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ORIGINAL RESEARCH

Seroprevalence of some bovine viral respiratory diseases among non vaccinated cattle in Saudi Arabia

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Abstract

Aim: Four viral pathogens, bovine viral diarrhea virus (BVDV), and bovine herpes virus type 1 (BHV-1), bovine parainfluenza type 3 virus (PI-3V), bovine respiratory syncytial virus (BRSV) are mainly associated with bovine respiratory diseases that cause major economic losses in the dairy cattle industry. This study aimed to document exposure of cattle in Saudi Arabia to infectious BVDV, BHV-1, PI-3V and BRSV viruses in non vaccinated cattle in order to obtain epidemiological and immunological information.

Materials and Methods: In the present study, 460 random serum samples obtained from non vaccinated cattle in five districts (Riyadh, Eastern Province, Jizan, Najran, Asir) of Saudi Arabia between January to March 2011. These samples were tested for presence of antibodies against BVDV, BHV-1, BRSV and PIV-3 by commercial indirect ELISA kits.

Results: Our findings displayed that Seropositivity rates were 26 % for BVD, 17.4 % for BHV-1, 69.1 % for PI-3V and 75.6 % for BRSV in the sampled population. In addition, coinfections with more than one virus were considerably common among non-vaccinated dairy cattle.

Conclusion: These results indicate that exposure to these agents is common within the study areas. Preventive and control measures against these infectious agents should therefore be adopted.

Key Words: BHV-1; BRSV; BVDV; PI-3V; Saudi Arabia; seroprevalence.

Introduction

Respiratory disorders are major concern for *Bovidae*. They occur in all countries that practice intensive livestock farming. Bovine respiratory diseases (BRD) complex is a major cause of economic losses in the dairy cattle industry. 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 intervene, whereas bacteria act as the second invaders to worsen the ill-animal's condition [1,2]. The most important viral agents are bovine viral diarrhea virus (BVDV), bovine herpes virus type 1 (BHV-1), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (PI-3V) and bovine adenovirus (BAV)[3]. Four viral pathogens, BVDV, BHV-1, BRSV and PI-3V are mainly associated with bovine respiratory diseases. These agents cause severe disruption of the respiratory tract and are associated with shipping fever in growing cattle, as well as weaned and transported calves to feedlots for finishing. Moreover, BHV-1 and BVDV can suppress the immune system of the host and increase the risk of secondary bacterial infections and/or mycoplasmas outbreaks of respiratory diseases [1].

BVDV, a member of the genus *Pestivirus* in the family *Flaviviridae*, is associated with various diseases of cattle including respiratory infections, gastrointestinal infections, and reproductive problems such as infertility, abortion, still birth, and weak calves [4,5,6]. The disease was first described in cattle in New York State in 1946 [7].

BHV-1 is a member of the *Varicellovirus* genus, *Alphaherpesvirinae* subfamily, *Herpesviridae* family and has a positive sense double stranded DNA genome. It creates different infections associated with respiratory symptoms such as infectious bovine rhinotracheitis (IBR), genital infection as pustular vulvovaginitis (IPV) and balanoposthitis (IBP) in bulls, conjunctivitis, encephalitis, abortions and fatal multi-systemic infections[8]. BHV-1 is an important viral disease of cattle worldwide and there are international restrictions to trade of seropositive animals or germ products from such animals [9].

PI-3V is an enveloped, non segmented negative-strand virus and a member of the family *Paramyxoviridae* within the genus *Respivirus*[10]. The PI-3V infection is commonly subclinical. Clinical disease may not occur until other pathogens are present or when adverse environmental conditions precipitate clinical disease[11].

BRSV is an enveloped RNA virus which, along with human respiratory syncytial virus (HRSV) and

Table-1. Seroprevalence of BVD, BHV-1, PI-3V and BRSV.

District	No. of samples	Seropositive for BVD (%)	Seropositive for BHV-1(%)	Seropositive for PI3V (%)	Seropositive for BRSV (%)
Riyadh	116	26(22.4)	18(15.5)	86(74)	78(67)
Eastern Province	92	48(52)	38(41.3)	64(69.5)	48(52)
Asir	106	26(24.5)	14(13.2)	74(69.8)	94(88.6)
Najran	60	0(0)	4(6.6)	56(93.3)	58(96.6)
Jizan	86	20(23.2)	6(6.9)	38(44.2)	70(81.4)
Total	460	120(26)	80(17.4)	318(69.1)	348(75.6)

Table-2 Serum antibody distribution to one or multiple infection in cattle

Virus	Number of multiple infections							
	I	II			III			IV
		BHV-1	PI3	BRSV	BHV-1/PI3	PI3/BRSV	IBR/BRSV	BHV-1/PI3/BRSV
BVD	14	2	8	16	5	52	--	23
BHV-1	–	--	5	2	--	45	–	--
PI-3V	41	--	--	139	--	–	–	--
BRSV	71	–	–	–	–	–	–	--
Total	126(27.4%)	172(37.4%)			102(22.2%)			23(5%)

pneumonia virus of mice, belongs to the genus *Pneumovirus* of the family *Paramyxoviridae*. BRSV and HRSV are similar in gene and protein compositions [12].

Although BRSV is a major cause of respiratory disease in calves, resulting in substantial economic losses to the cattle industry [13-16], cattle of all ages can be infected with BRSV, and severe morbidity and mortality has been described in adult animals [17].

The present study was conducted to document exposure of cattle in Saudi Arabia to infectious BVDV, BHV-1, PI-3V and BRSV viruses in non vaccinated cattle.

Materials and Methods

Samples: Experiments were carried out in accordance with the guidelines laid down by the International Animal Ethics Committee and in accordance with local laws and regulations.

According to the case history collected from the owners, none of the cattle herds in this study were vaccinated against BVD, BHV-1, BRSV, and PI-3V. Blood samples were randomly collected from 460 apparently healthy, 1-4 years old dairy cattle in small private cattle raising units (having between 5 to 25 cattle) at various parts of Saudi Arabia (Riyadh, Eastern Province, Jizan, Najran, Asir) between January and March 2011.

Blood samples (5 ml) were collected aseptically from jugular vein of each animal using anticoagulant free vacutainer tubes and transported on ice to the laboratory. Serum was separated by centrifugation of blood at 3000 rpm for 10 min at room temperature; the aliquots were transferred into 1.5 µl sterile microtube (Eppendorf®).These samples were submitted to the Central Veterinary Diagnostic Laboratory in Riyadh where stored at –20°C until tested.

Serological tests: Commercial indirect ELISA kits developed by Bio-X Diagnostics®, Belgium, were used to determine the presence of antibodies to BVDV, BHV-1, PI-V3 and BRSV. Microtiter plates coated with the respective viral antigens were used according to the manufacturer's instructions. Briefly, serum samples were diluted in PBS (1:100) and 100 µl volumes were dispensed into each well, incubated at 21°C +/- 3°C for one hour (all samples and controls were tested in duplicate) and then rinsed 3 times in washing buffer. Then anti-bovine immunoglobulin-peroxidase conjugate solution was dispensed into each well, incubated at 21°C +/- 3°C for another hour. After the second incubation, the plate is washed again and the chromogen (tetramethyl benzidine) is added to each well on the plate, incubated in the dark at room temperature for 10 min. If specific immunoglobulins are present in the test sera the conjugate remains bound to the microwell that contains the viral antigen and the enzyme catalyses the transformation of the colorless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The reaction was stopped by addition of 50 µl of stop solution, and the optical density (OD) was measured at 450 nm.

Results

460 serum samples were screened for the presence of antibodies to BVDV, BHV-1, PI-V3 and BRSV by commercial indirect ELISA kits. 37 out of 460(8%) samples were determined as negative for antibodies against the four tested viruses. In Table-1 Seropositivity rates were 26 % for BVD, 17.4 % for BHV-1, 69.1 % for PI-3V and 75.6 % for BRSV in the sampling population. The rates of seropositivity for each infection determined in the five districts were shown in Table-1. The Eastern province showed the highest seropositivities for BVD and BVH-1 while Najran showed the highest seropositivities for PI-3V and BRSV. We have also noticed that serum samples from animals in Najran were determined as negative for antibodies against BVDV.

In Table-2, data were evaluated in respect with single or multiple seropositivity. In 27.4 % (126/460) of animals, antibodies for a single virus were detected. In 37.4 % (172/460) animals were seropositive for two diseases, and in 22.2 % (102/460) were positive for 3 virus infections. In 5 % (23/460), antibodies were

simultaneously found for 4 viruses. The principal viral combinations were frequently associated PI-3V with BRSV (Table-2).

Discussion

Bovine respiratory disease (BRD) is a major health problem of cattle worldwide. It inflicts considerable financial losses in beef herds [18,19] and is the most common cause of mortality in dairy cattle [20]. Infections with BVDV are endemic in cattle populations in most parts of the world. The high prevalence in combination with the negative effects on reproduction and the general health condition in affected herds result in significant economic losses to the cattle industry globally [21]. Seroprevalence in non-vaccinated herds differs among areas or countries, ranging between 20% and 90% [22,23]. Area differences could in part explained by factors such as cattle density, herd size and management or livestock trade [24,25].

No vaccines against the four tested viruses (BHV-1, BRSV, BVDV and PI-3V) are used in small private cattle raising units in Saudi Arabia (F. Bayoumi, personal communi-cation, Dec. 2010). Our results in table-1 demonstrate a moderate level of exposure to BVDV and BHV-1 in the studied population with prevalences of antibody positive of 26% and 17.4%, respectively. These prevalences do not differ greatly from those reported previously in other parts of the world as the estimated prevalence of BVDV exposure among unvaccinated beef cattle in the Yucatan, Mexico was 14%[26] and that estimated in non vaccinated dairy cattle in Asturias region of Spain was 21%[27]. However, our findings appeared slightly lower than those reported in Uruguay where approximately 37% of beef cattle have been exposed to BHV-1 and 69% to BVD virus [28]. A similar survey performed in USA on American bison (*Bison bison*) bulls for detection of antibodies to BVDV, BHV-1, and BRSV reported 55.3% against BVDV, 43.8% against BHV- 1 and 92% against BRSV[29].The prevalence of BHV-1 seropositive cows may reflect the proportion of BHV-1 carriers because after a primary infection, the virus stays latent in neural ganglions that innervate genital or respiratory mucosae and may be re-excreted upon immuno-suppressive stimuli, such as corticosteroid injection or stress after shipment, calving and etc. The immunity against BHV-1 has no direct effect on the latency state and it modulates the re-excretion of the virus [30,31].For these latent infections, positive serology means that the animal is a potential carrier of the virus[32].

Viruses such as PI-3V and BRSV sometimes cause severe disease as single agents; also they can predispose the animal to bacterial infections of the lung[1].This is the first survey carried out to detect antibodies to PI-3V and BRSV in Saudi Arabian cattle. Our results revealed high PI-3V and BRSV sero-positivities in all the explored provinces that indicates most adult cattle have been exposed to PI-3V and BRSV. These results were in agreement with study for detection of antibodies against PI-3V and BRSV in beef cattle of Yucantan, Mexico showed seroprevalences of 90.8% and 85.6% for PI-3V and BRSV, respectively [2]. A serological survey on bovine respiratory syncytial virus in Chahar Mahal Bakhtiary province (Iran) showed that the infection rate was 80.98%[33].

The high seroprevalence of PI-3V virus found in this study is in agreement with the ubiquitous nature of the virus and with its world-wide distribution[34]. Also, BRSV demonstrates a seasonal incidence of disease, most cases occurring in late autumn and winter [15].

Our findings in Table-2 displayed that the multiple infections were common among non-vaccinated dairy cattle. The principal viral combinations were frequently associated PI-3V with BRSV. In another study, 123 cattle from 45 herds that had respiratory system symptoms was sampled and determined that the 1/4 of the animals had an infection because of one viral factor and the 3/4 of the animals had multiple infections[35]. Alkan *et al., (*1997) conducted an investigation to determine the presence of specific antibodies against 9 viruses (IBR, PI-3V, BRSV, BVDV, BAV-1, BAV-2, BAV-3, Enterovirus 1 and Enterovirus 2) and found the infection rates against one, two, and 3-8 viruses as 9.38%, 11.46% and 72.01%, respectively[36]. Also, Okur-Gümüşova *et al.*, (2007) conducted an investigation to explore the existence of specific antibodies against 5 viruses (BHV-1, BVDV, PI-3V, BAV-1 and BAV-3), and found one, double, threefold, quadruple and fivefold virus infection rates as 6.91%, 59.04%, 58.5%, 39.3% and 35.8%, respectively[37].

Conclusion

No vaccines are used in small private cattle raising units against the four tested viruses and only adult cattle were sampled in the present study so, the presence of antibodies indicates that exposure to these agents is common in the study areas. Larger scale studies which will enable more information to be gathered about these viruses in large industrial dairy herds are therefore warranted. In addition, studies to quantify the impacts of these viruses on animal health and production should be undertaken.

Authors' contributions

MRY conceived the study, carried out the laboratory work, analyzed the data and drafted the manuscript. MFM, SMA& MHB helped to draft the manuscript, performed the field work, collected the samples of the study and helped to carry out the laboratory work. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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