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REGULAR ARTICLES

Endless variety for bovine virus diarrhea viruses: new members of a novel subgroup into *Pestivirus A* from Turkey

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Abstract

As ubiquitous pathogens, bovine virus diarrhea viruses (BVDVs) in cattle have been reported several times in Turkey. Over time, the frequency and importance of this infection has increased for the livestock industries. A total of 1291 animals were sampled from a dairy herd in Turkey suspected of BVDV clinical signs, for instance, reproductive failures (abortion, congenital malformations in calves, repeat breeding, etc.) and interdigital phlegmon in adult animals. Reverse transcription polymerase chain reactions (RT-PCRs) were made by using targeted 5′ untranslated region (UTR), N^{pro}, E2, and NS2-3 pestiviral gene region primers for antigen ELISA-positive samples ($n = 20$). The obtained amplicons were sequenced. Sequence results showed the presence of a new subgroup in *Pestivirus A* species. This paper describes the nucleotide sequences of a new BVDV 1 (BVDV 1-v) subgroup member.

Keywords BVDV · Cattle · New subgroup · Turkey

Introduction

Bovine pestiviruses, known as bovine viral diarrhea viruses (BVDVs), have been classified into two genotypes, BVDV 1 and BVDV 2. Nowadays, these are classified by the *Flaviviridae* Study Group of the International Committee on Taxonomy of Viruses (ICTV) as *Pestivirus A* and *Pestivirus B*, respectively (Smith et al. 2016; Smith et al. 2017; Simmonds et al. 2017). These were divided into several subgroups, with over 20 in BVDV 1 (*Pestivirus A*) and four in BVDV 2 (*Pestivirus B*). These have also been given new names according to the letters of the alphabet. BVDV 1 and BVDV 2 have been described worldwide as atypical pestiviruses responsible for this infection in ruminants. Since the first identification of atypical pestiviruses, which infect other species although they are originated from different species (pronghorn antelope pestivirus, *Pestivirus E*; Bungowannah virus, *Pestivirus F*; Giraffe-1 H138, *Pestivirus G*) or could be found in commercial fetal bovine sera (HoBi-like *Pestivirus H*), they have been increasingly reported (Schirrmeier et al. 2004; Bauermann et al. 2012; Kirkland et al. 2007). Additionally, local pestivirus isolates from cattle as new members of novel subtypes have been reported from different countries in the last decade (Gao et al. 2013; Khodakaram-Tafti and Farjanikish 2017; Han et al. 2018). In addition to novel pestiviruses obtained from cattle hosts, novel pestivirus sequences have also been reported in rats and bats (Wu et al. 2012; Firth et al. 2014). This genetic variability is important not only for ICTV but also for the execution of prevention and control programs of BVDV.

It is known that the subtyping of BVDVs is associated with the geographical origin of these viruses (Flores et al. 2002). In Turkey, the presence of BVDV 1 and BVDV 2 subtypes has been reported several times (Oğuzoğlu et al. 2004, 2010a, 2012; Yesilbag et al. 2008, 2014). However, in Turkey, BVDV 1 subtypes (1-a, 1-b, 1-c, 1-d, 1-e, 1-f, 1-*l*, 1-p, 1-r) are characterized by a limited genetic diversity (Oğuzoğlu

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et al. 2010b; Yesilbag et al. 2014), as common local subtypes also have been reported. In this study, we have detected a new local subgroup (1-v) of the BVDV obtained from cattle samples in Turkey. We think that after investigating, the pathogenicity and the prevalence of novel BVDV types identified in the country and could be included them in the composition of vaccines that would be suitable on national disease prevention programs for effective immunity against BVDV if necessary after evaluation of the results.

Materials and methods

A herd of 1291 cattle (951 cows, 2 years or older, and 340 female calves under 5 months of age; male animals are sold after the feed is taken) from a private herd in Turkey was investigated in terms of the presence of BVDV infection. These animals consistently showed interdigital phlegmon, arthritis, repeated breeding, pneumonia, sudden deaths in dairy cows, diarrhea, abortion, still births, weak calf births, blindness in newborn calves, some limbs undeveloped (congenital absence of tail), arthrogryposis, etc. At the request of the herd's owner, all animals were tested by BVDV antigen ELISA (Idexx, USA). Positive samples were molecularly tested by PCR for 5′ UTR, N^{pro}, E2, and NS2-3 gene regions of pestiviruses and the obtained amplicons were sequenced. The obtained sequences have been compared to worldwide reference sequences available in the GenBank database.

Viral RNAs were extracted from whole blood samples using a commercial kit (Invitrogen, Thermo Fischer Scientific, USA) according to the manufacturer's instructions. Reverse transcription reaction was performed by using a Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Germany) according to the manufacturer's protocols. Four different gene regions (5′ UTR-288 bp, N^{pro}-428 bp, E2-1179 bp, and NS2-3) were used in PCR by using primer pairs, as described by previous studies (Vilcek et al. 1994; Vilcek et al. 2001; Oğuzoğlu et al. 2017; Greiser-Wilke et al. 1993). The amplicons, obtained from PCR reactions performed for each sample, were sequenced by Beckman Coulter CEQ 8000. Both direction sequences were compared in the BLAST program and the consensus sequences were generated using the CAP option in the BioEdit software program (version 7.0.5.3) (http:// www.mbio.ncsu.edu/bioedit/bioedit.html) (Hall 1999). Phylogenetic analyses were conducted using MEGA 6.0 (Tamura et al. 2016).

Results

Twenty animals (20/1291, 1.55%) were detected as persistently infected (PI) in this herd. After sampling time, an additional seven newborn calves were found positive with a quick antigen test for BVDV, but they were not sampled for laboratory confirmation. The dams of three of the seven calves were BVDV positive. All seven calves died because of recurrent pneumonia and diarrhea.

New pestiviruses from Turkey in this study were characterized by sequencing of 5′ UTR and N^{pro} partial and full E2 gene regions. Interestingly, positive samples based on 5′ UTR, N^{pro}, and E2 in PCR did not react in terms of the NS2-3 gene region. Maximum likelihood unrooted phylogenetic trees were constructed according to the Kimura-2 parameter model with 1000 bootstrap replicates based on the mentioned sequences. The accession numbers have been deposited in GenBank. The 5′ UTR consisted of 17 sequences: MH673439-MH673455; N^{pro}, 18 sequences: MH758720-MH758737; and E2, 19 sequences: MH673456-MH673474, respectively.

Topology of constructed trees indicated 20 isolates are localized at the pestivirus species, which is closely related to BVDV 1. According to the results of 5′ UTR, N^{pro}, and E2 phylogenetic analyses, it was found that all samples were nearly of BVDV 1 local dominant strains which described previous studies from Turkey. However, all isolates were clustered in a separate branch in all analyses (Fig. 1a, b, and c). A high level of genetic heterogeneity between new Turkish BVDV 1 strains with other BVDV 1 subtype strains was detected in this study.

Discussion

The members of the pestivirus genus, belonging to the family *Flaviviridae* (Simmonds et al. 2017), infect the ruminant species, which leads to dramatic events that directly affect herd health and reproductive efficiency. Recently, several novel pestivirus species or subgroups have been recognized worldwide. An important question for pestivirologists is how many new pestivirus subtypes/genotypes already exist in cattle; in this context, having an answer to this question would be helpful in the control and prevention of pestivirus infections in affected country.

In January 2018, various clinical signs (especially, abortion, congenital malformations, sudden deaths, and interdigital phlegmon in adult animals) in a dairy cattle herd in central Anatolia were reported. Subsequent to the mentioned clinical findings that were sampled at the owner's request, one animal (calf, 2 weeks old) that had a congenital absence of tail was tested against BVDV by using the antigen ELISA. It was found to be positive antigenically for BVDV. Based on this result, all animals in this dairy herd were sampled and tested for the BVDV antigen. Of this group, 20 were positive. After 21 days, all the positive animals were sampled for the second time and all were found to be repeatedly positive. This is the sign of a high ratio (1.55%) of PI animals in

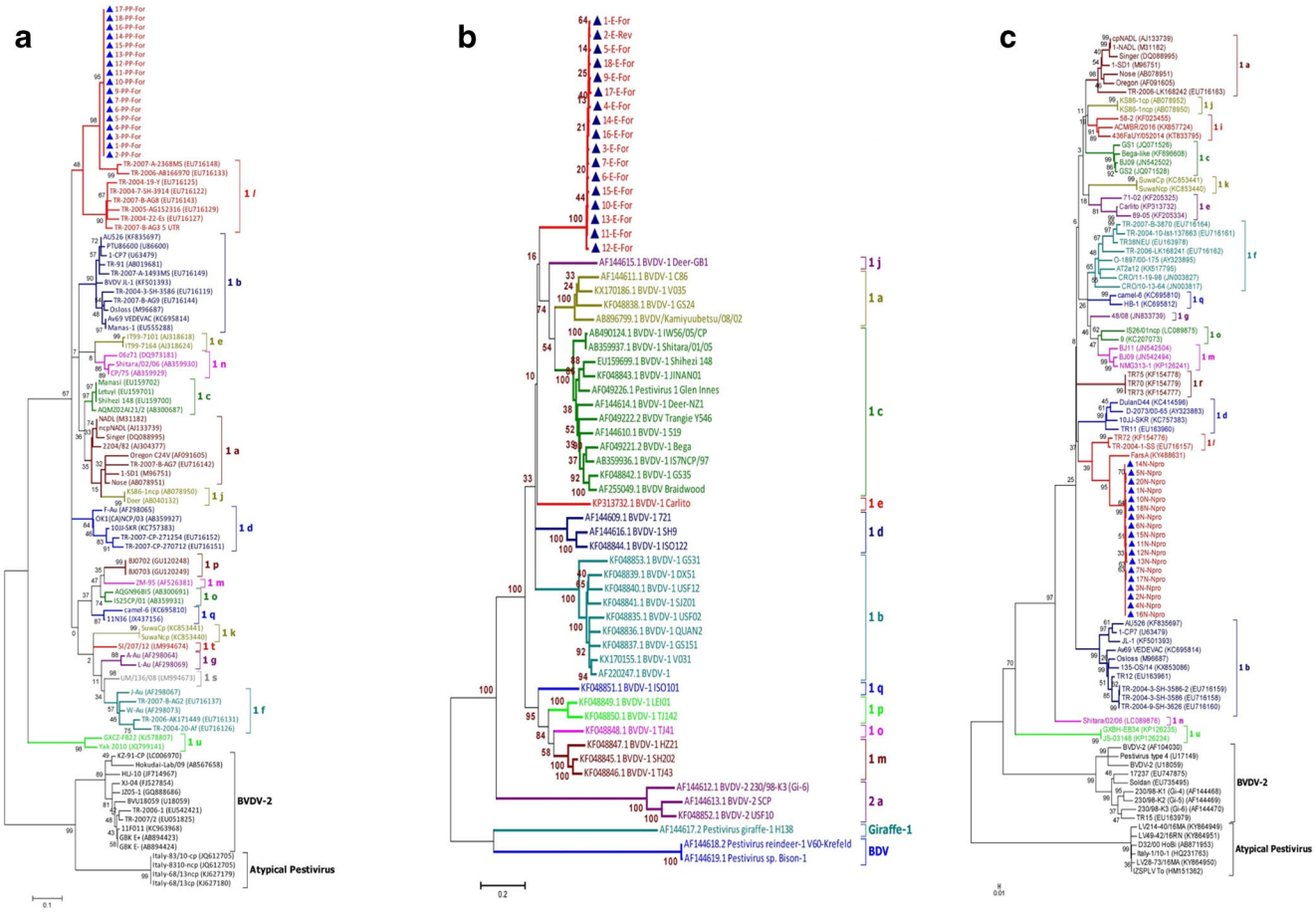

Fig. 1 **a**. Phylogenetic tree of new Turkish local subgroup and other BVDV strains obtained from GenBank database by using 5′ UTR sequences. Accession numbers deposited in GenBank are Turkish BVDV 1-v strains for 5′ UTR sequences: MH673439-MH673455. **b**. Phylogenetic tree of new Turkish local subgroup and other BVDV strains obtained from GenBank database by using E2 gene region sequences. Accession numbers are Turkish BVDV 1-v strains for complete E2 gene: MH673456-MH673474. **c**. Phylogenetic tree of new Turkish local subgroup and other BVDV strains obtained from GenBank database by using N^{pro} sequences. Accession numbers are Turkish BVDV 1-v strains for N^{pro} gene region: MH758720-MH758737

the herd. Precautions for preventing the disease continue in that herd. In this context, the aim is to prevent the increase of new PI individuals by controlling newborn animals with rapid antigen testing. As a result of an antigen screening test, seven new BVDV-positive calves, which were born after the first sampling date in this herd, were identified. Five of 20 PI animals were dams and these "Trojan" dams breed PI offspring ($n = 7$). The most important clinical findings in PI animals in this study were interdigital phlegmon and arthritis. This examination was found important in terms of the relationship between the new local virus strains obtained in this study and clinical signs.

To date, nine BVDV 1 subtypes including one local subtype have been identified in Turkish cattle. Our molecular characterization findings in this study showed that besides the common BVDV 1 viruses, new pestiviruses could have been present among the cattle in Turkey. The new members of the new subtype identified in this study were related to cattle BVDV-1 isolates obtained from Turkey, but nucleotide homology ratio was maximally 65% (data not shown). We believe that these are members of a new subgroup. We recommend the letter "v" in the naming of this new group.

Based on the phylogenetic analysis in this study, it has been observed that there have been significant differences especially in E2 gene region (Fig. 1b), which is an immune dominant major envelope protein of pestiviruses, during the comparison between the global reference strains and our sequences. Our opinion, the genetic divergence in 35% rates may lead to significant changes in immunity. Therefore, we suppose that it has been required to perform studies on their immunogenicity and pathogenicity for viruses in new types. It is suggested that these genetic differences and variations of virus strains be taken into account when developing control and prevention programs for BVDV.

BVDV infection negatively affects cattle health and livestock industries. The prevalence of PI cattle in herds should be considered when choosing prevention and control programs in a country or herd. In addition, for designing an effective

BVDV immunization protocol, local BVDV strains could be included in the production of relevant vaccines. Our group keeps abreast of pestivirus infections in ruminant herds in Turkey. We hope that the mentioned works will serve to help control the disease.

Conclusion

For the detection and control of these new viruses, it is important to improve and promote the knowledge of diversity of BVDV infection caused by atypical or novel subtype pestiviruses around the world for improved diagnostic tools and reformulation of current vaccines. This study described and antigenically characterized novel ruminant pestiviruses in Turkey for the first time. Our results show that different pestivirus species (for instance BVDV-1) have been in circulation between cattle herds in Turkey.

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Compliance with ethical standards

Ethical approval This article does not contain experimental animal studies.

Conflict of interest The authors declare that they have no conflicts of interest.

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References

Bauermann, F.V., Flores, E.F., Ridpath, J.F. 2012. Antigenic relationships between Bovine viral diarrhea virus 1 and 2 and HoBi virus: possible impacts on diagnosis and control. J Vet Diagn Invest 24:253–261.

Firth, C., Bhat, M., Firth, M.A., Williams, S.H., Frye, M.J., Simmonds, P., Conte, J.M., Ng, J., Garcia, J., Bhuva, N.P., Lee, B., Che, X., Quan, P.L., Lipkin, W.I. 2014. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal Rattus norvegicus in New York City. MBio 5:e01933–01914

Flores E.F., Ridpath J.F., Weiblen R., Vogel F.S.F. & Gil L.H.V.G. 2002. Phylogenetic analysis of Brazilian bovine viral diarrhea virus type 2 (BVDV-2) isolates: evidence for a subgenotype within BVDV-2. Virus Res. 87:51-60.

Gao, S., Luo, J., Du, J., Lang, Y., Cong, G., Shao, J., Lin, T., Zhao, F., Belak, S., Liu, L., Chang, H., Yin, H.. 2013. Serological and molecular evidence for natural infection of Bactrian camels with multiple subgenotypes of bovine viral diarrhea virus in western China. Vet Microbiol.; 163:172 –6.

Greiser-Wilke, I., Haas, L., Dittmar, K. E., Liess, B. & Moennig, V. 1993. RNA insertions and gene duplications in the nonstructural protein p125 region of pestivirus strains and isolates in vitro and in vivo. Virology 193, 977–980.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.

Han, D.G., Ryu, J.H., Park, J. and Choi, K.S. 2018. Identification of a new bovine viral diarrhea virus subtype in the Republic of Korea. BMC Vet Res.;14(1):233.

Khodakaram-Tafti, A. and Farjanikish, G.H. 2017. Persistent bovine viral diarrhea virus (BVDV) infection in cattle herds. Iranian Journal of Veterinary Research, Shiraz University, Vol. 18, No. 3, Ser. No. 60, Pages 154-163.

Kirkland, P.D., Frost, M.J., Finlaison, D.S., King, K.R., Ridpath, J.F. , Gu, X. 2007. Identification of a novel virus in pigs—Bungowannah virus: A possible new species of pestivirus. Virus Research 129, 26–34.

Oğuzoğlu, T. C., Alkan, F. and Burgu, I. 2004. Detection of Bovine Virusdiarrhoea Virus (BVDV) Infection in a naturally infected dairy herd. V. Middle European Buiatrics Congress, Hajduszoboszlo, Hungary, June 2 and 5, 2004.

Oğuzoğlu, T.C., Muz, D., Yılmaz, V., Alkan, F., Akça, Y., Burgu İ. 2010a. Molecular characterization of Bovine virus diarrhoea viruses species 2 (BVDV-2) from cattle in Turkey. Trop Anim Health Prod., 42: 1175–1180.

Oğuzoğlu, T.Ç., Muz, D., Karapınar, Z., Toplu, N., Timurkan, M.Ö., Şişman, E., Burgu, İ. 2010b. Untersuchungen über die viralen Ursachen der Kälberverluste aus einem Rinderzuchtbetrieb. Türk-Alman Tarım ve Tabii Bilim Araştırıcıları Derneği. 9. Uluslararası Sempozyumu. 22-26.Mart.2010, Hatay-Türkiye. Symposium über Wissenschaftliche Ergebnisse Deutsch–Türkischer Universitätspartnerschaften im Agrar Bereich. In: Symposiumsbuch.

Oğuzoğlu, T.C., Muz, D., Yılmaz, V., Timurkan, M.Ö., Alkan, F., Akça, Y., Burgu, I. 2012. Molecular Characteristics of Bovine Virus Diarrhoea Virus 1 Isolates from Turkey: Approaches for an Eradication Program. Transboundary and Emerging Diseases, Aug;59(4):303-10.

Oğuzoğlu, T.C., Muz, D., Timurkan, M.Ö., Koç, B.T., Özşahin, E., Burgu, İ., Akça, Y., Demirbağ, Z. 2017. Expression and production of recombinant proteins from immunodominant E gene regions of Bovine Viral Diarrhoea Virus 1 (BVDV-1) Turkish field strains for prophylactic purpose. *Revue Méd. Vét.*, 168, 7-9, 183-191.

Schirrmeier, H., Strebelow, G., Depner, K., Hoffmann, B., Beer, M. 2004. Genetic and antigenic characterization of an atypical *Pestivirus* isolate, a putative member of a novel *Pestivirus* species. J. Gen. Virol., 85, 3647-3652.

Simmonds, P., Becher, P., Bukh, J., Gould, E.A., Meyers, G., Monath, T., Muerhoff, S., Pletnev, A., Rico-Hesse, R., Smith, D.B., Stapleton J.P., ICTV Report Consortium 2017. ICTV virus taxonomy profile: *Flaviviridae*. J. Gen. Virol., 98, 2–3.

Smith, D.B., Becher, P., Bukh, J., Gould, E.A., Meyers, G., Monath, T., Muerhoff, A.S., Pletnev, A., Rico-Hesse, R., Stapleton, J.T., Simmonds, P. 2016. Proposed update to the taxonomy of the genera hepacivirus and pegivirus within the flaviviridae family. J. Gen. Virol., 97, 2894–2907.

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, B. 2017. Proposed revision to the taxonomy of the genus pestivirus, family flaviviridae. J. Gen. Virol., 98, 2106–2112.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2016. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.*, 30**,** 2725-2729.

Vilcek, S., Herring, A. J., Herring, J. A., Nettleton, P. F., Lowings, J. P. and Paton, D. J. 1994. Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three gen- ogroups using polymerase chain reaction and restriction endonuclease analysis. Arch. Virol. 136, 309–323.

Vilcek, S., Paton, D.J., Durkovic, B., Strojny, L., Ibata, G., Moussa, A., Loitsch, A., Rossmanith, W., Vega, S., Scicluna, M.T., Paifi, V. (2001). Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. Arch. Virol. 146, 99–115.

Wu, Z., Ren, X., Yang, L., Hu, Y., Yang, J., He, G., Zhang, J., Dong, J., Sun, L., Du, J., Liu, L., Xue, Y., Wang, J., Yang, F., Zhang, S., Jin, Q. 2012. Virome analysis for identification of novel mammalian viruses in bat species from Chinese provinces. J Virol 86:10999–11012

Yesilbag, K., Förster, C., Bank-wolf, B., Yılmaz, Z., Alkan, F., Ozkul, F., Burgu, I., Cedillo Rosales, S., Thiel, H.-J., König, M. 2008. Genetic heterogeneity of bovine viral diarrhoea virus (BVDV) isolates from Turkey: identification of a new subgroup in BVDV-1. Vet. Microbiol. 130, 258– 267

Yesilbag, K., Förster, C., Ozyigit, M.Ö., Alpay, G., Tuncer, P., Thiel, H-J., König, M. 2014. Characterisation of bovine viral diarrhoea virus (BVDV) isolates from an outbreak with haemorrhagic enteritis and severe pneumonia. Veterinary Microbiology 169 (2014) 42–49.