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EPITOPIC CHARACTERISATION OF TURKISH BOVINE VIRAL DIARRHOEA VIRUS (BVDV) ISOLATES USING MONOCLONAL ANTIBODIES

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In this study, 15 bovine viral diarrhoea viruses (BVDV) isolated from the field in Turkey were characterised for their biotype, cloned and eventually analysed for their epitopic composition in terms of glycoprotein E2. Immunoplaque assay, plaque assay, limiting dilution and streptavidin-biotin-peroxidase techniques were used for biotype characterisation, cloning of cytopathic (cp) and noncytopathic (ncp) biotypes and epitope analysis, respectively. While 14 out of 15 BVDV isolates were distinguished as ncp biotype, 1 isolate was found to be containing both biotypes (cp + ncp). According to the reactivity patterns of isolates with 15 monoclonal antibodies, 4 different antigenic groups could be formed. There were no antigenic differences between the isolates derived from the same animal with various time intervals. On the other hand, biotype clones isolated from the same animal exhibited difference in one epitope. This is the first study describing antigenic characterisation of BVDV field isolates in Turkey.

Key words: BVDV, cloning, antigenic characterisation, monoclonal antibodies

Bovine Viral Diarrhoea Virus (BVDV), a common pathogen of cattle around the world, is an enveloped positive-stranded RNA virus and is classified in the *Pestivirus* genus of the family *Flaviviridae.* Two biotypes of the virus, cytopathic (cp) and noncytopathic (ncp), are distinguished according to their effects on cultured cells, and two genotypes have been defined by sequence analyses of 5'UTR region of the viral genome (Pellerin et al., 1994). The BVDV genome is about 12.5 kb in length and induces many polypeptides in infected cells (Donis and Dubovi, 1987). Among the structural proteins, glycoprotein E2 (gp53) has an important role in virus neutralisation. Unfortunately, mutation-originated changes in this protein occur at a high rate. This leads to antigenic variation among BVD viruses.

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Monoclonal antibodies (mAbs) show specificity to a single epitope and are thus very useful tools for the detection of antigenic diversity in viruses. During the last decades many investigators in Europe, the USA and Japan have produced mAbs to determine antigenic characteristics of BVDV isolates and laboratory strains (Peters et al., 1986; Cay et al., 1989; Corapi et al., 1990; Nishimori et al., 1996).

Clinical manifestations caused by BVDV can cover respiratory, digestive and genital disorders. Severity of the disease may range from mild to fatal (Baker, 1987). In pregnant cows, fetal infections by ncp biotype may result in the birth of immunotolerant persistently infected (IPI) calves which shed live virus throughout their life and are the main source of infection in the population (Brownlie, 1991). Infection is generally asymptomatic in IPI animals, though some IPI cattle will develop a lethal illness, mucosal disease, after superinfection with a cytopathic BVDV strain antigenically homologous or semi-homologous to the persistent ncp strain. It was hypothesised that the presence of IPI animals could lead to the existence of herd-specific strains in cattle herds (Paton et al., 1995; Hamers et al., 1998).

The aim of this study was to characterise Turkish BVDV field isolates. Antigenic changes of BVDV in infected animals and the relationship between BVDV isolates from various herds were also studied.

Materials and methods

Viruses and cell line

The list of 15 BVDV isolates and some clones used is given in Table 1. Of them, 14 strains were isolated from leukocyte samples of 7 IPI cattle in two sampling periods. There were 85- to 140-day intervals between initial and sequential isolates from IPI animals used in the study. IPI cattle had been identified by two consecutive virus isolations three weeks apart. Another strain was isolated from necropsy materials of an animal which died of mucosal disease. Isolates originated from 5 different herds (abbreviated as DLM, KRK, ALT, BL and CKR) located in different parts of Turkey. NADL strain of BVDV originated from the Virology Institute of Hannover Veterinary School, Germany.

Madin-Derby Bovine Kidney (MDBK) cells, previously proved to be free from BVDV, were used in tests in Dulbecco's MEM (Seromed, Germany) by addition of 10% fetal calf serum (FCS) (Seromed, Germany). FCS used in the study was also examined to be negative for BVDV-specific antigen and antibody before use.

Monoclonal antibodies

A total of 15 mAbs were kindly provided by the Virology Institute of Hannover Veterinary High School, Germany. The mAbs included in this research are widely being used by different investigators for antigenic characterisation of BVDV isolates (Table 2).

Table 1

mAb reaction patterns of BVD viruses and their distribution to the groups

No.	Isolate*	Biotype	Binding with mAb															Group
			C 16	CT 2	CT 3	CT 6	CT 9	CA 1	CA 3	CA 25	CA 34	CA 36	CA 39	CA 73	CA 78	CA 80	CA 82	
1	DLM.17-96a	ncp	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	I
2	DLM.17-96b	ncp	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	
3	DLM.3-96a	ncp	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	
4	DLM.3-96b	ncp	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	
5	DLM.319-95a	ncp	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	II
6	DLM.319-95b	ncp	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	
7	DLM.266-96a	ncp	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	
8	DLM.266-96b	ncp	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	
9	KRK.60-95a	ncp	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	
10	KRK.60-95b	ncp	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	
11	ALT.151-94a	ncp	+	–	+	+	–	+	+	–	+	–	+	+	–	+	+	III
12	ALT.151-94b	ncp	+	–	+	+	–	+	+	–	+	–	+	+	–	+	+	
13	BL.30-96a	ncp	+	–	+	+	–	+	–	–	+	–	–	–	–	+	+	IV
14	BL.30-96b	ncp	+	–	+	+	–	+	–	–	+	–	–	–	–	+	+	
15	CKR.111-96	cp/ncp	+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	II
16	cp clone		+	–	+	+	+	+	+	–	+	–	+	+	–	+	+	
17	ncp clone		+	–	+	+	–	+	+	–	+	–	+	+	–	+	+	III
18	NADL	cp	+	–	+	+	–	+	+	–	+	–	+	+	–	+	+	

*First letters indicate farm code, numbers indicate animal ID and the last letter shows initial (a) or sequential (b) sampling

Table 2

Monoclonal antibodies used

Monoclonal antibody	Homologue strain	Protein specificity	Reference
C16	NADL	NS23	Peters et al. (1986)
CT2, CT3, CT6, CT9	A1138/69	E2	Körke (1989)
CA1, CA3	NADL	E2	Bolin et al. (1988)
CA25, CA34, CA36, CA39	7443	E2	Mateo-Rosell (1988)
CA73, CA78, CA80, CA82	Singer	E2	

Biotype characterisation

Detection of biotypes of isolates was done using immunoplaque assay as previously described elsewhere (Liess et al., 1993).

Purification of mixed biotypes

Plaque assay was performed as described by Sanders (1991) for purification of cp biotype from the mix. After incubation for 5 days, cells were stained with neutral red (1/10,000 w/v) to visualise the cp virus growth areas, which remained unstained. Individual plaques were sampled by punching the agar overlaying infected areas and they were used for inoculating the cells for the next step of purification. This was repeated 5 times to ensure the purity of viruses to be worked on.

Purification of the ncp biotype was carried out by using limiting dilution technique as practised by Grieser-Wilke et al. (1991). The final purity of purified biotypes was checked using immunoplaque assay at the last step.

Epitopic characterisation

For this purpose, 100 $TCID_{50}$ dilutions of isolates as well as cp and ncp biotype clones purified from isolate no. 15 were placed into microtitre plate wells in 0.1 ml volume and mixed with MDBK cells to obtain 15,000 cells per well. Plates were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h and heat-fixed before 1-h incubation with mAbs. Binding of mAbs was determined by using biotinylated anti-mouse IG and streptavidin-biotinylated peroxidase complex (Amersham, Germany). The substrate solution included 2 mg of 3-amino-9-ethyl-carbazole (Sigma, Germany) dissolved in 0.3 ml dimethyl-formamide (Merck, Germany), diluted to 5 ml by adding 0.05 M acetate buffer, pH 5, and 0.03% H_2O_2 as a final concentration. The results were evaluated with a light microscope.

Similarity of groups and statistical analysis

Reactivity percentages of individual groups with mAbs specific to glycoprotein E2 were accepted as a measure of antigenic similarity among those groups. Student's *t*-tests were performed to affirm significance of antigenic differences and to discuss herd specificity.

Results

Biotype characterisation

Out of 15, one isolate recovered from a mucosal disease suspected animal was found to be a mixture of both biotypes (cp and ncp) of BVDV, while 14 isolates were comprised of only the ncp biotype (Table 1).

Epitopic characterisation

Viruses used in the study showed various degrees of reactivity in binding to mAbs (Table 1). Some of the viruses (DLM 17–96a, 17–96b, 3–96a and 3–96b) gave narrow mAb binding spectrum whereas lots of them exhibited a broad spectrum. According to the patterns of reactivity with the mAbs, the following four groups of viruses were generated. Group I: includes isolates to which just mAbs C16, CA34 and CA82 were bound (narrow mAb spectrum). Group II: includes isolates and cp biotype clone, which reacted with all mAbs except CT2, CA25, CA36 and CA78 (broadest mAb spectrum). Group III: includes isolates and ncp biotype clone. These viruses showed no reaction with mAb CT9 and the mAbs which did not react with viruses in Group II. NADL also belonged to this group. Group IV: includes isolates which gave no reaction with mAbs CA3, CA39, CA73 and the mAbs which did not react with viruses in Group III.

On the other hand, some of the mAbs (C16, CA34 and CA82) reacted with all of the viruses tested; nevertheless, a few mAbs (CT2, CA25, CA36 and CA78) showed specificity to none of them.

Similarity of groups and statistical analysis

In terms of the mAbs specific to glycoprotein E2, the closest antigenic relation was detected between Group II (71%, 10/14) and Group III (64%, 9/14) using the binding percentages of mAbs to the isolates. Viruses of Group I (14%, 2/14) showed less antigenic similarities as compared to Groups II and III (Fig. 1).

Student's *t*-tests showed that Group I was significantly different from the others ($P < 0.01$ for Groups II and III, and $P = 0.043$ for Group IV), but there were no significant differences between Groups II and III, as well as between Groups III and IV.

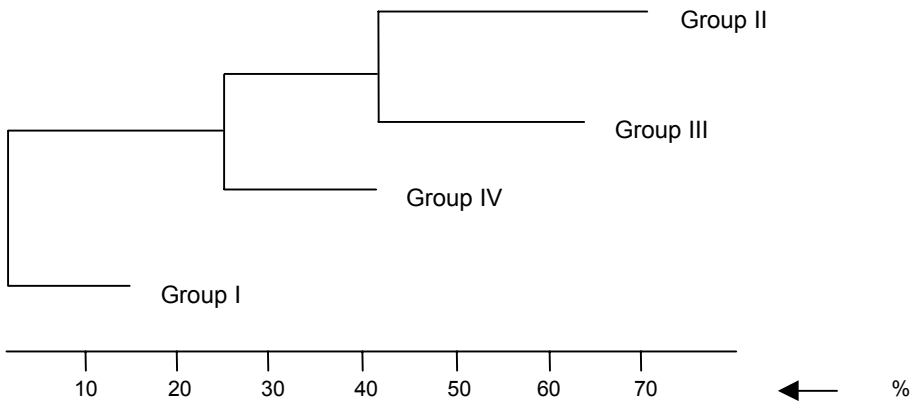

Fig. 1. Reactivity percentages of groups with mAbs specific to glycoprotein E2

Discussion

Due to mutations, antigenic variation among BVDV isolates is common. These changes are more important if they affect epitopes on glycoprotein E2, which is the largest envelope glycoprotein containing neutralising epitopes (Donis et al., 1988). Unfortunately lots of mutations affect that part of the genome (Donis, 1995).

In this study, all of the isolates and biotype clones gave a binding reaction with mAb C16 known as a pan-pestivirus specific mAb (Peters et al., 1986). The data taken from mAb CA34 are similar to the results of Pituco (1995), but the reactivity pattern of mAb CA82 was a little surprising, because this mAb is known to bind lots but not all of the BVDV field isolates (Pituco, 1995; Mateo-Rosell, 1988).

According to reactivity patterns, isolates could be assigned into four antigenic groups. There were definite diversities between some of them (Groups I and II). Viruses assigned to Groups II and III differed in a single epitope. Distribution of viruses into the groups and mAb binding percentages in each group indicated that the BVDV isolates used in the study had a high rate of antigenic diversity.

Several investigators (Paton et al., 1995; Hamers et al., 1998) reported that IPI animals lead to the occurrence of herd-specific strains of BVDV. Although there were antigenically diverse isolates from the farms KRK, ALT and BL, no significant differences could be shown. Meanwhile, in Group II all isolates had the same antigenic pattern despite the fact that they originated from different herds. Further studies with additional isolates need to be performed to determine the stability of strains in these herds. As reported earlier (Paton, 1995), the existence of BVDV herd-specific strains could be related to the immune elimination of newly emerged variants.

On the other hand, in the herd DLM there are two antigenically distinct field strains circulating in the same herd. The farms studied are closely managed state farms in which vaccination against BVDV or the purchase of new animals are not allowed. At this point, the only possible speculation is virus transmission from farm KRK by semen, since this farm has a 'frozen sperm production centre' which provides service to other farms as a sperm bank.

Mucosal disease may occur via superinfection of IPI animals with a cp strain of BVDV which is antigenically homologous (Howard et al., 1987; Shimizu et al., 1989) or semi-homologous (Fritzemeier et al., 1997) to the persisting ncp strain. Four mAbs (C12, C42, CA82 and CT9) are very important to point out antigenic differences between biotype couples. In this study, variation between purified cp and ncp biotype couples was detected in an epitope specific to mAb CT9. This epitope can be originally absent or lost during the purification procedure (Reinecke, 1993).

The mAb binding results of consecutive isolates from IPI cattle showed the same patterns. No antigenic changes were shown in strains obtained from IPI animals in a 140-day interval.

This study is a preliminary description of the antigenic characteristics of Turkish BVDV field isolates. Results of the study are believed to help in opening a new way to characterise strains and to trace their origin. Examination of more isolates will provide understanding of the spread of BVDV in cattle herds in Turkey.

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