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Seroprevalence of Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus Type 1 (BHV-1), Parainfluenza Type 3 Virus (PI-3V) and Bovine Respiratory Syncytial Virus (BRSV) among non Vaccinated Cattle

M.A. Mahmoud and A.M. Allam

Department of Parasitology and Animal Diseases,
Veterinary Division National Research Center,Dokki, Giza, Egypt

Abstract: Four hundred and sixty serum samples were collected between January and March 2011 obtained from non vaccinated cows in five districts of Saudi Arabia. The samples were tested for presence of antibodies against bovine viral diarrhea virus (BVDV), bovine herpes virus type 1 (BHV-1), parainfluenza type 3 virus (PI-3V) and bovine respiratory syncytial virus (BRSV) by commercial indirect ELISA.This finding displayed that seropositivity rates were 26 % for BVD, 17.4 % for BHV-1, 67.6 % for PI-3V and 75.5 % for BRSVin the sampling population. In addition the coinfection with more than one virus is considerably common among the tested dairy cattle. The results demonstrate a moderate level of exposure to BVDV and BHV-1 in the studied dairy populations while most of the cows were seropositive to PI-3V and BRSV.

Key words: BVDV · BHV-1 · BRSV · PI-3V · Cows · ELISA · Saudi Arabia

INTRODUCTION

Respiratory disorders are of 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 that worsen the ill-animal's condition [1-3].

The most important viral agents are bovine viral diarrhea virus (BVDV), bovine herpes virus type 1 (BHV-1), the causative agent of infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PI-3V) and bovine adenovirus (BAV) [4]. 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, 5].

Bovine herpesvirus-1 (BHV-1) is a member of the *Varicellovirus* genus, *Alpha herpesviridae* subfamily, *Herpesviridae* family and has double stranded DNA genome [6-8]. The virus creates different infections associated with respiratory symptoms such as *infectious bovine rhinotracheitis* (IBR), genital infection as *pustule vulvovaginitis* (IPV) and *balanoposthitis* (IBP) in bulls, conjunctivitis, encephalitis, abortions and fatal multi-systemic infections [7,8]. Bovine Herpes Virus 1 (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 [9-11]. Bovine herpes virus type 1 can became latent in the trigeminal ganglia and tonsils; latency allows the virus to persist, so that the introduction of a carrier into a non-infected herd is the principle way for the spread of the virus. For these latent infections, positive serology means the animal is a potential carrier of the virus [12].

Bovine viral diarrhea virus (BVDV), a member of the genus *Pestivirus* in the family *Flaviviridae*, is associated with various diseases of cattle including respiratory

Corresponding Author: M.A. Mahmoud, Department of Parasitology and Animal Diseases, Veterinary Branch, National Research Center, Dokki, Giza, Egypt.

infections, gastrointestinal infections and reproductive problems such as infertility, abortion, still birth and weak calves [13, 14]. The disease was first described in cattle in New York State in 1946 by [15]. BVDV infection was formerly listed as a group-B disease (milder infectious disease) on the list of notifiable animal diseases [16].

The parainfluenza-3 virus (PI-3V) is an enveloped, nonsegmented negative-strand virus and a member of the family Paramyxoviridae. It has been placed within the genus Respivirus [17]. 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 [18].

Bovine respiratory syncytial virus (BRSV) is a major cause of respiratory disease in calves, resulting in substantial economic losses to the cattle industry [19, 20]. BRSV is an enveloped RNA virus which, along with human respiratory syncytial virus (HRSV) and pneumonia virus of mice, belongs to the genus *Pneumovirus* of the family *Paramyxoviridae*. BRSV and HRSV are similar in gene and protein compositions [21].

The present study was conducted to document exposure of cattle in Saudi Arabia to infectious bovine rhinotracheitis, parainfluenza 3, bovine respiratory syncytial and bovine viral diarrhea viruses in non vaccinated cattle in Saudi Arabia for the first time in 2011. All work was done in the Parasitology and animal diseases department, Veterinary research Division, National research Center.

MATERIALS AND METHODS

Samples: Serum samples were obtained from animals breeding in various parts of Saudi Arabia (Riyadh, Eastern Province, Jizan, Najran, Asir) between January and March 2011 Fig. (1) and Table (I). In this study, Blood samples were randomly collected from 460 apparently healthy, 1-4 years old cattle from several private herds having between 5 to 25 cattle. The sera were separated from blood samples and stored at -20°C until use. None of the cattle in this study were vaccinated against BVDV, BHV-1, BRSV or PI-3V.

Serological Tests: Commercial indirect ELISA kits developed by Bio-X Diagnostics®, Belgium, were used to determine the presence of antibodies against BVDV, BHV-1, PIV-3 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 one 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 color is proportionate to the titer of specific antibody in the sample. The reaction was stopped by addition of 50 µl of stopping solution and the optical density (OD) was measured at 450 nm.

Fig. 1: A map of Saudi Arabia showing the sampling areas

RESULTS

460 serum samples were screened for the presence of antibodies to BVDV, BHV-1, PIV-3 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 IBR 69.1 % for PI-3V and 75.6 % for BRSVin the sampling population. Also, Rates of seropositivity for each infections determined in the five districts were shown in Table 1. Eastern province showed the highest seropositivity for BVD and IBR while Najran showed the highest seropositivity for PI3 and BRSV. We have also noticed that serum samples from animals in Najran were determined as negative for antibodies against BVD.

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

District	No.of samples	Seropositive for BVD(%)	Seropositive forBHV-1(%)	Seropositive for PI3 (%)	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

	Number of multiple infections							
		II			III			IV
Virus	I	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%)

In Table (2), Data were evaluated in respect with single or multiple seropositivity. 27.4 % (126/460) of animals were seropositivefor a single virus. 37.4% (172/460) of animals were seropositive for two viruses and 22.25 % (102/460) were seropositive for 3 viruses while 5 % (23/460) of animals were seropositive for 4 viruses. BRSV and PI-3V were the most frequently associated together 30.2% (139/460).

DISCUSSION

Bovine respiratory disease (BRD) is a major health problem of cattle worldwide. It inflicts considerable financial losses in beef herds [22,23] and is the most common cause of mortality in dairy cattle [24].

Infections with bovine viral diarrhea virus (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 [25]. Seroprevalence in non-vaccinated herds differs among areas or countries, ranging between 20% and 90% [26, 27]. Area differences could in part explained by factors such as cattle density, herd size and management or livestock trade [25-30].

Results in Table (1) demonstrate a moderate level of exposure to BVDV and BHV-1 in the studied population with prevalence of antibodiesin 26% and 17.4% of samples respectively. These ratios not differ greatly from those reported in other parts of the world as that estimated among unvaccinated beef cattle in the Yucatan, Mexico which was14%[31] and that estimated in non-vaccinated dairy cattle in Asturias region of Spain which was 21% [32]. However, our findings appeared slightly lower than those reported in Uruguay which indicated that approximately 37% of beef cattle have been exposed to BHV-1 and 69% to BVD virus [33].

A similar survey performed in the USAon American bison (*Bison bison*) bulls for detection of antibodies to BVDV, BHV-1 and BRSV reported that detectable anti bodies were found against all viruses; 55.3% against BVDV, 43.8% against BHV-1 and 92% against BRSV. These data indicate that a high percentage of bison sampled were seropositive for BVDV, BHV-1and BRSV [34]. Presence of antibodies against BVDV in sera of non vaccinated cattle in the kingdom indicates exposure to infection which may be gained through introduction of subclinically infected or carrier animals to the kingdom through importation from endemic countries to the large industrial dairy herds.

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 immunosuppressive 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 [35]. For these latent infections, positive serology means that the animal is a potential carrier of the virus [14].

Viruses such as bovine respiratory syncytial virus and bovine herpes virus type 1 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 BRSV and PI-3V in Saudi Arabian cattle.The high PI-3V and BRSV seropositivity in the explored provinces agreed with a recent study for detection of antibodies against BRSV and PIV-3 in beef cattle of Yucantan, Mexico which revealed that 90.8 and 85.6% of cattle were seropositive for BRSV and PIV-3, respectively [2]. The high seroprevalence of PI-3V found in this study agreed the ubiquitous nature of the virus and its world-wide distribution [36]. Our results agreed with s serological survey on bovine respiratory syncytial virus in Chahar Mahal Bakhtiary province (Iran) which showed that the infection rate was 80.98% [37]. BRSV demonstrates a seasonal incidence of disease, most cases occurring in late autumn and winter [18]. The high PI-3V and BRSV seropositivities in all the explored provinces indicate that most adult cattle have been exposed to BRSV and PI-3V.

Findings in Table (2) displayed that the multiple infections were common among non-vaccinated dairy cattle. Lauchli *et al.* [38] had sampled 123 cattle from 45 herds that had respiratory system symptoms 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. Alkan *et al.* [39] conducted an investigation to determine the presence of specific antibodies against 9 viruses (IBR, PI-3 virus, 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 [40] had reported the seropositivity rates against one, two, three, four, five, six and seven viruses as 14.7%, 36.22%, 29.92%, 14.56%, 3.93%, 1.57% and 0.39%, respectively. Okur Gümüşova *et al.* [41] conducted an investigation to explore the existence of specific antibodies against 5 viruses (BHV-1, BVDV, PI-3 virus, 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.

CONCLUSION

Because no vaccines are used in small private cattle raising unit against these 4 viruses and only adult cattle were sampled in the present study, the presence of antibodies indicates that exposure to these agents is common in these regions; this is probably due to the lack of control measures against these infections. 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.

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