The investigation of the herpesviruses (BoHV-1 and BoHV-4) on the occurrence of the reproductive disorders in dairy cattle herds, Turkey

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SUMMARY

In this study, the effects of Bovine Herpesvirus 1 (BoHV-1) and Bovine Herpesvirus 4 (BoHV-4) on reproductive disorders such as postpartum and chronic metritis, abortus and repeat breeder were investigated serologically and virologically in dairy cows, classified as Group I (n=8 herds) and Group II (n=5 herds) according to the presence of the animals with and without reproductive disorders, respectively. For this purpose, a totally 1000 sera (781 from herds in Group I and 219 from herds in Group II) were tested for antibodies to BoHV-1 and BoHV-4 by commercial ELISA. Additionally vaginal discharge samples (n=65), tissue samples from aborted foetus (n=7) and leukocyte samples (n=119) from cattle in Group I and/or Group II were examined for BoHV-1 and BoHV-4 by Polymerase Chain Reaction (PCR).

Data showed that the seropositivity rates were 52.3% and 47.9% for BoHV-1 in Group I and II, respectively. However, the rates were detected as 47.2% and 5.4% for BoHV-4 in Group I and II, respectively. Virological results are in concordance with serological results, which pointed the etiological role of BoHV-4 in reproductive disorders of Group I cattle alone or with some other agents such as BoHV-1.

Keywords: Bovine herpesvirus 1, bovine herpesvirus 4, reproductive disorders, antibody, polymerase chain reaction.

RÉSUMÉ

Présence des infections à Herpes virus 1(BoHV-1) et Herpes virus 4 (BoHV-4) chez les bovins avec des troubles de la reproduction dans les troupeaux laitiers.

Dans cette étude, les effets des infections à BoHV-1 et BoHV-4 sur les troubles de la reproduction (métrites post partum et métrites chronique, avortements et vaches nécessitant plusieurs saillies avant de concevoir) ont été étudiés par une approche sérologique et virologique chez les vaches laitières réparties en 2 groupes I (n = 8 élevages) et II (n = 5 élevages) selon la présence d'animaux présentant ou non des troubles reproductifs, respectivement. Pour cela, une recherche d'anticorps anti- BoHV-1 et anti-BoHV-4 a été réalisée par ELISA à partir des échantillons de sérum de 1000 vaches (781 issues des élevages du groupe I et 219 issues des élevages du groupe II). La présence de virus BoHV-1 et BoHV-4 a été recherchée par PCR dans des échantillons des écoulements vaginaux (n = 65), des échantillons de tissus d'avortons (n = 7) et des échantillons de leucocytes (n = 119) des vaches des groupes I et/ou II.

Les résultats montrent que les taux de séropositivité sont de 52,3 % et 47,9 % pour BoHV-1 dans les groupes I et II, respectivement. Pour BoHV-4, ces taux sont de 47,2 % et 5,4 % dans les groupes I et II, respectivement. Les données virologiques sont cohérentes avec les résultats des sérologies qui suggèrent l'implication de BoHV-4 dans l'incidence des troubles reproductifs des vaches du groupe I seul ou en association avec d'autres agents comme BoHV-1.

Mots clés : Herpès virus bovin 1, herpès virus bovin 4, troubles de la reproduction, sérologie, réaction en chaîne par polymérase.

Introduction

Reproductive disorders are common health problems of dairy farming in the world and lead to considerable economic losses. A number of viruses have been presumed to play a role in reproductive disorders of cattle. Bovine herpesviruses (BoHV-1 and BoHV-4) are known to cause reproductive disorders in cattle. Herpesviruses are agents of a wide range of disease syndromes in cattle [14, 17, 20, 26, 30] and the main objects of control and eradication programs worldwide because of the considerable economic losses they cause [7,8,10].

BoHV-1 is the causative agent of infectious bovine rhinotracheitis, which is a severe and highly contagious respiratory disease. From this regard, abortion and fatal systemic diseases in neonates are the most severe consequences of BoHV-1 [21]. It is also responsible for latent infection, mainly in sensory neurons, innervating their multiplication site [1].

BoHV-1 is currently eradicated in several European countries. Similarly, control programs based on the use of marker vaccines have been initiated in other European countries, especially those experiencing a high seroprevalence. These vaccines allow serological differentiation between naturally infected and vaccinated cattle [28].

BoHV-4 is a worldwide distributed gammaherpesvirus which is antigenically and biologically distinct from all other bovine herpesviruses. In primary and latent infections, their target cells are essentially mononuclear cells. Like other herpesviruses, animals experimentally infected with BoHV-4 develop a latent infection, and virus reactivation and reexcretion occurs in these animals following the dexamethasone treatment

[7, 19]. The persistent infection with BoHV-4 that was established in each species has been shown to interfere with the immune response [22]. This effect may be a major contributory factor to the impaired defense system of animals against other pathogens during acute and latent BoHV-4 infections [13]. BoHV-4 establishes persistent infections in blood leukocytes, spleen macrophages and endothelial cells [18, 22, 23] and can be reactivated by dexamethasone treatment in various tissues [7].

The association between viral infections and reproductive disorders of dairy cattle a lot of studies [9, 24] reported in Turkey. ÖZKUL *et al*. [24] reported that out of tested sera from cows with fertility problems, 22.8% and 21.0% were positive for BVDV and BoHV-1, respectively. In the other study [9], the seroprevalence of BoHV-1 was reported as 68.1% in cows with fertility problems from 19 closed dairy herds.

Similarly, the presence of antibodies against BoHV-4 in cattle with reproductive problems has been reported by several researchers [14, 17, 20]. Also, MONGE *et al*. [20] detected BoHV-4 in 83% of the cases with clinical signs of acute postpartum metritis by virus isolation and BoHV-4 gB-PCR. In Turkey, studies [3,4] focused the interaction between BoHV-4 and fertility problems had also been reported. BILGE DAĞALP *et al.* [3] noted the BoHV-4 seropositivity as 56.8% and 44.9% in the selected dairy herds with reproductive disorders and with healthy appearance, respectively. BILGE DAĞALP *et al*. [4] also determined that 29% (16/55) of the vaginal swab samples obtained from cows with postpartum metritis were positive for BoHV-4 DNA by gB-PCR in a dairy herd in Turkey.

The aim of this study was to investigate the role of the BoHV-1 and BoHV-4 on the occurrrence the reproductive disorders in dairy cattle herds, which including 500 animals per herds at least, as a pilot project for future studies on the control of these infections.

Material and Methods

SAMPLED ANIMALS

A total of 1000 cattle aged ≥2 years old, housed in 13 different dairy herds, were sampled. The dairy herds which have a lot of animals with reproductive disorders like metritis, abortus or repeat breeder (No:I, II, III, IV, V, VI, VII, VIII) were included in Group I as well as the other dairy herds housing animals without reproductive disorders (No: IX, X, XI, XII, XIII) were included in Group II (Table I). All sera samples were tested for antibodies to BoHV-1 gB and BoHV-4 by commercial ELISAs. Besides, sera from animals vaccinated with BoHV-1 gE(-) inactivated marker vaccine (Pfizer, France) (Herds No I and III) were tested for antibodies for gE antigen of BoHV-1 because they had been vaccinated 2 months before the sampling. For virological studies, vaginal discharges (n=65) and tissue samples (liver, spleen and lungs) from aborted fetus (n=7) animals and leukocyte samples (n=119) were sampled from cattle in Group I and/or Group II. Then, they tested for BoHV-1 and BoHV-4 by PCR. The information of the presence / absence of the reproductive disorders and their rates in the herds with reproductive problems, and also of the treatment applications to animals, had been obtained from the veterinarian of these herds. A number of the samples taken from the herds and remarks were given in Table I.

Group No	Herd no	Province	Test materials				Remarks	Vaccination
			Serum	Leukocyte	Vaginal swab samples	Aborted foetus		
I	I	Balıkesir[1]	176	36	24	-	M, A, HA	Inactivated IBR-Marker
	II	Balıkesir[2]	48	33	1	2	M, A	-
	III	Kırklareli	148	5	15	-	M, A,HA	Inactivated IBR-Marker
	IV	Aydın[1]	96	21	7	-	RB	-
	V	Kayseri[1]	23	-	1	-	A	-
	VI	Kırşehir	28	3	-	-	A, RB	-
	VII	Aksaray	260	9	13	3	M, A, HA	-
	VIII	Sakarya	2	-	-	2	A	-
	Subtotal		781	107	61	7		
II	IX	Aydın[2]	79	-	-	-	HA	-
	X	Kayseri[2]	45	1	4	-	HA	-
	XI	Yalova	50	11	-	-	HA	-
	XII	Malatya	30	-	-	-	HA	-
	XIII	Elazığ	15	-	-	-	HA	-
	Subtotal		219	12	4			
	Total		1000	119	65	7		

M: metritis A: abortus HA:healthy appearance RB: Repeat breeder

TABLE I: The distribution of the sample according to the herds.

VIRUSES

BoHV-1 Cooper and BoHV-4 DN-599 strain were used as control viruses for PCR. BoHV-4 reference strain was kindly provided by Dr.G.J.Wellenberg (Lelystad, Netherlands). Madin Darby Bovine Kidney and Bovine turbinatae (BT) cell cultures were used for propagation of reference viruses, respectively.

DNA EXTRACTION AND POLYMERASE CHAIN REACTION (PCR) TECHNIQUE (FOR BOHV-1/4)

DNA extractions from vaginal discharge samples were carried out according to SAMBROOK *et al*. [25]. The oligonucleotide primers specific for the BoHV-4 glycoprotein B (gB1 and gB2) [16] were used in the PCR amplification of the target fragments. The detection of BoHV-1 DNA was performed using the BoHV1-glycoprotein C (gC) PCR as described by VAN ENGELENBURG *et al*. [27] with minor modifications. The detection of BoHV-4 DNA was performed using the BoHV-4-gB PCR as described by WELLENBERG *et al*. [31] with some modifications.

Briefly, 3 µl DNA was subjected to thermocycling in a 30 µl reaction mixture. The reaction mix contained 2.5 U Taq Polymerase, 3.5 mM dNTP mix, primers at 10 pmol concentrations, 1.5 mM $MgCl_2$, 1X PCR buffer and 6% DMSO. Thermal cycling conditions were 6 min at 96°C followed by 40 cycles at 56°C for 45 sec, 72°C for 2 min, and 95°C for 1 min, followed by a final 10 min extension at 72°C for both primer sets. 3-5 µl each of the amplified PCR products from the final reaction were visualized in 1% agarose gel containing ethidium bromide.

ELISAs

A commercial indirect ELISA kits (Bio X, Belgium, Bio K 066 for BoHV-4; Bio K 027 for BoHV-1) were used to detect antibodies against the BoHV-1 and BoHV-4. Additionally, sera from animals in Herd I and III were tested with gE blocking ELISA kit (IDEXX, Westbrook, Maine, cat no: BGVV-B174). All ELISAs were carried out as recommended by manufacturer.

Results

PCR

The expected size of amplicon (615 bp) was obtained from leukocyte and vaginal swab samples. The rates of the positivity for BoHV-4 gB gene were detected as 26.1% (17/65) and 33.6% (40/119) in vaginal discharge and leukocyte samples, respectively (Table II). No fragment was amplified from vaginal discharge samples for BoHV-1. Data for cattle which their vaginal swab samples were detected positive for BoHV-4 were summarized in Table III.

ELISA

Out of 1000 blood sera tested, 51.4% (514/1000) and 38.1% (381/1000) were found to be positive for BoHV-1 and BoHV-4, respectively. The positivity rates for BoHV-1 were 52.3% and 47.9% in Group I and II while the rates for BoHV-4 were 47.2% and 5.4 % in Group I and II, respectively. According to the herds and Groups, the seropositivity rates for the natural infections with BoHV-1 and BoHV-4 were given Table IV.

Discussion

In this study, the role of BoHV-1 and BoHV-4 on the reproductive disorders were investigated serologically and virologically in cattle housed in different herds included in Group

Herd	Vaginal swab samples		Leukocyte samples	
no	The number of materials	PCR + (%)	The number of materials	PCR+ (%)
I	24	7 (29.1)	36	16(44.4)
II	1	1(100)	33	8(24.2)
III	15	3 (20.0)	5	4(80.0)
IV	7	3 (42.8)	21	7(33.3)
V	1	0	-	-
VI	-	-	3	3(100)
VII	13	3 (23.07)	9	1(11.1)
VIII	-	-	-	-
Subtotal	61	17 (27.8)	107	39(36.4)
IX	-	-	-	-
X	4	0	1	1(100)
XI	-	-	11	-
XII	-	-	-	-
XIII	-	-	-	-
Subtotal	4	0	12	1
Total	65	17(26.1)	119	40(33.6)

TABLE II: The results of PCR for BoHV-4 according to herds

Herd no	Animal no	BHV1 Ab(+)	BHV4 Ab(+)	Vaginal swab PCR	Leukocytes PCR	Clinical signs	Age
I	T9	+	+	+	+	Metritis	6
	T17	+	+	-	+	Metritis	NI
	T53	-	+	-	+	Metritis	NI
	T58	-	+	+	-	Metritis	4
	T61	-	+	+	+	Metritis	3
	T82	-	+	+	NT	Metritis	7
	T157	-	+	+	NT	Metritis	3
	T141	+	+	+	NT	Abortus	6
	T209	+	+	+	NT	Abortus	7
III	TG1	-	+	-	+	Metritis	NI
	TG3	-	+	-	+	Metritis	NI
	TG4	-	+	+	+	Metritis	2
	TG9	-	+	+	+	Metritis	2
	TG100	-	+	+	NT	Metritis	2
II	E205	-	+	+	NT	Abortus	NI
V	A80	-	+	+	+	RB	NI
	A67	+	+	+	-	RB	NI
	A78	-	-	+	-	RB	NI
VIII	KÇ12	+	-	+	NT	Metritis	9
	KÇ15	+	-	+	NT	Metritis	3
	KÇ21	+	+	+	NT	Metritis	3

NT: Not tested RB: Repeat breeder NI: No information

TABLE III: The individual results of some cattle with reproductive disorders.

Groups	Herd No	The number of materials	BoHV-1 (+) (%)	BoHV-4 (+) (%)	BHV1 / BHV-4 (+/+) (%)	BHV1/BHV4 (+/-) (%)	BHV1/BHV4 (-/+) (%)	BHV1/BHV4 (-/-) (%)
I	I	176	65 (36.9)	88 (50.0)	39 (22.0)	26 (14.7)	49 (27.8)	62 (35.2)
	II	48	18 (37.5)	37 (77.0)	17 (35.0)	1 (2.0)	20 (41.6)	10 (20.8)
	III	148	33 (68.7)	49 (33.1)	11 (7.0)	22 (14.8)	38 (25.6)	75 (50.6)
	IV	96	37 (38.5)	21 (21.8)	16 (16.6)	21 (21.8)	5 (5.0)	54 (56.0)
	V	23	19 (82.6)	11 (47.8)	10 (43.4)	9 (39.1)	1 (4.0)	3 (13.0)
	VI	28	16 (57.1)	3 (10.7)	1 (3.0)	15 (53.5)	2 (7.0)	10 (35.7)
	VII	260	221 (85)	160 (61.5)	157 (60.3)	64 (24.6)	3 (1.0)	36 (13.8)
	VIII	2	-	-	-	-	-	2 (100.0)
	Subtotal	781	409(52.3)	369(47.2)	251	158	118	254
II	IX	79	7 (8.8)	-	-	7 (8.0)	-	72 (91.0)
	X	45	32 (71.1)	1 (2.2)	1 (2.0)	31 (68.0)	-	13 (28.8)
	XI	50	44 (88.0)	11 (22.0)	9 (18.0)	35 (70.0)	2 (4.0)	4 (8.0)
	XII	30	17 (56.6)	-	-	17 (56.6)	-	13 (43.3)
	XIII	15	5 (33.3)	-	-	5 (33.3)	-	10 (66.6)
	Subtotal	219	105(47.9)	12(5.4)	10	95	2	112
	Total	1000	514 (51.4)	381 (38.1)	261 (26.1)	253 (25.3)	120 (12)	366 (36.6)

TABLE IV: The seroprevalence of BoHV-1 and BoHV-4 infections according to the sampled herds.

I and Group II. The seroprevalences detected in indicated that BoHV-1 and BoHV-4 infections are widespread in cattle/herds in Turkey as reported elsewhere [2,3,4,5].

The positivity rates were detected as 52.3% and 47.9% for BoHV-1 and 47.2% and 5.4% for BoHV-4 in Group I and II, respectively. When the serological data from Group I and Group II were compared, the differences of the rates for BoHV-4 were found to be statistically important (*P<0,01*). Similarly, the positivity (26.1%) of the vaginal swab samples from cattle in Group I for BoHV-4 have been had us reflect its etiological importance on the clinical remarks (Table II) described in Group I, along or with other pathogens as BoHV-1, BVDV, etc. Also, BoHV-4 was detected in leukocyte samples

from Group I in 36.4% (39/107) while it was detected as 8.3% (1/12) in Group II. It is important that 4 cattle were identified as infected with BoHV-4 depend on the positive results from leukocyte samples while their vaginal swab samples were found to be negative for BoHV-4 nucleic acid (Table III). In this study, no BVDV infection were investigated in herds because many of them have a control program of BVDV infection for a long time (depend on the elimination persistently infected animals with or without vaccination the rest of the animals in herd after their removing). Although BoHV-1 was not detected by PCR in cattle with reproductive disorders, the presence of high BoHV-1 seroprevalence should be considered. IBR vaccination in herds numbered I and III with high reproductive disorders can be helpful in reducing the virus spread in accordance with antibody titers.

Despite the absence of satisfactory knowledge regarding the coexistence of BoHV-1 and BoHV-4 viruses, a possibility of interaction between these two viruses could be a concern (Table IV) as shown by different researchers for individual viruses [2, 5, 6].

It is known that BoHV-4 may concurrently cause persistent infection in leukocytes, spleen macrophages and endothelial cells [11, 18, 22]; on the other hand, dexamethasone application in tissues can reactivate the infection [7]. It was reported that pregnancy may cause BoHV-4 reactivation [20, 29]. Thus, the virus can be an important agent in postpartum metritis. In addition, BoHV-4 reactivation can play a role in opportunistic infections [12]. After infection, BoHV-4 replicates in the epithelial cells of the upper respiratory tract and intestines. The virus may also replicate in peripheral blood leukocytes and spread throughout the body in these infected cells. Lymphoid organs, mononuclear cells (monocyte-macrophage lineage) and lymphocytes have been suggested as loci of viral latency in cattle as the virus was recovered from co-cultures and organ explants of spleen from all latently infected cattle [7, 22]. For this purpose, a totally 119 leukocyte samples, which 107 leukocyte samples obtained from Group I and 12 from Group II, were analysed for BoHV-4 by PCR and 33.6% were found to be positive although a sample was from cattle from healthy appearance. The results from animal No . T17, T53, TG1, TG3 (Table III) were pointed the long term viremia as reported elsewhere [13] because of the differences of the results from vaginal swap and leukocyte samples. Even we have no this result, it is noted that virus would not be present in leukocytes in each case, or its level could be lower than the detection limit during a local infection with BoHV-4.

In conclusion, it may say that BoHV-4 causes reproductive problems along and/or the other agents and it is possible that the interaction between BoHV-1 and BoHV-4 viruses could increase reproductive disorders in dairy cows. The use of marker vaccines offers good prospects for the eradication of BoHV-1 while the vaccine has not yet been developed against BoHV-4. However it is remained that the potential of lifelong infection necessitates the detection of positive animals within a herd in order to control of endemic infections. Further studies on the epidemiological analyses of herpesviruses and their interactions with together and with other pathogens in the different clinical cases will be needed to understand their effects on economical losses in cattle husbandry in Turkey.

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