Transboundary and Emerging Diseases

ORIGINAL ARTICLE

Molecular Characteristics of Bovine Virus Diarrhoea Virus 1 Isolates from Turkey: Approaches for an Eradication Programme

T. Ç. Oğuzoğlu[1], D. Muz[2], V. Yılmaz[3], M. Ö. Timurkan[4], F. Alkan[1], Y. Akça[1] and İ. Burgu[1]

[1] Department of Virology, Faculty of Veterinary Medicine, Ankara University, Dışkapı-Ankara, Turkey
[2] Department of Virology, Veterinary Faculty, Mustafa Kemal University, Antakya-Hatay, Turkey
[3] Department of Virology, Veterinary Faculty, Kafkas University, Paşaçayırı-Kars, Turkey
[4] Department of Virology, Veterinary Faculty, Atatürk University, Yakutiye-Erzurum, Turkey

Keywords:
molecular characterization; bovine virus diarrhoea virus; Turkey

Correspondence:
T. Ç. Oğuzoğlu. Department of Virology, Faculty of Veterinary Medicine, Ankara University, 06110 Dışkapı-Ankara, Turkey.
Tel.: +90 312 317 0315/448;
Fax: +90 312 3164472;
E-mail: oguzoglu@ankara.edu.tr

Received for publication August 25, 2011

doi:10.1111/j.1865-1682.2011.01272.x

Summary

Forty pestivirus isolates sampled from cattle in Turkey between 2002 and 2007 were characterized according to 5′ untranslated region (5′UTR) sequences and autoprotease (N^{pro}) gene sequences. The sampling of Bovine virus diarrhoea viruses (BVDVs) from 15 farms in five different regions indicated that BVDV 1-*l* (18/40, 45%) was the predominant genotype in Turkey; the samples also contained the genotypes 1-f (10/40, 25%), 1-b (7/40, 17.5%), 1-d (3/40, 7.5%), and 1-a (2/40, 5%), respectively.

Introduction

Bovine pestiviruses, namely the Bovine virus diarrhoea virus (BVDV), a ubiquitous major pathogen of cattle, are enveloped, positive-strand RNA viruses that belongs to the *Pestivirus* genus within the family *Flaviviridae* (Heinz et al., 2000).

Two genotypes of BVDV, BVDV 1 and BVDV 2, have been recognized based on genomic sequence differences, especially in the 5′ untranslated region (UTR) (Pellerin et al., 1994; Ridpath et al., 1994). Bovine virus diarrhoea virus 1 has been classified into 11 subgroups (Vilcek et al., 2001). In recent years, several novel species and genetic groups of BVDVs have been defined by genetic typing (Yamamoto et al., 2008; Yesilbag et al., 2008; Robesova et al., 2009; Xue et al., 2010). Bovine virus diarrhoea virus 1-a and 1-b have been reported in North American, South American and European countries (Vilcek et al., 2001; Fulton et al., 2005). Bovine virus diarrhoea virus 1-c is a local strain in Australia (Mahony et al., 2005) and has also been isolated from cattle in Japan (Nagai et al., 2008) and in China (Zhong et al., 2011). Subtypes 1-d, 1-e, 1-f, 1-g, 1-h, 1-i, 1-k are circulating in European countries (Hornberg et al., 2009; Robesova et al., 2009). Recently, 1-m and 1-p were detected in China (Xue et al., 2010), as was 1-n in Japan (Nagai et al., 2008); 1-o was found as a subgenotype in both Japan and China (Yamamoto et al., 2008).

Bovine virus diarrhoea virus infection can cause important economic losses to the cattle industry. Therefore, molecular genetic typing of BVDV isolates is important for designing and constructing effective eradication strategies involving vaccination. For a vaccine to be effective in an entire country, major antigenic components from field viruses must be included.

In this study, bovine pestivirus isolates (some of which may be vaccine candidates) circulating in Turkish cattle were collected from 2002 to 2007 and typed for 5′ UTR ($n = 37$). Some selected isolates ($n = 8$, three of them were not typed for 5′UTR) were also typed in the autoprotease (N^{pro}) coding region. The results of this study will allow better understanding of the genetic variability of BVDV 1 field isolates in Turkey and will contribute

towards development of more effective vaccines that may provide adequate protection against BVDV infection in Turkish cattle and improve currently existing programmes to nationally eradicate BVDV.

Materials and Methods

Some clinical signs consistent with BVDV infection were reported in the cattle herds sampled, including abortions, births with congenital anomalies (Arthrogryposa and Hydranencephaly-AH syndrome), reproductive failure (infertility), oral lesions, diarrhoea, and early calf mortality (that is, death in the second week after birth). In this study, BVDV isolates representing different herds/geographical origin from blood samples collected between 2002 and 2007 and their cell culture supernatants (which were obtained from foetal bovine turbinata cells) were used (Table 1 and Fig. 1).

Table 1. The characteristics of Turkish bovine virus diarrhoea virus 1 isolates were feature in this study

No	Isolate names	Accession no	Origin	BVDV 1 subgroup	Remarks
1	TR-2004-1-SS	EU716117	SİVAS	/	r.d.m.
2	TR-2004-2-SH-3895	EU716118	KAYSERİ	/	Abortions, infertility, high mortality rate
3	TR-2004-3-SH-3586	EU716119	KAYSERİ	b	on calfs
4	TR-2004-4-SH-3304	EU716120	KAYSERİ	/	
5	TR-2004-6-SH-3586-2	EU716121	KAYSERİ	b	
6	TR-2004-7-SH-3914	EU716122	KAYSERİ	/	
7	TR-2004-8-SH-3626**	EU716160	KAYSERİ	b	
8	TR-2004-9-SH-3926	EU716123	KAYSERİ	b	
9	TR-2004-10-Ist-137663	EU716124	İSTANBUL	f	r.d.m.
10	TR-2004-19-Y	EU716125	YOZGAT	/	
11	TR-2004-20-Af	EU716126	AFYON	f	
12	TR-2004-22-Es	EU716127	ESKİŞEHİR	/	
13	TR-2005-A141896	EU716128	ANKARA/MERKEZ	b	
14	TR-2005-AG152316	EU716129	ANKARA/GÖLBAŞI	/	Abortions, stillbirths
15	TR-2005-AG152318	EU716130	ANKARA/GÖLBAŞI	/	
16	TR-2006-AK171449*	EU716131	ANKARA/KIRKLARELİ	f	Abortions, stillbirths
17	TR-2006-AK171450*	EU716132	ANKARA/KIRKLARELİ	/	
18	TR-2006-AB166970	EU716133	ANKARA	/	Infertility
19	TR-2006-LK168241	EU716134	KIRKLARELİ	f	Abortions, stillbirths, AH syndrome
20	TR-2006-LK168242**	EU716163	KIRKLARELİ	a	
21	TR-2006-LK168243	EU716135	KIRKLARELİ	f	
22	TR-2006-LK168244	EU716136	KIRKLARELİ	f	
23	TR-2007-B-3870**	EU716164	BURSA	f	Abortions, infertility, high mortality on early
24	TR-2007-B-AG2	EU716137	BURSA	f	weeks after birth
25	TR-2007-B-AG3	EU716138	BURSA	/	
26	TR-2007-B-AG4	EU716139	BURSA	f	
27	TR-2007-B-AG5	EU716140	BURSA	f	
28	TR-2007-B-AG6	EU716141	BURSA	/	
29	TR-2007-B-AG7	EU716142	BURSA	a	
30	TR-2007-B-AG8	EU716143	BURSA	/	
31	TR-2007-B-AG9	EU716144	BURSA	b	
32	TR-2007-A-1500MS	EU716145	ANKARA	/	Abortions, stillbirths, AH syndrome,
33	TR-2007-A-8500MS	EU716146	ANKARA	/	diarrhoea, high mortality rate on calfs
34	TR-2007-A-2361MS-175475	EU716147	ANKARA	/	
35	TR-2007-A-2368MS	EU716148	ANKARA	/	
36	TR-2007-A-1493MS	EU716149	ANKARA	b	
37	TR-2007-Gu-175454-4695	EU716150	GÜMÜŞHANE	/	Abortions, diarrhoea
38	TR-2007-CP-270712	EU716151	ŞANLIURFA	d	r.d.m.
39	TR-2007-CP-271254	EU716152	ŞANLIURFA	d	
40	TR-2007-CP-271016	EU716153	ŞANLIURFA	d	

r.d.m., the isolate was acquired from routine diagnostic materials. Clinical symptom was not notified.

*Two isolates (AK171449 and AK171450) which achieved from a herd in Ankara belong to imported animals from Kırklareli.

**Three isolates were typed only for N^{pro}.

Fig. 1. The geographical origin of Turkish bovine virus diarrhoea virus 1 isolates characterized in this study.

RNA extraction and reverse transcription- polymerase chain reaction (RT-PCR)

RNA was extracted from field samples (serum or EDTA blood) or cell culture supernatants using the High Pure Viral RNA Kit (Roche, Mannheim, Germany) according to the manufacturer's recommendations. Complementary DNA (cDNA) synthesis was carried out using a first-strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania) as described in manufacturer's protocol. The 5′UTR amplifications from cDNA were performed by RT-PCR using the primer pairs 324 and 326 (Vilcek et al., 1994). A semi-nested PCR with primers BD1, BD2 and BD3 was used for the N^{pro} region as described by Vilcek et al. (2001).

DNA purification, sequencing, alignment and phylogeny

The primers generated amplicons of 288 bp for 5′UTR and of 428 bp for the N^{pro} gene. The PCR products were purified using the DNA Purification Kit (Roche) according to manufacturer's description. The nucleotide sequences were performed on Beckman Coulter CEQ 8000 (Beckman Coulter, Brea, CA, USA) and aligned by Bioedit with ClustalW algorithm (Hall, 1999). Phylogenetic and bootstrap analysis (Figs 2 and 3) was performed using the Molecular Evolutionary Genetics Analysis (MEGA) software program version 4.0 (Tamura et al., 2007).

Results

For molecular typing of Turkish pestiviruses from cattle, 5′ UTR and N^{pro} PCR products were used. Turkish BVDV isolate sequences were deposited in GenBank with the following accession numbers: EU716117–EU716153 for 5′ UTR and EU716157–EU716164 for N^{pro}.

All virus isolates were non-cytopathic on the cell culture passages.

The results of phylogenetic analysis (Figs 2 and 3) indicated that the predominant subgenotypes in Turkey are BVDV 1-*l* (18/40), 1-f (10/40), 1-b (7/40), 1-d (3/40) and 1-a (2/40). When pylogenetic results were evaluated geographically, BVDV 1-*l* was detected in cattle from Ankara for the years 2005 through 2007; BVDV 1-f (8/10) was frequently present in the Marmara region, and BVDV 1-b (6/7) dominated in the central Anatolian region. Of the subgenotypes that were less frequently detected, BVDV 1-d (3/3) was only detected in samples obtained from Şanlıurfa and BVDV 1-a (2/2) only appeared in cattle in the Marmara region.

Discussion

Turkey, a large (814.578 km^2) country situated between Asia and Europe that is bordered by Iran, Armenia and Georgia in the East, Iraq and Syria in the South, and Greece and Bulgaria in the West, has an animal husbandry industry with about 11 million cattle in seven major regions. In recent years, Turkey has imported animals from other countries in large numbers due to a decrease in the number of domestic cattle. Bovine virus diarrhoea virus strains likely to be seen in other countries are now being seen in Turkey due to the importation of animals. In support of this concept, we found that BVDV 1c (which is a local Australian

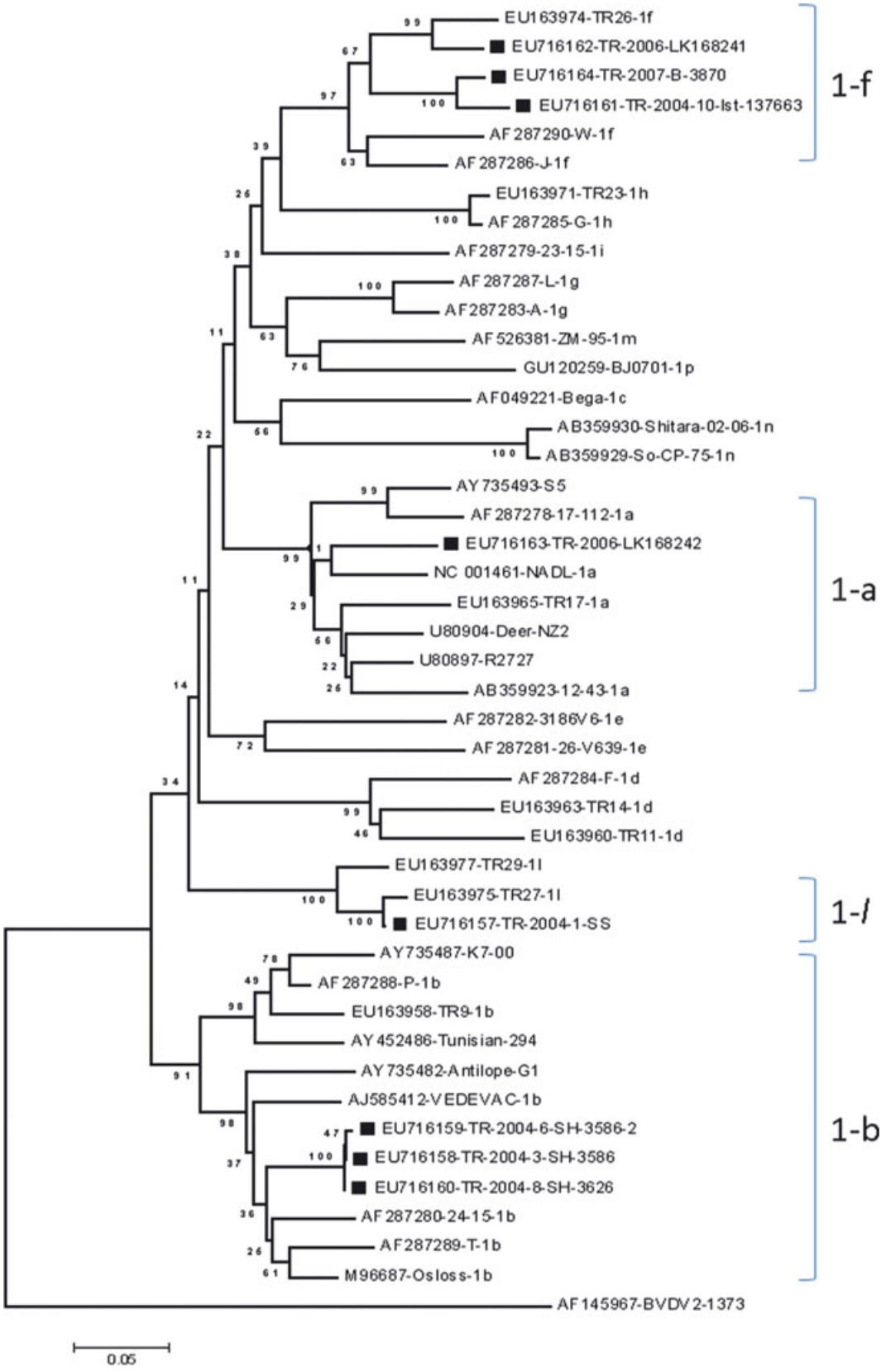

Fig. 2. The phylogenetic tree based on N^{pro} sequences.

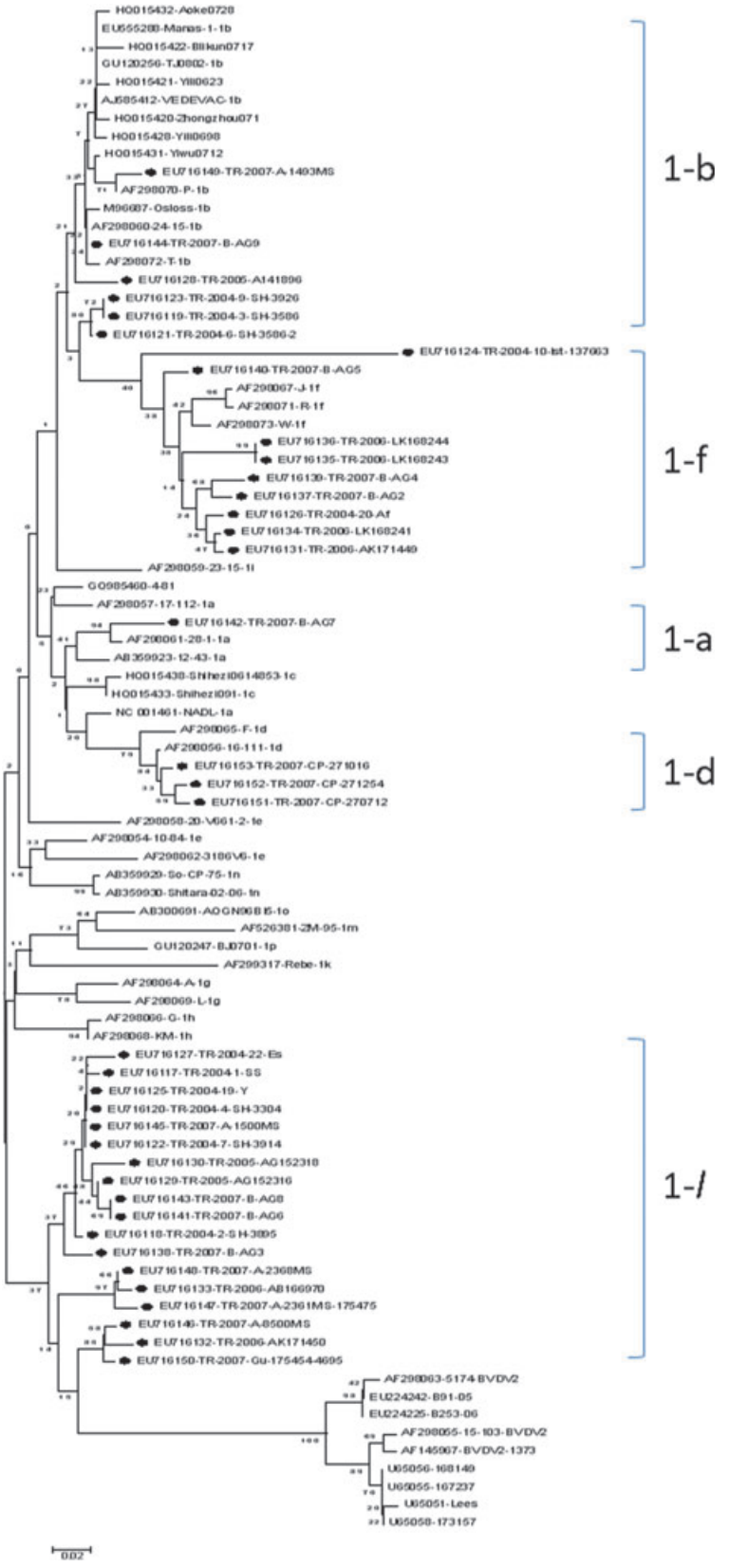

Fig. 3. Phylogenetic tree based on the 5′ untranslated region sequences.

strain) was detected from a herd that imported animals from Australia; this is one of several previously undetectable strains of BVDV that have now been reported in Turkey (Oguzoglu et al., 2010a). The subtypes usually remain limited to a geographical region in Turkey; however, the 1-f subtype (isolate name TR-2006-AK171449), which is seen with particular regularity in the Marmara region, was detected from a herd in the central Anatolian region, indicating that different genotypes can be transmitted between regions/countries by animal transport/trade. In addition, a remarkable association exists between clinical symptoms and BVDV 1 subtypes. In particular, BVDV 1-*l* subtypes from herds in the central Anatolian region were clinically related to persistent infection, congenital AH syndrome, diarrhoea and mucosal lesions in the mouths of infected animals (Table 1).

Use of mostly 5′UTR sequences was preferred for molecular characterization of pestiviruses, because it is easy to obtain by RT-PCR and provides greater possibility for comparison with other reference sequences available in GenBank. At present at least eleven genetic groups of BVDV 1 are recognized based on 5′UTR sequences, indicating the genetic diversity of BVDV. In this study, identification of BVDV 1 subtypes in Turkey was achieved using both 5′UTR ($n = 37$) and some selected N^{pro} ($n = 8$) sequences. The isolates from 5′UTR and N^{pro} sequence analysis were characterized and determined for the same subtype. With reference to sequences, we detected five subgroups of BVDV in Turkey (1-a, 1-b, 1-d, 1-f and 1-*l*). Bovine virus diarrhoea virus 1-*l* has been reported to date by Yesilbag et al. (2008) in Turkey, and by Jackova et al. (2008) in France. Also, Giammarioli et al. (2009) presented one isolate from Italy that was identified as BVDV 1-*l* after comparison in the phylogenetic tree with 71-03 and 71-15 from France (Jackova et al., 2008). However, Minami et al. (2011) reported that these BVDV 1-*l* subtypes which reported by Yesilbag et al. (2008) and by Jackova et al. (2008) were placed in different clusters. Turkish 1-*l* field pestiviruses identified in this study were clustered in the same branch as reported by Yesilbag et al. (2008) in the phylogenetic tree shown in Figs 2 and 3. These results show that Turkish BVDV 1-*l* subtype might be geographically restricted.

Bovine virus diarrhoea virus infection has a worldwide distribution and continues to have an adverse economic impact on the cattle industry. Prevention and control of this infection occurs by screening the herds, detecting persistent infection and removing cattle that are persistently infected from herds. In addition, female cattle should be vaccinated for foetal protection (Frey et al., 2002); this vaccination may not be necessary if large-scale herds do not have many persistently infected animals, as with the Scandinavian BVDV eradication model; Houe et al., 2006). In 2005, a programme was started in many European countries for the purpose of BVDV control, and it is known to date that several of these countries have accomplished the goals set out in BVDV eradication campaigns. Bovine virus diarrhoea virus circulation in Turkish cattle has been reported several times (Alkan et al., 1997, 2000; Oguzoglu et al., 2004, 2010b; Yesilbag et al., 2008), but there is no effective national eradication programme. However, a pilot project on the control and eradication of BVDV infection depends on the strategy of detecting persistently infected animals and removing them from herds, and then the use of the BVDV vaccine for the protection of herds where infection has been detected (Alkan et al., 2011). It is clear that, to improve the control and eradication programme in Turkey, the subgenotypes common in Turkey must be known, as must the role of persistently infected animals in transmission and the prevalence of infection. The introduction of new animals to herds with imports (and the probable introduction of new strains) due to international trade provides another risk factor.

Given the data that has been obtained in recent years in Turkey, one might say that the implementation of an eradication programme including vaccine use should have started at the national level in Turkey. The vaccines were based on different combinations of identified variations among BVDV isolates in the country. Most BVDV vaccines on the market in many countries, including in Turkey, contained BVDV 1-a and 1-b strains, some of them in combination with a BVDV 2 strain (Ridpath, 2005). Ideally, a pestivirus control programme for Turkey should use a vaccine that contains not only BVDV 1 strains but also BVDV2 (Oguzoglu et al., 2010b). For efficacious BVDV immunization and protection in Turkey, predominant BVDV 1 subgenotypes (1-*l* and 1-f) are clear vaccine candidates. The results of the present study identify a selection of vaccine strains for supporting a BVDV eradication programme in Turkey. We hope that the molecular studies will be expanded for the purpose of starting such an eradication programme.

Acknowledgements

This project was supported by TÜBİTAK-TOVAG-Carrier programme (No. 105O479). The accession numbers in this study are EU716117–EU716153 for 5′ UTR and EU716157–EU716164 for N^{pro}. T.Ç.Oğuzoğlu have a position in Turkish Academy of Science in the framework of young scientist award programme (TUBA-GEBİP-2010).

References

Alkan, F., A. Ozkul, T. Karaoglu, S. Bilge, Y. Akca, I. Burgu, K. Yesilbag, and T. C. Oguzoglu, 1997: Seroepidemiological studies on respiratory infections of cattle caused by viruses. *Ankara Univ. Vet. Fak. Derg.* 44, 73–80.

Alkan, F., A. Ozkul, S. Bilge-Dagalp, K. Yesilbag, T. C. Oguzoglu, Y. Akca, and I. Burgu, 2000: Virological and serological studies on the role of PI-3 virus, BRSV, BVDV and BHV-1 on respiratory infections of cattle. I. The detection of etiological agents by direct immunofluorescence technique. *Dtsch. tierärztl. Wschr.* Mai 107, 193–195.

Alkan, F., T. Ç. Oğuzoğlu, M. T. Karaoğlu, D. Muz, Z. Karapınar, M. Ö. Timurkan, A. Z. Akkutay, H. M. Turan, and I. Burgu, 2011: Investigation of BVDV infection in herds as first step of the control/eradication program. In: *Proceeding: 8th ESVV Pestivirus Symposium*, September 25–28, 2011, p. 116. Hannover, Germany.

Frey, H. R., K. Eicken, B. Grummer, S. Kenklies, T. C. Oguzoglu, and V. Moennig, 2002: Foetal protection against bovine virus diarrhoea virus after two-step vaccination. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 49, 489–493.

Fulton, R. W., J. F. Ridpath, S. Ore, A. W. Confer, J. T. Saliki, L. J. Burge, and M. E. Payton, 2005: Bovine viral diarrhoea virus (BVDV) subgenotypes in diagnostic laboratory accessions: distribution of BVDV1a, 1b, and 2a subgenotypes. *Vet. Microbiol.* 111, 35–40.

Giammarioli, M., E. Canelli, S. Ciulli, E. Rossi, and G. M. De Mia, 2009: Genetic Diversity of Bovine Viral Diarrhoea Virus Isolates From Italy. In: *ESVV 8th ICVV Proceedings*, p. 144. Budapest-Hungary.

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

Heinz, F. X., M. S. Collett, R. H. Purcell, E. A. Gould, C. R. Howard, M. Houghton, R. J. M. Moormann, C. M. Rice, and H.-J. Thiel, 2000: Family Flaviviridae in Virus Taxonomy. In: Van Regenmortel, M. H. V., C. M. Fauquet, D. H. L. Bishop, E. Carstens, M. K. Estes, S. Lemon, J. Maniloff, M. A. Mayo, D. McGeogh, C. R. Prinale and R. B. Wickner (eds), *Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 859–868. Academic Press, San Diego.

Hornberg, A., S. Revilla-Fernandez, C. Vogl, S. Vilcek, M. Matt, M. Fink, J. Koefer, and K. Schoepf, 2009: Genetic diversity of pestivirus isolates in cattle from Western Austria. *Vet. Microbiol.* 135, 205–213.

Houe, H., A. Lindberg, and V. Moennig, 2006: Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. *J. Vet. Diagn. Invest.* 18, 427–436.

Jackova, A., M. Novackova, C. Pelletier, C. Audeval, E. Gueneau, A. Haffar, E. Petit, L. Rehby, and S. Vilcek, 2008: The extended genetic diversity of BVDV-1: typing of BVDV-1 isolates from France. *Vet. Res. Commun.* 32, 7–11.

Mahony, T. J., F. M. McCarthy, J. L. Gravel, B. Corney, P. L. Young, and S. Vilcek, 2005: Genetic analysis of bovine viral diarrhoea viruses from Australia. *Vet. Microbiol.* 106, 1–6.

Minami, F., M. Nagai, M. Ito, T. Matsuda, H. Takai, Y. Jinkawa, T. Shimano, M. Hayashi, Y. Seki, Y. Sakoda, K. Sugiura, and H. Akashi, 2011: Reactivity and prevalence of neutralizing antibodies against Japanese strains of bovine viral diarrhea virus subgenotypes. *Comp. Immunol. Microbiol. Infect. Dis.* 34, 35–39.

Nagai, M., M. Hayashi, M. Itou, T. Fukutomi, H. Akashi, H. Kida, and Y. Sakoda, 2008: Identification of new genetic subtypes of bovine viral diarrhea virus genotype 1 isolated in Japan. *Virus Genes* 36, 135–139.

Oguzoglu, T. Ç., F. Alkan, and I. Burgu, 2004: Symposium über Wissenschaftliche Ergebnisse Deutsch – Türkischer universitätspartner schaften im Agrocr Bereich. In: Symposiums buch. *Detection of Bovine Virusdiarrhoea Virus (BVDV) Infection in a naturally infected dairy herd. V*. Middle European Buiatrics Congress, Hajdúszoboszló, Hungary, June 2 and 5, 2004.

Oguzoglu, T. C., D. Muz, Z. Karapinar, N. Toplu, M. Ö. Timurkan, E. Şişman, and İ. Burgu, 2010a: *Untersuchung Abhängig vom Bovine Virusdiarrhoea Virus (BVDV) der Kälberverluste aus einem Rinderzuchtbetrieb*. March 23–25, 2010, Antakya-Hatay.

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

Pellerin, C., J. van den Hurk, J. Lecomte, and P. Tussen, 1994: Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities. *Virology* 203, 260–268.

Ridpath, J. F., 2005: Practical significance of heterogeneity among BVDV strains: impact of biotype and genotype on U.S. control programs. *Prev. Vet. Med.* 72, 17–30.

Ridpath, J. F., S. R. Bolin, and E. J. Dubovi, 1994: Segregation of bovine viral diarrhea virus into genotypes. *Virology* 205, 66–74.

Robesova, B., K. Kovarcik, and S. Vilcek, 2009: Genotyping of bovine viral diarrhoea virus isolates from the Czech Republic. *Vet. Med.* 54, 393–398.

Tamura, K., J. Dudley, M. Nei, and S. Kumar, 2007: MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24, 1596–1599 (publication PDF at http://www.kumarlab.net/publications).

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

Vilcek, S., D. J. Paton, B. Durkovic, L. Strojny, G. Ibata, A. Moussa, A. Loitsch, W. Rossmanith, S. Vega, M. T. Scicluna, and V. Palfi, 2001: Bovine viral diarrhoea virus genotype 1 can be separated into at least 11 genetic groups. *Arch. Virol.* 146, 99–115.

Xue, F., Y.-M. Zhu, J. Li, L.-C. Zhu, X.-G. Ren, J.-K. Feng, H.-F. Shi, and Y.-R. Gao, 2010: Genotyping of bovine viral diarrhea viruses from cattle in China between 2005 and 2008. *Vet. Microbiol.* 143, 379–383.

Yamamoto, T., T. Kozasa, H. Aoki, H. Sekiguchi, S. Morino, and S. Nakamura, 2008: Genomic analyses of bovine viral diarrhea viruses isolated from cattle imported into Japan between 1991 and 2005. *Vet. Microbiol.* 127, 386–391.

Yesilbag, K., F. Christine, B. W. Barbara, Z. Yildirim, F. Alkan, A. Ozkul, I. Burgu, S. Cedillo-Rosales, H.-J. Thiel, and M. Köning, 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.

Zhong, F., N. Li, X. Huang, Y. Guo, H. Chen, X. Wang, C. Shi, and X. Zhang, 2011: Genetic typing and epidemiologic observation of bovine viral diarrhea virus in Western China. *Virus Genes* 42, 204–207.