SERO-PREVALENCE OF COMMON BOVINE RESPIRATORY VIRAL DISEASES IN SAUDI ARABIA

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ABSTRACT

Respiratory viral diseases in cattle are an important cause of mortality especially in young calves. Of this group of viruses, present study chooses to focus on Infectious Bovine Rhinotracheitis virus (IBR), Bovine viral diarrhea (BVD), Bovine Parainfluenza-3 (BPI-3), Respiratory syncytial virus (RSV), and Bovine Adenoviruses (BAdVs). 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 regions in Saudi Arabia (Eastern, Central, Northern, and Western regions). Commercially available ELISA kits were used on these samples. The results showed the high seroprevalence of the indicated viruses in Saudi Arabia. The seroprevalence of IBR, BVD, RSV, PI-3 and BAdV-3 were 50 %, 35%, 30%, 39% and 49% respectively. To the best of our knowledge this is the first study to report the high seroprevalence of BVAdV-3 in the Gulf region, or especially in Saudi Arabia. Since the animals used in the current study have not been vaccinated against the indicated viruses, it is assumed that those herds were exposed to these viruses previously, and thus seroconverted to against these viruses. Further molecular studies are needed to examine these viruses, and the application of science- based vaccination programs is highly recommended to protect these animals from the indicated pathogens.

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), bovine herpesvirus-1 (BoHV-1), bovine viral diarrhea virus (BVD) and bovine Adenoviruses (BAdVs) are detected in clinical cases (Ellis, 2001). While usually considered a respiratory pathogen, infection with BoHV-1 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 (Hodgson *et al.,* 2005). Infectious bovine rhinotracheitis, caused by BoHV-1, is a disease of domestic and wild cattle. BoHV-1 is a member of the genus Varicellovirus in the subfamily *Alphaherpesvirinae*, which belongs to the *Herpesviridae* family (Nandi *et al.,* 2009). The BoHV causes respiratory disease, abortion, conjunctivitis, and other clinical forms of disease complex. Genetic analyses of various clinical isolates have found at least three distinct BHV-1 subtypes; a respiratory subtype, a genital subtype, and an encephalitic subtype designated as BoHV-1.1, BoHV-1.2, and BoHV-1.3, respectively (Fuchs *et al.,* 1999) .

BVDV is classified in the virus 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 BRD.

BPI-3V is in the genus Respirovirus of the subfamily *Paramyxovirinae*, order Mononegavirales, of the family *Paramyxoviridae* (Ellis, 2010). 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 BoHV-1, 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).

Bovine adenoviruses (BAdVs) belong to the family *Adenoviridea*, and. appear to be related to other diseases. and BAV-3 is related to respiratory infections of cattle (Castano and Eisenbarth, 1990). BAdVs are a group of viruses which cause various symptoms in cattle including pneumonia, conjunctivitis, diarrhea and polyarteritis (Castano and Eisenbarth, 1990). Based on their serological and biological criteria, BAdVs can be classified in two groups, the first belongs to the Mastadenovirus genera including BAdVs (Hodgson *et al.,* 2005; Horwood *et al.,* 2008) while the other group belongs to genus Atadenovirus including BAdVs (Zhu *et al.,* 2011). BAdV-3 is one of the most important causes of respiratory manifestations in cattle especially newborn calves (Zhu *et al.,* 2011).

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.

MATERIALS and METHODS

Sampling
Serum samples used in the current study were originally collected to perform a serosurveillance of Rift Valley Fever virus in Saudi Arabia (Al-Afaleq *et al.,* 2012). A random sample of of 359 bovine serum samples was collected randomly from four different regions (Table 1). 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 till tested.

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. 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.

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. Net OD of the positive serum control must exceed the following threshold for the test to be acceptable: BHV-1 > 0.700; BVD > 1.000; BRSV > 0.800; BPI3 > 0.800; BAd-V-3 > 0.600. If the plate passed internal quality control criteria, sample positivity was calculated using the following equation:-

$$Percentage\ Positivity\ (PP) = \frac{Sample\ Serum\ Net\ OD}{Positive\ Serum\ Net\ OD} * 100$$

Samples were considered positive if PP exceeded 30% for BHV-1, 20% for BVD, BRSV, BPI3; and 10% for BAdV-3 as indicated by the kits instructions.

Statistical analysis

RESULTS

Seroprevalence of BHV-1 in KSA
According to recorded results high seroprevalence of BHV-1 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 1). In total, the overall seroprevalence in the five tested areas was as high as 50% (Figure 1 and Table 2).
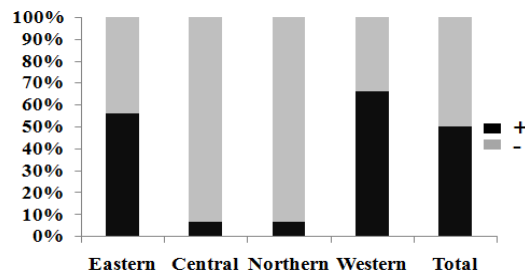
Fig. 1: Seroprevalence of IBR in KSA

Seroprevalence of BVD 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 BVD across regions was 35% (Figure 2 and Table 2).
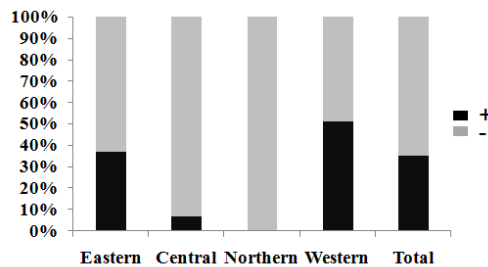

Fig. 2: Seroprevalence of BVD in KSA

Seroprevalence of RSV in KSA

The lowest seroprevalence for BPI-3 virus was reported among herds in the central region (1%). The other regions including the Eastern, Northern and the Western were showing positive rates as of 33%, 12%, and 40% respectively. The overall prevalence of PI-3 among the tested samples was 38%.
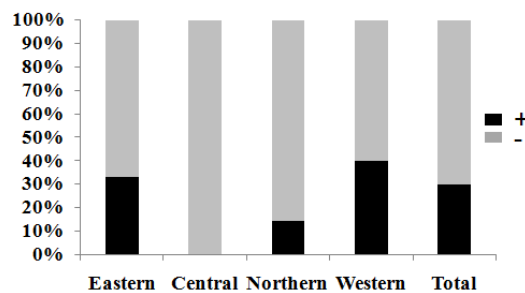

Fig. 3: Seroprevalence of RSV in KSA

Seroprevalence of BPI-3 in KSA

The highest seroprevalence of BPI-3 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-3 in KSA was 39% (Figure 4 and Table 2).
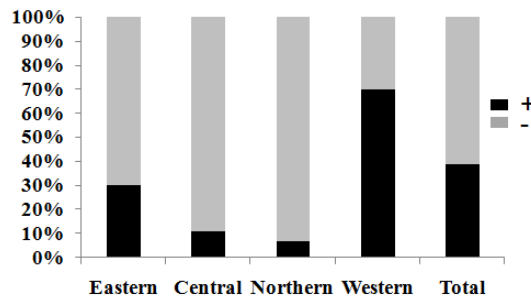
Fig. 4: Seroprevalence of PI-3 in KSA

Seroprevalence of BAdV-3 in KS
The highest seroprevalence reported among all cattle flocks against BAdV-3 was reported in the cattle flocks of the Western region. The second most affected region was the Northern region as (50%) of the tested animals were seropositive for BAdV-3. The Eastern and Central regions were had variable results (22% and 29%) respectively (Figure 5). The overall seroprevalence of BAdV-3 in all tested animals was 49% (Figure 5 and Table 2).
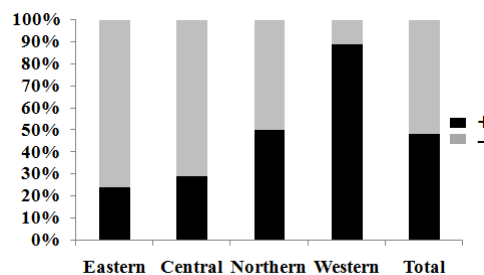

Fig. 5: Seroprevalence of BAdV-3 in KSA

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. The overall seroprevalence of different regions were (50%, 35%, 30%, 39% and 49%) in the following regions (Eastern, Central, Northern, Western and Western) respectively.

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.

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

Region	Eastern	Central	Northern	Western	Total
No. of samples	94	92	84	89	359

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

Region	Eastern	Central	Northern	Western	Total
BHV-1	53	5	6	64	50
BVD	38	8	1	50	35
BRSV	33	1	12	40	30
BPI-3	30	10	6	70	39
BAdV—3	22	29	50	90	49

Table 3: Percentages of the animals reactive to several viruses.

Region	A	B	C	D	E
Eastern	25	24	11	7	11
Central	22	30	9	10	13
Northern	32	29	30	27	26
Western	11	18	30	12	9

A- Animals reacted to BHV-1 +BVD
B- Animals reacted +BAdV-3
C- Animals reacted to BHV to BHV-1+RSV
D- Animals reacted to BVD -1 + BVD + RSV + BPI3
E- Animals reacted to BHV-1 + BVD + RSV + BPI3

DISCUSSION

Bovine respiratory viruses are among the most common causes of high morbidity and mortality in bovine species worldwide (Yesilbag and Gungor, 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 Gungor, 2008). This study was designed to investigate the immune status of the most common respiratory viruses affecting cattle (BHV-1, BVD, BPI-3, RSV, and BAdV-3). Samples in the current study were collected from small flocks of 4 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 and BAdV-3 (50 % and 49 %) respectively. This is in contrast to another study had been done in Uruguay as 37% of the tested animals were seropositive to IBR (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 Yuctan (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 BHV-1, BRSV, and BVD in American bulls. These studies showed higher seroprevalence of the indicated viruses than our results. For example, the incidence of IBR was up to 92% among the tested animals (Sausker and Dyer, 2002). Interestingly enough, some viruses such as BVDV and BRSV were almost negative in some areas such as Northern and Central regions respectively. This is in contrast to high seroprevalence of BAdV-3 in the Western region (90%), (Table 2 and Figure 5). It is worthwhile to report that the current study is the first study to investigate the seroprevalence of BAdV-3 in Saudi Arabia. Interestingly enough, we identified 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 is 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.

ACKNOWLEDGMENT

CONCLUSIONS

High seroprevalence of the common respiratory viruses of cattle including BHV-1, BVD, RSV, BPI-3 and BAdV-3 in 4 geographic locations in Saudi Arabia was reported by the current study. Since those animals were non-vaccinated against the indicated viruses, this indicates the exposure of the tested animals to these viruses at certain point.

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مسوحات سيرولوجية على بعض الأمراض الفيروسية التى تصيب الجهاز التنفسى للأبقار فى المملكة العربية السعودية

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تعتبر الأمراض الفيروسية التي تصيب الأبقار من أهم أسباب النفوق خاصة في الأعمار الصغيرة. يعد الالتهاب الرغامي للقصبات الهوائية (أي بي ار) البي في دي-الالتهاب الفيروسي المخلوي - نظير الأنفلونزا الثالث - وفيروس الادينو المصيب للأبقار العترة الثالثة من أهم المسببات الفيروسية للالتهاب الرئوي في الأبقار. علي الرغم من ذلك فلا توجد معلومات كافية عن هذه الفيروسات في منطقة الخليج بصفة عامة والمملكة العربية السعودية بشكل خاص. تهدف الدراسة الحالية إلي استقصاء مدي تعرض قطعان الأبقار في أربعة مناطق من المملكة وتشمل المنطقة الشرقية-الوسطي-الشمالية- والغربية. ولتحقيق هذا الهدف فقد قمنا بتجميع عدد 359 عينة مصل دم من المناطق السالفة الذكر. بفحص هذه العينات باستخدام تقنية الاليزا المتوفرة تجاريا.   أوضحت النتائج وجود الأجسام المضادة للفيروسات سالفة الذكر في أمصال قطعان الأبقار في المناطق المختلفة في المملكة. و أشارت النتائج إلي النسب الآتية 50%-35%-30%-39%-49% في حالات ال أي بي ار-البي في دي-الالتهاب الفيروسي المخلوي-   نظير الأنفلونزا الثالث - وفيروس الادينو المصيب للأبقار العترة الثالثة علي التوالي. وحيث إن هذه الأبقار لم يسبق لها التحصين ضد هذه الفيروسات من قبل فنحن نعتقد بان هذه القطعان من   الأبقار سبق لها العدوي بالفيروسات محل الدراسة. هناك حاجة ماسة   إلي التوصيف البيولوجي والجزيئي للفيروسات محل الدراسة وذلك للتاكد من وجود هذه الفيروسات في المملكة. توصي هذه الدراسة بتحصين قطعان   الأبقار ضد هذه الفيروسات لضمان حمايتها.

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