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Comparative Evaluation between Nested RT-PCR and Mono-Screen Antigen ELISA for Diagnosis of Bovine Respiratory Syncytial Virus in Cattle

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THE MOST frequent cause of bovine respiratory illness in the world is respiratory syncytial virus (BRSV) of cattle. The objective of the present investigation was to detect the bovine respiratory syncytial virus (BRSV) utilizing nested RT-PCR and a mono-screen antigen-enzyme-immunosorbent assay (Antigen-ELISA). It also searched for to evaluate the success of the diagnostic techniques. A total of four hundred and fifty nasal swabs from suspected respiratory infection cattle of local breeds aged 1-4 years were obtained. Out of the Four hundred and fifty nasals wabs, 168(37.3%) samples were positive to BRSV using RT-PCR technique (P< 0.05), while 139 (30.8%) samples were positive to BRSV using direct ELISA. However, 29 (6.4%) animals were negative results for the direct ELISA test but positive results for the RT-PCR technique. Considering antigen ELISA as the gold standard, the sensitivity and specificity of RT-PCR technique were 82.70% and 97% respectively. Based on Kappa value was (0.838), a perfect agreement between the two diagnostic methods. In conclusion , the One Tube RT-PCR System Script RT-PCR Premix Kit had the best sensitivity, specificity, and agreement when compared to the mono screen Antigen enzyme-linked immunosorbent assay BRSV /Sandwich double well (Antigen -ELISA). This implies that BRSV infection is detected using both assays. Antigen-ELISA is a sensitive, inexpensive test that may be used for routine diagnosis.

Keywords: Cattle, ELISA, Respiratory syncytial virus, Nested RT-PCR, Nasal swabs.

Introduction

Respiratory syncytial virus or recently the bovine orthopneumovirus is a frequent reason of respiratory illness in animals of all ages. The virus can lead to enzootic pneumonia in calves. [1-2]. The nose is where BRSV predominantly replicates. The virus invades the upper and lower airway epithelium, resulting in inflammation, cellular damage, and blockage of the airways. [3]. The negative-strand RNA virus BRSV is an enveloped, non-segmented member of the Orthopneumovirus genus [4]. Lung lavage, tracheal washes, and nasal swabs taken from live cattle or post-mortem samples for viral isolation and identification of cytopathic effects in cell cultures are some of the specialized methods for BRSV infection detection that have been established [5].The most widely used techniques for the diagnosis of BRSV are virus isolation, serum neutralization test (SNT), complement fixation test (CFT), immunoprecipitation (IP), and enzyme-linked immunoassay (ELISA) [6–7]. Immunofluorescent antibody (IF) and enzyme-linked immunosorbent (ELISA) assays, which have 47% and 60% sensitivity for BRSV, respectively, are serological tests that are used to identify viral infection[5]. The gold standard

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for BRSV diagnosis at the moment, reverse transcription-polymerase chain reaction (RT-PCR) study, offers 99% sensitivity and specificity [8]. Some veterinary diagnostic laboratories are now unable to perform real-time PCR on a regular basis. As a result, sensitive and quick BRSV diagnosis techniques are required for everyday usage [9]. The BRSV ELISA kits that are offered for sale are easy to use and interpret[10-11]. The current study's objective was to identify the bovine respiratory syncytial virus (BRSV) using nested RT-PCR and a monoscreen BRSV antigen ELISA (Bio-X, Rochefort, Belgium) .Additionally, a comparison was conducted to assess the effectiveness of the two tests performed in identifying the BRSV infection.

Material and Methods

Ethical approval

This study was ethically permitted by the animal ethics committee of the College of Veterinary Medicine at the University of Mosul, Iraq (15 decision number in 1-9-2017) has acknowledged the current study.

Sampling

A total of four hundred and fifty nasal swabs were collected from local breeds cattle aged 1-4 years that were not received the BRSV vaccination and thought to be infected with bovine respiratory diseases, during 2018 from different cattle farms in Nineveh province, Iraq.

Two nasal swabs were simultaneously utilized, one for PCR and one for antigen ELISA. After cleaning the nostrils with a paper towel, the swab was placed into each nostril to a depth of 5 cm, switching between the right and left nostrils. The nasal septum was gently moved up and down four times as the swab was pushed against it. All swabs were kept at 20 °C until examined.

Laboratory analysis

By applying the PrimePrep Viral RNA/ DNA Extraction Kit (Genet BioInc., Korea), nested RT-PCR RNA was extracted from the swabs closely according to the manufacturer's instructions. Utilize a Nanophotometer TM P-Class (Implen, Germany) to measure the purity and concentration of the extracted DNA.Using the One Tube RT-PCR System Script RT-PCR Premix Kit (GeNet Bio Inc. in South Korea), nested reverse transcriptase polymerase chain reactions were produced. The RT-PCR mixture comprised 10 microliter of SuPrime Script RT-PCR Premix, 3.5 microliter of PCR-grade water, 1 microliter each of forward and reverse primers, 1 microliter of magnesium chloride, and 3.5 microliter of extracted RNA.

There were utilized two pairings of each of the following primers: the first pairs of primers were F: B5A (5'-CCA CCC TAG CAA TGA TAA CCT TGAC-'3), and a R: B6A (5'-AAG AGA GGA TGC (T/C) TT GCT GTGG'3), were used to amplify a 603 bp region in the G protein gene, and the second pair of primers were F: B7 (5'-CAT-CAATCCAAAGCACCACACTGTC-'3) and a R: B8 (5'-GCTAGTTCTGTGATTGTTGTC'-3) were used to amplify a 372 bp region in the G protein gene [12].

After 30 minutes of reverse transcription at 50 °C, the sample was denaturated for 5 minutes at 95 degrees Celsius. A 35 cycles of amplification were carried out at the following temperatures: thirty seconds at 95 degrees Celsius, thirty seconds at 57 degrees Celsius, and sixty seconds at 72 degrees Celsius. After 5 minutes of final extension at 72 degrees Celsius, the reaction was stopped. Both the first and second PCR reactions involve these steps. On a 1.5 % agarose gel stained with ethidium bromide, the results of the polymerase chain reaction were detectable [13].

Enzyme-Linked Immunosorbent Assay

A Sandwich Double Wells (single Screen antigen ELISA BRSV). The Mono Screen Antigen ELISA Kit (Bio-X, Rochefort, Belgium) was also used to test all samples. The analysis was proceeded in accordance with the manufacturer of the kit's instructions.

The plates were examined at 450nm using an ELISA reader (Thermo USA). The optical density (OD) in well coated with viral antibody was corrected by subtracting the OD value of corresponding negative control. Percent positivity values of sample were calculated as given below:

$$\text{Val(ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

Samples value was then compared with positive reference supplied with manufacturing kit.

Statistical analysis

All of the data from the current study underwent statistical analysis utilizing an online statistical tool (2-way Contingency Table Analysis) , In https://statpages.info/ctab2x2.html.

Results

Out of the 450 swabs, 168 (37.3%) were positive using RT-PCR (Figs 1-2), while 139 (30.8%) were positive using direct ELISA detected viral antigen. However, 29 animals (6.4%) were both positive in the RT-PCR technique and negative in the direct ELISA test. (Table 1). Considering antigen ELISA as the gold standard, the sensitivity of RT-PCR was 82.70%, the specificity of RT-PCR was 97%, and based on the kappa value was (0.838), a perfect agreement between the two diagnostic methods (Table 2).

Discussion

Viral respiratory illnesses in cattle can result in a herd's continued infection and generate large financial losses [2-14-15]. In order to control and eradicate the disease, it is crucial to apply accurate diagnostic tools to identify the infection's causal agent [16–17]. Using RT-PCR and antigen ELISA, a total of 168 (37.3%) and 139 (30.8%) samples from 450 nasal swabs were found to be positive in the current analysis. According to the findings of our research, the diagnosis of viral infection in cow herds should be made using a number of severely ill animals and

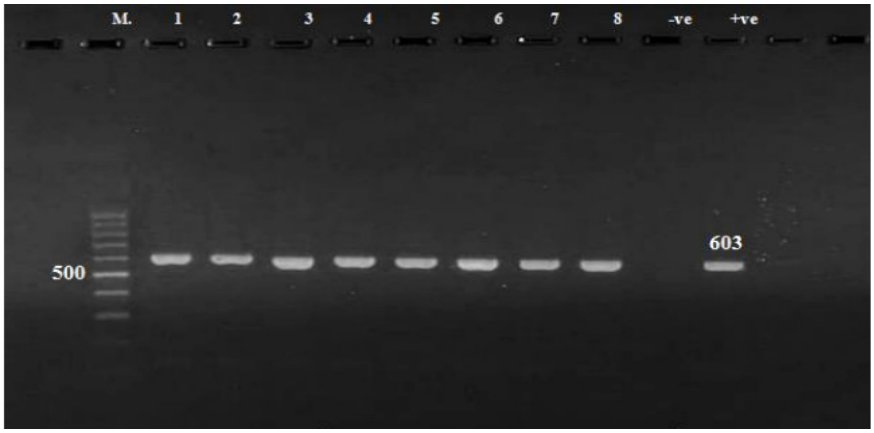

Fig. 1. Reveals results of PCR in 1.5 % agarose gel stained with Ethidium bromide under UV. of first RT-PCR reaction products using specific primers B5A and B6A of G protein gene for detection of Bovine Respiratory Syncytial Virus (BRSV) from nasal swabs of cows .Expected band size is 603 bp. Lanes 1 -8 are the positive samples; ve + and ve- are the control positive and negative samples; M is the molecular DNA ladder (100-1000 bp

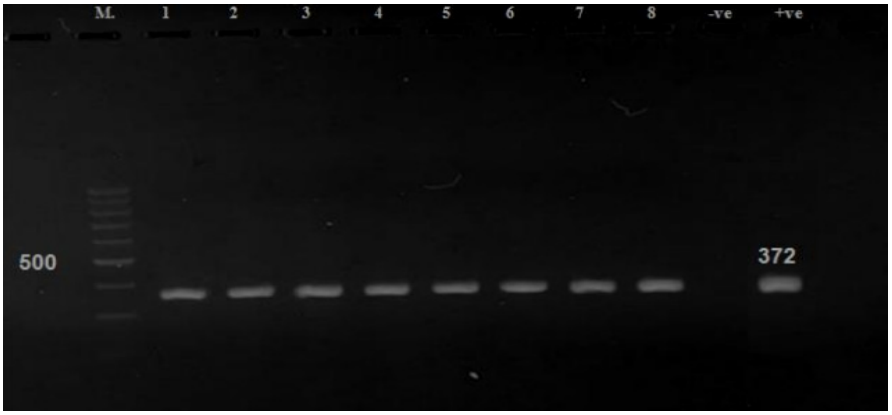

Fig. 2. Reveals results of PCR in 1.5 % agarose gel stained with Ethidium bromide under UV. of second RT-PCR reaction products using specific primers B7 and B8 of G protein gene for detection of Bovine Respiratory Syncytial Virus (BRSV) from nasal swabs of cows .Expected band size is 372 bp. Lanes 1 -8 are the positive samples; ve + and ve- are the control positive and negative samples; M is the molecular DNA ladder (100-1000 bp

several tests on each animal [17]. A commercial respiratory syncytial virus ELISA was released recently. It has been demonstrated to be effective in the direct identification of RSV antigens with bovine characteristics in nasopharyngeal washes and nasal swabs [9–18]. These examinations are quick, precise, and simple [19]. Viral isolation techniques are insufficient for identifying infectious BRSV from clinical samples because BRSV is too unstable to survive in transit circumstances [20]. This finding is in agreement with the observation reported by Masot et al. [21] who thought that ELISA was the best method for detecting BRSV antigen in lung tissue . Oguzhan et al. [19] attempted antigen capture direct ELISA for the detection of viral antigen from pneumonic lung tissue, and they found 16.6% positive for BRSV antigen but were unable to identify BoHV-1, BPI3V, or BVDV. Our findings differed from those of Quinting et al. [22] who investigated several antigen detection methods for the diagnosis of BRSV antigen and proposed that ELISA is less sensitive than RT-PCR for the detection of BRSV antigen in lung tissues. The sample size and seasonal variance in comparison to earlier findings might potentially account for the lack of BRSV antigen.

The popularity of molecular approaches is rising as a result of their great accuracy, sensitivity, and specificity [10–23]. On the other hand, conducting these exams is usually inconvenient, time-consuming, and expensive. With antigen ELISA serving as the gold standard, the relative RT-PCR sensitivity and specificity were 82.7% and 97% higher when compared to direct ELISA. Our findings showed that the RT-PCR had the highest sensitivity and specificity

TABLE1. Results of Mono Screen Antigen- ELISA and Nested RT-PCR.

Tests	Examined swabs	Positive swabs (%)	Negative swabs (%)
Direct ELISA	450	139 (30.8)	311(69.1)
Nested RT-PCR	450	168 (37.3) *	282 (62.6)

* significantly P< 0.05.

TABLE 2. Comparative estimation of Nested RT-PCR technique with Mono Screen Antigen- ELISA in diagnosis of viral antigens in Nasal swabs

Serial number	Parameters Gold standard:- Sandwich ELISA: a = 139, b = 0 ,c = 29 ,d = 197	
1	Kappa value	0.838
3	Sensitivity	82.7%
4	Specificity	97%
5	Positive predictive value	1
6	Negative predictive value	0.872
7	Positive Likelihood Ratio	37.6
8	Negative Likelihood Ratio	0.137
9	Diagnostic Odds	9
10	Error Odds Ratio	0.0
11	Overall fraction correct	0.921
12	Overall agreement	92.1%

A = Samples positive to tests , b = Samples that positive to the traditional test but fail the gold standard test, c =Samples negative to traditional, but positive to the gold standard test, d = samples negative for both tests.

percentages, and that its kappa value was (0.838) indicated to a perfect agreement between the two tests. According to earlier research, ELISA is less sensitive than RT-PCR for the detection of BRSV antigen [14,15-18]. These antigen detection assays' dependability and accuracy are influenced by the reagents employed, especially the sensitivity and specificity of the anti-BRSV antibody utilized.

Conclusion

In conclusion, the One Tube RT-PCR System Script RT-PCR Premix Kit had the best sensitivity, specificity, and agreement when compared to the mono screen Antigen enzyme-linked immunosorbent assay BRSV /Sandwich double well (Antigen -ELISA). This implies that BRSV infection is detected using both assays. Antigen-ELISA is a sensitive, inexpensive test and can be applied to routine diagnosis

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Conflicts of interest

The authors didn't disclose any conflicts of interest.

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التقييم المقارن مابين فحص RT-PCR المتداخل والمقايسة الممتز المناعي المرتبط بالإنزيم المستضد أحادي المستضد في تشخيص الاصابة بالفايروس المخلاوي البقري

خضر جاسم حسين و مآب ابراهيم الفروه جي
فرع الطب الباطني والوقائي - كلية الطب البيطري - جامعة الموصل - العراق.

يعد الفيروس المخلوي التنفسي البقري السبب الأكثر شيوعًا لمرض الجهاز التنفسي البقري في جميع أنحاء العالم. وللمقارنة بين استخدام RT-PCR المتداخل والمقايسة الممتز المناعي المرتبط بالإنزيم المستضد أحادي المستضد BRSV (Antigen -ELISA) لتشخيص الاصابة بالفايروس المخلاوي البقري. تم جمع ٤٥٠ مسحة أنفية من الأبقار المشتبه بها بالاصابات التنفسية من مختلف الأعمار والسلالات. تم فحص جميع العينات بواسطة مستضد Nested RT-PCR (Script RT-PCR و ELISA (Bio-X، Rochefort ، بلجيكا) Suprime kit، GENET شركة بيو ، كوريا). من بين ٤٥٠ مسحة ، كانت ١٦٨ مسحة إيجابية بواسطة RT-PCR (37.3٪) ، لكن 139 مسحة فقط (30.8٪) أظهرت مستضدًا فيروسيًا بواسطة ELISA المباشر. في المقابل ، كانت 29 حيوانًا (6.4٪) سلبية في اختبار ELISA المباشر وإيجابية في اختبار RT-PCR. أظهرت الدراسة وجود اتفاق بين كلا الاختبارين في الكشف عن BRSV باستخدام قيمة Kappa والتي بلغت (0.838). يشير هذا إلى أن اختبارات RT-PCR و antigen-ELISA المتداخلة متكافئة في تشخيص تشخيص الاصابة بالفايروس المخلوي البقري. نستنتج من هذه الدراسة ، أن استخدام RT-PCR المتداخل كانت له حساسية وخصوصية واتفاق أفضل عند مقارنتها بالمقايسة الممتز المناعي المرتبط بالإنزيم BRSV / المستضد (Antigen-ELISA). هذا يعني أنه يمكن تشخيص الاصابة بالفيروس المخلوي التنفسي البقري باستخدام كلا الاختبارين. وان المقايسة الممتز المناعي المرتبط بالإنزيم / المستضد هو اختبار حساس وغير مكلف يمكن استخدامه للتشخيص الروتيني.