Serological Surveillance of Infectious Bovine Rhinotracheitis virus, Bovine viral diarrhea virus and Bovine Parainfluenza-3 virus in Saudi Arabia

Mohammed Ali Al-Hammadi
Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, Saudi Arabia

ABSTRACT

Keywords: IBRV, BVDV, PI-3V, ELISA, Cattle, serodiagnosis, seroconversion

Monitoring and surveillance of respiratory viral infections in different animals is essential to build up the future bovine animal health and control strategies. The aim of present study was to document the prevalence of some common bovine respiratory viral infections in different regions in Saudi Arabia. Infectious Respiratory viral diseases in cattle are an important cause of mortality especially in young calves. Out of this group of viruses, present study choses to focus on Infectious Bovine Rhinotrachietis virus (IBRV), Bovine viral diarrhea virus (BVDV), and Bovine Parainfluenza-3 virus (BPI-3V). Little is known about the prevalence of these viruses throughout the Gulf and so the primary goal of the study was to test the exposure history of different cattle herds in Saudi Arabia to the indicated viruses. To achieve this goal, 359 serum samples were collected from four different regions in Saudi Arabia (Eastern, Central, Northern, and Western regions). Commercially available ELISA kits were used to evaluate the immune response of animals against these viruses. The results showed the high seroprevalence of the indicated viruses in Saudi Arabia. The seroprevalence of IBRV, BVDV and PI-3 were 50 %, 35%, and 39% respectively. Since the animals used in the current study have not been vaccinated against the indicated viruses, it is highly likely that those herds were exposed to these viruses previously, and thus seroconverted to against these viruses. More molecular studies are urgently needed to do further characterization these viruses in Saudi Arabia. Meanwhile, application of science- based monitoring systems is highly recommended to mitigate the risk of exposure of different animals for such viruses.

*Author of correspondence: Mohammed Ali Al-Hammadi: malhammadi@kfu.edu.sa

1. INTRODUCTION

Bovine respiratory disease (BRD) is a major health problem for cattle worldwide, and research on the area has long been priority. In economic terms, BRD leads to decreased productivity due to, high levels of mortality and morbidity, increased veterinary and labor costs and reduced carcass value. The causation is multifactorial and the disease appears to be a result of the interaction of infectious micro-organisms and such predisposing factors as host defense, environment and stress. Viruses and bacteria in combination with stress play a key role in triggering acute respiratory infections. It is generally accepted that viruses are the first pathogens to play a role in infecting animals, whereas bacteria act as second invaders to worsen the ill animal’s condition (Solis-Calderon et al., 2007). Several viruses such as bovine respiratory syncytial virus (BRSV), bovine parainfluenza type 3 (BPI3), Infectious Bovine Rhinotrachietis (IBRV), bovine viral diarrhea virus (BVDV) and bovine Adenoviruses (BAdVs) are detected in clinical cases (Ellis, 2001). While usually considered a respiratory pathogen, infection with IBRV can also cause abortion in pregnant cattle. These agents may represent risks to camels, other livestock and even human population (Teshome et al., 2003). Infection with these viruses can also facilitate invasion of opportunistic secondary pathogens such as Mannheimia haemolytica, Pasteurella multocida, Haemophilus somnus and a number of mycoplasma species such as M. bovis and M. dispar (Teshome et al., 2003).

IBRV is a disease of domestic and wild cattle. IBRV is a member of the genus Varicellovirus in the subfamily Alphaherpesvirinae, which belongs to the Herpesviridae family (Nandi et al., 2009). The IBRV causes respiratory disease, abortion, conjunctivitis, and other clinical forms of disease complex. Genetic analyses of various clinical isolates have found at least three distinct IBRV subtypes; a respiratory subtype, a genital subtype, and an encephalitic subtype designated as IBRV.1,
IBRV.2, and IBRV.3, respectively (Fuchs et al., 1999).

BVDV is classified in the family Flaviviridae and is a member of the genus Pestivirus. This virus is detected in most CLINICAL cases that are investigated early after the onset of clinical signs (Lanyon et al., 2013). On farms where BVDV is not well controlled, this can lead to immunosuppression and influence the progression of Bovine Respiratory diseases (BRD).

BPI-3V is in the genus Respirovirus of the subfamily Paramyxovirinae, order Mononegavirales, of the family Paramyxoviridae (Ellis, 2001). This virus causes clinical symptoms in the respiratory system of cattle and sheep. Antibodies of BPI-3V have been found in humans, cow, sheep, and other animals. Like IBRV, BPI-3V is a viral agent of shipping fever. The highly conserved partial matrix (M) protein has been used recently for the rapid identification of PI-3 in feedlot cattle (Horwood et al., 2008).

An episode of BRD, can cause permanent lung damage, making animals more susceptible to subsequent episodes of respiratory disease compromising growth rates and economic returns for the farmer (Thonur et al., 2012). Few reports are available on the respiratory diseases of farm animals in Saudi Arabia (Barbour et al., 1997). Main goal in this current study was to test the exposure history of cattle population in several regions in KSA to the indicated major respiratory viruses.

2. MATERIALS AND METHODS

2.1. Sampling

A total of 359 random sample of bovine sera were collected from four different regions across the kingdom (Figure 1 and Table 1). Samples were collected from apparently healthy animals with no obvious clinical respiratory manifestations. Serum samples were collected by venipuncture from the jugular vein, left overnight at 4°C. Serum samples were then processed by centrifugation at 5000 rpm for 10 min. Serum samples were heat inactivated at 56°C for 30 min then stored at (-80°C) for further testing.

2.2. Animal Ethics statement

Serum sample collection was done according to The King Abdul-Aziz City of Science and Technology, Royal Decree No. M/59, (http://www.kfsh.med.sa/KFSH_WebSite/usersuploadedfiles%5CNCBE%20Regulations%20ENGLISH.pdf).

2.3. Enzyme linked immunosorbant assay (ELISA)

Testing of the bovine sera was conducted using the commercial available, RESPIRATORY ELISA PENTAKIT® (BIO K 028/5 from Bio-X Diagnostics, Belgium) used in accordance with the manufacturer’s instructions. Briefly, ELISA plates were coated with monoclonal antibodies and inactivated viruses. Tested sera were diluted at a ratio of 1:500 in the dilution buffer. Samples were added to the corresponding wells, incubated at 37°C for 1 hr. Plates then washed three times with the washing buffer. The conjugate was diluted 1:50 in 1X dilution buffer and added to each wells. Plates were then lidded, incubated at room temperature for one hour, and washed as stated above. Undiluted chromogen was added to each well; plates were then incubated in dark at room temperature for 10 minutes. The reaction was stopped by adding 50 μl per well of the undiluted stop solution followed by reading Optical Density (OD) at 450 nm.

2.4. Interpretation of test results

Net OD was calculated by subtracting the OD value of the negative control column (no. 6) from corresponding values of columns 1, 2, 3, 4 and 5. The Net OD of the positive serum control must exceed the following threshold for the test to be acceptable: IBRV > 0.700; BVDV > 1.000; BPI3V > 0.800. If the plate passed internal quality control criteria. sample positivity was calculated using the following equation:-

\[
\text{Percentage Positivity (PP)} = \frac{\text{Sample Serum Net OD}}{\text{Positive Serum Net OD} \times 100}
\]

Samples were considered positive if PP exceeded 30% for IBRV, 20% for BVDV and BPI3V as indicated by the kits instructions.
3. RESULTS

3.1. Seroprevalence of IBRV in KSA

According to recorded results, high seroprevalence of IBR among cattle was reported in the Western and Eastern regions (64 and 53 %) respectively. The lowest seroprevalence was reported in the central areas (5%). However, central regions Riyadh was showing moderate seroprevalence (6%) (Figure 2). In total, the overall seroprevalence in the five tested areas was as high as 50% (Figure 2 and Table 2).

3.2. Seroprevalence of BVDV in KSA

The highest reported rates of seropositivity was found in the Western region (50%), followed by the Eastern region (38%). Both the central and Northern areas showed (8% and 1%) positive results respectively. The overall seroprevalence of BVDV across regions was 35% (Figure 3 and Table 2).

3.3. Seroprevalence of BPI-3V in KSA

The highest seroprevalence of BPI-3V was reported among samples collected from the Western region (70%) while, the lowest seroprevalence was reported in the cattle flocks of the Northern region. Meanwhile, the Eastern and central regions showed seroprevalence of (30% and 6%) respectively. The obtained results are showing high seroprevalence among animals in the Western areas with (70%) followed by 30% at the Eastern region while the central and Northern regions were showing 10% and 6% respectively (Figure 4). The overall prevalence of BPI-3V in KSA was 39% (Figure 4 and Table 2).

Table 1: Number of the collected serum samples per each region within Saudi Arabia

<table>
<thead>
<tr>
<th>Region</th>
<th>Eastern</th>
<th>Central</th>
<th>Northern</th>
<th>Western</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>94</td>
<td>92</td>
<td>84</td>
<td>89</td>
<td>359</td>
</tr>
</tbody>
</table>

Table 2: Summary of the seroprevalence of common respiratory viruses among cattle flocks in different regions of Saudi Arabia

<table>
<thead>
<tr>
<th>Region</th>
<th>IBRV</th>
<th>BVDV</th>
<th>BPI-3V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern</td>
<td>53</td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td>Central</td>
<td>5</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Northern</td>
<td>6</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Western</td>
<td>64</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>35</td>
<td>39</td>
</tr>
</tbody>
</table>

Figure 1). Map of Saudi Arabia illustrating the geographical distribution of the collected samples throughout the kingdom. Regions under study are highlighted by asterisks.
Table 3: Simultaneous detection of antibodies against two or more viruses

<table>
<thead>
<tr>
<th>Region</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>Central</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>Northern</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>Western</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

A- Animals reacted to IBRV+BVDV
B- Animals reacted to IBRV+BVD +BPI-3V

3.4. Summary of the seroprevalence of common respiratory viruses among cattle flocks in different regions of Saudi Arabia

Table 2 summaries the seroprevalence of common respiratory viruses in adult cattle in several regions in Saudi Arabia. In general, almost most animals included in the current study were seroconvert to different viruses.

3.5. Identification of the animals reactive to several viruses

Among the interesting results of the current study was the identification of animals showed high seroprevalence of two or more viruses. Table 3 summarizes the reactive animals to two or more viruses in different locations within Saudi Arabia.

4. DISCUSSION

Bovine respiratory viruses are among the most common causes of high morbidity and mortality in bovine species worldwide (Yesilbag and Gundor, 2008). They cause major economic losses to the dairy industry due to the sharp reduction in milk and meat production (Potgieter, 1997). This is in addition to the high mortality rates especially among the young calves (Yesilbag and Gundor, 2008). This study was designed to investigate the immune status of the most common respiratory viruses affecting cattle (IBRV, BVDV, and BPI-3V). Samples in the current study were collected from small flocks of four different regions in Saudi Arabia. Those animals were non-vaccinated against the indicated viruses. Our results are showing moderate level of exposure to several viruses such as IBR (50 %). This is in contrast to another study had been done in Uruguay as 37% of the tested animals were seropositive to IBRV (Guarino et al., 2008). Similar
serosurveillance studies had been conducted in many countries in the world and showing variable seroprevalence among the tested cattle flocks. These studies included Yucatan (14%), Spain (21%), and Mexico (14%) (Mainar-Jaime et al., 2001; Solis-Calderon et al., 2007). Our data is very much closer to the previous records for many viruses especially BVDV (Table 2 and Figure 2). Similar studies had also been conducted in USA to detect the seroprevalence of IBRV, BRSV, and BVDV in American bulls. These studies showed higher seroprevalence of the indicated viruses than our results. For example, the incidence of IBRV was up to 92% among the tested animals (Sausker and Dyer, 2002). Interestingly enough, some viruses such as BVDV was almost negative in some areas such as Northern and Central regions respectively. Interestingly enough, we identified some animals exposed to two or several viruses at the same time as shown in (Table 3). It was found that the highest seroprevalence of two or more viruses was recorded in the Northern region of Saudi Arabia. One possible explanation for this that the area is relatively cold throughout the year. This cold weather may favor the spreading of respiratory viruses. This is consistent with other research that reported high prevalence of winter dysentery among calves during cold winter weather (Park et al., 2007). High seroprevalence of major respiratory viruses was reported in several regions in Saudi Arabia. The authors strongly suggest that study to evaluate the present situation of these viruses and perform intensive vaccination programs against the indicated viruses.

5. CONCLUSIONS

High seroprevalence of the common respiratory viruses of cattle including IBRV, BVD, and BPI-3V in four major geographic locations across Saudi Arabia was reported. Since those animals were nonvaccinated against the indicated viruses, this indicates the exposure of the tested animals to these viruses at certain point.

ACKNOWLEDGMENT

This work was funded by a grant from the deanship of Scientific Research (Grant no130248), King Faisal University, Kingdom of Saudi Arabia. Thanks are also directed to Mr. Anwar Al-Kubati for his technical laboratory assistance.

Conflict of Interest Statements

The author declare, there is no conflict of interest.

Contribution of each author statement

M.A.H collected samples, conducted experiment, analyzed data and wrote the paper.

9. REFERENCES


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