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Research article

Assessment of human and ovine respiratory syncytial virus in some respiratory cases in Al-Qadisiyah City, Iraq

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Abstract

The purpose of this study was to use real-time PCR to verify the detection of RSV in nasopharyngeal and throat swabs from hospitalized children less than 5 years who had influenza-like disease but moderate-to-severe pneumonia, and bronchial swabs from carcasses of sheep in Diwaniyah slaughterhouse. Viral RNA was detected in 28% (5/18) of children and 9% (4/45) according to real-time RT-PCR results. There was a noticeable circulating infection with HRSV and ORSV 2019, in which the findings demonstrated a substantial viral load. Finally, it should be noted that RT-PCR can help reduce severe disease and is advised for the diagnosis of RSV virus infections in pneumonia cases involving younger patients and sheep.

Keywords: ARTI, HRSV, ORSV, RT-PCR

Introduction

Globally, acute respiratory tract infection (ARTI) is a major cause of illness and mortality, with respiratory syncytial virus (RSV) constituting the most common cause. In both people and calves, respiratory syncytial virus causes serious respiratory infections. HRSV is a significant cause of pulmonary disease in newborns, children, immunosuppressed adults, and the elderly (1). In the absence of a vaccine, preventing and treating HRSV disease is a big issue. Less is understood regarding the way RSV affects sheep, however in some regions of the world, sheep are frequently seropositive for the virus, with 35–64% of animals having antibodies (2). Bovine, human, and ovine strains of RSV can infect sheep; in fact, newborn lambs have been suggested as a model for researching respiratory disorders in humans (3). Sheep virus infection with ovine genotypes of the virus is not widely recognized (36). According to their respiratory system, sheep are susceptible to respiratory disorders (4). Infection with ovine genotype of the virus has not been extensively researched in sheep. The illness manifests as yearly winter outbreaks in temperate climates. Re-infections occur throughout life, however the initial episode is usually the most severe (5).

Pneumovirus, RSV is an RNA virus, with a single-stranded RNA molecule of negative-sense orientation that is classified in the genus of the Paramyxoviridae family (6). It is called RSV because F proteins in the outer coat cause the cell membranes of adjacent cells to fuse and become syncytia. It has Genera A and B, which are distinguished by shifts in antigens in the G, F and N domains, and the initial steps of infection are governed by two major surface glycoproteins in the RSV virion: the attachment glycoprotein (G) and the fusion glycoprotein – F – which fuses the virion membrane with the membrane of the target cell (7,8). RSV isolates from both the A and B subgroups share a significant degree of amino acid sequence identity (at least 90%) in the F glycoprotein (7). The most popular

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procedures used in laboratories to diagnose RSV infections include ELISA and the rapid test, RT-PCR can also be used for carrying out an assessment (5).

This work aims to identify RSV infections using an real time PCR assay from particular clinical samples of throat swabs and nasal secretions exhibiting in upper respiratory tract infection from hospitalized children and from lungs of sheep carcasses soon after slaughter create risks to the safety and health of workers as well as the welfare of livestock.The purpose of this study was to use real-time PCR to verify the detection of RSV in nasopharyngeal and throat swabs from hospitalized children less than 5 years who had influenza-like disease but moderate-to-severe pneumonia, and bronchial swabs from carcasses of sheep in Diwaniyah slaughterhouse. Viral RNA was detected in 28% (5/18) of children and 9% (4/45) according to real-time RT-PCR results. There was a noticeable circulating infection with HRSV and ORSV 2019, in which the findings demonstrated a substantial viral load. Finally, it should be noted that RT-PCR can help reduce severe disease and is advised for the diagnosis of RSV virus infections in pneumonia cases involving younger patients and sheep.

Keywords: ARTI, HRSV, ORSV, RT-PCR

Materials and methods

Ethical approval

The study was approved by the Ethical committee at the College of Veterinary Medicine, University of Al-Qadisiyah. The parents' consents were obtained to collect the samples from their children and use these samples in the current study. The approval No. was 46 on Novamber 12, 2018.

Samples collections

During one month (March to April 2019), the investigation comprised 23 children clinical specimens of nasopharyngeal secretions and swabs of the throat. They were gathered from a Hospital for Chest and Respiratory Diseases in Al Diwaniyah, Al-Qādisiyah Governorate. Children less than 5 years who had been admitted with breathing difficulties symptoms with different clinical features showed that wheeze, fever, and cough were more prevalent than other signs. Samples were collected in 5 ml of transport buffer (phosphate-buffered saline with 10% glycerol, 1 mg/ml gentamicin, 8 IU/ml penicillin, 8 µg/ml streptomycin, and 0.02 IU/ml amphotericin B) and stored at 4°C (Invitrogen, Carlsbad, CA) (9). According to (10), 49 Awassi sheep lung samples less than 1 year old were collected from the Diwaniyah Slaughterhouse, the animals demonstrating severe pathological symptoms of pneumonia, such as inflated pleura, areas of consolidation, substantial lung mottling, and cranio-ventricular consolidation. Trained veterinarians found tired lungs in the corpse trays and took samples for analysis. After exposing the dorsal section of the trachea with a scalpel, proceed clockwise from the right cranial lobe to clean each bronchi tree using a sterile, viscose-tipped sample collection swab. In a slaughterhouse, the knife was temporarily cleaned at a hot water station after each set of lungs. The bronchial swabs were immediately collected and submerged in 1 mL of RPMI, placed on damp ice, and frozen.

We tested all 72 samples from human and domestic ruminants using RT-PCR in the Zoonotic Diseases and Research's Unit / Veterinary Medicine College, and Al-Qadisiyah University during 2019.

Molecular methods

QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was employed for RNA extraction. At -120 °C, the RNA was stored. The F gene primers (11) and RSV A and B were F:

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5′AACAGATGTAAGCAGCTCCGTTATC -′3 and R: 5′-CGATTTTTATTGGATGCTGTACATTT-′3 ordered from Macrogen, Inc., Korea. In 20 µl total of reaction, 10 µl 16-30.8 ng/µl RNA, 0.5 pmol for each primer were used. In an Applied Biosystem 7500, one cycle of 30 minutes at 50°C and 15 minutes at 94°C, followed by 45 cycles of 10 seconds at 95°C and 1 minute at 60°C.

Results

Result of real-time reverse transcription-QPCR (RT-PCR) assay for children samples (5 positive, 18 negative) 28% and domestic ruminant (sheep) samples (4 positive, 45 negative) 9% for RSV in comparison with positive control and healthy cases. Despite the fact that all patients were treated with different antibiotics and none of the sheep had received immunizations, Figures 1 and 2 represent the positive samples compared to the control, and healthy samples.

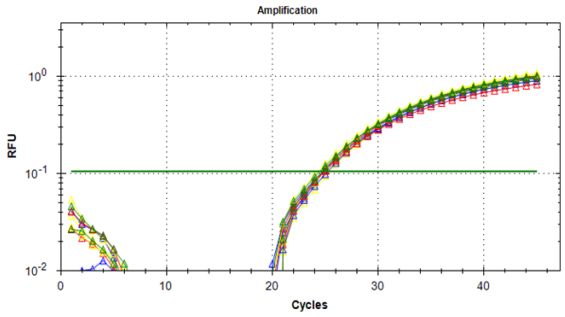

Figure 1: Real-time PCR amplification plots of the F gene in respiratory syncytial virus clinical isolates. Where the red plot (control group), the green plots (humans), the blue plots (sheep), and the yellow plots (healthy samples from humans and sheep).

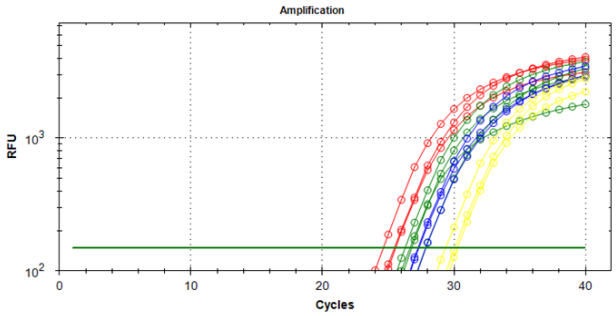

Figure 2: Real-time PCR amplification plots of the F gene in respiratory syncytial virus clinical isolates. Where the red plot (control group), the green plots (humans), the blue plots (sheep), and the yellow plots (healthy samples from humans and sheep).

Discussion

ARTI is one of the world's most serious public health issues because of its high prevalence and ease of transmission in society (12). Respiratory viruses are among the most prevalent pathogens causing ARTI (14, 12). The investigation of respiratory virus incidence is critical to the prevention and management of these diseases (13, 12). Lambs are a suitable model for studying RSV in people due to parallels in lung alveolar development, immunological responses, and resistance to infection (14–16). Pulmonary dysfunction in lambs following RSV infection is comparable to that seen in humans (3, 17). RSV spreads by direct contact with infected people or their contaminated subjects (18) of 4-6 days of incubation to show mild cold-like symptoms in children (19). Stressful conditions such as hot, dry weather, dust, summer storms, first shearing, and grain feeding can lead to illness epidemics in lambs. Four viruses have been linked to respiratory disease in sheep: adenovirus, reovirus, parainfluenza virus type 3, and respiratory syncytial virus (20, 21). Little is known about the incidence

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of infections caused by viruses in sheep in Iraq, including RSV.

One of the most crucial instruments in veterinary and medical diagnosis is RT-qPCR, which offers a 100 times more accurate approach than traditional PCR for the quick, precise, and sensitive detection of ORSV (22, 23). Therefore, we assessed the prevalence of RSV by employing RT-PCR, and several studies (25, 35) have examined the molecular epidemiological status of RSV employing the F gene sequences, but with certain limitations (24, 25).

The current study rate was 28% in children. Al-Charrakh et al. (26) showed a percentage of 18.75% in Wasit City, and Hassan et al. (27) revealed (20.4%) in the Kurdistan region (28) and (17.33%) in Baghdad (29).

RSV was studied in 2020 in Iraq, in cattle in the Ninawa district, and in people in multiple studies conducted in 2018. However, neither Iraq nor the majority of Arab nations have conducted sufficient molecular research on the disease ORSV (28, 30). This study found numerous ORSV clinical manifestations, and these clinical indicators are consistent with findings from investigations of experimental and natural infections. In a 1995 investigation on experimental infections in Japan, fatigue, coughing, and serous nasal discharge were among the moderate clinical signs (37) additionally in accordance with the findings of an additional study carried out in Turkey (2017) (2). In addition, bighorn sheep in the USA that have an ORSV infection may exhibit signs such as coughing and nasal and eye discharge (31, 32). The results of the molecular prevalence of ORSV in Awassi sheep were 9%. In the present research, which indicates that the rate by using the F gene was 9%, which is less than that in Mexican sheep (38.4%) (2), another comparable investigation carried out in Turkey in 1996 found that the ORSV infection rate was 35.77%, which was lower than the findings of the previous study (33). Additionally, the overall incidence of ORSV infection was much lower in Turkey's Marmara region, where it reached 50% based on immunohistochemical approaches (34).

Conclusion

RSV is a very important infection that results in mild to catastrophic respiratory complaints and, in severe cases, leads to mortality. There are studies confirming its presence in the middle and south of Iraq. This study is the first molecular study to detect the presence of RSV and ORSV in children and sheep in Diwaniyah province by ustilizing F gene as a target for molecular detection.

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Conflict of Interest

The authors disclose no conflicts of interest.

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