Trop Anim Health Prod (2018) 50:353–363
DOI 10.1007/s11250-017-1440-3

REGULAR ARTICLES

Long-term study (2005–2010) on the vaccination with BoHV-1 glycoprotein E-deleted marker vaccine in selected two dairy herds in Turkey

Feray Alkan[1] · **S. Bilge-Dagalp**[1] · **Z. Karapınar**[2] · **M. O. Timurkan**[3] · **N. Coskun**[4] · **I. Burgu**[1]

Received: 25 April 2017 / Accepted: 26 September 2017 / Published online: 25 October 2017

Abstract A follow-up study from 2005 to 2010 was carried out in two herds where eradication programme for the bovine herpes virus-1 (BoHV-1) infection depends on the vaccination with inactivated glycoprotein E-deleted vaccine that was started in 2001 following the vaccination with inactivated conventional vaccine between 1999 and 2001. For serological screening, a total of 12,976 sera sampled over several sampling times approximately 6 months of interval during 5 years (2005–2010) were tested for glycoprotein E (gE)- and glycoprotein B-specific antibodies using ELISA. According to the serological evidence, the long-term persistence of BoHV-1 antibodies, success of marker vaccine, first vaccination time of the calves in herds regularly vaccinated, etc. were discussed in this paper. In conclusion, the vaccination programme using gE (−) marker vaccines, with making efforts to prevent the other factors about transmission of infection, was suggested for the eradication of BoHV-1 infection in Turkey as many EU countries. This is the first report on the BoHV-1 eradication programme in some dairy cattle in Turkey.

Keywords BoHV-1 · Marker vaccine · ELISA

✉ Feray Alkan
falkan@ankara.edu.tr

[1] Faculty of Veterinary Medicine, Department of Virology, Ankara University, Ankara, Turkey

[2] Faculty of Veterinary Medicine, Department of Virology, Balıkesir University, Balıkesir, Turkey

[3] Faculty of Veterinary Medicine, Department of Virology, Atatürk University, Erzurum, Turkey

[4] Faculty of Veterinary Medicine, Department of Virology, Kafkas University, Kars, Turkey

Introduction

Bovine herpes virus-1 (BoHV-1) belongs to the genus *Varicellovirus* under *Alphaherpesvirinae* subfamily of the *Herpesviridae* family, which causes a wide range of disease syndromes including severe and highly contagious respiratory disease named IBR (infectious bovine rhinotracheitis), fertility problems like repeat breeding and abortion and fatal systemic diseases in neonates (Jones and Chowdhury 2010; Raaperi et al. 2012; Radostits et al. 2007). By spreading intracellularly, BoHV-1 can exist in the presence of specific antibodies to this virus (Srikumaran et al. 2007). Hence, the cattle infected with BoHV-1 remain carriers of the virus for the rest of their lives. It is known that the occult virus remains in sensory neurons which innervate its multiplication site (Ackermann et al. 1982) and also remain in non-neural sites such as tonsillar lymphoid cells (Winkler et al. 2000). It can reactivate following some stress factors such as calving, transports, parasite infestation, and some drug administration (Kook et al. 2015; Winkler et al. 2000). After reactivation of virus, re-excretion of BoHV-1 from the infected animals causes the spreading of the virus in a population (Pastoret and Thiry 1985). Thus, in the framework of an eradication programme, all seropositive animals should be considered as potential carriers of the virus. It is known that there are two approaches in BoHV-1 infection control and eradication. One of them depends on the strategy to test the animals and then to slaughter/remove the seropositive animals. This approach is useful in the countries which have an initial low prevalence (Ackermann and Engels 2006). The other strategy is to use a marker vaccine including glycoprotein E (gE) deletion mutants of bovine herpes virus type 1 (BoHV-1) and to remove the naturally infected animals from herd when the rate of them could be acceptable economically for removing. Latter approach has been preferred

because of the ability to differentiate animals between vaccinated with marker vaccine or naturally infected, using specific detection systems such as ELISA (Van Oirschot et al. 1996) in a lot of EU countries with higher prevalence (Bosch et al. 1997, 1998; Ackermann and Engels 2006; Raaperi et al. 2014). EU legislation (Decision 2004/558/CE) defines the requirements to be fulfilled in order to obtain approval for such an IBR eradication programme. As known, a lot of EU countries have finished the control programme and certificated as BoHV-1 free either on a region (province of Bolzano, Italy) or on a nationwide basis (as Denmark, Finland, Sweden, Austria, Norway, Sweden, Switzerland) (Ackermann and Engels 2006; Muylkens et al. 2007; Raaperi et al. 2014).

In Turkey, there is no official programme to eradicate BoHV-1 infection, while some rules have been asked for the bulls in Artificial Insemination (AI) centres. But, a lot of studies on the serological screening of private and state farms (Alkan et al. 2005; Tan et al. 2006; Gur 2011) and studies questioning BoHV-1 as an aetiological agent for different clinical cases (Alkan et al. 2000; Bilge-Dagalp et al. 2012) were reported. These studies showed that (i) BoHV-1 is common in a lot of herds with high the seropositivity rates (Alkan et al. 2005). (ii) BoHV-1 has caused some economical losses due to the fertility problems such as abortion, repeat breeding and respiratory tract disease (Alkan et al. 2000; Bilge-Dagalp et al. 2012) in herds.

For this reason, a pilot project was conducted in 31 herds which have some problems such as repeat breeding, metritis and different seropositivity rates focusing on the control/eradication, based on vaccination with the commercial inactivated gE-deleted BoHV-1 vaccine in the beginning of the 1997 (Alkan et al. 2005). These state farms were chosen as examples for the eradication of BoHV-1 in Turkey, because they have the sources of good management and they aim at providing a BoHV-1 negative cattle stock. The schedule of the vaccination was composed by the use of the inactivated vaccine between 1999 and 2000, for at least preventing the raising the new naturally infected animals, following the use of marker vaccine after that time due to the choice of managers of herds. In this paper, the results and detail of BoHV-1 control/eradication programme from two of them chosen a representative were discussed.

Materials and methods

History of herds and design of the study

In this study, 2 of 31 herds in organized farms, tested for BoHV-1 antibodies to determine the presence/seroprevalence of the infection in a previous study (Alkan et al. 2005), were used. These herds are closed and had been restocking only from internal animal source. Animals over 1 year of age are kept under same roof, although new borns are grouped under different roof for every 3 months of age. However, they do mingle freely during the grazing. In the previous study, authors showed that 53.2% (6930/13011) of the sampled cows and 97% of the herds (30/31) were naturally infected with BoHV-1 (Alkan et al. 2005). In positive herds with high seropositivity, the animals had been vaccinated with conventional inactivated BoHV-1 vaccine during 2 years (from 1999 to 2000) for reducing of the spreading of virus, because the suggestion on using the marker vaccine with periodically sampling and removing the seropositive animals was economically unacceptable. After that, use of the BoHV-1 marker vaccine was started for eradication of the infection in these herds in 2001. The vaccination programme was carried out as recommended by manufacturer (Rispoval® IBR Marker Inactivated, Pfizer). All the animals aged 3 months or more were vaccinated subcutaneously. A booster vaccination was given after 21 days. They were revaccinated every 6–8 months. At time interval of 4 years (2001–2005), we have monitored these herds for clinical signs without serological analysis because the antibodies related the inactivated BoHV-1 vaccine used in 1999–2000 would be detectable for the at least 3–4 years.

The BoHV-1 seroprevalence in two herds used in this study were 50.2 and 17.3% in the sampling in February 1999 and December 1998 for herd I and herd II, respectively (Alkan et al. 2005). The seroprevalence of BVDV infection were estimated at 55.4 and 0.6% in the same sampling times in these herds which had high pregnancy rates (95 and 96%) and birth rates (93.8 and 93.0%).

For the investigation of the results of vaccination with marker vaccine, all animals in these two herds are periodically sampled with 4–6 months of interval between 2005 and 2010 (Table 1). Some reproductive disorders like metritis, abortion or repeat breeding were detected in herd I, while no specific clinical symptoms were noted about BoHV-1 infection in herd II by herd veterinarians during the eradication programme.

Serum samples

A total of 12,976 blood samples were collected and tested in this follow-up study covering the time period from 2005 to 2010 (Table 1). Blood samples collected directly into polystyrene tubes with clot activator (Greiner, 455071) were centrifuged at low speed, and the serum samples were stored at − 20 °C until use. The serum samples were analysed for BoHV-1 antibodies using commercially available ELISAs for the detection of antibodies specific to glycoprotein B (gB) and gE of BoHV-1.

Table 1 The number of sampled animals according to the sampling times in herds

Sampling no.	Herd no. I				Herd no. II			
	Date	Animals			Date	Animals		
		n	≤ 6 months	> 6 months		*n*	≤ 6 months	> 6 months
I	16.May.2005	636	80	556	13.May.2005	555	144	411
II	30.March.2006	684	71	613	04.April.2006	581	13	568
III	06.December.2006	707	121	586	26.December.2006	632	97	535
IV	26.September.2007	782	164	618	29.May.2007	633	121	512
V	19.March.2008	732	89	643	14.November.2007	707	124	583
VI	30.October.2008	748	55	693	24.April.2008	730	122	608
VII	27.May.2009	715	–	715	5.November.2008	720	161	559
VIII	18.November.2009	738	103	635	26.May.2009	667	124	543
IX	05.May.2010	816	116	700	02.November.2009	654	99	555
X					14.May.2010	704	124	580
Total		6559	799	5760		6417	1129	5288

Serological monitoring by ELISA

A commercial indirect ELISA kit (Bio K 027 for gB BoHV-1) and gE blocking ELISA kit (IDEXX, USA) were used to detect antibodies against gB and gE of BoHV-1, respectively. All ELISAs were carried out as recommended by manufacturer.

Data analysis

For each sampling time, seroprevalence of the infection was estimated considering ratio of the number of gE-seropositive cattle to the number of cattle sampled. The success of vaccine was determined evaluating the ratio of the number of gB seropositive without gE antibodies of the cattle sampled. Additionally, the suggestion for the first vaccination age was determined according to data on the duration of maternal antibodies in calves aged 1–11 months in every sampling time.

Results

At the end of first sampling time (in May 2005; Table 1), the percentages of the seropositive animals for gE antibodies were found to be 28.77 and 25.7% for herd I and herd II, respectively. As reported in the material and method part of this paper, all animals with gE antibodies were accepted as naturally infected although it is possible that a fraction of the animals can establish latency without immediately presenting detectable anti-gE antibodies and, also, some young animals may have maternal gE antibodies without (presently) being infected. The results showed that the rates of animals with detectable antibodies against gB were reduced in the following sampling times according to the data from all sampled animals (Table 2; Fig. 1). Also, seropositivity rates calculated for animals aged ≥ 6 months were shown in Fig. 2 to exclude seropositivity related to the maternal antibodies. On contrary, the rates of the animals had anti-gB antibody without anti-gE-antibody have increased steadily in the following sampling times for both all sampled animals (from 55.81 to 89.9% for herd I and from 64.5 to 93.3% for herd II) (Table 2; Fig. 1) and for animals aged ≥ 6 months (Fig. 2). These rates showed that BoHV-1 marker vaccine being used in these herds has the high quality of the immunization to animals although quantitative reaction for antibodies in animals that have been vaccinated single or multiple times could not be investigated.

The rates of animals with detectable antibodies against gB and with or without gE according to the age at the sampling times are presented in Tables 3, 4, 5 and 6. These tables show that positivity of animals that have antibodies against gB without gE was increased in older age in the further sampling times. Contrastly, positivity of animals that have antibodies against both gB and gE was decreased. This result pointed that the use of marker vaccines was able to prevent the spreading of infection in these herds. Additionally, the rate of the animals without antibodies related to both gB and gE gradually fell down in the further sampling times (data not shown).

When assessing the efficacy of the vaccine, the rates of the exposure to natural infection in newborns are important to design the eradication programme in the herd, the same is also true for the rates of animals aged ≥ 6 months with antibodies to gE and rates of animals have antibodies related to vaccine. The results of tests performed on animals aged 1–11 months

Table 2 The positivity for antibodies to gB and/or gE according to the sampling times in herds

Sampling no.	Animals in herd no. I				Animals in herd no. II			
	Sample numbers (*n*)	Both gE and gB positive (%)	gB positive without gE (%)	Both gB and gE negative (%)	Sampling numbers (*n*)	Both gE and gB positive (%)	gB positive without gE (%)	Both gB and gE negative (%)
I	636	183 (28.77)	355 (55.81)	98 (15.40)	555	143 (25.7)	358 (64.5)	54 (9.7)
II	684	144 (21.05)	497 (72.66)	43 (6.28)	581	134 (23.0)	447 (77.0)	–
III	707	128 (18.10)	499 (70.57)	80 (11.31)	632	132 (20.8)	457 (72.3)	43 (6.8)
IV	782	119 (15.21)	641 (81.96)	22 (2.81)	633	125 (19.7)	469 (74)	39 (6.3)
V	732	83 (11.35)	617 (84.28)	32 (4.37)	707	91 (12.87)	559 (79.0)	57 (8.1)
VI	748	61 (8.15)	641 (85.69)	46 (6.30)	730	86 (11.78)	598 (81.91)	46 (6.40)
VII	715	33 (4.61)	625 (87.41)	57 (7.97)	720	62 (8.61)	617 (85.69)	41 (5.69)
VIII	738	31 (4.20)	656 (88.88)	51 (6.91)	667	21 (3.14)	621 (93.10)	25 (3.74)
IX	816	23 (2.81)	735 (90.07)	58 (7.10)	654	27 (4.12)	620 (94.80)	7 (1.07)
X					704	19 (2.69)	657 (93.32)	28 (3.97)

also pointed the success of the vaccination in these herds (Appendix). When age-related distribution of the positivity for antibodies against to gE and gB in sampling animals ≤ 1 year was investigated, a higher ratio of the presence of gB antibodies was observed in the calves with 1–4 months of age in the first four to five sampling times when positivity rates for the gB antibodies were also high in adult animals. Thus, the positivity for gB antibodies in these calves decreased gradually in parallel with that in adults in the further sampling times. To investigate the duration of maternal antibodies related to vaccine and the first vaccination time for calves, the seropositivity rates for gE and gB antibodies were determined. As a result, the rates of maternal antibodies in calves and the number of vaccination had a positive correlation. Briefly, we can say that maternal antibodies related to vaccine were reduced approximately 5–6 months. Our results are parallel with the suggestion for the first vaccination time with marker vaccine recommended by manufacturer.

Discussion

IBR, listed by the World Organization of Animal Health, has been responsible for important economic losses due to decreased production and fertility (Sayers 2017; Rola et al. 2014; Hage et al. 1998; Statham et al. 2015; Graham 2013). It is known that vaccination with conventional or marker vaccine, and also others like DNA and subunit, has been the most important control strategy for the purpose to decrease level of the naturally infected animals and prevent the economical losses or eradicate the infection (Bosch et al. 1998; Ackermann and Engels 2006). Although the conventional vaccines confer effective clinical protection against clinical symptoms induced by BoHV-1, they have disadvantages in the framework of an eradication programme because of the impossibility of the distinguishing of the antibody response, induced by either these vaccines or from that after natural

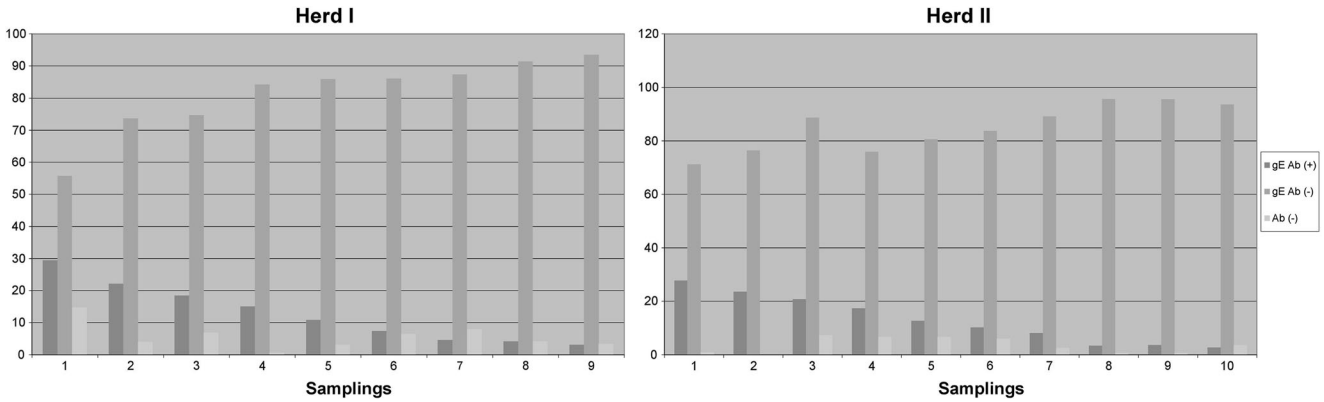

Fig. 1 The results of ELISAs for antibodies to gE and gB in herds

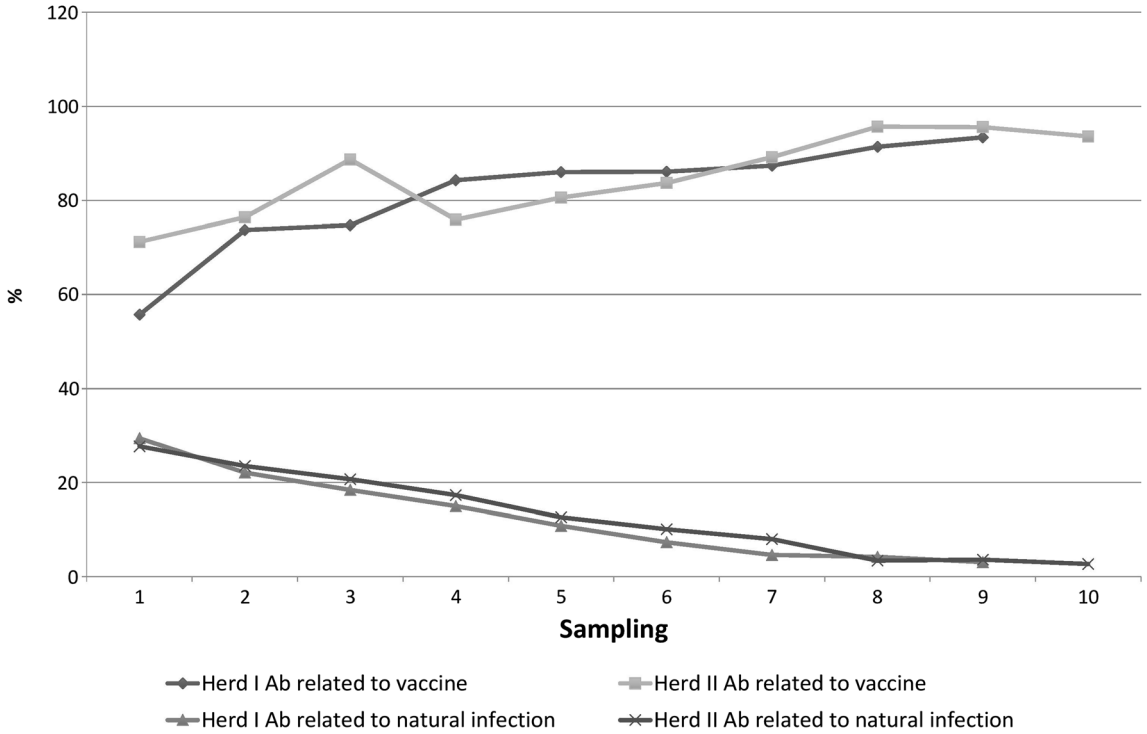

Fig. 2 The positivity rates for antibodies to gE and gB for animals aged ≥ 6 months according to the sampling times

infection. It is known that eradication programmes that make use of glycoprotein E (gE) deletion mutants of bovine herpes virus type 1 (BoHV-1), so-called IBR marker vaccines (Kaashoek et al. 1995), have an important advantage for the possibility to differentiate the animals that have only antibody-related vaccine. European authorities support IBR eradication programmes according to additional guarantees to IBR-free countries (Directive CEE64/432 is amended by 2011/674/EU). Several countries like Finland, Norway, Sweden, Austria, Denmark, Switzerland and the Bolzono region of Italy in Europe have been declared IBR-free by the OIE (Raaperi et al. 2014), in which the eradication programme of BoHV-1 infection carried out by prohibiting marker vaccination and removing seropositive animals and by using additional preventive measures like movement control (Batza 2003; Makoschey and Keil 2000).

It is not known when BoHV-1 infection entered Turkey as the virus firstly isolated in 1980s (Burgu and Akca 1987). But its prevalence, geographic distribution and a lot of clinical/subclinical cases from BoHV-1 infection were reported in a lot of studies (Alkan et al. 2000; Tan et al. 2006). In some state farms which have a large dairy cattle population, very high seropositivity rates and clinical cases were reported (Alkan et al. 2005). Unfortunately, there is not a regional or national eradication programme of this disease, yet.

In this pilot project, glycoprotein E (gE)-deleted BoHV-1 vaccine was used, in combination with an ELISA detecting gE specific antibodies, and herds were monitored for the clinical cases and investigated for the circulation of BoHV-1. Data show that the rates of the animals with antibodies related to gE were reduced gradually in the further sampling times and that the use of marker vaccines limited new naturally infected animals. During the study, animals with antibodies to gE were not removed from herd because of the high seropositivity rates, especially in early sampling times, and also for economical reasons. But, animals having some problems, especially with fertility, were removed from herds. A lot of them were detected as positive for gB antibodies. It may be said that the decreasing of the transmission of infection is also partially supported by this situation. But it is known that antibodies related to the field virus are detectable for at least 3 years at high levels. In the further sampling times in our study, the rate of animals with gE antibodies were reduced because of the herd dynamic (deaths and slaughtered animals, the culling of gE positive animals with low milk secretion and/or fertility problems) and also the transmission rate decreased due to the increasing of the vaccine related antibody in herds. Our data show that transmission rate is very low in these two herds. The rates of the animals with vaccine-related antibody (gE-negative, gB-positive) have increased steadily in the following sampling times (Fig. 2). On the other hand, the results from calves aged 1–11 months also pointed the success of the vaccination in these herds, because of the detection of the increasing antibody rates to only gB and of decreasing the detection rate of the gE antibodies while

Table 3 The rates (%) of animals with antibodies both gB and gE according to the sampling times in herd I

Sampling no.	Age											
	1	2	3	4	5	6	7	8	9	10	11	12
I	– (0/73)[a]	– (0/56)	2.1 (2/95)	1.7 (1/58)	52.9 (27/51)	89.0 (57/64)	100 (19/19)	87.5 (14/16)	93.7 (15/16)	94.1 (16/17)	100 (10/10)	
II	2.2 (1/44)	– (0/141)	2.9 (2/68)	1.3 (1/73)	3.8 (2/52)	70.9 (44/62)	90.6 (39/43)	93.3 (14/15)	100 (8/8)	94.1 (16/17)	80.0 (4/5)	100 (5/5)
III	– (0/63)	– (0/135)	– (0/50)	1.5 (1/66)	2.8 (2/69)	14.2 (4/28)	82.4 (47/57)	93.3 (14/15)	92.8 (13/14)	80.0 (4/5)	80.0 (4/5)	100 (8/8)
IV	2.6 (2/76)	– (0/126)	– (0/105)	– (0/60)	– (0/60)	1.8 (1/54)	14.2 (4/28)	94.8 (37/39)	92.0 (23/25)	87.5 (7/8)	66.6 (2/3)	100 (11/11)
V	– (0/66)	0.9 (1/110)	0.9 (1/110)	2.2 (2/90)	– (0/54)	6.6 (3/45)	3.1 (1/32)	71.8 (23/32)	96.0 (24/25)	100 (5/5)	100 (10/10)	
VI	– (0/80)	– (0/129)	1.1 (1/90)	– (0/99)	– (0/51)	– (0/41)	1.7 (1/56)	18.7 (3/16)	87.8 (29/33)	87.5 (7/8)	100 (4/4)	100 (6/6)
VII	– (0/124)	– (0/147)	– (0/79)	0.9 (1/102)	– (0/71)	– (0/37)	2.8 (1/35)	5.0 (1/20)	63.1 (12/19)	86.6 (13/15)	100 (1/1)	100 (2/2)
VIII	– (0/73)	– (0/178)	– (0/107)	– (0/67)	– (0/79)	– (0/31)	– (0/32)	6.8 (2/29)	18.1 (2/11)	80.9 (17/21)	80.0 (4/5)	100 (1/1)
IX	– (0/5)	– (0/187)	– (0/133)	– (0/69)	– (0/89)	– (0/58)	– (0/32)	3.8 (1/26)	– (0/15)	47.0 (8/17)	85.7 (12/14)	100 (1/1)

[a] Animal numbers with Ab to both gB and gE/animals sampled are shown in paranthesis

Table 4 The rates (%) of animals with antibodies to gB without gE according to the sampling times in herd I

Sampling no.	Age											
	1	2	3	4	5	6	7	8	9	10	11	12
I	83.5 (61/73)[a]	96.4 (54/56)	96.8 (92/95)	96.5 (56/58)	47.0 (24/51)	10.9 (7/64)	– (0/19)	12.5 (2/16)	6.2 (1/16)	5.8 (1/17)	– (0/10)	
II	97.7 (43/44)	98.5 (139/141)	97.0 (66/68)	98.6 (72/73)	96.1 (50/52)	29.0 (18/62)	9.3 (4/43)	6.6 (1/15)	– (0/8)	5.8 (1/17)	20.0 (1/5)	– (0/5)
III	74.6 (47/63)	95.5 (129/135)	98.0 (49/50)	95.4 (63/66)	97.1 (67/69)	85.7 (24/28)	17.5 (10/57)	6.6 (1/15)	7.1 (1/14)	20.0 (1/5)	20.0 (1/5)	– (0/8)
IV	97.3 (74/76)	98.4 (124/126)	100 (105/105)	100 (60/60)	100 (60/60)	98.1 (53/54)	85.7 (24/28)	5.1 (2/39)	8.0 (2/25)	12.5 (1/8)	33.3 (1/3)	– (0/11)
V	96.9 (64/66)	99.0 (109/110)	99.0 (109/110)	97.7 (88/90)	100 (54/54)	93.3 (42/45)	96.8 (31/32)	40.6 (13/32)	4.0 (1/25)	– (0/5)	– (0/10)	
VI	93.7 (75/80)	99.2 (128/129)	98.8 (89/90)	100 (99/99)	100 (51/51)	100 (41/41)	98.2 (55/56)	81.2 (13/16)	12.1 (4/33)	12.5 (1/8)	– (0/4)	– (0/6)
VII	66.9 (83/124)	100 (147/147)	100 (79/79)	99.0 (101/102)	100 (71/71)	100 (37/37)	97.1 (34/35)	95.0 (19/20)	36.8 (7/19)	13.3 (2/15)	– (0/1)	– (0/2)
VIII	64.3 (47/73)	99.4 (177/178)	100 (107/107)	100 (67/67)	100 (79/79)	100 (31/31)	100 (32/32)	93.1 (27/29)	81.8 (9/11)	15.0 (3/20)	20.0 (1/5)	– (0/1)
IX	100 (5/5)	100 (187/187)	100 (133/133)	100 (69/69)	100 (89/89)	100 (58/58)	100 (32/32)	96.1 (25/26)	100 (15/15)	52.9 (9/17)	14.2 (2/14)	– (0/1)

[a] Animal numbers with Ab to only gB/animals sampled are shown in paranthesis

Table 5 The rates (%) of animals with antibodies both gB and gE according to the sampling times in herd II

Sampling no.	Age											
	1	2	3	4	5	6	7	8	9	10	11	12
I	3.7 (3/79)[a]	9.7 (10/103)	8.1 (4/49)	39.0 (16/41)	63.0 (29/46)	56.5 (26/46)	33.3 (3/9)	100 (8/8)	90.0 (9/10)	100 (3/3)	100 (2/2)	
II	7.4 (11/147)	3.1 (3/96)	10.9 (14/128)	10.0 (3/30)	39.4 (15/38)	58.3 (28/48)	67.3 (31/46)	55.5 (5/9)	90.0 (9/10)	90.0 (9/10)	100 (4/4)	100 (2/2)
III	1.4 (1/70)	3.9 (5/127)	10.3 (9/87)	11.2 (8/71)	12.5 (3/24)	63.6 (21/33)	67.8 (19/28)	76.0 (19/25)	88.8 (8/9)	100 (11/11)	80.0 (4/5)	100 (2/2)
IV	– (0/80)	1.0 (1/100)	6.6 (5/75)	12.8 (9/70)	7.1 (3/42)	51.7 (15/29)	73.9 (17/23)	72.7 (24/33)	80.0 (4/5)	100 (5/5)	100 (5/5)	
V	– (0/62)	– (0/117)	3.4 (3/86)	12.6 (11/87)	8.3 (5/60)	26.3 (5/19)	55.5 (15/27)	69.5 (16/23)	80.0 (12/15)	66.6 (2/3)	100 (5/5)	100 (1/1)
VI	1.5 (1/64)	– (0/132)	1.9 (2/104)	3.3 (2/60)	9.8 (5/51)	13.3 (4/30)	40.0 (8/20)	80.9 (17/21)	70.5 (12/17)	66.6 (2/3)	100 (3/3)	100 (2/2)
VII	1.0 (1/94)	4.0 (3/74)	0.9 (1/110)	4.3 (3/69)	8.4 (5/59)	9.4 (5/53)	26.6 (4/15)	63.1 (12/19)	62.5 (5/8)	80.0 (4/5)		
VIII	– (0/62)	– (0/118)	– (0/97)	2.6 (2/76)	– (0/40)	6.6 (3/45)	11.1 (2/18)	42.8 (6/14)	57.1 (4/7)	50.0 (2/4)		
IX	– (0/109)	– (0/97)	– (0/58)	1.0 (1/95)	3.5 (2/56)	10.5 (4/38)	– (0/25)	45.4 (5/11)	66.6 (6/9)	50.0 (1/2)	50.0 (1/2)	
X	– (0/103)	– (0/140)	– (0/78)	– (0/88)	3.0 (2/65)	– (0/37)	13.0 (3/23)	16.6 (2/12)	41.6 (5/12)	66.6 (2/3)	50.0 (3/6)	

[a] Animal numbers with Ab to both gB and gE animals sampled are shown in paranthesis

Table 6 The rates (%) of animals with antibodies to gB without gE according to the sampling times in herd II

Sampling no.	Age											
	1	2	3	4	5	6	7	8	9	10	11	12
I	93.6 (74/79)[a]	89.3 (92/103)	89.7 (44/49)	60.9 (25/41)	36.9 (17/46)	43.4 (20/46)	66.6 (6/9)	– (0/8)	10.0 (1/10)	– (0/3)	– (0/2)	
II	92.5 (136/147)	96.8 (93/96)	89.0 (114/128)	90.0 (27/30)	60.5 (23/38)	41.6 (20/48)	32.6 (15/46)	44.4 (4/9)	10.0 (1/10)	10.0 (1/10)	– (0/4)	– (0/2)
III	81.4 (57/70)	96.0 (122/127)	89.6 (78/87)	88.7 (63/71)	87.5 (21/24)	36.3 (12/33)	32.1 (9/28)	24.0 (6/25)	11.1 (1/9)	– (0/11)	20.0 (1/5)	– (0/2)
IV	91.2 (73/80)	99.0 (99/100)	93.3 (70/75)	87.1 (61/70)	92.8 (39/42)	48.2 (14/29)	26.0 (6/23)	27.2 (9/33)	20.0 (1/5)	– (0/5)	– (0/5)	
V	96.7 (60/62)	100 (117/117)	96.5 (83/86)	87.3 (76/87)	91.6 (55/60)	73.6 (14/19)	44.4 (12/27)	30.4 (7/23)	20.0 (3/15)	33.3 (1/3)	– (0/5)	– (0/1)
VI	96.8 (62/64)	100 (132/132)	98.0 (102/104)	96.6 (58/60)	90.1 (46/51)	86.6 (26/30)	60.0 (12/20)	19.0 (4/21)	29.4 (5/17)	33.3 (1/3)	– (0/3)	– (0/2)
VII	96.8 (91/94)	95.9 (71/74)	99.0 (109/110)	95.6 (66/69)	91.5 (54/59)	90.5 (48/53)	73.3 (11/15)	36.8 (7/19)	37.5 (3/8)	20.0 (1/5)		
VIII	100 (62/62)	100 (118/118)	100 (97/97)	97.3 (74/76)	100 (40/40)	93.3 (42/45)	88.8 (16/18)	57.1 (8/14)	42.8 (3/7)	50.0 (2/4)		
IX	100 (109/109)	100 (97/97)	100 (58/58)	98.9 (94/95)	96.4 (54/56)	89.4 (34/38)	100 (25/25)	54.6 (6/11)	33.3 (3/9)	50.0 (1/2)	50.0 (1/2)	
X	89.3 (92/103)	100 (140/140)	100 (78/78)	100 (88/88)	96.9 (63/65)	100 (37/37)	86.9 (20/23)	83.3 (10/12)	58.3 (7/12)	33.3 (1/3)	50.0 (3/6)	

[a] Animal numbers with Ab to only gB/animals sampled are shown in paranthesis

the same is also true for adult animals. Similarly, Bosch et al. (1998) showed that an inactivated gE- negative BoHV-1 vaccine reduced the incidence and transmission of BoHV-1 infections in the field. They reported that the transmission ratio (Ro) of BoHV-1 in vaccinated herds is an important factor to prevent major outbreaks on a farm and the reduced transmission, especially when the Ro between herds is below 1, which makes farms less susceptible thus less infectious. It is known that various herd-level risk factors like large herd size, high density of farms in a geographical area, participation of infected animals to herd, etc. have been important for presence of BoHV-1 infection. In this study, the new natural infections and/or recurrent infections were detected in very limited animals when seroconversions are examined, but it did not contribute to the increasing of the positivity for gE antibodies in the following sampling time in herds.

It is known that the BoHV-1 remains latent through lifetimes of infected cattle and may be reactivated by factors causing stress or immunosuppression (Ackermann et al. 1982; Winkler et al. 2000; Kook et al. 2015). The goal of the vaccination is to prevent their capacity to re-excrete BoHV-1 by repeated vaccination. When the prevalence of latent carriers is low, it is safer to cull them. The immune status of a BoHV-1 latent carrier is the key factor to control the virus re-excretion under reactivation stimulus. Therefore, vaccination of latent carriers must be carefully addressed by repeated vaccination schemes at regular 6-month intervals in order to decrease the risk of re-excretion (Dispas et al. 2004). In this study, the vaccinations were also applied every 6–8 months. The decreasing of the naturally infected animals in the following sampling times and the lack of detection for signs of recurrent or reinfection, except in a few animals, indicated that these schemes have a positive effect in the success of results as reported before (Vitale et al. 2004; Van Schaik et al. 2002; Nardelli et al. 2008). Briefly, we conclude that main effects of the using marker vaccines to BoHV-1 infection are to (i) protect against infection or at least reduce the clinical signs very efficiently, (ii) reduce virus circulation and allow to control and/or eradication of infection and (iii) support the reducing economical losses due to the decreasing of especially fertility problems (unpublished data).

In this study, there are two main limiting factors due to the decision by managers. One of them is the lack of the separation of infected animals from herd. However, this situation did not contribute largely to new naturally infected animals; it caused the persistence of the animals with gE antibodies in herds. But the completion of the eradication programme suffered delay in the expected time. Other factor is the lack of the further sampling after 2010 while vaccination with gE-deleted vaccine was being continued in the herds. This situation is a cause of grief for us because we were not able to monitor the herds and to share the further knowledge on finishing the programme.

In conclusion, we suggest that the BoHV-1 eradication programme in Turkey has to be initiated with government decision as soon as possible. It should not be forgotten that the eradication of BoHV-1 from some herds without vaccination can be possible if some rules like separation of infected animals can be applied. In this point, it is important that small family herds, constituting the great proportion of Turkey's animal population, should have to be also included in BoHV-1 control/eradication programmes as well as organized herds. Briefly, we may say that vaccine-related control and eradication programme can be favourable for our country when current organization of cattle breeding is considered. However, further studies on economical analysis of different programmes on the eradication of BoHV-1 infection are needed to settle for this subject. Experiences from a lot of EU countries in which the eradication programme of BoHV-1 infection finished obviously show that the eradication of this infection needs a very long time. By this way, if the eradication programme should begin as soon as possible, economic expenses and needed time due to control/eradication programmes would be minimized. It is known that the status of "BoHV-1 free" infection is being imposed to the international trade of animals and their products in the countries of EU. Turkey is not a member of EU, but the rules of EU are very important because Turkey is a candidate for the EU. Even if we ignore it, it is important that BoHV-1 causes economical losses related to fertility problems, high mortality rates in calves with systemic infection, etc. We suggest that current data, including prevalences of the infection in herds managed differently as organized and small family herds, has to be immediately created in epidemiological studies countrywide together with the monitorization of the control measures as the introduction of new animals to the herd, etc. and the education of the veterinarian/farmer, etc. and that the best eradication programme for Turkey has to be started as soon as possible.

Acknowledgements Preliminary findings of this study were presented at the 4th ESVV, Veterinary Herpes Symposium, held during January 17–18, 2013, Zurich, Switzerland.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Table 7 The number of animals with antibodies to gB and/or gE in animals aged 1–11 months according to the sampling times in herd I (%)

Age (m)		Sampling								
		I	II	III	IV	V	VI	VII	VIII	IX
1	Animals (*n*)	54	16	19	41	15	–	–	27	25
	gB (+) and gE (+)	14 *(25.9)*	–	4 (21.0)	8 (19.5)	1 (6.6)	–	–	3 (11.1)	1 (4.0)
	gB (+) and gE (−)	28 *(51.8)*	14 (87.5)	15 (78.9)	33 (80.4)	14 (93.3)	–	–	24 (88.8)	24 (96.0)
	gB (−) and g E(−)	12 (22.2)	2 (12.5)	–	–	0 (0)	–	–	–	–
2	Animals (*n*)	10	17	10	37	23	–	–	15	18
	gB (+) and gE (+)	3 (30.0)	4 (23.5)	2 (20.0)	11 (29.7)	6 (26.0)	–	–	1 (6.6)	–
	gB (+) and gE (−)	5 (50.0)	13 (76.4)	7 (70.0)	26 (70.2)	16 (69.5)	–	–	14 (93.3)	16 (88.8)
	gB (−) and gE (−)	2 (20.0)	–	1 (10.0)	–	1 (4.3)	–	–	–	2 (11.1)
3	Animals (*n*)	6	4	18	26	24	–	–	25	24
	gB (+) and gE (+)	–	–	6 (33.3)	4 (15.3)	4 (16.6)	–	–	–	–
	gB (+) and g E(−)	5 (83.3)	4 (100)	8 (44.4)	19 (73.0)	17 (70.8)	–	–	23 (92.0)	22 (91.6)
	gB (−) and gE (−)	1 (16.6)	–	4 (22.2)	3 (11.5)	3 (12..5)	–	–	2 (8.0)	2 (8.3)
4	Animals (*n*)	8	7	20	22	5	–	–	19	16
	gB (+) and gE (+)	2 (25.0)	2 (28.5)	5 (25.0)	3 (13.6)	–	–	–	–	–
	gB (+) and gE (−)	5 (62.5)	3 (42.8)	8 (40.0)	13 (59.0)	4 (80.0)	–	–	10 (52.6)	9 (56.2)
	gB (−) and gE (−)	1 (12.5)	2 (28.5)	7 (35.0)	6 (27.2)	1 (20.0)	–	–	9 (47.3)	7 (43.7)
5	Animals (*n*)	–	4	17	11	9	25	–	10	20
	gB (+) and gE (+)	–	2 (50.0)	1 (5.8)	–	2 (22.2)	7 (28.0)	–	–	–
	gB (+) and gE (−)	–	–	7 (41.1)	8 (72.7)	5 (55.5)	18 (72.0)	–	2 (20.0)	5 (25.0)
	gB (−) and gE (−)	–	2 (50.0)	9 (52.9)	3 (27.2)	2 (22.2)	–	–	8 (80.0)	15 (75.0)
6	Animals (*n*)	2	23	37	27	13	30	–	7	13
	gB (+) and gE (+)	–	–	2 (5.4)	–	–	3 (10.0)	–	–	–
	gB (+) and gE (−)	2 (100)	11 (47.8)	16 (43.2)	21 (77.7)	8 (61.5)	26 (86.6)	–	2 (28.5)	5 (38.4)
	gB (−) and gE (−)	–	12 (52.1)	19 (51.3)	6 (22.2)	5 (38.4)	1 (3.3)	–	5 (71.4)	8 (61.5)
7	Animals (*n*)	2	35	12	14	6	30	31	0	24
	gB (+) and gE (+)	–	–	4 (33.3)	1 (7.1)	–	–	2 (6.4)	–	–
	gB (+) and gE (−)	2 (100)	16 (45.7)	1 (8.3)	12 (85.7)	3 (50.0)	18 (60.0)	26 (83.8)	–	10 (41.6)
	gB (−) and gE (−)	–	19 (54.2)	7 (58.3)	1 (7.1)	3 (50.0)	12 (40.0)	3 (9.6)	–	14 (58.3)
8	Animals (*n*)	3	7	10	12	20	25	14	1	18
	gB (+) and gE (+)	–	–	1 (10.0)	–	–	–	–	–	–
	gB (+) and gE (−)	3 (100)	3 (42.8)	6 (60.0)	10 (83.3)	15 (75.0)	6 (24.0)	11 (78.5)	1 (100)	11 (61.1)
	gB (−) and gE (−)	–	4 (57.1)	3 (30.0)	2 (16.6)	5 (25.0)	19 (76.0)	3 (21.4)	–	7 (38.8)
9	Animals (*n*)	7	39	14	–	18	7	18	0	10
	gB (+) and gE (+)	–	–	–	–	–	–	–	–	–
	gB (+) and gE (−)	7 (100)	37 (94.8)	6 (42.8)	–	9 (50.0)	3 (42.8)	8 (44.4)	–	9 (90.0)
	gB (−) and gE (−)	–	2 (5.2)	8 (57.2)	–	9 (50.0)	4 (57.1)	10 (55.5)	–	1 (10.0)
10	Animals (*n*)	–	–	–	–	4	12	–	0	1
	gB (+) and gE (+)	–	–	–	–	–	–	–	–	–
	gB (+) and gE (−)	–	–	–	–	3 (75.0)	10 (83.3)	–	–	–
	gB (−) and gE (−)	–	–	–	–	1 (25.0)	2 (16.6)	–	–	1 (100)
11	Animals (*n*)	–	–	–	–	12	6	–	0	1
	gB (+) and gE (+)	–	–	–	–	–	–	–	–	–
	gB (+) and gE (−)	–	–	–	–	12 (100)	4 (66.6)	–	–	–
	gB (−) and gE (−)	–	–	–	–	–	2 (33.3)	–	–	1 (100)

Table 8 The number of animals with antibodies to gB and/or gE in animals aged 1–11 months according to the sampling times in herd II (%)

Age (m)	BoHV-1 Ab	Sampling									
		I	II	III	IV	V	VI	VII	VIII	IX	X
1	Animals (*n*)	48	–	29	30	17	11	15	–	–	15
	gB (+) and gE (+)	18 (37.5)	–	12 (41.3)	16 (53.3)	5 (29.4)	2 (18.1)	2 (13.3)	–	–	–
	gB (+) and gE (−)	30 (62.5)	–	16 (55.1)	14 (46.6)	12 (70.5)	9 (81.8)	13 (86.6)	–	–	15 (100)
	gB (−) and gE (−)	–	–	1 (3.4)	–	–	–	–	–	–	–
2	Animals (*n*)	23	–	13	25	29	30	21	–	–	34
	gB (+) and gE (+)	2 (8.6)	–	4 (30.7)	5 (20.0)	9 (31.0)	7 (23.3)	6 (28.5)	–	–	1 (2.9)
	gB (+) and gE (−)	20 (86.9)	–	9 (69.2)	20 (80.0)	20 (68.9)	23 (76.6)	15 (71.4)	–	–	33 (97.0)
	gB (−) and gE (−)	1 (4.3)	–	–	–	–	–	–	–	–	–
3	Animals (*n*)	9	6	12	26	31	24	26	34	–	33
	gB (+) and gE (+)	3 (33.3)	–	1 (8.3)	9 (34.6)	1 (3.2)	5 (20.8)	3 (11.5)	–	–	1 (3.0)
	gB (+) and gE (−)	4 (44.4)	6 (100)	11 (91.6)	17 (65.3)	29 (93.5)	19 (79.1)	23 (88.4)	34 (100)	–	31 (93.9)
	gB (−) and gE (−)	2 (22.2)	–	–	–	1 (3.2)	–	–	–	–	1 (3.0)
4	Animals (*n*)	30	7	9	12	23	17	38	33	26	8
	gB (+) and gE (+)	–	–	2 (22.2)	3 (25.0)	1 (4.3)	1 (5.8)	4 (10.5)	2 (6.0)	2 (7.6)	–
	gB (+) and gE (−)	5 (16.6)	7 (100)	7 (77.7)	9 (75.0)	17 (73.9)	16 (94.1)	31 (81.5)	31 (93.9)	23 (88.4)	7 (87.5)
	gB (−) and gE (−)	25 (83.3)	–	–	–	5 (21.7)	–	3 (7.8)	–	1 (3.8)	1 (12.5)
5	Animals (*n*)	21	–	15	14	16	22	28	35	36	34
	gB (+) and gE (+)	5 (23.8)	–	1 (6.6)	1 (7.1)	–	5 (22.7)	–	–	4 (11.1)	–
	gB (+) and gE (−)	3 (14.2)	–	14 (93.3)	10 (71.4)	7 (43.7)	14 (63.6)	20 (71.4)	25 (71.4)	32 (88.8)	28 (82.3)
	gB (−) and gE (−)	13 (61.9)	–	–	3 (21.4)	9 (56.2)	3 (13.6)	8 (28.5)	10 (28.5)	–	6 (17.6)
6	Animals (*n*)	13	–	19	14	8	18	33	22	37	–
	gB (+) and gE (+)	1 (7.6)	–	–	2 (14.2)	–	4 (22.2)	2 (6.0)	–	1 (2.7)	–
	gB (+) and gE (−)	3 (23.0)	–	15 (78.9)	10 (71.4)	4 (50.0)	8 (44.4)	16 (48.4)	11 (50.0)	34 (91.8)	–
	gB (−) and gE (−)	9 (69.2)	–	4 (21.0)	2 (14.2)	4 (50.0)	6 (33.3)	15 (45.4)	11 (50.0)	2 (5.4)	–
7	Animals (*n*)	3	–	2	11	31	21	13	19	22	–
	gB (+) and gE (+)	1 (33.3)	–	1 (50.0)	–	–	2 (9.5)	1 (7.6)	–	–	–
	gB (+) and gE (−)	2 (66.6)	–	–	4 (36.3)	11 (35.4)	9 (42.8)	3 (23.0)	15 (78.9)	20 (90.9)	–
	gB (−) and gE (−)	–	–	1 (50.0)	7 (63.6)	20 (64.5)	10 (47.6)	9 (69.2)	4 (21.1)	2 (9.0)	–
8	Animals (*n*)	1	–	11	7	15	32	11	23	12	–
	gB (+) and gE (+)	–	–	–	–	–	2 (6.2)	–	–	–	–
	gB (+) and gE (−)	1 (100)	–	4 (36.3)	–	6 (40.0)	15 (46.8)	8 (72.7)	23 (100)	10 (83.3)	–
	gB (−) and gE (−)	–	–	7 (63.6)	7 (100)	9 (60.0)	15 (46.8)	3 (27.2)	–	2 (16.6)	–
9	Animals (*n*)	–	–	29	6	14	17	13	20	19	13
	gB (+) and gE (+)	–	–	1 (3.4)	–	–	–	–	–	–	–
	gB (+) and gE (−)	–	–	10 (34.4)	–	8 (57.1)	11 (64.7)	13 (100)	20 (100)	19 (100)	4 (30.8)
	gB (−) and gE (−)	–	–	18 (62.0)	6 (100)	6 (42.8)	6 (35.2)	–	–	–	9 (69.2)
10	Animals (*n*)	3	–	1	13	11	18	7	–	–	–
	gB (+) and gE (+)	–	–	–	1 (7.6)	–	–	–	–	–	–
	gB (+) and gE (−)	3 (100)	–	1 (100)	5 (38.4)	11 (100)	15 (83.3)	6 (85.7)	–	–	–
	gB (−) and gE (−)	–	–	–	7 (53.8)	–	3 (16.6)	1 (14.2)	–	–	–
11	Animals (*n*)	8	–	–	14	7	14	9	–	–	–
	gB (+) and gE (+)	–	–	–	–	–	–	1 (11.1)	–	–	–
	gB (+) and gE (−)	8 (100)	–	–	8 (57.1)	6 (85.7)	12 (85.7)	8 (88.8)	–	–	–
	gB (−) and gE (−)	–	–	–	6 (42.8)	1 (14.2)	2 (14.2)	–	–	–	–

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