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Original Article

Coinfections of Sudanese dairy cattle with bovine herpes virus 1, bovine viral diarrhea virus, bluetongue virus and bovine herpes virus 4 and their relation to reproductive disorders

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ABSTRACT

Objective: The aim of this study was to investigate the seroprevalence of coinfections with bovine herpes virus-1 (BHV-1), bovine viral diarrhea (BVD), bluetoungue virus (BTV) and bovine herpes virus-4 (BHV-4) in cattle with reproductive problems in Sudan, and to determine the relation of single or mixed seropositivity with abortion, infertility and death after birth.
Materials and methods: Meta-analysis of the results from our earlier serosurveys conducted with a total of 688 dairy cattle was carried out in order to determine prevalence of seropositivity of single and mixed (coinfection) viral infections.
Reults: The meta-analysis of the data indicated high seroprevalence of coinfections with various combinations of these agents; only few animals were singly infected. An infection with BHV-1 was observed to be higher than the prevalence of associations between BHV-1 and the other three viral agents. Prevalence of seropositivities to coinfection with BHV-1/BTV; BHV-1/BVD; BHV-1/BTV/BVD were the highest while seropositivities prevalences that involved BHV-4 were much lower. The highest abortion rates were encountered in coinfections with BHV-1/BVD/BTV (31%) and BHV-1/BVD/BTV/BHV-4 (30%) while most infertility cases were noticed in coinfection with BHV-1/BVD/BTV (44%) and BHV-1/BVD/BTV/BHV-4 (21%), and coinfections with the four viruses were encountered in most of the death after birth cases (25%). Overall mixed infections with BHV-1/BVD/BTV (34%) and BHV-1/BVD/BTV/BHV-4 (22.5%) were involved in the majority of reproductive problems studied.
Conclusion: Mixed infections constitutes the vast majority of cases and are involved in the majority of reproductive disorders investigated. The high prevalence of seropositivity to all of the four viruses should call for an intervention strategy to reduce the impact of these viruses.

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KEYWORDS

BHV-1, BVD, BTV, BVH-4, Coinfections, Reproductive disorders

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INTRODUCTION

Numerous bacterial, viral, protozoan, and fungal pathogens had been associated with reproductive problems in cattle (Givens and Marley, 2008). Of the viral pathogens, bovine herpes virus-1 (BHV-1), bovine diarrhea virus (BVD), bluetongue virus (BTV), and bovine herpes virus-4 (BHV-4) are well known to be involved in such reproductive problems in livestock (Kirkbride, 1992; Kelling, 2007; Muylkens et al., 2007; Khezri, 2015).

BHV-1 infection in cattle may result in various clinical forms including severe respiratory disease, genital infections with reduced reproducetive performance and abortion (Muylkens et al., 2007). Following primary infection, BHV-1 can become latent, and may be reactivated under stress conditions. In Sudan, BHV-1 was isolated since 1953 (Ali and El Amin, 1983).

BVD causes abortion and congenital defects especially when infection occurs during the first 100-150 days of gestation (Kelling, 2007). BVD is also known to disrupt the pregnancy before conception (Allen et al., 2005). Neutralizing antibodies to BVD were detected in different areas of Sudan (Ali and El Amin, 1983). BHV-4 has been isolated from animals with various clinical manifestations including pneumonia, meteritis, enteritis, conjunctivitis and bladder infections (Monge et al., 2006). On the other hand, BHV-4 may cause infertility and/or early abortion that are undetected and are rarely submitted for diagnostic purposes. Antibodies against BHV-4 were first detected in Sudanese dairy cattle in 2011 (El Hassan et al., 2011).

BTV is the prototype species of the genus Orbivirus, family Reoviridae. BT disease is characterized by various clinical forms with symptoms ranging from acute, subacute, mild or inapparent. The virus is responsible for fetal death, congenital defects and reproductive failure in cattle (Murray, 1999). The sequelae of abortion and the birth of calves with congenital defects like dwarfism, crooked legs and gingival hyperplasia may occur in cattle naturally infected with BTV in early stages of pregnancy (Luedke et al., 1970).

Several surveys indicated high prevalence of infections in cattle with BHV-1, BTV, BHV-4 and BVD in Sudan. The aim of the present study was to assess the prevalence of coinfections with these viruses and their associations in dairy cattle with reproductive problems in Sudan, and to assess the effect of these coinfections on reproductive disorders (*e.g.*, abortion, infertility and death after birth) in dairy cattle.

MATERIALS AND METHODS

Serum samples collected from dairy herds with reproductive problems from Khartoum and Gezira States (Central Sudan) have previously been investigated by us for seroprevalence of BHV-1, BVD virus, BHV-4 (Elhassan et al., 2011) and BTV (Elhassan et al., 2014a). Data generated from these studies in a total of 688 samples (of which 120 samples were from animals with known reproductive disorders) were collectively examined in order to identify prevalence of seropositivity of single and mixed (coinfection) viral infections. Also the relation of single or mixed seropositivity to reproductive problems was studied in 120 samples with known specific reproductive (abortion, infertility and death after birth) disorders.

Statistical analyses: Data were analyzed using package for social science (SPSS). To check the relation between seropositivity prevalence for BHV-1 and seropositivity of the other three viral agents the chi-square was determined. *P* value of $\leq$0.05 was considered significant.

RESULTS

The seropositivity prevalence of each of the four viruses (BHV-1, BTV, BVD and BHV-4) are shown in **Table 1**. BHV-1 seropositivity (84.4%) showed the highest prevalence followed by BTV and BVD (83.7% and 76.2% respectively). BHV-4 seropositivity, on the other hand, indicated the lowest (44.3%) prevalence. No significant differences between seropositivity of BHV-1 and BTV or BTV and BVD were noted while such differences were significant between the prevalence of BHV-1and BVD and between the seropositivity prevalence of BHV-4 and that of the other 3 viruses (**Table 1**). Pair wise compari-sons of the seropositivities of the 4 viruses indicated strong association of seropositivity between coinfections with BTV/BVD and BHV-1/BTV while such association were lacking among other combinations (**Table 2**).

The seropositivities of single and mixed infections with BVD, BTV and BHV-4 in relation to the seropositivity of BHV-1 are presented in **Table 3**. Infection by BHV-1 was observed to be higher than the prevalence of associations between BHV-1 and the other 3 viral agents.

In addition, prevalences of seropositivities due to coinfection with BHV-1/BTV, BHV-1/BVD, BHV-1/BTV/BVD were the highest while seropositivities prevalence that involved BHV-4 were much lower (**Table 4**). Generally; except for coinfection that involved BHV-4; other coinfection combinations were higher than singular infections (**Table 4**).

Table 1: Seropositivity to the four viruses (BHV-1, BTV, BVD and BHV-4) in the tested animals.

Parameters	BHV-1	BTV	BVD	BHV-4
+ve samples	581	576	524	305
%	84.4%[a]	83.7%[ab]	76.2%[b]	44.3%[c]

Percentage followed by different letters in the same line are different ($P<0.05$).

Table 2: Pairwise association of seropositivity to the four viruses (BHV-1, BVD, BTV, BHV-4) in the tested animals.

Parameters	BHV-1/BTV	BHV-1/BVD	BHV-1/BHV-4	BTV/BVD	BTV/BHV-4	BVD/BHV-4
+ve samples	489	473	270	439	267	244
P value	0.462	0.0001*	0.008*	0.942	0.015*	0.038*

*means the pair is not associated.

Table 3: Comparison between the number of samples seropositive for BHV-1, BVD, BTV, and BHV-4 and the percentage of samples positive for more than one virus in the tested animals.

Virus	+ve samples	Percentage
BHV-1	581	84.4[a]
BHV-1/BTV	489	71.1[b]
BHV-1/BVD	473	68.8[c]
BHV-1/BHV-4	270	39.2[f]
BHV-1/BTV/BVD	401	58.3[e]
BHV-1/BTV/BHV-4	236	34.3[i]
BHV-1/ BHV-4/BVD	229	33.3[j]
BHV-1/BHV-4/ BVD/BTV	197	28.6[k]

Percentage followed by different letters in the same line are different ($P<0.05$).

Table 4: Frequency of animals seropositive for single and mixed infections and their percentages in the tested animals.

Virus	Positive samples	Percentage
BHV-1	18	2.6
BVD	10	1.5
BTV	30	4.4
BHV-4	1	0.1
BHV-1/BTV	49	7.2
BHV-1/BVD	40	5.9
BHV-1/BHV-4	2	0.3
BVD/BTV	26	3.8
BVD/BHV-4	3	0.4
BTV/BHV-4	19	2.8
BHV-1/BVD/BTV	204	29.9
BHV-1/BVD/BHV-4	32	4.7
BHV-1/BTV/BHV-4	39	5.7
BVD/BTV/BHV-4	12	1.8
BHV-1/BVD/BTV/BHV-4	197	28.9
Total*	682	100

*6 Samples out of 688 were antibodies negative for any of the tested viruses.

Table 5: Frequency of abortion, infertility and death after birth in animals with seropositivity to single & multiple infections with BHV-1, BVD, BHV-4 and BTV.

Virus	Abortion (%)	Infertility (%)	Stillbirth (%)	Total (%)
BHV-1	-	-	-	-
BVD	-	-	1 (8.3%)	1(0.8%)
BHV-4	-	-	-	-
BTV	3 (4.3%)	3 (7.9%)	-	6 (5%)
BHV-1/BTV	3 (4.3%)	3 (7.9%)	1 (8.3%)	7 (5.8%)
BHV-1/BVD	6 (8.6%)	5 (13.2%)	-	11 (9.2%)
BHV-1/BHV-4	1 (1.4%)	-	-	1 (0.8%)
BVD/BHV-4	2 (2.8%)	-	-	2 (1.7%)
BVD/BTV	2 (2.8%)	-	1(8.3%)	3(2.5%)
BHV-4/BTV	2(2.8%)	1(2.6%)	-	3(2.5%)
BHV-1/BVD/BTV	22(31.4%)	17(44.7%)	2(16.7%)	41(34.2%)
BHV-1/BHV-4/BTV	2(2.8%)	-	-	2(1.7%)
BHV-1/BVD/BHV-4	9(12.9%)	-	3(25.0%)	12(10.0%)
BVD/BHV-4/BTV	2(2.8%)	1(2.6%)	1(8.3%)	4(3.3%)
BHV-1/BVD/BHV-4/BTV	16(22.9%)	8(21.0%)	3(25.0%)	27(22.5%)
Total	70(100%)	38(100%)	12(100%)	120(100%)

Prevalence of abortion, infertility and death after birth in the 120 cases of known reproductive problem in relation to seropositivity to single or multiple infections with BHV-1, BVD, BHV-4, and BTV are shown in **Table 5**. The highest abortion rate was found to be associated with mixed infections with BHV-1/BVD/BTV (31%) and BHV-1/BVD/BHV-4/BTV (30%). On the other hand, most infertility cases were encountered in multiple infections with BHV-1/BVD/BTV (44%) and BHV-1/BVD/BHV-4/BTV (21%) while multiple infections with the four viruses were responsible for most of the death after birth cases (25%). Overall mixed infections with BHV-1/BVD/BTV (34%) and BHV-1/BVD/BHV-4/BTV (23%) were involved in the majority of the productive problems. Seropositivity to single infections was found in minority of cases (6%) involving only BTV and BVD (**Table 5**).

DISCUSSION

Numerous studies in domestic and wild animals and in humans have shown that coinfection (multiple or mixed infections) with pathogenic agents is the rule rather than the exception (Bordes and Morand, 2011). Coinfecting pathogens can compete among each other or with host symbionts for host resources and can modulate host immune responses in a way that may or may not favor the emergence of invading pathogens or decrease the incidence of an established pathogen (Graham, 2008; Rynkiewicz et al., 2015). Hence, viral coinfection can lead to increased risk of hospitalization of children with respiratory infections compared with mono-infection (Diaz et al., 2015), however, in another study there was no evidence for or against respiratory viruses coinfection and risk of bronchiolitis in children (Goka et al., 2014). Infection of African lions with canine distemper virus; that is known to be immunosuppressive; led to high *Babesia spp*. parasitemia that led to high mortality in the infected lions (Munson et al., 2008). Mortality rate was also higher among chickens coinfected with chicken anemia virus (also a well-known immunosuppressive agent) and the malaria parasite *Plasmodium juxtanucleare* due to the very high parasitemia levels of the parasite induced by immunosuppressant effect of the virus (Silveira et al., 2013).

Our present data indicated that mixed infections constituted the vast majority of cases, only a minority of seropositive animals showed seropositivity to single infections with one of the 4 viruses tested (**Table 4**). Infection by BHV-1 was noted to be higher (P>0.05) than the prevalence of associations between BHV-1 and the other three viral agents (**Table 1**). This may be due to the high infectivity of BHV-1 and its ease of transmission especially under conditions of crowdedness that is prevalent in our peri-urban dairy farms.

The investigated viruses in our study are known to be immunosuppressive (BVD, BTV) or can be reactivated under stress condition (BHV-1, BHV-4) (Cavirani et al., 2005). This may explain the high prevalence of seropositivity to BHV-1 in animals coinfected with either BVD or BTV or by both BVD and BTV. These coinfections may lead to reactivation of BHV-1 and subsequent detectable levels of seroconversion. Similar phenomenon was observed in coinfections involving *Neospora caninum*, BTV and BVD (de Mello et al., 2004; Cavirani et al., 2005). Interestingly, in our study, sero-positivity to BHV-4 either singly or in combination with BVD and BTV was low but higher in coinfection that included BHV-1 (**Table 4**). This may indicate; unlike BHV-1; absence of reactivation of BHV-4 by BVD and BTV or otherwise that the additional stress by BHV-1 is required for its reactivation. It may also indicate that BHV-4 is not readily transmitted under field conditions in Sudan.

Our data showed that most of the abortion cases were found in animals seropositive to BHV-1/BVD/BTV (31.4%) or BHV-1/BVD/BTV/BHV4 (22%) which are the most prevalent coinfections (**Table 5**). The same was also true for the cases of infertility and death after birth where the same combinations of infection were involved in most of the cases. This might be attributed to the combined immunosuppressive effects of BVD, BTV and pregnancy which can allow easier infection of placental or fetal tissues (Murray, 1999) with consequent pathogenesis by the BHV-1 and/or BHV-4 in the infected mothers or fetuses. Compared to the other pair-wise concomitant BTV/BVD and BHV1/BTV infections; were according to our results; highly associated (**Table 2**). It is worth mentioning that mycotoxins contamination is highly prevalent in animal feeds in Sudan (Elzupir et al., 2009) and this may add to the immunosuppressive effects of the viral and other infectious agents in these animals.

Of interest is that single infections with either BHV-1 or BHV-4 were not responsible for any of the three reproductive problems studied while single infection with BTV was involved in cases of abortion and infertility (4.3% and 7.9%, respectively) and that of BVD was involved in cases of death after birth (8.3%). These results further support our earlier findings of association between prevalence of antibodies to BHV-1 and BVD and abortion, infertility and death after birth problems (Elhassan et al., 2011).

The cases of reproductive problems constituted 17% (n=120/688) of our study population which is relatively high and represents a major economic loss. This in addition to the high prevalence of antibodies to all of the viruses investigated in our study would call for adoption of intervention strategies to reduce the impact of these viruses. A key component of this strategy would be the wide use of vaccines that contain appropriate serotypes or genotypes. This will require that more efforts should be directed toward isolation, identification and characterization of the studied viruses in order deploy the appropriate vaccines.

However, one should anticipate that other widespread agents in Sudan such as Akabane virus (Elhassan et al., 2014b) and *Toxoplasma gondii* (Elfahal et al., 2013) and *Neospora caninum* (Ibrahim et al., 2012; Hussein et al., 2012) will undoubtedly be of significance in the many syndromes of reproductive disorders in Sudan.

CONCLUSION

The present study shows the detrimental effects of coinfections in terms of reproductive problems; however, other aspects such as morbidity, mortality and losses due to reduction in body weight, body weight gain, milk production and culling rates demand for further investigations. Finally, we only restricted our study to 4 viral agents of importance in bovine reproductive disorder.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHORS CONTRIBUTIONS

Elhussein AM and Elhassan AM conceived of the work and wrote the manuscript, Musa AB, Elsadig M and El Hussein AM did the statistical analysis.

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