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SHORT COMMUNICATION

Effect of Different Storage Temperatures on the Stability of Bovine Viral Diarrhea Virus RNA in Blood Samples

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ABSTRACT

The present study was conducted to determine stability of Bovine viral diarrhea virus (BVDV) RNA stored at different temperatures. A total of 6 blood samples obtained from a private cattle farm, which were found to be antigen positive (Ag+) by direct ELISA method, were used in this study. BVDV Ag+ samples were stored separately at 4, 21 and 37°C for 1 month. The samples were analyzed on the 0, 1st, 2nd, 3rd and 4th weeks by ELISA for the presence of BVDV Ag and by RT-PCR for the presence of BVDV RNA. Stability of BVDV RNA was calculated using maximum concentration (C_{max}) and area under the curve (AUC) as kinetic parameters of each sample. All of the samples were found positive both by ELISA and RT-PCR on each week. C_{max} values of BVDV RNA for the storage temperatures of 4, 21 and 37°C were 356, 346 and 338 ng/µL respectively, and $AUC_{0\rightarrow4}$ values for the same temperatures were 1151, 1106 and 1077 week.ng/µL respectively. It was determined that storage at different temperatures for one month does not statistically influence the kinetic parameters of BVDV RNA ($P>0.05$). In conclusion, it can be expressed that storage of BVDV RNA at 4, 21 and 37°C for one month has no effect on the stability of BVDV RNA.

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INTRODUCTION

Bovine viral diarrhea virus (BVDV) is one of the viral agents that cause economic losses all over the world (Tiwari *et al*., 2007). The virus, which involves digestive and respiratory systems, causes economic losses mainly by leading to reproductive disorders such as infected fetus, fetal reabsorption, abortion, congenital defects, and persistent infected (PI) calf born (Loy *et al*., 2013). The agent is a member of *Pestivirus* genus of *Flaviviridae* family (Fernandez-Sirera *et al*., 2012). The virion with single-stranded + polarity RNA is 12.5 kb in length (Zemke *et al*., 2010). It contains a total of 4 structural proteins consist of 3 glycoproteins-Erns, E1, E2 and a capsid protein (Loy *et al*., 2013).

Isolation of virus in cell cultures is considered as the gold standard for the diagnosis of BVDV. However, it has been reported that false negative results can be obtained due to autolytic activities likely to occur in the studies that will examine tissue samples (Ellis *et al*., 1995). Immunofluorescence (IF) or immunoperoxidase (IP) tests are also used to identify BVDV Ags (Ozkul *et al*., 2002). In addition to these tests in the diagnosis of BVDV, reverse transcriptase-polymerase chain reaction (RT-PCR) and real-time PCR methods are also preferred (Kosinova *et al*., 2007).

There are studies in which different tests were used to determine the stability of BVDV RNA by storing tissues (Ridpath *et al*., 2006; Liu *et al*., 2013) and blood samples at different temperatures and on different days (Ozkul *et al*., 2002). Basic pharmacokinetic parameters ($C_{max,}$ AUC) are successfully used to identify the motility of drug in the living organism. Nevertheless, any motility that have time-concentration relationship and is defined by a curve can be explained by means of kinetic parameters. Thus, cumulative increase and decrease in motility (AUC), maximum concentration in this curve (C_{max}), and after how long the curve will increase and/or decrease to which level could be detected mathematically (Altan *et al*., 2010).

The present study was conducted to determine stability of RNA, which is found in BVDV Ag+ leukocyte

samples, at different temperatures by calculating kinetic parameters (C_{max} ng/µL, $AUC_{0\rightarrow4}$ week.ng/µL).

MATERIALS AND METHODS

Leukocyte samples that were found to be Ag+ by ELISA method were stored at different temperatures (4, 21 and 37°C) for 4 weeks and then extraction was performed and $RNA_{260/280}$ absorbance ratios were measured on each week. Extraction products were analyzed by RT-PCR for the presence of BVDV viral genome. All procedures of the research were performed in the Virology Laboratory of Faculty of Veterinary Medicine, Selcuk University. The present study used 6 leukocyte samples obtained from private cattle farm (Afyonkarahisar), which were found to be BVDV Ag+ by direct ELISA. Commercially available direct ELISA (Idexx, USA) kit was performed in accordance with the test procedure. RNA extraction from leukocytes was performed using RNeasy Mini Kit (Qiagen, 74106). Viral $RNA_{260/280}$ absorbance ratios (ng/µL) were measured by NanoDrop (Thermo Scientific, USA).

Calculation of kinetic parameters: RNA concentration-time curve was obtained for each sample. Kinetic parameters were analyzed using a non-compartmental model for each sample. C_{max} and AUC kinetic parameters of the groups were determined using Phoneix 6.4 (WinNonlin 6.3, Pharsight Certara Company, USA) program. Kinetic parameters were presented as Mean±SD.

Statistical analysis: Statistical evaluation of the data obtained was done by one way analysis of variance (ANOVA), whereas the difference between the groups was determined using Duncan test (SPSS 16.0, SPSS for Windows, SPSS Inc., USA). $P<0.05$ was considered to the limit for statistical significance.

RESULTS AND DISCUSSION

All of the samples stored at 4, 21 and 37°C for 4 weeks were found to be positive by ELISA in terms of the presence of BVDV antigen. Moreover, all of the samples were found to be positive via RT-PCR in terms of presence of BVDV viral genome at the end of the 4th week. C_{max} and AUC values are demonstrated in Table 1. No statistical difference was determined between the groups in terms of stability of BVDV RNA stored at different temperatures ($P>0.05$). BVD-Mucosal Disease, which has clinical and pathological variations, is a multi-systemic infection that causes huge economic losses in cattle breeding (Tiwari *et al*., 2007). Cattle rising business with reproductive problems must certainly be examined in terms of BVDV, and obtaining rapid results is of critical importance. Controlling business enterprises in terms of BVDV infection plays an important role in preparing regional or national control programs.

All samples were found to be positive in terms of presence of BVDV antigen and viral genome in this study (Fig. 1). Ozkul *et al*. (2002) compared different tests in the diagnosis of BVDV and found that RT-PCR is the most appropriate test in detecting BVDV in leukocyte samples stored as frozen for more than a year. In the present study, it was found that storing the samples, which were positive for viral genome via RT-PCR, at 4, 21 and 37°C for 1 month, has no effect on BVDV RNA stability ($P>0.05$, Table 1). Moreover, C_{max} values for BVDV RNA stored at 4, 21 and 37°C were 356±64.7, 346±65.6 and 338±69.1 ng/µL, respectively (Table 1). Based on the C_{max} values obtained, it was determined that the amount of BVDV RNA in the positive samples stored at specified temperatures remained detectable until the end of the 4th week and that the lowest RNA loss was in the samples stored at 4°C (Fig. 1).

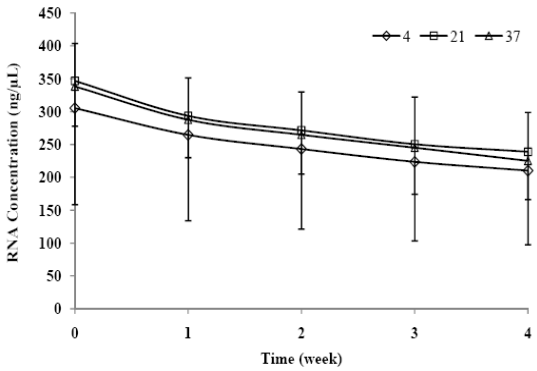

Fig 1: RNA concentration-time curves of leukocyte samples stored at 4, 21 and 37°C (Mean±SD).

Table 1: Kinetic parameters of BVDV RNA stored at different temperatures (Mean±SD)

RNA Storage temperatures	C_{max} (ng/µL)	$AUC_{0\rightarrow4}$ (week.ng/µL)
4°C n:6	356±64.7	1151±271
21°C n:6	346±65.6	1106±270
37°C n:6	338±69.1	1077±272

C_{max}=Maximum RNA concentration, AUC= Area under the curve RNA concentration. No significant difference in the same column.

Not only obtaining under optimum conditions as possible is important for the materials that would be transferred to the laboratories, but also storage temperature, solutions for dilution and type of transportation are also of critical importance. The present study revealed that storage of leukocyte samples that would be analyzed for the presence of BVDV antigen at 4, 21 and 37°C for one month would not influence the laboratory results. Nevertheless, considering $AUC_{0\rightarrow4}$ values, the highest RNA (1151±271 week.ng/µL) was obtained from the samples stored at 4°C even though the laboratory results of samples stored at different temperatures for 4 weeks showed no change. The amount of RNA in the samples stored at 21 and 37°C were 1106±270 and 1077±272 week.ng/µL, respectively.

In the present study, even though the amount of BVDV RNA in the leukocyte samples containing BVDV RNA stored at 4, 21 and 37°C for 1 month decreased at the end of the 4th week versus day 0, BVDV genome was detected via RT-PCR. Ridpath *et al*. (2006) stated that BVDV remained stable in the samples that they stored between -20 and 25°C for 7 days. Results of the present study carried out at three different temperatures (4, 21 and 37°C) used to determine stability of BVDV RNA is consistent with the result of the study, in which the researchers (Ridpath *et al.,* 2006) reported stability for 7 days.

Conclusion: Based on the stability of BVDV RNA continued for 4 weeks when stored at 4, 21 and 37°C, it can be stated that storage of the samples, which would be sent from the field to be analyzed for BVDV, at various temperatures for a certain time (4 weeks) due to inappropriate conditions is unlikely to influence the laboratory results, but, nonetheless, it would be beneficial to store the samples at 4°C where available.

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Author's contribution: Authors will declare that OA, OB, OY and SY conceived and designed the review. ID and KA executed the experiment and analyzed the samples, ELISA and RT-PCR applications. AS analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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