

Neutralizing antibody titers against field strains of bovine viral diarrhea virus after vaccination with three commercial vaccines

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Abstract: Modified killed vaccines against bovine viral diarrhea virus (BVDV) are used worldwide. In the present study, the cross-neutralization antibody responses against field strains from seven subgenotypes of BVDV-1 and one subgenotype of BVDV-2 were evaluated using sera obtained by three inactivated commercial vaccines. One vaccine contained both BVDV-1 and BVDV-2 strains, while the others contained only BVDV-1a. Three vaccine groups, each containing five calves, were vaccinated two times with 30-day intervals. The antibody titers were evaluated by virus neutralization assay. The monovalent vaccine induced the highest antibody titers. Significant levels of neutralizing antibody titers were maintained up to the last sampling time. Although one vaccine contained the BVDV-2 strain, the lowest antibody titers were detected against field strains from BVDV-2, BVDV-1b, and BVDV-1r. This study indicated that cross-protective immune responses with the most common international BVDV vaccine strains need to be evaluated with challenge experiments against field strains for efficient protection.

Key words: Bovine viral diarrhea, inactivated vaccine, field strains, subgenotype, neutralizing antibody

1. Introduction

Infection control of bovine viral diarrhea virus (BVDV) is important due to the negative effect on cattle management and production. The management of BVDV infections generally consists of the detection and elimination of persistently infected animals and vaccination as an optional step (1). Although there are different factors that affect the control of the disease, the potential effects of antigenic and serological differences between the strains are significant (2).

Two species, known as BVDV-1 and BVDV-2, were reported by the International Committee on Taxonomy of Viruses (ICTV). However, there are at least 21 subgenotypes in BVDV-1 (1a–1u) based on 5' UTR and N^{pro} regions (3–6) and 4 subgenotypes in BVDV-2 (2a–2d) (7), which are not recognized by the ICTV. Apparently, BVDV-1 is the dominant species of BVDV. The diversity of BVDV is not only at the genetic level but also in antigenic composition. Antigenic differences were also demonstrated between both BVDV species (8) and subgenotypes (9). Complications arise from differences among BVDV strains that restrict the efficiency of diagnosis (2) and vaccination protocols (10) and negatively affect the control and management programs. The effect of having more subgenotypes on disease control programs was reviewed (11). There are a number of

studies that demonstrate the effects of immunization by different kinds of vaccines. Although some side effects of vaccines, such as clinical signs or infection development, were indicated, there is progress in vaccine production. It has been suggested that a modified live vaccine developed by mutation reduces the risk of persistent infection (12). Therefore, new approaches on live vaccines are promising in terms of reducing the risk associated with live vaccines.

Besides the increasing numbers of subgenotypes, interactions between the effect of vaccines and recently identified subgenotypes are still unknown.

The aim of this study was to investigate neutralizing antibody titers against field strains from different subgenotypes of BVDV in cattle vaccinated with commercial vaccines. For that purpose, neutralizing antibody titers raised by three internationally widely used commercial vaccines, including either BVDV-1a alone or BVDV-1 and BVDV-2 strains together, were evaluated against field strains (10 BVDV-1 and 1 BVDV-2) from eight different subgenotypes of BVDV.

2. Materials and methods

2.1. Animals

Twenty Holstein-Friesian calves aged between 6 and 10 months were selected from a dairy farm located in

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northwest Turkey. The farm uses an intensive management system (Dataflow, SCR), including a programmed BVDV eradication schedule adapted to follow by personal records. All animals on the farm were monitored under surveillance for a few years and BVDV-free status was confirmed prior to the start of this study. The animals were selected from well-conditioned male calves in a young stock. Confirmation of BVDV-negative status of the studied calves for BVDV antigen and antibodies was achieved by blood testing using antigen-capture ELISA (Herdcheck Idexx, 99-43830, Sweden) and a serum neutralization assay against the reference strain BVDV-NADL (5). Unvaccinated calves were randomly selected from the tested calves for the study. Animals were grouped according to their ages and maintained under equivalent conditions. Twenty calves were divided into one control and three experimental (vaccine) groups, where each group consisted of five animals. Data on the animals and

study groups are shown in Table 1. Experimental studies on animals were authorized by the Uludağ University Local Ethic Committee for Animal Experiments (UÜ-HADYEK 2008-5/6). After immunization, the animals were housed in the same facility under natural management conditions provided by the farm.

2.2. Vaccine administration and sample collection

Three commercial vaccines were used for animal immunization. All three vaccines were inactivated whole virion vaccines and were applied as recommended by the manufacturers. One of the vaccines (Vaccine-1) was monovalent while the other vaccines, Vaccine-2 and Vaccine-3, were polyvalent, consisting of different viruses and bacteria (Table 1). A booster dose was applied for all three vaccines at day 30 after prime vaccination (pi). Beginning at prime vaccination (day 0), blood samples were collected into vacutainer tubes. Samples including control groups were collected 6 times within 15-day intervals at

Table 1. Properties of vaccines and animals used in the experiment.

Experimental groups	Vaccines				Animals	
	Properties	Type of BVDV content	Other pathogen compositions	Usage	Numbers	Ages (months)
Group 1 (Vaccine-1)	Inactivated, monovalent	BVDV-1a (5960)	-	2 mL, SC, 30 days interval, 2 doses	1.1	10
					1.2	9
					1.3	8
					1.4	7
					1.5	6
Group 2 (Vaccine-2)	Inactivated, polyvalent	BVDV-1a (KY22), BVDV-2a (TN131)	BHV-1, BRSV, PI-3, <i>H. somnus</i>	5 mL, IM, 30 days interval, 2 doses	2.1	10
					2.2	9
					2.3	8
					2.4	7
					2.5	6
Group 3 (Vaccine-3)	Inactivated, polyvalent	BVDV-1a (Singer)	BHV-1, BRSV, PI-3, <i>Phaemolytica</i> , <i>L.pomona</i> , <i>L. hardjo</i> , <i>L. grippotyphosa</i> , <i>L. canicola</i> , and <i>L. icterohaemorrhagiae</i>	5 mL, IM, 30 days interval, 2 doses	3.1	10
					3.2	9
					3.3	8
					3.4	7
					3.5	6
Control group	-	wPBS	-	2 mL, SC, 30 days interval, 2 doses	C.1	10
					C.2	9
					C.3	8
					C.4	7
					C.5	6

BHV-1: Bovine herpesvirus type 1; BRSV: Bovine respiratory syncytial virus; PI-3: Parainfluenza virus type 3; SC: Subcutaneous injection; IM: Intramuscular injection; wPBS: white phosphate buffered saline.

- : There are no other pathogens given by the vaccination.

days 0, 15, 30, 45, 60, and 75 pi. At prime and booster dose applications, blood samples were collected from all animals before immunization. Sera were inactivated at 56 °C for 30 min and stored at -20 °C until further use in the neutralization assay.

2.3. Viruses and cell line

Viruses were selected among field strains gathered from Turkey. There were eight published subgenotypes of BVDV (BVDV-1a, -1b, -1d, -1f, -1h, -1l, -1r, BVDV-2b) circulating in Turkey at the time of the study. Thus, we evaluated the neutralizing antibody levels against 10 BVDV-1 field strains from subgenotypes of BVDV-1a, -1b, -1d, -1f, -1h, -1l, and -1r, and one BVDV-2 strain (BVDV-2b), which were selected according to their phylogenetic analyses (5,6) (Table 2). Because BVDV-1l is the most prevalent BVDV subgenotype in Turkey (3,5), four representative strains were selected from this subgenotype, while only one strain was selected for the other subgenotypes included in this study (Table 2). All of the 11 BVDV strains used in this study were of noncytopathogenic (ncp) biotype. A cytopathogenic reference strain BVDV-NADL was used as a control virus in neutralization tests and a noncytopathogenic strain TR-19 (BVDV-1l) isolate was chosen as a control virus for indirect immunoperoxidase monolayer assay (I-IPMA). All viruses used for the assays were propagated in the Madin-Derby Bovine Kidney (MDBK) cell line grown in Dulbecco's Modified Eagle's Medium (DMEM, Biochrom AG, T041-05) supplemented with 5% fetal bovine serum (PAA, A11-151), amphotericin B (250 mg/mL, PAA, p11-001), and penicillin/streptomycin (PAA, p11-010). Cell lines and fetal bovine serum were also checked by antigen ELISA, I-IPMA, and neutralization testing to confirm they were not contaminated prior to the start of this study. Viral growth was determined using an indirect immunoperoxidase monolayer assay (I-IPMA). Viruses were harvested, frozen-thawed twice, and centrifuged at 3000 × g for 10 min. The MDBK cell line was also used in the virus titration and cross-neutralization tests. Virus titers were calculated using the Spearman-Kärber method. Properties of the viruses and estimated titers are shown in Table 2.

2.4. Neutralization test and indirect immunoperoxidase monolayer assay (I-IPMA)

To evaluate cross-reactivity between the vaccine-induced antibody and BVDV isolates, indirect immunoperoxidase monolayer assay was applied combined with neutralization assay, as recommended before (5,6) with minor alterations. Briefly, 2-fold dilutions of heat-inactivated sera from all animals collected at 6 different time points were prepared in DMEM. Two serial 2-fold dilutions of each sample were prepared in 24-well plates in 400 µL and mixed with an equal volume of 100 TCID₅₀ of the tested BVDV strain.

Table 2. Virus strains and their titers.

BVDV subgenotype	Virus strain	Titer (TCID ₅₀ /0.1 mL)
BVDV-1a	TR-2	10 ^{-4.7}
BVDV-1b	TR-12	10 ^{-4.45}
BVDV-1d	TR-11	10 ^{-4.2}
BVDV-1f	TR-38	10 ^{-2.45}
BVDV-1h	TR-23	10 ^{-4.95}
BVDV-1l	TR-1	10 ^{-4.95}
BVDV-1l	TR-16	10 ^{-5.2}
BVDV-1l	TR-21	10 ^{-3.7}
BVDV-1l	TR-29	10 ^{-3.7}
BVDV-1r	TR-73	10 ^{-4.2}
BVDV-2b	TR-15	10 ^{-2.75}

Plates were incubated for 1 h at 37 °C. After the addition of an equal volume of MDBK cell suspension (200,000 cells/mL), the plates were incubated at 37 °C in an atmosphere of 5% CO₂ for 3 days. An indirect immunoperoxidase monolayer assay was performed to determinate the virus-neutralization reactions. For this purpose, after heat fixation of the test plates by dry heat at +80 °C for 3 h, 0.5% O-D-glucopyranoside (Sigma, St. Louis, MO, USA) solution in w-PBS (without calcium and magnesium) was added and plates were incubated for 10 min at room temperature. After rinsing with Tween-PBS (0.05% Tween 20 in PBS), the monoclonal mouse antibody (pool 1/4/7) specific to NS3 protein of pestiviruses (13), biotin-labeled antimouse antibody (Pierce, Rockford, USA), and streptavidin-biotinylated-HRPO conjugates (Pierce, Rockford, USA) were used respectively and each were incubated for 90 min at 37 °C. The rinse and antibody dilution were performed with Tween-PBS (0.05% Tween 20 in PBS) at the end of each step. Development of an intracytoplasmic reddish stain after the addition of substrate solution (2 mg AEC in 0.3 mL DMF, 4.7 mL Na-acetate buffer [pH 5.5], and 0.05% H₂O₂) was considered virus-positive, but negative for specific antibodies. In addition, the highest serum dilution inhibited specific viral growth in 50% of the related wells and was considered as the neutralization titer.

2.5. Statistical analyses

The model included the fixed effects of group, time of blood sampling, virus strain, and all other interactions on the antibody titers; the effect of calves was taken into account as a random effect. The PROC GLIMMIX procedure of the software SAS 9.4 was used for statistical analyses of antibody titers. All the values are expressed as least square means ± SEM. Statistical differences were considered significant for P < 0.05 and a tendency for P < 0.10.

3. Results

All animals in the control group remained seronegative (< 1:5 antibody titer) until the end of the study, and all of the vaccinated animals had seroconverted. Neutralizing antibodies specific to BVDV were detected in sera collected from all immunized animals during the five sampling periods at days 15, 30, 45, 60, and 75 pi. In this study, individual antibody responses to BVDV-1a, -1b, -1d, -1f, -1h, -1l, -1r, and BVDV-2b strains were determined on days 0, 15, 30, 45, 60, and 75. Interaction of sampling times and neutralizing antibody titers of vaccine groups were statistically analyzed (Table 3). Geometric means of antibody titers against ten BVDV-1 strains and one BVDV-2 strain detected in five animals in the group were used to determine the mean titer against a defined virus strain, as presented in Table 4. Geometric means of neutralizing antibody titers were calculated for each sampling time for comparison of the groups.

According to the geometrical means of antibody titers obtained in 5 samplings (Table 4), the highest antibody titer was detected against BVDV-1h by Vaccine-2 (mean titer 1577.6). The second highest titer was against BVDV-1a by Vaccine-1 (mean titer 1460.8). Considering that the four strains belong to BVDV-1l (TR-1, TR-16, TR-21, TR-29), the highest antibody titer was obtained with different vaccines. While Vaccine-1 induced the highest antibody titers for TR-21 (445) and TR-29 (1311.9), Vaccine-3 induced the same conditions for TR-1 (666.3) and TR-16 (874.2). Besides the higher responses induced by Vaccine-3 among BVDV-1 subgenotypes, titers against BVDV-1r and BVDV-1d at Vaccine group 3 were prominently low. Vaccine-3-induced antibody titers were between 147.0 and 388.0 against TR-73 (BVDV-1r) and between 194.0 and 445.7 against TR-11 (BVDV-1d). For strain TR-12 (BVDV-1b), not only Vaccine-3 but also Vaccine -2 led to low levels of neutralization antibody titers. Titers for Vaccine-3 were between 55.7–84.4.

Antibody responses of strain TR-15 (BVDV-2 genotype) induced by all vaccines were between titer 32.0 and 294.1 (Table 4). However, individual observations of antibody titers against TR-15 (BVDV-2 genotype) by each of the three vaccines were 128.0–294.1, 64–84.4, and 32.0–64.0, respectively.

4. Discussion

Because of the risk of live vaccines in pregnant cows, investigations for the control of BVD have mostly focused on the improvement of inactivated vaccines (14). However, an inactivated BVDV vaccine PregSure[®] BVD containing cytopathogenic BVDV-1a (strain 5960) was retracted from the market due to having a correlation with bovine neonatal pancytopenia (BNP) (15).

Although the efficiency of inactivated vaccines is strongly and directly related to antigen concentration, the

Table 3. Least square means of antibody titers of all the subgenotypes (including BVDV-1 and BVDV-2) obtained with neutralization assay at different sampling times.

Group	Sampling day	Antibody titers
1	15	1266.91 ± 151.04
1	30	963.20 ± 151.04
1	45	1247.71 ± 151.04
1	60	1203.49 ± 151.04
1	75	1245.96 ± 151.04
2	15	828.22 ± 151.04
2	30	841.02 ± 151.04
2	45	890.47 ± 151.04
2	60	929.75 ± 151.04
2	75	1204.65 ± 151.04
3	15	712.96 ± 151.04
3	30	627.64 ± 151.04
3	45	469.96 ± 151.04
3	60	874.18 ± 151.04
3	75	908.80 ± 151.04

number and route of administration, individual immunity discrepancies, specificity of detection methods, type of adjuvant, and strains of virus selected for vaccination are also critical factors for the level of antibodies detected after BVDV immunization (16–21). In previous studies, effects of vaccine types on the level of immune response (22), differences in cross-protective immune response between different BVDV subgenotypes (23), or adverse effect, i.e. leucopenia induced by BVDV vaccination (24), have also been reported. Although it has been suggested that antibody titers may not be predictive criteria (22), neutralization responses are still important criteria to discuss the level of cross-protection among various BVDV strains/subgenotypes. Hence, due to high diversity among BVDV strains, a special interest has been focused on cross-protection between vaccine strain and field strains over the last decade (17,20).

In this study, neutralizing antibody responses developed by three commercial inactivated BVDV vaccines were investigated against 11 field strains of BVDV obtained in Turkey. The individual antibody titers in calves evaluated by virus neutralization assays ranged between 8 and 8192 (data not shown). Although the interaction between vaccine groups and sampling times was not significant ($P = 0.11$), especially in groups 2 and 3, antibody titers did not increase quickly following the second administration of vaccines. This outcome can also be seen in different studies in which killed vaccines were used (21,25). In a

Table 4. Geometric mean values of neutralizing antibody titers against local BVDV strains*.

Experimental group	Sampling day	BVDV-1a	BVDV-1b	BVDV-1d	BVDV-1f	BVDV-1h	BVDV-1l				BVDV-1r	BVDV-2b
		TR-2	TR-12	TR-11	TR-38	TR-23	TR-1	TR-16	TR-21	TR-29	TR-73	TR-15
1	15	1176.3	337.8	675.6	776.0	445.7	388.0	1024.0	256.0	1782.9	675.6	128.0
	30	1176.3	73.5	294.1	1176.3	388.0	256.0	891.4	337.8	1552.1	1176.3	194.0
	45	2048.0	294.1	512.0	1024.0	588.1	337.8	445.7	294.1	1024.0	1351.2	294.1
	60	1552.1	512.0	588.1	891.4	776.0	256.0	776.0	891.4	1024.0	1351.2	147.0
	75	1351.2	256.0	588.1	2048.0	512.0	445.7	776.0	445.7	1176.3	1552.1	147.0
	Mean**	1460.8	294.7	945.5	1183.1	542.0	336.7	782.6	445.0	1311.9	1221.3	182.0
2	15	588.1	194.0	445.7	1024.0	2352.5	256.0	445.7	445.7	1024.0	294.1	84.4
	30	512.0	147.0	445.7	1351.2	1176.3	294.1	891.4	337.8	891.4	222.9	64.0
	45	256.0	222.9	588.1	675.6	1024.0	388.0	512.0	512.0	1024.0	294.1	73.5
	60	445.7	147.0	588.1	675.6	1552.1	675.6	891.4	337.8	588.1	675.6	84.4
	75	675.6	222.9	675.6	588.1	1782.9	675.6	891.4	337.8	776.0	388.0	55.7
	Mean**	495.5	186.8	370.4	863.9	1577.6	567.9	726.4	394.2	860.7	374.9	72.4
3	15	512.0	55.7	222.9	388.0	1176.3	891.4	588.1	388.0	1024.0	168.9	32.0
	30	588.1	84.4	294.1	588.1	891.4	588.1	388.0	388.0	891.4	194.0	32.0
	45	388.0	64.0	194.0	512.0	776.0	588.1	588.1	256.0	1024.0	147.0	42.2
	60	891.4	73.5	337.8	776.0	891.4	675.6	1024.0	512.0	588.1	388.0	64.0
	75	675.6	55.7	445.7	588.1	776.0	588.1	1782.9	675.6	776.0	388.0	55.7
	Mean**	611.0	66.7	298.9	570.4	902.2	666.3	874.2	443.9	860.7	257.2	45.2

*Each value of titers represents the geometric mean of titers from five animals allocated in the subjected experimental group.

**Means of antibody titer obtained in five consecutive samples.

general approach, using killed whole virion vaccines is expected to have a considerably higher titer after booster vaccination. Unexpected data obtained in the study may be related to technical problems or antigenic dissimilarity between vaccinal strain and test viruses.

Fulton et al. (26) reported that when using a live attenuated vaccine containing BVDV-1a and BVDV-2a, the obtained neutralizing antibody titer of 64 against BVDV-1b was not sufficient for protection against infection with BVDV-1b. In another experimental study that evaluated the efficiency of fetal protection using an inactivated vaccine containing BVDV-1a and BVDV-2a, it was noted that mean antibody titers of 724 against BVDV-1a and the titer of 351 against BVDV-2a could be inadequate for protection against new infections (27). These results showed that cross-neutralizing titers gained with the vaccines, including BVDV-1a and -2a, could be inadequate for protection against BVDV-1b strains. In the present study, the lowest geometrical mean titers in vaccinated animals were detected against the field strain from BVDV-1b among BVDV-1 subgenotypes (Table 4). The average of geometrical mean titers against BVDV-1b was extremely low (55.7 at day 75 pi) in the group of

animals vaccinated with Vaccine-3. Containing different BVDV-1a strains in each of the three vaccines could be the main reason for diversity in antibody titers against BVDV field strains. Thus, the obtained results support the idea that vaccines containing international vaccine strains of BVDV subgenotypes can be less efficacious in different countries or geographies where a different subgenotype is predominant.

Bolin et al. (28) suggested that a neutralizing antibody titer of 256 or higher is required for protection against the clinical appearance of BVDV infections, although Beer et al. (14) reported another protective titer of 512. Considering both studies, the lowest neutralizing antibody titer for protection against BVDV is 256 under experimental conditions. Although most of the antibody titers detected in this study were at a considerable level, some results were not satisfying according to the previous studies. Regarding titer 256 as a reference titer, results against BVDV-1b and some other subgenotypes in BVDV-1 appear to be critical. Lower antibody titers were observed against BVDV-1d with the sera developed by Vaccine-3 and against BVDV-1r by both Vaccines 2 and 3. When considering strain BVDV TR-73 (BVDV-1r), which was

isolated from a BVDV vaccinated herd (6), antibody titers lower than 256 support the previously suggested data (28).

The highest antibody responses are generally detected against homologous strains of the virus (29,30). Differences between antibody titers induced against homologous subgenotypes are another striking issue in this study. For Vaccine-2 and Vaccine-3, the highest antibody titers were observed against BVDV-1h; however, the highest antibody reaction with Vaccine-1 was produced by the homologous subgenotype BVDV-1a. Previous indicated serological similarities between BVDV-1a and BVDV-1h (30) may be related to higher antibody reactions detected in this study; however, antigenic dissimilarities between BVDV-1a strain TR-2 and the strain included in the vaccine could be taken into account.

The geometrical means of antibody titers against BVDV-2 detected in this study were between 128.0 and 294.0 by Vaccine-1, and between 32.0 and 64.0 by Vaccine-3. There are different studies with BVDV-1 vaccines that reported low antibody responses against BVDV-2 strains compared to BVDV-1 (31,32). Interestingly, Vaccine-2 also induced low antibody titers (56.0–84.0) against BVDV-2, although it contains the BVDV-2 strain in addition to BVDV-1a. Antigenic differences between the vaccine strain and BVDV-2b field strain could be the reason for low reactions obtained at the cross-neutralization test. These parameters noted the importance of antigenic differences between BVDV-2 subgenotypes, as accepted in BVDV-1.

The geometrical mean values of antibody titers obtained by all three vaccines against BVDV-1 subgenotypes were between 502.0 and 836.0, 443.0 and 606.0, and 273.0 and 455.0, respectively (Table 5). Vaccine-1 used in this study was monovalent, while the other two vaccines were polyvalent. In an experiment in which the BVDV-1a-

Table 5. Geometric mean values of antibody titers against BVDV-1 and BVDV-2.

Vaccine group	Sampling day	BVDV-1 (total)	BVDV-2
1	15	633.5	128.0
	30	501.9	194.0
	45	784.9	294.1
	60	835.9	147.0
	75	803.4	147.0
2	15	478.7	84.4
	30	484.9	64.0
	45	443.5	73.5
	60	532.7	84.4
	75	605.9	55.7
3	15	272.7	32.0
	30	360.2	32.0
	45	268.7	42.2
	60	454.6	64.0
	75	426.3	55.7

containing monovalent vaccine and the polyvalent vaccine containing five BVDV-1 strains were compared, higher neutralization titers against the NADL strain have been stated by the monovalent vaccine (33). Similar results were obtained in this study. According to the geometric mean values, Vaccine-1 commonly yielded higher antibody titers compared to the other two vaccines. This situation was observed not only in BVDV-1 subgenotypes but also in BVDV-2b subgenotypes (Figure). The effect of group

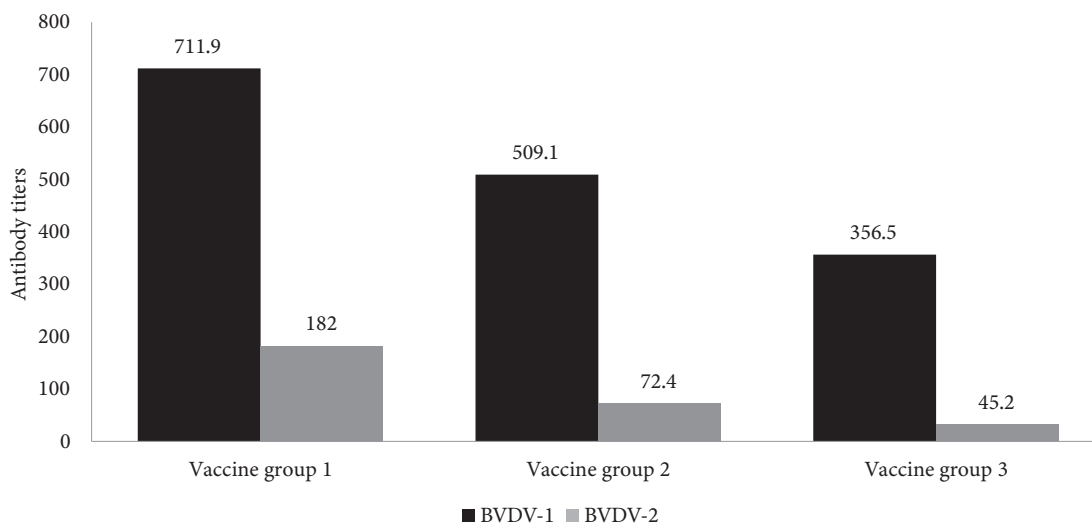


Figure. Comparison of mean values for antibody titers against BVDV-1 and BVDV-2.

(vaccine type) was found to be significant ($P < 0.01$). The difference among groups was a result of vaccine type (monovalent vs. polyvalent) and the monovalent vaccine (group 1) had higher antibody titers when compared to group 2 ($P = 0.09$) and group 3 ($P < 0.01$). The results also support the idea of monovalent vaccines having better immunity results compared to polyvalent vaccines. This outcome may arise not only from variations of strains used in vaccines but also due to different combinations of additional organisms in the vaccine. The efficiency of monovalent and polyvalent vaccines against heterologous subgroups may be a matter for future work.

In conclusion, abundant research studies have evaluated the protective efficiency of commercial vaccines against field isolates. A considerable amount of these investigations were performed with BVDV-1a, -1b, and BVDV-2 strains, which are widespread in most

geographic areas (4,27,34). There is only a small number of investigations on immunological reactions against newly reported BVDV isolates. The aim of this study was to evaluate the antibody titers against current BVDV subgenotypes. This study concluded that in addition to the subgenotype BVDV-1b, which is frequently seen in Europe (3), low antibody titers could be induced against BVDV-1d, BVDV-1r, and BVDV-2b by commonly used inactivated BVDV vaccines.

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