Molecular Characterization of Bovine Viral Diarrhea Virus Infection from Boer Goats

Ji-Hyoung Ryu[1#]

Seung-Uk Shin[1#]

Joon-Seok Chae[2]

Kyoung-Seong Choi[1]*

[1]Department of Animal Science and Biotechnology,
College of Ecology and Environmental Science,
Kyungpook National University, Sangju 37224, Republic of Korea

[2]Laboratory of Veterinary Internal Medicine,
BK21 PLUS Program for Creative Veterinary Science Research,
Research Institute for Veterinary Science and College of Veterinary Medicine,
Seoul National University, Seoul 08826, Korea

[#]These authors equally contributed to this work

*Correspondence should be addressed to:
Kyoung-Seong Choi, DVM, MS, PhD
College of Ecology and Environmental Science
Kyungpook National University
Sangju 37224, Republic of Korea
Tel: 82-54-530-1222
Fax: 82-54-530-1959
E-mail: kschoi3@knu.ac.kr

KEY WORDS: bovine viral diarrhea virus, goats, BVDV-1b, genetic variation

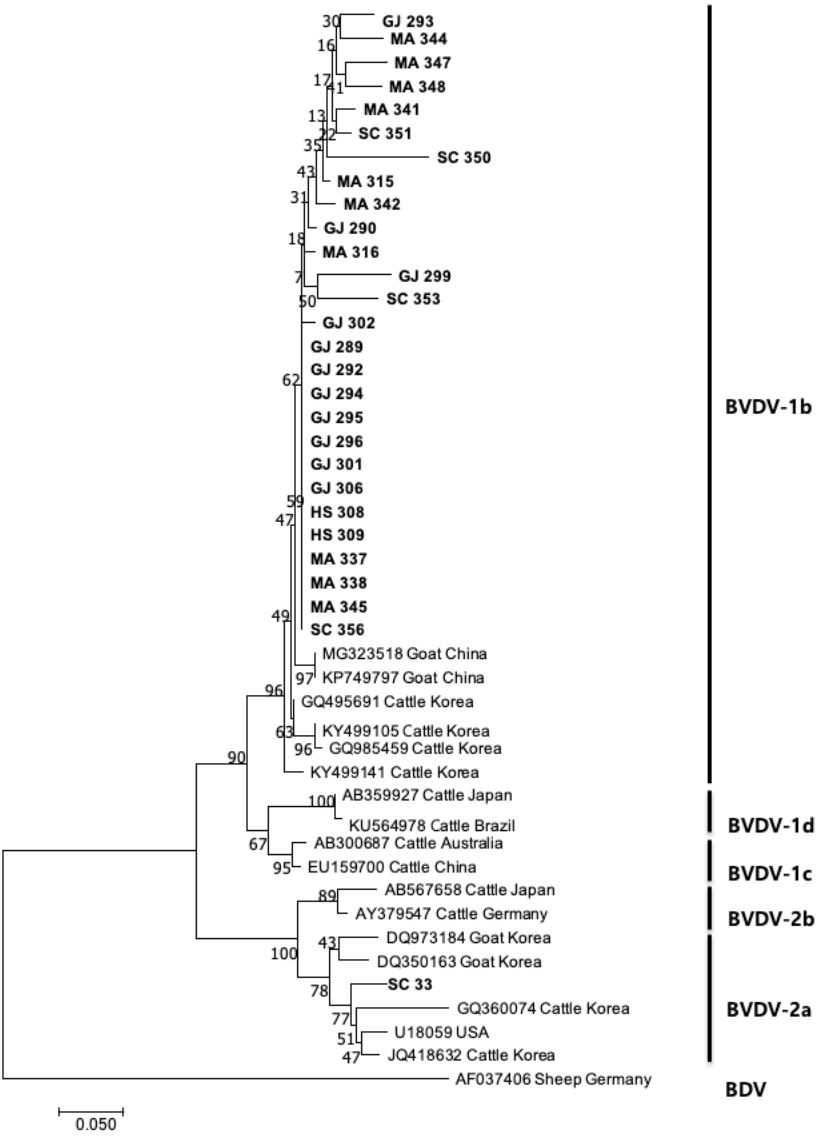
ABSTRACT

Bovine viral diarrhea virus (BVDV) is an economically important viral pathogen of the cattle industry worldwide. BVDV has a very broad host range and can infect domestic and wild ruminants as well as swine. Recently, the goat industry has increasingly developed in the Republic of Korea (ROK). However, very limited information is available about BVDV infection in goats. The objective of this study was to investigate the prevalence of BVDV infection in goats and determine the subtype of BVDV circulating in the ROK. Between 2018 and 2019, a total of 659 blood samples were collected from Boer goats in the ROK. All these goats did not exhibit any clinical signs. Thirty-one (4.7%) samples were identified to be positive for BVDV infection by RT-PCR. Phylogenetic analysis based on the 5'- untranslated region revealed that 27 samples and 1 sample were classified into BVDV-1b and BVDV-2a, respectively. BVDV-1b isolates identified in goats exhibited genetic variation. This may have occurred under the following scenarios: interspecies transmission, pre-adaptation in goat, and goat-to-goat transmission. To our knowledge, this is the first report of BVDV-1b infection in goats in the ROK. The results have important implications for goat production. These results suggest that goats may act as virus reservoir hosts for BVDV infection. The information highlights the importance for the control and prevention of BVDV infection in goats.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is a member of the Pestivirus genus be-

Figure 1. *Phylogenetic analysis of bovine viral diarrhea virus isolated from goats based on the 5'-UTR. A phylogenetic tree was constructed using MEGA7 software and by employing the maximum likelihood method; the numbers over branches indicate bootstrap values (1000 replicates) as percentages, supporting each phylogenetic branch. The Korean isolates identified in this study are shown in boldface.*



longing to the family Flaviviridae. The most common species of the genus Pestivirus are BVDV-1, BVDV-2, Classical swine fever virus (CSFV), and Border disease virus (BDV), and these are characterized by an interspecies transmission. Additionally, other species of Pestivirus have been proposed as BVDV-3 or HoBi-like pestivirus, Bungowannah virus, pronghorn virus, giraffe virus, and other atypical viruses[4,16]. Recently,[11] recognized species of Pestivirus have been re-designated from A to K, wherein BVDV-1, BVDV-2, CSFV, and BDV are Pestivirus A, B, C, and D, respectively[3, 11, 15].

BVDV has a very broad host range and can infect both domestic and wild ruminants as well as swine, causing a serious impact on the domestic livestock industry. Infections with BVDV are associated with subclinical infection, immunosuppression, acute diarrhea, respiratory diseases, and reproductive failures such as infertility, abortion, and stillbirth. Transplacental infection of the fetus with BVDV can result in the birth of weak calves or persistently infected (PI) animals. PI cattle are the main source of BVDV; however, persistent infections also occur in sheep and deer. BVDV infection in goats typically results in reproductive diseases and viable PI goats are rare.[13]

Based on sequence analysis of 5'-UTR, to date, 21 subtypes (1a-1u) of BVDV-1, four subtypes of BVDV-2 (2a-2d), and two genotypes of BVDV-3 (Brazilian and Thai origin) have been identified.[9] Several epidemiological surveys on BVDV have beeconducted in goats in the Republic of Korea (ROK), and to date, BVDV-1a and BVDV-2a have been detected in goats in the ROK.[6,7] However, the transmission route of BVDV in these goats still remains unclear. Recently, the goat industry has developed increasingly in the ROK. Approximately 4.5 million goats were reared in the ROK during 2017 according to the official Livestock Statistics. The number and size of goat flocks differed accordingly to the region. A recent study performed by our group showed that BVDV infection in goat was not necessarily accompanied by clinical symptoms[12] and above all, goats could act as reservoir host of BVDV. Therefore, we investigated the prevalence and subtypes of BVDV circulating in goat farms. These findings highlight the importance of controlling and preventing BVDV infections.

MATERIALS AND METHODS

In 2019, blood samples were collected from 659 Boer goats by a veterinarian and delivered to Seoul National University. All goats were clinically healthy. Some of blood were transferred to Kyungpook National University and RNA was extracted from individual blood samples by using TRIzol reagent (Takara Bio, Otsu, Japan) according to the manufacturer's instructions, following which the extracted RNA was immediately frozen at -80°C until use.

Primers specific for the 5'-UTR and N^{pro} regions were used for PCR amplification. RT-PCR was performed to amplify BVDV using the DiaStar™ One-Step RT-PCR Smart Mix (Solgent, Daejeon, ROK), as described previously.[5, 12] The predicted size of the amplified PCR product was 288 bp for 5'-UTR and 425 bp for N^{pro}. The PCR products were purified using the AccuPrep® PCR Purification Kit (Bioneer, Daejeon, ROK) as per the manufacturer's instructions, and the amplicons were directly sequenced (Macrogen Inc., Seoul, ROK).

All the sequences obtained were analyzed using Chromas software (version 2.33, http://www.technelysium.com.au/chromas.html) and aligned with GenBank BVDV reference strains, which were representative of BVDV-1 and BVDV-2, using Clustal X (version 1.8); manual editing was performed using BioEdit software (version 7.2.1). Phylogenetic tree was constructed on the basis of 5'-UTR using MEGA7 software.[8] The evolutionary history of the aligned sequences was inferred using maximum-likelihood method.[15]

RESULTS

All goats examined in this study did not exhibit any clinical signs. Thirty-one goats (4.7%) out of 659 were positive for BVDV using 5'-UTR, whereas two goats were positive for BVDV using N^{pro} region. It was difficult to accurately estimate the age of these experimental goats, but most goats might be at least ≥15 months old. Twenty-eight good sequences from 31 PCR-positive samples were obtained and included in the phylogenetic tree. These sequences showed 83.4%-95.5% homology with each other. The phylogenetic tree based on 5'-UTR showed that 27 isolates and one isolate belonged to BVDV-1b and BVDV-2a, respectively (Fig. 1). All isolates belonging to BVDV-1b formed the same cluster with goat isolates

identified from China (MG323518 and KP749797), but were diverged from Korean cattle isolates (Fig. 1). One BVDV-2a isolate showed 94.6%-95.0% identity with goat isolates previously detected in the ROK but formed a separate clade from Korean goat isolates (Fig. 1). Additionally, this BVDV-2a isolate was closely related to Korean cattle isolates (JQ418632 and GQ360074) (Fig. 1). There were genetic variations in BVDV-1b found in goats. Thus, to our knowledge, this is the first report of BVDV-1b infection in Boer goats.

DISCUSSIONI

In this study, two genes were used to detect BVDV infection. Interestingly, BVDV was identified at a higher rate in goats by using the 5'-UTR than by using Npro region. We cannot explain the reason for the difference in BVDV detection between the two genes. Moreover, we could not obtain sequencing results for the two PCR-positive samples by targeting the Npro region. To date, no published study has compared BVDV detection between these two genes. Consequently, our results have demonstrated that 5'-UTR is suitable for BVDV detection in goats.

Until now, few studies have investigated BVDV infection in goats, and thus, the epidemic situation of BVDV in goats remained unclear. In the present study, BVDV infection has been investigated throughout the ROK and the results revealed that the prevalence of BVDV infection was 4.7% in the blood samples. BVDV infection was found only in goats in the southern region. These farms where BVDV was detected are mainly located in the plain area and had no beef or dairy farms in the vicinity. We could not reach a conclusion regarding the manner in which BVDV infection occurred in these goats. Because the blood of these goats was taken from a slaughterhouse, we did not know whether these goats were PI or not. It is speculated that BVDV-infected goats are present at these farms and transmit the virus to other goats. The possibility that pregnant goats are infected with BVDV via unintentional exposure and may give birth to PI kids cannot be excluded. Because BVDV infection in goats does not necessarily exhibit any clinical signs,9, 10 the farmer may overlook a BVDV-infected goat. Although the route of BVDV transmission was not identified in these goats, goats may possibly act as a reservoir of BVDV in non-bovine hosts.

The present results revealed that BVDV-1b is the prevalent subtype in goats in the ROK. This is consistent with a recent result from a cattle-based study.[14] BVDV-1b isolates identified in goats showed genetic variations and these sequences were different from cattle isolates. According to a previous study performed by our group, BVDV infection in goats led to the changes in nucleotide sequences.[12] It is speculated that interspecies transmission may have occurred first and then the virus become pre-adapted in goats.[1] As a result, this phenomenon is the consequence of goat-to- goat transmission of a viral genome, although it is not confirmed, BVDV may have already existed among goats for a long time. Interestingly, BVDV-2a subtype was identified in only one goat. It is unclear at this time whether BVDV-2a had a low infection rate or BVDV-2a infection was low among the goats which we sampled. However, BVDV-2a infection in cattle was not as high as BVDV-1b infection, which can be attributed to a similar situation. Based on the phylogenetic tree, BVDV-2a was grouped together with Korean cattle isolates, which is the result of interspecies transmission and might be undergoing a process of pre-adaptation in goats. Moreover, the present study did not show any association between clinical symptoms and BVDV infection. Further study should be necessary to investigate the prevalence and the subtypes of BVDV in goats with various clinical signs, understand BVDV infections in goats, and design and develop effective control strategies.

CONCLUSION

Our results show that BVDV-1b is predominantly detected in goats. To date, three BVDV subtypes (1a, 1b, and 2a) have been identified in goats in the ROK. Genetic

variations seen in goats can be occurred under scenarios such as interspecies transmission, pre-adaptation in goats, and goat-to-goat transmission. Although BVDV-infected goats did not exhibit any clinical manifestation, BVDV infections may cause economic loss to the developing goat industry. These results suggest that goats may act as virus reservoir hosts for BVDV infection. Because BVDV cross-infections already occurred between goats and cattle, the present findings highlight the importance of controlling and preventing BVDV infection in goats.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (NRF), funded by the Korea government (MSIP) (No. 2018R1D1A1B07048271). This research was also supported by Kyungpook National University Development Project Research Fund, 2019.

REFERENCES

1. Bachofen, C., Vogt, H.R., Stalder, H., Mathys, T., Zanoni, R., Hilbe, M., Schweizer, M., Peterhans, E., 2013. Persistent infections after natural transmission of bovine viral diarrhoea virus from cattle to goats and among goats. *Vet. Res*. 44, 32.
2. Deng, Y., Wang, S., Liu, R., Hao, G., 2018. Genetic Diversity of Bovine Viral Diarrhea Virus Infection in Goats in Southwestern China. *J. Vet. Med*. 827, 4397.
3. Feknous, N., Hanon, J.B., Tignon, M., Khaled, H., Bouyoucef, A., Cay, B., 2018. Seroprevalence of border disease virus and other pestiviruses in sheep in Algeria and associated risk factors. *BMC Vet. Res.* 14, 339.
4. Gomez-Romero, N., Basurto-Alcantara, F.J., Verdugo-Rodriguez, A., Bauermann, F.V., Ridpath, J.F., 2017. Genetic diversity of bovine viral diarrhea virus in cattle from Mexico. *J. Vet. Diagn. Invest.* 29, 362-365.
5. Han, D.G., Ryu, J.H., Park, J., Choi, K.S., 2018. Identification of a new bovine viral diarrhea virus subtype in the Republic of Korea. *BMC Vet. Res.* 14, 233.
6. Han, Y.J., Chae, J.B., Chae, J.S., Yu, D.H., Park, J., Park, B.K., Kim, H.C., Yoo, J.G., Choi, K.S., 2016. Identification of bovine viral diarrhea virus infection in Saanen goats in the Republic of Korea. *Trop. Anim. Health Prod.* 48, 1079-1082.
7. Kim, I.J., Hyun, B.H., Shin, J.H., Lee, K.K., Lee, K.W., Cho, K.O., Kang, M.I., 2006. Identification of bovine viral diarrhea virus type 2 in Korean native goat (Capra hircus). *Virus Res*. 121, 103-106.
8. Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* 33, 1870-1874.
9. Mao, L., Li, W., Yang, L., Wang, J., Cheng, S., Wei, Y., Wang, Q., Zhang, W., Hao, F., Ding, Y., Sun, Y., Jiang, J., 2016. Primary surveys on molecular epidemiology of bovine viral diarrhea virus 1 infecting goats in Jiangsu province, China. *BMC Vet. Res.* 12, 181.
10. Mishra, N., Rajukumar, K., Vilcek, S., Tiwari, A., Satav, J.S., Dubey, S.C., 2008. Molecular characterization of bovine viral diarrhea virus type 2 isolate originating from a native Indian sheep (Ovies aries). *Vet. Microbiol.* 130, 88-98.
11. Neill, J.D., Workman, A.M., Hesse, R., Bai, J., Porter, E.P., Meadors, B., Anderson, J., Bayles, D.O., Falkenberg, S.M., 2018. Identification of BVDV2b and 2c subgenotypes in the United States: Genetic and antigenic characterization. *Virology* 528, 19-29.
12. Oem, J.K., Han, D.G., Choi, K.S., 2019. Experimental infection of Korean native goats (Capra aegagrus hircus) with bovine viral diarrhea virus 1b. *BMC Vet. Res.* 15, 202.
13. Passler, T., Riddell, K.P., Edmondson, M.A., Chamorro, M.F., Neill, J.D., Brodersen, B.W., Walz, H.L., Galik, P.K., Zhang, Y., Walz, P.H., 2014. Experimental infection of pregnant goats with bovine viral diarrhea virus (BVDV) 1 or 2. *Vet. Res.* 45, 38.
14. Ryu, J.H., Choi, K.S., 2019. Genetic analysis of bovine viral diarrhea virus in pre-weaned native Korean calves. *Trop. Anim. Health Prod.* 51, 2085-2090.
15. Smith, D.B., Meyers, G., Bukh, J., Gould, E.A., Monath, T., Scott Muerhoff, A., Pletnev, A., Rico-Hesse, R., Stapleton, J.T., Simmonds, P., Becher, P., 2017. Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae. *J. Gen. Virol*. 98, 2106-2112.
16. Yesilbag, K., Alpay, G., Becher, P., 2017. Variability and Global Distribution of Subgenotypes of Bovine Viral Diarrhea Virus. *Viruses* 9.