**The Most Frequent Cause of Bovine Respiratory Illness**

Respiratory syncytial virus (RSV) is one of the most common causes of respiratory illness in cattle. The objective of this investigation was to detect the bovine respiratory syncytial virus (BRSV) utilizing nested RT-PCR and a mono-screen antigen-enzyme-immunosorbent assay (Antigen-ELISA). The study also aimed to evaluate the success of the diagnostic techniques.

A total of 450 nasal swabs from suspected respiratory infection cattle of local breeds aged 1-4 years were obtained. Of these, 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.
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 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’-AAD AGA GGA TGC (T/C) TT GCT GTGG3), 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’-GCTAGTTCTGTGATTGTGGTGC-’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 denatured 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:

$$\text{Val}(\text{ur}) = \frac{\Delta \text{OD Sample} \times 100}{\Delta \text{OD positive}}$$

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](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 pneumatic 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.

### TABLE 1. Results of Mono Screen Antigen-ELISA and Nested RT-PCR.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Examined swabs</th>
<th>Positive swabs (%)</th>
<th>Negative swabs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct ELISA</td>
<td>450</td>
<td>139 (30.8)</td>
<td>311 (69.1)</td>
</tr>
<tr>
<td>Nested RT-PCR</td>
<td>450</td>
<td>168 (37.3) *</td>
<td>282 (62.6)</td>
</tr>
</tbody>
</table>

* 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

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

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

### Acknowledgments

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### Conflicts of interest

The authors didn’t disclose any conflicts of interest.

### References


المتداخل والمقايسة الممتز المناعي المرتبط
بالإنزيم المستضد أحدى المستضد في تشخيص الإصابة بالفأرير المخارفي البقرى

خضير جاسم حسين و مأب إبراهيم الفروه جي
فرع الطب الباطني والوقائي - كلية الطب البيطري - جامعة الموصل - العراق

يفيد الفيروس المخارفي التنفسي البقرى سبب الأكثر شيوعًا لمرض الجهاز التنفسي البقرى في جميع أنحاء العالم. والمقارنة بين استخدام Nested RT-PCR والمتداخل والمقياس الممتز المناعي المرتبط بالإنزيم المستضد أحدى المستضد (Antigen-ELISA) BRSV المستضد تقييم المقارن مابين فحص بالإنزيم المستضد أحدي المستضد في تشخيص الإصابة بالفأرير المخارفي البقرى. تم جمع 450 مسحة أندية من الأبقار المشتبه بها بالإصابات التنفسية بسبب الفيروس المخارفي بقرى. تم فحص جميع العينات المستضد Antigen-ELISA BRSV بواسطة مستضد Biostar ELISA (Bio-X،纶り، Rochefort)، Nested RT-PCR (Script RT-PCR، بلجيكا(، وSuprime kit GENET، كوريا( لتشخيص الإصابة بالفيروس المخارفي البقرى. تم جمع 450 مسحة، كانت 118 مسحة إيجابية بواسطة ELISA المباشرة، لك توب كتاست مستضدًا فيروسياً بواسطة ELISA المباشرة. RT-PCR المتأخرة، كانت 29 حية (6.4٪) سلبية في اختبار RT-PCR المتأخر، وأظهرت الدراسة وجود اتفاق بين كلا الاختبارين في الكشف عن BRSV في الدم و 168 مسحة، كانت 450 من شركة بي، كوريا(، كانت 139 مسحة فقط (30.8٪) أظهرت مستضدًا فيروسياً بواسطة RT-PCR (37.3٪) وRT-PCR المباشر وأظهرت 0.838 Kappa باستخدام قيمة Kappa التفاعل LLC (antigen-ELISA و RT-PCR). يشير هذا إلى أن اختبارات RT-PCR المتأخرة في تشخيص الفيروس المخارفي البقرى. تستنتج من هذه الدراسة أن استخدام RT-PCR المتأخر في تشخيص الفيروس المخارفي البقرى. و BRSV مستضد LLC (antigen-ELISA). هذا يعني أنه يمكن تشخيص الإصابة بالفيروس المخارفي التنفسي البقرى باستخدام كلا الاختبارين. وأن المقياس الممتز المناعي المرتبط بالإنزيم/ المستضد هو اختبار حساس وغير مكلف يمكن استخدامه للتشخيص الروتيني.