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Molecular evidence of schmallenberg virus associated by ovine abortion with fetal anomalies in Nineveh province, Iraq

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Abstract

In late 2011, Schmallenberg virus (SBV) was observed in Germany using genomic analysis. The virus is transmitted through insect vectors and vertically from females to their offspring across the placenta. In adult sheep, the virus causes a short viremia followed by lethargy, abortion, and dystocia when giving birth to malformed lambs. RT-PCR for virus detection and commercial ELISAs for antibody detection were rapidly developed. No previous studies have detected SBV in sheep in Nineveh province. Thus, this study intended to investigate the presence of SBV in aborted fetuses and describe the macroscopic lesions. Fifteen aborted lambs aged between 70 to 135 days were collected between October 2021 and January 2022. Brain stem, spinal cord, spleen, liver, lung, and abdominal fluid were collected and stored at -20ºC for molecular analysis. Viral RNA was extracted from these collected samples, and reverse transcription was performed in one step. RT-PCR was applied to amplify the SBV gene (S segment). Three of fifteen lambs showed marked malformations in the vertebral column, arthrogryposis, hydranencephaly, cerebral and cerebellar hypoplasia, and porencephaly. SBV was detected in malformed aborted lambs by RT-PCR with 474bp product size. These findings indicate that SBV causes abortion with malformations. Further studies on this topic should include the isolation and characterization of the virus and SBV epidemiology.

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Introduction

Schmallenberg virus (SBV) is a negative-sense RNA virus with a single-stranded and belongs to the Bunyaviridae family, which includes hundreds of viruses that are pathogenic to vertebrates and invertebrate hosts (1,2). The viral genome is made up of three RNA segments called small (S), medium (M), and large (L). The RNA replicase is encoded by the L segment, while the M segment encodes the surface glycoproteins. The nucleocapsid and nonstructural proteins encoded by the S segment are involved in complement fixation and influencing host cell innate immunity (3). SBV has spread throughout large parts of the European continent since it was first detected in autumn 2011 in northwestern Europe (4-9). China, Lebanon, and Iran are affected (10-12). SBV antibodies were also detected in the blood obtained from slaughtered cattle, sheep, goats, and buffalo in Turkey between 2006 and 2010, showing that SBV was present in Turkey prior to the European outbreak (13). Antibodies against SBV were also detected in sheep in Duhok, Iraq (14). The virus is transmitted by insect vectors, particularly *Culicoides midge* bites (15-17). It is also transmitted vertically from females to their offspring across the placenta (18). In adult sheep, SBV causes a short viremia (5-6 days) followed by lethargy, abortion, diarrhea, and dystocia due to malformed lambs (19,20). Malformed vertebrae, joint spasms (arthrogryposis), neurological symptoms, and skeletal muscle hypoplasia can all be seen in utero infected lambs (21-26). SBV can be diagnosed by virus isolation,

neutralization tests, and detection of serological response in animals after virus infection. At the same time as the virus was detected, RT-PCR protocols were being developed (27-31). Primers that amplified a portion of the L gene were applied as a template for detecting the SBV genome for the first time. The approach for selecting the S region was optimized and found to have better diagnostic sensitivity (19,32). SBV can be detected in the cerebrum, placental fluid, and umbilical and spinal cord, according to studies, and a high concentration of SBV has been found in the brainstem (18,27). However, the SBV has not been identified as a causative agent for ovine abortion and fetal abnormalities in Nineveh province. As a result, the study's objectives were to explore the occurrence of SBV in aborted ovine fetuses and describe the accompanying macroscopic abnormalities.

Materials and methods

Ethical approve

The Institutional Animal Care and Use Committee, College of Veterinary Medicine, University of Mosul, accepted the sample collection methods on August 23, 2021, with approval issue number UM.VET.2021.27.

Samples collection

All samples tested were obtained from fifteen aborted lambs aged between 70 to 135 days from 15 farms in different parts of Nineveh province between October 2021 and January 2022. The brain stem, spinal cord, spleen, liver, lung, and abdominal fluid were collected from these aborted lambs and preserved at -20^{o}C until molecular analysis.

Extraction of RNA and synthesis into cDNA

A commercial kit (FavorPrep^{TM} viral RNA, Favorgen, Taiwan) was used to extract viral RNA from tissues and abdominal fluids according to the manufacturer's instructions. According to the manufacturer's instructions, reverse transcription was achieved in one step (AddBio Inc., South Korea). A 20-microliter reaction mixture comprised 7µl of RNA, 10µl of AddScript RT master (2x conc.), and 3µl of nuclease-free water. The thermal profile employed was 60 minutes at 50°C.

Amplification and gel electrophoresis

The RT-PCR was applied to amplify the S segment of SBV. A 20 µl PCR reaction consisted of 5µl of cDNA, 10µl of master mix (2x conc., AddBio Inc., South Korea), and one microliter of forward ($^{5`}$-AGTAGTGAACTCCAC-$^{3`}$) and reverse ($^{5`}$-GCCCCAGGTGCAAAT-$^{3`}$) primers, and three microliters of nuclease-free water (32). Amplification was carried out using a Bio-Rad thermocycler (Bio-Rad, USA) under the following conditions: one cycle at 95°C for 10 minutes, followed by 35 cycles at 95°C for 45 seconds, 56°C for 45 seconds, and 72°C for 45 seconds. Then, one cycle at 72°C for 7 minutes was set for the final extension. Finally, the reactions were cooled at 4°C until the gel electrophoresis proceeded. The amplified products were verified in a 1.5 % agarose gel prepared with 1x Tris-Borate-EDTA buffer and stained with a red safe DNA staining solution (GeNetBio, South Korea). The results were visualized using a UV transilluminator and digital camera (Bio-Rad, USA). DNA molecular weight marker 100bp (AddBio Inc., South Korea) was introduced in all electrophoresis.

Results

Macroscopic findings of aborted lambs

Three of the fifteen aborted lambs had significant spinal column abnormalities, including torticollis, curvature, kyphosis, and/or lordosis. Scoliosis and kyphosis were primarily found in the thoracic spinal column and were linked to various thoracic malformations, including a flattened ribcage and a reduced chest cavity. Furthermore, the three lambs had varying degrees of arthrogryposis multiplex congenital (AMC). Both the forelimbs and the hindlimbs were afflicted, with the majority being bilaterally symmetric. Hydranencephaly (absence of cerebral hemispheres in the brain), hydrocephaly, cerebral and cerebellar hypoplasia, and porencephaly were also discovered during the necropsy (Figure 1).

RT-PCR test

SBV, S segment gene, was detected in three malformed aborted lambs. A 474bp product size revealed that the brain stem, spinal cord, spleen, liver, lung, and abdominal fluid were all positive in RT-PCR with a negative control; there was no amplification (Figure 2).

Discussion

The occurrence of stillborn deformed lambs in our province and the identification of SBV in neighboring countries led us to explore the existence of SBV in ovine malformed aborted fetuses. There has been no report of SBV detection in aborted ovine fetuses in Iraq's Nineveh province. As a result, the study aimed to investigate samples from malformed aborted fetuses to determine the presence of SBV and describe the macroscopic lesions. SBV is a new emerging arthropod-borne virus reported as a novel viral disease in cattle, sheep, and goats worldwide (6). Embryonic loss, abortion, and stillbirth have all been associated with SBV infection. Fetal death can be caused by abnormalities in the placenta, embryo, or fetus (19). The CNS, skeletal muscle, and axial skeletons are the most affected organs or systems (21). For the first time, we described SBV abnormalities in aborted ovine fetuses. The results of the postmortem revealed severe abnormalities. The CNS abnormalities were thought to be the cause of

musculoskeletal deformities. Arthrogryposis and spinal column abnormalities would develop in these fetuses due to this pathogenesis because skeletal muscle motor units without their innervating lower motor neuron fail to develop correctly and become hypoplastic. Arthrogryposis of joints and vertebral column deformity would occur if the appendicular and axial muscles were denervated. Arthrogryposis in the forelimbs and/or hindlimbs was linked to neuronal absence in the cervical and lumbar intumescences (33).

Figure 1: SBV macroscopic findings. Aborted lambs with varying degrees of arthrogryposis multiplex congenital (AMC). Both the forelimbs and the hindlimbs were afflicted, with the majority being bilaterally symmetric (A, B, C). Torticollis, scoliosis, kyphosis, and/or lordosis were spinal column malformations (D, E, F). Hydrocephaly, hypoplasia, and porencephaly of cerebral and cerebellar hemispheres replaced the cerebral hemispheres (G, H, I).

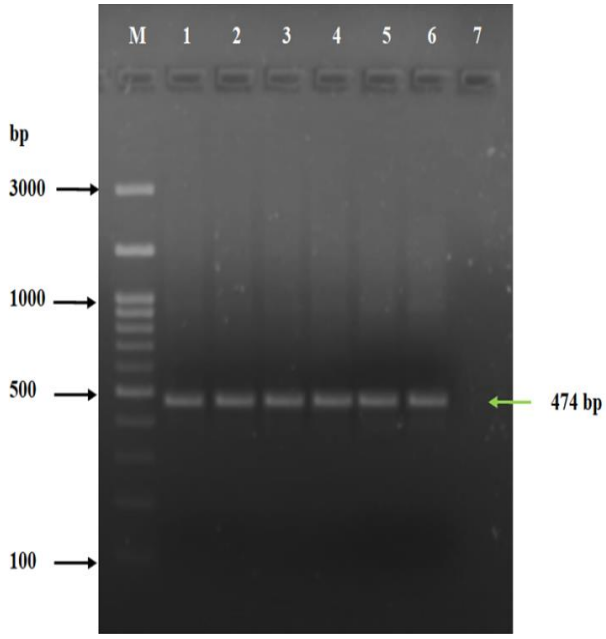

Figure 2: Gel electrophoresis of RT-PCR products. Lanes M, reference marker; lanes 1-6, positive samples of S segment gene for Schmallenberg virus; lane 7 negative control.

Furthermore, hydranencephaly, hydrocephaly, cerebral and cerebellar hypoplasia, and porencephaly have been observed during necropsy (Figure 1). These findings are consistent with those of others (34,35), who found hydranencephaly, porencephaly, hydrocephalus, cerebellar hypoplasia, and micromyelia in aborted lambs. This type of lesion could be caused by inflammation in the central nervous system (36,37). The explanation of the RT-PCR result is presented in figure 2. The three deformed lambs' brain stem, spinal cord, spleen, liver, lung, and abdomen fluid were all positive for the S segment of SBV RNA. Our results concur with other studies; they also detected SBV in aborted fetuses, with neurological signs and malformed fetuses (4,27,38). Several SBV RT-PCR assays were carried out using different primers that target the M, L, and S segments of RNA. After comparing the results, the SBV-S assay was shown to be the best for identifying the SBV genome, with excellent sensitivity and accuracy. These findings led to S segment targeting primers in this study (39,40). The emergence of SBV in Nineveh province, Iraq, is likely due to several factors, including climate and ecological changes, economic interchange, and trading changes. All of these variables combined to produce ideal conditions for spreading infected vertebrate hosts and invertebrate vectors across large geographic areas, particularly in our country, which was forced to import a large number of livestock (41,42).

Conclusion

The musculoskeletal and neurological systems detected the most common abnormalities in naturally infected ovine malformed aborted fetuses with SBV. The virus was first detected using RT-PCR from aborted fetuses in Nineveh province. SBV appears to be the cause of deformed abortions, according to these findings.

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Conflict of interest

According to the authors, there are no conflicts of interest in publishing this work.

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الدليل الجزيئي لفيروس شمالنبرغ المرتبط بالضأن المجهضة للأجنة المشوهة في محافظة نينوى، العراق

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الخلاصة

في أواخر عام ٢٠١١، لوحظ فيروس شمالنبرغ في ألمانيا باستخدام التحليل الجيني. حيث ينتقل الفيروس من خلال الحشرات الماصة للدم فضلا عن انتقاله عموديًا من الإناث إلى مواليدها عبر المشيمة. في الضأن البالغة، يبقى الفيروس في الدم لفترة قصيرة يتبعها الخمول والإجهاض وعسر الولادة عندما تكون الحملان مشوهة. طور اختبار تفاعل البلمرة المتسلسل- النسخ العكسي للكشف عن الفيروس واختبار الاليزا التجاري للكشف عن الأجسام المضادة. لا توجد دراسات سابقة حول الكشف عن الفيروس في الضأن في محافظة نينوى. لذلك عدت هذه الدراسة للتحري عن وجود فيروس شمالنبرغ في أجنة الضأن المجهضة ووصف الآفات العيانية المرتبطة به. تم جمع خمسة عشر حملًا مجهضا، يتراوح أعمارهم بين ٧٠ إلى ١٣٥ يومًا، ما بين تشرين الأول ٢٠٢١ وكانون الثاني ٢٠٢٢. حيث جمع جذع الدماغ والحبل الشوكي والطحال والكبد والرئة وسوائل البطن وتم حفظها بدرجة حرارة -٢٠°م لحين إجراء التحليل الجزيئي. تم استخلاص الحمض النووي الريبي الفيروسي من العينات التي تم جمعها ومن ثم اجري النسخ العكسي بخطوة واحدة. كما تم إجراء تفاعل البلمرة المتسلسل- النسخ العكسي لتضخيم الجين (جزء س) الخاص بفيروس شمالنبرغ. أظهرت ثلاثة من خمسة عشر حملًا تشوهات ملحوظة في العمود الفقري واعوجاج المَفْاصِل وموه الدماغ ونقص تنسج الدماغ والمخيخ وتَنَخُرُبُ الدِّماغ. تم الكشف عن فيروس شمالنبرغ في الحملان المجهضة والمشوهة باستخدام اختبار تفاعل البلمرة المتسلسل- النسخ العكسي بحجم ٤٧٤ زوج قاعدي. أشارت النتائج إلى أن فيروس شمالنبرغ تسبب في الإجهاض مع التشوهات. دراسات أخرى حول الموضوع يجب أجراءها وتشمل عزل وتوصيف ووبائية فيروس شمالنبرغ.