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RESEARCH ARTICLE

Vaccine Induced Antibody Response to Foot and Mouth Disease in Infectious Bovine Rhinotracheitis Seropositive Cattle

Murat Şevik

Molecular Microbiology, Veterinary Control Institute, Meram 42080, Konya, Turkey
*Corresponding author: dr_muratank@hotmail.com; msevik@kkgm.gov.tr

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ABSTRACT

Foot and mouth disease (FMD) and infectious bovine rhinotracheitis (IBR) are two important infectious diseases of cattle. Inactivated FMD vaccines are the most powerful tools to protect animals against FMD. Previous studies showed that recombinant IBR-FMD viruses protected cattle from virulent BHV-1 challenge and induced protective levels of anti-FMDV antibodies. FMD is considered to be endemic in Turkey and inactivated oil adjuvanted vaccines are used for the immunization of cattle. Previous studies showed that seroprevalence of IBR in the Turkey's dairy herd more than 50%. In this study, antibody response in IBR seropositive cattle following vaccination against FMD was investigated. IBR seropositive (n=208) and IBR seronegative (n=212) cattle were vaccinated with oil-adjuvanted bivalent vaccine (containing O_1 Manisa, A_{22} Iraq FMDV strains). Solid-phase competitive ELISA (SPCE) was used to measure antibodies produced in cattle. Protective level of antibody against serotype O was detected in 77.4% and serotypes A in 83.6% of IBR seropositive cattle. Protective level of antibody against serotype O antibody was detected in 49% and serotypes A in 66.9% of IBR seronegative cattle. The differences between the protection rates against both serotype O (P=0.0001) and serotype A (P=0.0001) in IBR seropositive and seronegative animals were statistically important (Fisher's exact test, P<0.01). Results showed that after FMD vaccination, IBR seropositive animals produced high titres of antibodies than seronegative animals.

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INTRODUCTION

Infectious bovine rhinotracheitis (IBR) is a highly contagious viral disease of cattle that causes serious economic losses in the cattle industry. The disease is characterized by clinical signs in the upper respiratory tract such as purulent nasal discharge and by conjunctivitis (Nandi *et al.*, 2009). The causative agent of IBR, bovine herpes virus type 1 (BHV-1), belongs to the Herpesviridae family in the Alphaherpesvirinae subfamily (van Regenmortel *et al*., 2000). BHV-1 has been associated with a variety of clinical syndromes including rhinotracheitis, vulvovaginitis, balanoposthitis, abortion, conjunctivitis and generalized systemic infection (Fulton, 2009; Shabbir *et al.*, 2013). BHV-1 infection is widely distributed in beef and dairy cattle herds around the world, and very few countries (Norway, Finland, Sweden, Austria, Denmark, the Bolzano Province of Italy and parts of Germany) have eradicated it (OIE, 2012). Previous studies revealed that the seropositivity rate of BHV-1 in dairy cattle herds in Turkey was between 7.2 and 74% (Bulut *et al*., 2003; Bilge-Dagalp *et al*. 2007; Gur, 2011).

Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals, characterized by fever, excessive salivation, vesicular lesions, and erosions of the epithelium of the mouth, tongue, nares, feet, and teats (Alexandersen *et al*., 2003). Foot and mouth disease virus (FMDV) belongs to the genus Aphthovirus in the Picornaviridae family and it has seven antigenically distinct serotypes (O, A, C, Asia 1, SAT1, SAT2, and SAT3) with a large number of subtypes (van Regenmortel *et al*., 2000). The disease is endemic in many countries of Asia, Africa, South America and Anatolia Region of Turkey (World Reference Laboratory, 2011; Knight-Jones and Rushton, 2013).

FMD control in endemic areas is implemented by systematic vaccination programs (Rodriguez and Grubman, 2009). Protection against FMD is often

associated with the induction of high levels of neutralizing antibodies in serum (Doel, 2005). FMD vaccines formulated with the adjuvant of aluminium hydroxide gel-saponin (AS) or oil, and can be monovalent, bivalent and multivalent, including viruses of different strains and/or serotypes. Oil adjuvant FMD vaccines have been shown to induce higher antibody titres than AS vaccines, and may initiate protection against disease within 4 to 5 days of vaccination (Iyer *et al*., 2000; Barnett and Carabin, 2002; Dar *et al*., 2013). But, the antigenic variation of FMDV is a direct consequence of its genetic variation, affects vaccine efficiency and effectiveness of vaccination programs (Haydon *et al*., 2001; Alam *et al*., 2013). Previous studies reported that BHV-1 (given intranasally) provided protection against FMD viral challenge in calves, and vaccination with recombinant IBR-FMD viruses can elicit protective immune response against FMD (Ren *et al.*, 2009). Previously no reports are available in accessible literature about the seroprevalence of BHV-1 in Eastern Black Sea Region. In this paper, it was first time that the prevalence of antibodies to BHV-1 in Eastern Black Sea Region and affect of previous BHV-1 infection on serum neutralizing antibody titres against FMDV in vaccinated cattle were studied.

MATERIALS AND METHODS

Study area: This research was carried out in five different provinces (Ordu, Giresun, Trabzon, Rize and Artvin) in the Eastern Black Sea Region in Turkey. The Eastern Black Sea Region is located within 41° 10′ North and 40° 59′ North latitudes and 41° 49′ East and 37° 52′ East longitudes. In this region, annual precipitation over 200 cm and mean annual temperature is between 13 and 15°C. The study was performed during the rainy season (September-November) with a mean temperature of 15±1.5°C and humidity level was higher than 70%.

Sampling strategy and sample collection: Serum samples were randomly collected from small sized-family type farms with a history of respiratory or genital problems and no record of FMD for many years. The numbers of farms sampled were 15, 14, 13, 12 and 12 in Ordu, Giresun, Trabzon, Rize and Artvin, respectively. Numbers of animals in these farms were between 2 and 10; between the age of 1 and 177 months. The sampled farms, with land smaller than 0.5 hectare, had mostly Brown Swiss hybrid cattle. Intensive farming methods were used. Cattle did not receive vaccination against IBR. Purulent nasal discharge, conjunctivitis, reduced milk yield and reduced reproductive performance were the most common clinical signs in sampled animals. Also, owners of the selected farms reported having seen abortions in their farms. Serum samples were collected from 420 (estimated prevalence of 50% confidence level of 95% and acceptable error of 4.8%) cattle. Ages of animals learned from the animal registration system (Turkvet System). Then, the animals were divided into three groups according to the age (0-11 months, 12-35 months, and older than 35 months). Cattle in all groups were vaccinated against FMD with oil-adjuvanted bivalent vaccine (containing O_1 Manisa, A_{22} Iraq FMDV strains, payload of the antigens 6 µg and 4 µg, respectively) formulated in a double oil emulsion adjuvant. In this study, the same batches of a commercial vaccine were used. Serum samples were obtained 28 days after FMD vaccination.

ELISA to test the antibodies to BHV-1: Serum samples were tested for the detection of glycoprotein E (gE) specific antibodies to BHV-1 by using commercial ELISA test kit (IDEXX IBR gE Ab test, Institut Pourquier, Montpellier, France) according to manufacturers' instruction. The optical density (OD) of each well was read at 450 nm filter using a spectrophotometer (Bio-Tek Instruments Inc., Winooski, Vt.)

Solid-phase competition ELISA: The solid-phase competition ELISA was carried out as described by Mackay *et al.* (2001). OD values at 492 nm wavelength were read using spectrophotometer (Molecular Devices, Sunnyvale, CA). Serum giving ≥60% inhibition was considered positive (Paiba *et al*., 2004). This represents a titres ≥1:7.5 ($\log_2$=2.9). ELISA titres of 1:15 ($\log_2$=3.9) or more were considered protective (Berinstein *et al*., 2000; FMD Institute, 2009).

Statistical analysis: The Fisher's exact 2-tailed and Kruskal-Wallis tests were used for nonparametric analysis. The 95% confidence interval (CI) and odds ratios were used in case-control groups. A $P<0.01$ was considered statistically significant. All statistical analyses were performed with GraphPad InStat version 3.10 (GraphPad Software, San Diego, CA, USA) and StatPac version 4.0 (Statpac Inc., Bloomington, MN).

RESULTS

BHV-1 gE specific antibodies: Specific antibodies against BHV-1 were detected in 208 animals. The animals involved in this study were not vaccinated against IBR. Therefore, the serological cases were thought to have been the result of natural BHV-1 infection. The result showed that seroprevalence of BHV-1 was 49.5% in Eastern Black Sea Region. Seroprevalence of BHV-1 was found to increase (65.7%) with the age of the animals (Table 1), being highest in cattle older than three years of age (Kruskal-Wallis test, $P<0.0001$).

Table 1: Antibody response against BHV-1 according to age

Age (Months)	IBR positive		95% Confidence interval (CI)
	No	%	
1-11	44	31.4	24.31-39.54
12-35	72	51.4	43.22-59.56
≥36	92	65.7	57.52-73.07

In each age group 140 samples were tested.

Antibody response in BHV-1 seropositive and BHV-1 seronegative cattle following vaccination against FMD: Solid-phase competitive ELISA titres of BHV-1 seropositive and seronegative cattle are presented in Table 2. The differences between the antibody titres against both serotype O (χ^2=12.74, P=0.0004) and serotype A (χ^2=15.12, P=0.0001) in BHV-1 seropositive and seronegative cattle were statistically significant (Odds ratio [OR] 2.39, 95% CI 1.47-3.91 for serotype O, OR 2.99, 95% CI 1.69-5.28 for serotype A). Furthermore,

Table 2: Antibody response by SPCE in BHV-1 seropositive and seronegative cattle

Animal groups	Age (Months)	No. tested	Serotype O		95% Confidence interval (CI)[e]	χ^{2g}	Serotype A		95% Confidence interval (CI)[f]	χ^{2h}
			≥2.9-3.9[c]	≥3.9[d]			≥2.9-3.9[c]	≥3.9[d]		
	1-11	44	8	22	53.37-80.07	1.23	8	25	60.41-85.57	2.45
A[a]	12-35	72	2	62	79.33-94.51	1.22	3	66	87.97-99.06	1.26
	≥36	92	7	77	83.55-95.75	2.98	4	83	87.60-97.96	2.17
	Total	208	17	161	80.11-89.75	12.74	15	174	86.11-94.14	15.11
	1-11	96	28	28	48.33-67.69		7	52	51.45-70.58	
B[b]	12-35	68	12	44	71.48-89.76		9	53	81.73-96.22	
	≥36	48	7	32	67.83-90.03		5	37	74.93-94.51	
	Total	212	47	104	64.79-76.91		21	142	70.74-82.07	

[a]BHV-1 seropositive cattle; [b]BHV-1 seronegative cattle; [c]SPCE antibody titre (log_2) is considered as a positive; [d]SPCE antibody titre (log_2) is considered as a protective; [e]95% CI for Serotype O seropositivity rate; [f] 95% CI for Serotype A seropositivity rate; [g]Chi-square value for Serotype O seropositivity rate between A and B groups; [h]Chi-square value for Serotype A seropositivity rate between A and B groups.

differences between the protective antibody titres against both serotype O (χ^2=36.23, P=0.0001) and serotype A (χ^2=15.67, P=0.0001) in BHV-1 seropositive and seronegative cattle were statistically significant (OR 3.55, 95% CI 2.33-5.42 for serotype O, OR 2.52, 95% CI 1.58-4.01 for serotype A). The numbers of animals with positive (≥2.9 log_2) and protective (≥3.9 log_2) antibody titres to serotype O and serotype A in each age group (0-11 months, 12-35 months, and >35 months) are presented in Table 2. Animals in the same age groups of BHV-1 seropositive and seronegative cattle had same number of FMD vaccinations, but in all age groups BHV-1 seropositive animals tended to have higher positive and protective antibody titres to serotypes O and A than BHV-1 seronegative cattle.

DISCUSSION

FMD is one of the most important diseases causing significant economical losses in Turkey. Three serotypes of FMDV (O, A and Asia 1) have been isolated from Turkey field samples over the last 20 years. FMDV type O belongs to the PanAsia-2 lineage is the most common serotype in Turkey. The O/ME-SA/PanAsia-$^{2ANT-10}$ and more recently identified A-Iran-05^{HER-10} and A-Iran-05 $^{SIS-10}$ lineages continue to dominate in Turkey. The sequence data available shows that Turkish isolates were found to be very close to isolates from the Middle-East (Klein *et al*., 2006).

Previously no reports are available in accessible literature about the antibody responses of IBR seropositive cattle to vaccination against FMD. In this study, for the first time antibody serotype responses induced in BHV-1 seropositive cattle by oil-adjuvanted bivalent vaccine were studied. To determine whether there is any effect of previous IBR infection on antibody response, BHV-1 seropositive and BHV-1 seronegative animals were vaccinated with the same dose of FMD vaccine. It was determined that BHV-1 seropositive animals had higher positive and protective antibody titres to serotypes O and A than BHV-1 seronegative animals (Table 2). Also, cattle were classified according to age (0-11 months, 12-35 months, and >35 months). In all age groups, BHV-1 seropositive animals tended to have higher antibody response than BHV-1 seronegative animals to serotypes O and A (Table 2). Both seroprevalence of BHV-1 and protective level of antibody against serotype O and serotype A were found to increase with the age of the animals (Table 1 and Table 2).

It is generally believed that protection against FMD is mainly related to high levels of neutralizing antibody and has been correlated with IgG1 and IgG2 levels (Yadav *et al*., 2007). Furthermore, it has been shown that cellular immunity plays an important role in protection against FMDV (Rodriguez and Grubman, 2009; Carr *et al*., 2013). BHV-1 infection elicits both humoral and cellular immune responses within 5-10 days. The virus is not eliminated from the infected host upon recovery but establishes life-long latency in the sensory ganglia, from where it may be reactivated at intervals (Muylkens *et al*., 2007). Stresses such as vaccination, transport, dehorning and castration may result in excretion of BHV-1 (Hodgson *et al*., 2012). It has been reported that treatment with adrenocorticotropic hormone and trigeminal neurotomy resulted in recrudescence of BHV-1, but immunosuppression was not detected in BHV-1 seropositive animals (Fulton, 2009; Workman *et al*., 2012).

It was postulated that stress induced by FMD vaccination can cause reactivation of BHV-1 and reactivation of BHV-1 stimulates the release of inflammatory cytokines. Thus, activation of the T lymphocyte response by the inflammatory cytokines leads to an increase in antibody production in vaccinated cattle. This suggestion is consistent with previous results that B-cell and T-cell epitopes are required for production of neutralizing antibodies in FMDV infection (McCullough and Sobrino, 2004; Cubillos *et al*., 2012). Immunity induced by vaccination with inactivated FMDV vaccine is short-lived (Rodriguez and Grubman, 2009). Induction of stronger T-cell responses by recrudescence of BHV-1 and more efficient sequestration of antigen may improve the memory responses after vaccination and prolong the duration of protection.

Conclusion: Results from this study suggest that BHV-1 infection may modulates the cellular immune response and drives the production of increasing levels of neutralizing antibodies against FMD vaccination. Also many diseases and different immunizations can cause stress. In order to elucidate effect of viral or bacterial diseases and vaccinations on antibody responses in BHV-1 seropositive animals more studies are needed.

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