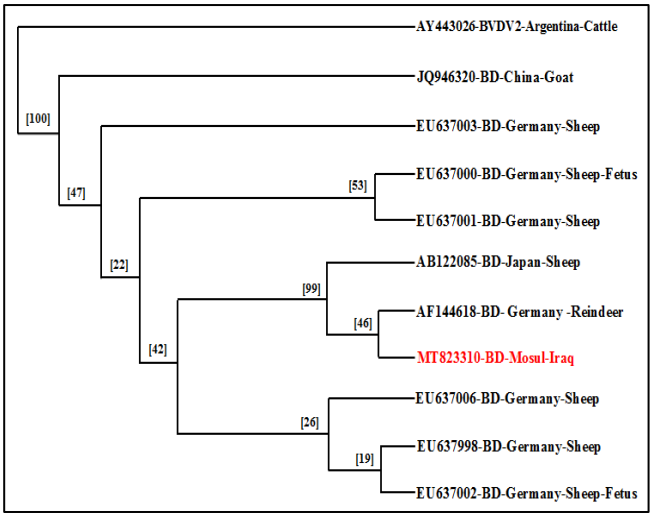

Figure 1: The partial sequences of the 5'UTR were used to build the evolutionary tree of BDV. The written code in red color represents the local BVD genotype, and the BVDV2 (AY443026) was used as an outgroup.

Discussion

Sheep and goats were infected with the border disease virus (BDV), which has a major effect on the reproductive health of different species (26). In this study, border disease was seen in sheep at a prevalence rate of 15.9%. The lack of immunization and/or control initiatives in Mosul City could be a critical factor in the disease prevalence. Additionally, importing animals from BDV-endemic regions such as Iran (24) and Turkey (27) is a significant cause of introducing diseased animals to Iraq (28).

According to the country or region of the inquiry, seroprevalence rates for BDV in sheep range from 5 to 50%. Prior serological studies in Iraq found that the BDV prevalence was 30.35% and 46.9% in sheep (22,29). Other potential factors include the possibility of interspecies transmission between sheep and other agricultural animals, including goats, beef cattle, and dairy cows, which may significantly contribute to increased disease prevalence. These results align with those of Braun *et. al.* (30), Karl *et al.* (31), and Hasan (32), who found that, while BDV is commonly thought to be a disease agent in sheep, it has no specific host and may infect a variety of domestic animals and wild animals' fauna. Furthermore, BDV can spread by fluid discharges from the diseased animals via aborted fetal excretions and blood. In some cases, PI animals in a farm are thought to contribute to the transmission of BDV. The findings listed above are potentially the reasons for the high incidence of BDV observed in the current study. These outcomes are in common with other researchers' conclusions of Oguzhan and Sibel (27), and-Yu *et al*. (33).

This study found that in large-size herds >40, the BDV was considerably more frequent than in small-size herds. This finding matches the result of Hasan and Alsaad (28), and Mohammadi *et al*. (34). This inconsistency in data might be due to various factors such as direct pasture contact, the ongoing introduction of animals, restricted space at housing, mortality, breeding, susceptibility, and the increased presence of PI in herds; the presence of more animals might imply a higher likelihood of infection. Also, these results are generally consistent with other investigations (35-37).
This work showed a significant difference in the prevalence of BDV in sheep between close and interspecies contacted animals, with the contacted animals being at higher risk (odds ratio = 3.03). This result aligned with those reported by Feknous *et al.* (38), and Fernandez *et al.* (39). Factors such as the viral transmission between cattle, sheep, and goats might partially account for differences. Natural BDV infections in cattle have been recorded in Austria (40), Italy (41), and New Zealand (42). According to previous studies, BDV and BVDV are not exclusively species-specific diseases. The latter could also contract BVDV acquired from PI animals with the potential to spread among them, as demonstrated in goats (43-45).

Following the configuration of the nucleotide sequences 100 times using Bootstrap analysis, the results of the phylogenetic tree of acquired sequence MT823310 of the 5'UTR sequence for BDV genotype revealed that it has mutual phylogenetic features and a substantial evolutionary (developmental) correlation between other viral genotypes in different regions around the globe, including Germany and Japan, with a percentage of 99% (11,46,47).

Conclusion

The results of this study show that sheep in Mosul City, Iraq, are highly susceptible to BDV. The disease frequency is greatly influenced by herd size and interspecies management. It is the first publication about the phylogenetic details of BDV in Mosul City, Iraq. Further advanced studies regarding pestivirus infection in ruminants in Mosul City are recommended by the present study.

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Conflict of interest

The authors claim that the paper has no conflicts of interest.

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عوامل الخطورة والتنوع الجيني لفيروس مرض الحدود في المجترات الصغيرة في محافظة نينوى، العراق

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الخلاصة

أن جمع المعلومات حول حالة فيروس مرض الحدود له دور في الحد من انتشار المرض، فضلا عن جعل جهود المراقبة والسيطرة عليه أكثر كفاءة. وفقا لتقارير مختلفة وموثقة، تنتشر العديد من الأنماط الوراثية لفيروس مرض الحدود على مستوى العالم. في محافظة نينوى - العراق، إن تحليل النشوء والتطور وبعض عوامل الخطورة المرتبطة بفيروس مرض الحدود في الأغنام كانت ضمن أهداف هذا العمل. تم جمع ٢٦٤ عينة دم من الأغنام في مناطق مختلفة من محافظة نينوى. تم إجراء تحليل تسلسل الحامض الرايبوسي باستخدام أداة محاذاة التسلسل المتعدد عبر الإنترنت .بعد ذلك، تمت مطابقة التسلسلات مع سلالات فيروس مرض الحدود الأخرى المتاحة في بنك جينات المركز الوطني لمعلومات التكنولوجيا الحيوية. كما تم استخدام وضع الالتحاق المتقارب لإنشاء شجرة النشوء والتطور. أظهرت النتائج أن ١٥,٩٪ (٢٦٤/٤٢) من الأغنام كانت موجبة لفيروس مرض الحدود، وكان لحجم القطيع والإدارة تأثيرا معنويا على معدل الانتشار. خضعت أثني واربعون تسلسل خضعت التسلسلات للتحليل الفردي، والذي حدد ولأول مرة واحدة من الأنماط الجينية لفيروس مرض الحدود في محافظة نينوى. يمكن أن يكون نتائج هذه الدراسة مفيدة في الدراسات المستقبلية وإدارة حالة هذا المرض في منطقة الدراسة.

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