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The Effects of Vaccination on the Immune Responses of Dairy Cattle Seropositive to Bovine Viral Diarrhea Virus (BVDV)

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Abstract: The aim of the study was to determine the effects of vaccination on the antibody titres of dairy cattle seronegative and seropositive to Bovine Viral Diarrhea Virus (BVDV). For this purpose, 23 cattle were used in the study. Before the vaccination, 23 cattle from two different dams (consisted of 15 and 8 in each) were tested for presence of BVDV specific antibodies and antigens. In the study, 16 (69.6%) and 2 (8.7%) animals were found to seropositive and persistently infected [antigen (+) but antibody (-)], respectively. Remaining 5 cattle (21.7%) were detected as seronegative. Sixteen seropositive and five seronegative cattle were vaccinated with an inactive commercial BVDV vaccine. In the study, serum and blood samples were collected before and after vaccination and analysed for the precence of BVDV specific antibodies and antigens using Enzyme Linked Immunosorbent Assay (ELISA). Serum Neutralization Test (SNT) was also used to detect BVDV neutralizing specific antibodies. Antibody titres in serum samples collected from vaccinated seropositive animals were significantly ($p = 0.001$, $p<0.001$) increased compare to those of samples collected before vaccination detected by ELISA. Finally, it was thought that vaccination of seropositive cattle for BVDV may result in long-lasting and strong immunity compared to those of seronegative animals which may benefical to protect cattle against BVDV infection.

Key words: Bovine viral diarrhea virus, seropositive, seronegative, vaccination, dairy cattle

INTRODUCTION

The agent of Bovine Viral Diarrhea Virus (BVDV) is pestivirus, a member of Flaviviridea family. BVDV infection is a major problem causing serious economical losses in cattle breeding (Xue *et al*., 2009). The virus biotypes are classified as cytopathogenic (cp) or non-cytopathogenic (ncp) (Houe *et al*., 2006; Fulton *et al*., 2009). The virus has various virulence and causes subclinical or clinical diseases in cattle. The disease causes serious lesions in respiratory, digestion and genital systems of infected animals. Furthermore, addition to abortion in pregnant cattle, malformations, congenital defect and neonatal mortality can also be seen. The agent has also known to have an immunsuppressive effect on immune system and causes serious diarrhea and mucosal diseases characterized with high mortality in some breeds of cattle (Greiser-Wilke *et al*., 2003; Baker, 1995; Rodning *et al*., 2010).

Enzyme Linked Immunosorbent Assay (ELISA) has been widely used for the detection of BVDV antigens and antibodies. Serum Neutralization Test (SNT) is also used for the detection of virus specific neutralizing antibodies in BVDV infected animals. Seroprevalance in non-vaccinated herds has been reported to range between 20 and 90% in different regions and countries (Bolin *et al*., 1985; Houe, 1999). In countries without BVDV control programs, about 1-2% animals were found Persistently Infected (PI) with BVDV (Duong *et al*., 2008). PI animals are known to play an important role to spread the virus between infected and healthy herds (Loneragan *et al*., 2005; Fernandez *et al*., 2009; Xue *et al*., 2009). For this reason, detecting and eradicating PI animals and vaccinating healthy animals are very important for the success of protection and controlling strategies (Xue *et al*., 2009; Rossmanith *et al*., 2010). For this purpose, many inactivated and live BVDV vaccines are used to immunize dairy cattle against BVDV infection (Brownlie *et al*., 1995; Dean and Leyh, 1999; Makoschey *et al*., 2001; Grooms *et al*., 2007; Rodning *et al*., 2010). It is necessary to clarify that if there is any difference in antibody titres between seropositive

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and seronegative animals vaccinated with BVDV vaccine. This point will help to clarify whether or not seropositive animals should or should not be vaccinated with BVDV vaccine.

The aim of the study was to determine the effects of vaccination on the antibody titres of dairy cattle seropositive and seronegative to BVDV.

MATERIALS AND METHODS

Animals, sample collection and vaccination: In this study, 23 dairy cattle, aged 2-4 years were used. All animals were not vaccinated against BVDV before the study. Two animals were brought to the Clinics of Internal Medicine with suspecious of BVDV. These two cattle from different dams were diagnosed as BVDV-PI infected and not used in the study. Sixteen seropositive and five seronegative cattle from the same dams with PI animals were used in the study. These animals were then vaccinated with an inactive commercial BVDV vaccine (containing cp type 1a, ncp type 1 and ncp type 2 of BVDV, Vira sheild 6+Somnus®, Novartis Animal Health US, Inc. Larchwood, IA, 51241 USA) twice with 3 weeks intervals. Blood samples were collected from each animal before and after first and second vaccination and then used to prepare serum and leucocyte samples. Serum samples and leucocyte samples were kept in -25 and -80°C until used, respectively.

ELISA (Antibody): Bovine viral diarrhea virus antibody-ELISA (Svanovir BVDV-Ab, Sweden) kit was used to detect BVDV specific antibodies according to the manufacturer instructions.

ELISA (Antigen): BVDV/MD antigen-ELISA (Bio-X Diagnostics, Belgium) kit was used to detect BVDV antigen in blood leucocyte samples collected from animals before and after each vaccination. The test was performed according to the procedures of the concerning company.

Serum Neutralization Test (SNT): Virus neutralizing antibodies specific to BVDV in serum samples were detected using microneutralization test (mNT) as described by Frey and Liess (1971). Neutralization test for positivity and SN_{50} titers were evaluated and calculated as described by Kaerber (1964).

Statistical analysis: The significances of ELISA OD values obtained from each vaccination were determined using ANOVA test. Pearson test of Minitab Program in the correlation was also used to detect sensitivity and specificity of the methods. The $p<0.001$ were accepted as significant (Minitab Inc., 1998).

RESULTS

Persistently Infected animals (PI): Antibody (-)/antigen (+) was detected in two dairy cattle from two different dams brought to the clinic with a complaint of bloody diarrhea. Same results were collected in the second sampling of these animals after 45 days. These two animals were accepted as BVDV PI and conducted to slaughter.

ELISA: In the study, 16 (69.6%) and 2 (8.7%) animals were found to seropositive and persistently infected [antigen (+) but a ntibody (-)], respectively. Remaining 5 cattle (21.7%) were detected as seronegative. The average OD values of vaccinated seropositive animals were 1.41±0.18 before the vaccination whereas the OD values were found to be 2.07±0.11 after the first and 2.27±0.36 after the second vaccination (Table 1, Fig. 1). Antibody titres in seronegative and antigen negative animals were also increased after the first and second vaccinations but the titres were significantly lower in this group compared to those of seropositive group ($p<0.001$) (Table 1, Fig. 1).

SNT: In SNT, 11 out of 16 serum samples were found to positive for BVDV antibody. The SN_{50} distributions of 11 serum samples were 1:2-1:89.1 before, 1:2.81-1:89.1 after the first and <1:2 to ≥1/256 after the second vaccination.

Serum neutralizing antibody titers were significantly increased in samples collected from the seropositive and seronegative animals after the first and second

Table 1: Mean and standard error of the mean (mean±SEM) OD values of serum samples collected on days 0, 21 and 42

Groups	Before vaccination (0th day)	1st vaccination (21st day)	2nd vaccination (42nd day)
Seropositive (n = 16)	1.41±0.180^{b,*}	2.07±0.110^{a,*}	2.27±0.360^{a,*}
Seronegative (n = 5)	0.42±0.003^{c}	0.75±0.005^{b,c}	0.93±0.005^{b,c}

$^{a-c}$The significances of the values obtained in various days were indicated by letters ($p<0.001$). *The significances of the values between seropositive and seronegative groups were indicated by symbol ($p<0.001$)

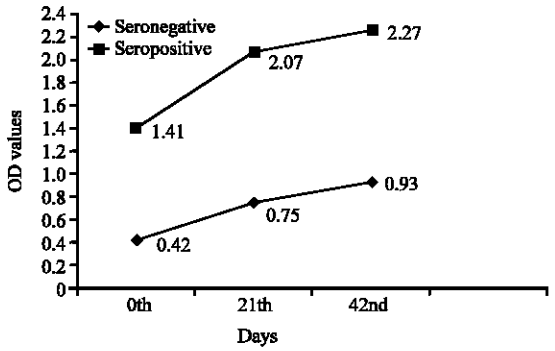

Fig. 1: OD values of the seropositive and seronegative animals on days 0, 21 and 42

vaccination compared to those of samples collected before vaccination ($p<0.001$). Significant differences in OD values were also obtained between first and second vaccination ($p<0.001$). Antibody titers detected by both ELISA and SNT were significantly higher in samples collected from seropositive animals after first and second vaccination compared to those of vaccinated seronegative animals ($p<0.001$).

According to the results of the study, antibody titers which detected in both ELISA and SNT were significantly increased in samples collected from the animal after the first and second vaccination compared to those samples collected before the vaccination. Despite 100% sensitivity and 69.5% correlation between both tests, no statistical significance ($p = 0.305$; $p<0.1$ NS) was detected between the number of seropositive animals detected by ELISA and SNT.

DISCUSSION

BVDV infection is an important disease of dairy cattle industry. It usually causes serious economical losses. BVDV has been reported that to invade respiratory, gastrointestinal, reproductive, immune and endocrine systems resulting subclinical and clinical disease. The infection occurs endemically world-wide according to the biosafety, diagnostic monitoring and vaccination studies based on controlling efforts. Today, BVDV controlling programs are applied in some European and Scandinavian countries (Houe, 2003; Gunn *et al*., 2005; Moennig *et al*., 2005a; Xue *et al*., 2009; Rodning *et al*., 2010).

In Europe, BVDV seroprevalance has been reported to vary as 95% in England and Wales (Paton *et al*., 1998), 64% in Denmark (Houe and Meyling, 1991) and 46% in Sweden (Niskanen *et al*., 1991), 19% in Norway (Loken *et al*., 1991) and 1% in Finland (Nuotio *et al*., 1999). Similarly, BVDV seroprevalance in Asia has been shown to range 66-100% in cattle (Garoussi *et al*., 2009).

BVDV seroprevalance has been reported to range between 14.3 and 100% in various regions of Turkey (Alkan *et al*., 1997; Cabalar and Karaoglu, 1999; Tan *et al*., 2006; Yildirim *et al*., 2011; Ozkanlar *et al*., 2012). In previous study, Ozturk *et al*. (2012) reported that BVDV seroprevalance was found to be 81.5% in dairy cattle having abortion problems in Burdur. The results and earlier studies performed in Burdur indicate that BVDV is still wide-spread in the region and a controlling program needs to be applied for cattle farming. High BVDV seroprevalance (69.6%) detected in the study might be the evidence of the presence of PI animals in dairy cattle in the Burdur region as reported elsewhere (Houe *et al*., 1995).

Vaccination is still the most common way in controlling BVDV in many European countries and it is considered as a supplementary biosafety tool to prevent reinfection in countries with high BVDV prevalence (Moennig *et al*., 2005a, b). BVDV vaccination is usually performed on seronegative and non-infected animals (Makoschey *et al*., 2007; Fernandez *et al*., 2009; Xue *et al*., 2009; Raue *et al*., 2011; Alvarez *et al*., 2012). However, in this study, seropositive animals were vaccinated to find out the possible alterations in antibody titers after the first and second vaccination. In the study, significant differences in antibody titers detected by both ELISA and SNT were obtained between the samples collected from seropositive and seronegative animals after the first and second vaccinations. Therefore, BVDV vaccination was proved to be effective for the development of high antibody titers specific to BVDV infection. It was thought that high antibody titers may result in long-lasting immunity and protection against BVDV infection in cattle. Therefore, two vaccination programs may have beneficial for long-lasting protection and control programs in both seropositive and seronegative cattle.

Controlling BVDV infection in a herd can be succeeded by detecting and eradicating PI animals and providing long-lasting protection by immunization (Kelling, 2004; Moennig *et al*., 2005a, b; Grooms *et al*., 2007; Fernandez *et al*., 2009). For dairy cattle inducing the development of antigen specific immune responses due to vaccination at least 3 weeks before pregnancy may provide fetal protection against BVD infection and fetopathogenic phenomenon can be prevented (Radostits and Littlejohns, 1988). It is well-known that diagnostic tests and eradication programs have not been sufficiently applied in Burdur and its vicinity. The results of present study indicate that all the animals without testing either seronegative or seropositive should be vaccinated twice in order to reduce the prevalence of the BVDV infection in the region like Burdur.

CONCLUSION

The present study indicated that long-lasting immunity and protection can be induced due to double vaccination in both seropositive and seronegative cattle. Therefore, all the dairy cattle, regardless of seropositivity should be vaccinated at least twice to reduce prevalence of BVDV infection in Burdur Region.

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