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RESEARCH ARTICLE

Seroprevalence of Bovine Herpes Virus-1, Bovine Herpes Virus-4 and Bovine Viral Diarrhea Virus in Dairy Cattle in Sudan

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ABSTRACT

A survey was conducted to determine prevalence of antibodies against Bovine herpes virus-1 (BoHv-1), Bovine herpes virus-4 (BoHv-4) and Bovine viral diarrhea (BVD) in dairy cattle in farms with reproductive problems in two areas in Sudan. Sera samples were collected from Khartoum state and central Sudan during 2005-2008 and analyzed using direct ELISA. The prevalence of antibodies was discussed with respect to age, season, sex, breed and locality BoHv-1 and BVD antibodies were highly prevalent in Khartoum state (51.7 and 50.4%, respectively) while in central Sudan BoHv-1 (32.7%) antibodies were the most prevalent followed by, BVD (25.7%) and BoHv-4 (19.3%). The highest prevalence of antibodies against the three viruses in both areas was found during the rainy season (July to October). The prevalence of antibodies to viruses studied was significantly associated with female sex except for BoHv-1. Prevalence of antibodies to BoHv-4 was significantly associated with breed while those of BoHv-1 and BVD were not. The present results indicated that older cattle were more likely to be seropositive in case of BoHv-4 but to BoHv-1 or BVD viruses. Furthermore, it was found that BoHv-1 and BVD antibodies were highly prevalent in aborted dams. While, infertility problems were highly associated with BoHv-1 antibodies. BVD antibodies showed the highest prevalence in case of death after birth. The results of this study provide better understanding of viral epidemics of reproductive disorders and represent the first report of BoHv-4 antibodies in cattle in Sudan.

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INTRODUCTION

A higher than expected frequency of fetal loss, due to abortion and infertility, present challenges to the farmer, veterinarians and diagnostic laboratories since these conditions cause calves losses to herd owners and it is difficult to diagnose the causative agent (Forar *et al*., 1995; Nazifi *et al*., 2011).

Numerous bacterial, viral, protozoan and fungal pathogens had been associated with infertility and abortions in cattle (Givens and Marley, 2008). Kirkbride (1992) and Kelling (2007) recorded bovine herpes virus-1 (BoHv-1), bovine viral diarrhea virus (BVD) and bluetongue virus (BTV) as known viruses associated with abortion in livestock. Bovine herpes virus-4 (BoHv-4) may cause infertility and/or early abortion that go undetected & are rarely submitted for diagnostic purposes.

BoHv-1 may result in various clinical consequences, including severe respiratory disease, venereal disease with reduced reproductive performance and abortion (Muylkens *et al*., 2007). BVD virus not only causes abortion but also cause congenital defects especially when the infection occurs during mid gestation (100-150 days) (Kelling, 2007). Regarding viral diseases that cause abortion at first trimester, BVD was known to cause the problem and disrupt the pregnancy before the conception (Allen *et al*., 2005). Compared to BVD, BoHv-1 abortion occurred at any stage of gestation, but was usually seen in the second half of gestation (Kelling, 2007).

In Sudan, BoHv-1 was isolated since 1983 (Eisa, 1983). An evidence of neutralizing antibodies to BVD in cattle had been detected in different Sudan states (Ali and El-Amin, 1982). However, no information about BoHv-4 in our country is available. The aim of this study was to perform a sero-epidemiological study of BoHv-1, BVD and BoHv-4 viruses as possible evidence regarding the causative agents of abortions & other infertility problems in dairy farms to assess the potential risk factors.

MATERIALS AND METHODS

Investigation of herds with reproductive disorders

Dairy herds with reproductive disorders from farms with history of abortions at different stages of pregnancy, infertility, stillbirth, congenital defects and neonatal death (death 1-2 days after birth) were investigated around Khartoum state (Khartoum, Khartoum north and Omdorman) and central Sudan (Alshukaba, Wad Madani, Alkamleen, Sinnar, and Kenana) during 2005- 2008. Bovine herds in these farms were tested for the presence of antibodies against BoHv-1, BVD and BoHv-4 viruses. The herds tested had no history of vaccination against any of these viral diseases.

Serum samples were collected from all animal (n = 688) of various age groups (1-6, 7-12, 13-24, 25- 36, 36 months and above) at different seasons, winter (November- February), dry hot (March-June) and rainy season (July- October). Sera were kept at -20^{0}C till they were tested using ELISA. Other information gathered during this investigation included locality, sex and breeds (indigenous and crossbred) of the sample animals.

In addition, one hundred and twenty sera from animals that were identified by owners of herds as suffering from reproductive problems (abortion, neonatal deaths or infertility) were compiled and analyzed to identify their associations with the prevalence of the antibodies investigated. The samples were tested using an indirect ELISA kit (Bio-X diagnostic, Belgium) for detection of BoHv-1, BVD and BoHv-4 antibodies. The procedure followed was as recommended by the manufacturer.

Statistical Analysis

The serological results and other information gathered during this investigation such as locality, sex and breeds (indigenous and crossbred) of the sampled animals were compiled and managed. The statistical computations were performed using statistical package (SPSS version 13). To identify the associations of the risk factors with the specific viral seroprevalence the Chi-square (χ^2) test was used.

RESULTS

Seroprevalence of BoHv 1 was found with significant variation across the season and locality with higher prevalence observed during the rainy season (48.1%) and in Khartoum state (51.7%) than in central Sudan (32.7%). But no significant differences were observed among breeds of cattle, age-groups and sexes (Table 1).

Sex and locality played significant role in the perpetuation of BVD while, breeds, age-groups and season had non-significant effect. The seroprevalence was significantly higher in female (67.72%) than in male (8.4%) and in Khartoum state (50.4%) than central Sudan (25.7%).

There was no significant variation between breeds in seroprevalence of BoHv-4 antibodies nevertheless the females showed significantly (40.4%) higher seroprevalence than males (3.9%). Besides, highly significant variation was also observed with the age-groups of animals with older animals being more frequently infected than younger animals particularly during the rainy seasons (29.9%) and in Khartoum state (25%).

Individual animals which suffered reproductive disorders including abortion, infertility and death after birth were studied. In 120 serum samples collected from such cases, BoHv-1 antibodies were highly prevalent in infertility cases (86.8%) followed by aborted cases (84.3%) and death after birth (75%) with non significant variation (Table 2). The BVD antibodies were high (91.67%) in cases of death after birth, aborted cases (84.3%) and in infertility (81.6%) with non-significant

Table 1: Comparative seroprevalence of bovine viral diarrhea (BVD), bovine herpesvirus-1 (BoHv-1) and bovine herpesvirus-1 (BoHv-4) in cattle as detected through ELISA test in Sudan

Factor	Group	BVD		BoHv-1		BoHv-4	
		No Positive (%)*	P-value	No Positive (%)*	P-value	No Positive (%)*	P-value
Breed	Local	20 (2.9)		18 (2.6)		6 (0.87)	
	Cross	504 (73.3)	0.2	563 (81.8)	0.1	299 (43.5)	0.3
Sex	Female	466 (67.7)		512 (74.4)		278 (40.4)	
	Male	58 (8.4)	0.02	69 (10)	0.1	27 (3.9)	0.005
Season	Winter	101 (14.7)		111 (16.1)		44 (6.4)	
	Dry	135 (19.6)		139 (20.2)		55 (8)	
	Rainy	288 (41.9)	0.2	331 (48.1)	0.03	206 (29.9)	0.000
Age	1-6	29 (4.2)		33 (4.8)		7 (1)	
(Months)	6-12	57 (8.3)		67 (9.7)		27 (3.9)	
	12-24	33 (4.8)		37 (5.4)		19 (2.8)	
	24-36	84 (12.2)		85 (12.4)		51 (7.4)	
	36	321 (46.7)	0.1	359 (52.2)	0.1	201 (29.2)	0.000
Locality	Khartoum State	347 (50.4)		356 (51.7)		172 (25)	
	Central Sudan	177 (25.7)	0.001	225 (32.7)	0.03	133 (19.3)	0.001

* % calculated from 688 of sera tested.

Table 2: Seroprevalence of BoHv-1, BVD and BoHv4 antibodies in animals afflicted with abortion, infertility and death of calves after birth among aborted cases in Sudan

Afflictions	Total No. of sera Tested	BoHv-1		BVD		BoHv4	
		+ve	%	+ve	%	+ve	%
Abortion	70	59	84.3	59	84.3	34	48.6
Infertility	38	33	86.8	31	81.6	10	26.3
Death after birth	12	9	75	11	91.7	7	*58.3

* Significant P value ≤05%

variations; however, antibodies of BoHv-4 were significantly (58.3%) prevalent among death after birth but less prevalent in aborted and infertility cases (48.6 and 26.3%) respectively.

DISCUSSION

Abortion and infertility are serious problems that cattle breeders face, because it is often difficult to determine the causes of bovine reproductive problems due to the numerous infectious and non infectious factors involved (Kirkbride, 1992; Jamaluddin *et al*., 1996). The present investigation showed that the studied viral diseases that may cause reproductive problems were prevalent as indicated by antibodies prevalence rates in dairy cattle in Sudan. It was also found that the studied viral antibodies were prevalent at all seasons of the year. The prevalence rates of BoHv-1, BVD and BoHv-4 antibodies in Khartoum state were 51.7%, 50.4% and 25% respectively, while in central Sudan BoHv-1 (32.7%) was highly prevalent followed by BVD (25.7%) and BoHv4 (19.3%).

A significant variation ($P \leq 0.03$) was observed in the prevalence of BoHv-1 in Khartoum state compared to central Sudan and in the rainy season compared to other seasons. This may be due to the fact that most of the farms in Khartoum state are intensively managed in contrast to central Sudan where semi intensive and extensive farm practices prevail. Gibbs and Rweyemamu (1977) explained that collection of cattle from diverse backgrounds in terms of previous exposure to the disease ensures the continual introduction of a wide variety of pathogens including BoHv-1. This may be obvious in Khartoum state where animals are recruited from different places and kept together and usually feedlot so as to satisfy many purposes including meat and milk production and external exports. This may expose animals to higher attack rates, more severe disease and higher fatality rates than in range or extensively raised dairy cattle.

In Sudan, no vaccination program for cattle against BoHv1, BVD or BHv-4 viruses is practiced. The result of this serological survey and the high overall antibodies prevalence rates observed (Table 1) would clearly indicate that these infectious viruses are part of the reproductive disorder problem in Sudanese cattle. Givens and Marley (2008) recorded that IBR and BVD viruses were infectious causes of embryonic and fetal mortality. In addition, BoHv-4 presents an important cause of abortion in cattle and could play a role in reproductive disorders (Deim *et al.,* 2007; Dagalp *et al*., 2007). While *in utero* infection with BVD virus may result in one of five outcomes: early embryonic death, abortion, persistent infection, congenital defects or birth of normal/weak serpositive calves (Grooms, 2004; Radostits *et al*., 2007)

The present study revealed that BoHv-1 infection was not significantly correlated with type of cattle, age or sex. This indicates that under the same circumstances, both male and female, all age groups and both indigenous and cross breed animals have an equal chance of acquiring infection. Earlier studies had also observed that BoHv-1 virus antibodies were prevalent in Sudanese cattle and that their prevalence was highest among animals more than three years old (El-Hussein *et al*., 2005).

Regarding BVD the prevalence of the virus antibodies was not significantly different among both breeds of cattle, age groups or seasons (Table 1). This finding indicates that the animals are exposed equally to infection during all seasons and at different ages. This can also be explained by the presence of many persistently infected animals (PI) which spread the disease by direct contact. Laureyns *et al*. (2009) stated that PI animals spread BVDV infection through the herd that; animals harbor the virus for life and shed it in high concentration without showing any immune response. The elimination of PI animals is, therefore, paramount in efforts to limit the spread of the virus, but a control program also requires other components such as determination of the herd's initial BVDV status and preventing the introduction of BVDV in non-infected herds

However, BVD antibodies prevalence was significantly correlated with sex with females having higher prevalence rate than males in Khartoum locality. This may be due to the point of view that BVD can be transmitted via semen especially from artificial insemination (AI) centers. In recent years, most of Khartoum farmers use AI as a tool for breeding. Meyling and Jensen (1998) reported that if PI bulls are used for AI, all or most of the seronegative females bred with the semen will be infected with BVD virus, although most will not produce PI calves.

During the course of the present work there was no significant variation between breed towards the prevalence of BoHv-4 antibodies but these antibodies were highly significantly correlated with the age of animal with older animals being more frequently infected than younger animals (Table 2). This finding fully agreed with those of Dagalp *et al*. (2007). Also highly significant variations were observed in the rainy season and in Khartoum state compared to central Sudan. Also females showed significantly higher BoHV-4 antibodies prevalence than males raising, once more, the issue of sexual transmission as mentioned for BoHv-1 above.

Examination of the 120 serum samples collected from individual animal with reproductive disorders including aborted cases, infertility and death after birth revealed that BoHv-1 antibodies were highly prevalent in infertility cases (86.8%) followed by aborted cases (84.3%) and death after birth (75%) with no significant variation (Table 1). On the other hand, BVD antibodies were high but not significant in cases of death after birth (91.67%), aborted cases (84.3%) and less in infertility cases (81.6%). BonDurant (2007) stated that both BoHv-1 and BVD viruses had been associated with clinical cases among herd outbreaks of abortion in cattle. Thus they must be considered as potential hazard in almost any cattle raising areas including Sudan.

BoHv-4 was significantly prevalent among death after birth cases (58.3% $P \leq 0.04$) followed by abortion and infertility cases (48.6% and 26.3% respectively). Deim *et al*. (2007) described the presence of BoHv-4 in 7 (29%) tissue of aborted bovine fetuses; the authors stated that BoHv-4 infection is considered as a risk factor for abortion in cows.

Conclusions

In conclusion, the results obtained in this study confirm the widespread nature of BoHv-1, BVD and BoHv-4 exposure among dairy cattle in Sudan. This indicates the need for further field and laboratory investigations to identify appropriate immunization or biosecurity measures to prevent infection and protect pregnant susceptible cows, thus reducing economical impact of these infections in the country. In addition this study documents for the first time the existence of BoHv-4 antibodies and its association with reproductive disorders of dairy cattle in Sudan.

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