CHARACTERIZATION OF A BOVINE VIRAL DIARRHEA VIRUS ISOLATED FROM ROE DEER IN GERMANY

Siglinda Fischer,1 Emilie Weiland,2 and Kai Frölich1
1 Institute for Zoo Biology and Wildlife Research Berlin, IZW, Alfred-Kowalke-Strasse 17, P.O. Box 601103, 10252 Berlin, Germany.
2 Bundesforschungsanstalt für Viruskrankheiten der Tiere, Paul-Ehrlich-Strasse 28, 72076 Tübingen, Germany.

ABSTRACT: The 5' untranslated region (5' UTR) of cytopathogenic pestiviruses isolated from two seronegative roe deer (Capreolus capreolus) in northern Germany was partially sequenced and compared with those of 28 other pestiviruses. Due to the occurrence within a narrow location and the complete identity of the sequenced fragments from both roe deer isolates (SH9 and SH11) they seem to belong to the same bovine virus diarrhea virus (BVDV) strain called SH9/11. This strain is highly homologous (up to 93% identity) to “classical” BVDV strains. However, SH9/11 has characteristic variations in its 5' UTR distinct from all other pestiviruses analyzed in this study. Strain SH9/11 is more similar to BVDV group I than to group II, although it is clearly separated from all other cattle isolates. In monoclonal antibody (mAb)-typing studies, isolate SH9 reacted with one pestivirus-specific mAb (C16), with two BVDV specific mAbs (N2B12 and D5), and with one mAb (48) raised against the E2 protein of classical swine fever virus out of a panel of 13 mAbs. The separate position of strain SH9/11 again was demonstrated by the unique reaction pattern of isolate SH9 when compared with other mAb 48-positive BVDV and BDV strains. All these results indicate that distinct BVDV strains might exist among free-ranging roe deer in Germany.

Key words: Bovine virus diarrhea virus, Capreolus capreolus, mAb-typing, roe deer, reverse transcription polymerase chain reaction, 5' untranslated region, virus characterization.

INTRODUCTION

According to present systematics, the bovine virus diarrhea virus (BVDV) belongs to the genus Pestivirus within the Family Flaviviridae (Horzinek, 1990). Recently, viruses classified by genetic analysis have been placed into the BVDV group, although they have been isolated from sheep. In another case, a pestivirus was grouped with border disease virus (BDV) despite its isolation from swine (Hofmann et al., 1994; Vilcek et al., 1994). Common hosts of pestiviruses are livestock such as cattle (BVDV), sheep (BDV), and pigs (classical swine fever virus; CSFV) (Thiel et al., 1996). Moreover, a variety of wild species seems to be susceptible to pestiviruses (Nettleton, 1990). The diseases caused by pestiviruses vary not only among the pestivirus species but also among different pestivirus strains (Pellerin et al., 1994).

The identification of a large number of pestivirus nucleic acid sequences indicates a wide variety of BVDV and BVDV-like viruses (Pellerin et al., 1994; Ridpath et al., 1994). Some researchers suggest that a new classification of pestiviruses, especially of BVDV, is required (Pellerin et al., 1994; Becher et al., 1995). According to Pellerin et al. (1994), the BVDV group consists of two distinct subgroups, group I (divided into Ia and Ib) and group II. Group Ib contains most of the “classical” BVDV strains, like NADL and Singer. While discrimination of BVDV strains into group I and II can be done both by comparison of nucleic acid sequences and antibody crossreactions, differentiation between group Ia and Ib is possible by analysis of 5' UTRs only. Recently, monoclonal antibody (mAb)-typing has been developed as a useful tool to characterize pestivirus isolates based on the knowledge of both conserved and non-conserved antigenic domains within the viral proteins E0, E2 or p125/80 (Corapi et al., 1990; Kosmidou et al., 1995).

In cervids, BVDVs have been isolated from roe deer (Romvár, 1965; Schellner, 1977), fallow deer (Cervus dama; Neumann et al., 1980), and red deer (Cervus elaphus; Nettleton et al., 1980). Frölich and Hofmann (1995) isolated cytopatho-
genic pestiviruses (isolates SH9 and SH11) from two most likely non-related seronegative roe deer from northern Germany. It is still unclear whether some deer populations might have their own BVDV strains or whether they are infected with cattle strains. Therefore, the objective of this paper was to further characterize these two roe deer isolates.

MATERIALS AND METHODS

Isolates SH9 and SH11 were derived from an adult female roe deer (54°35' N, 9°22' E; isolate SH9) and from a young buck (54°35' N, 9°30' E; isolate SH11) from northern Germany (Schleswig-Holstein). The animals were collected on 6 December 1990 (female) and 26 July 1991 (buck) within a distance of approximately 15 km. One hundred thirteen spleen samples from roe deer were examined for BVDV and two BVDV-positive samples were detected (Frölich and Hofmann, 1995). Both isolates have been cultivated only on BVDV-free bovine embryonic lung cells (BEL) kindly supplied by G. Wizigmann (Tierzusendheitsdienst Bayern, Grub, Germany), where they showed cytopathogenic effects. Supernatant (2 μl) from cultured BEL cells experimentally infected with isolates SH9 and SH11 were used in reverse transcription-polymerase chain reaction (RT-PCR) assays using the RNA PCR kit (Perkin-Elmer, Überlingen, Germany) according to the instructions for 20 μl reactions. Reverse transcription was performed with 0.75 μM reverse primer PEST2 (5'-TCAACTCCA-TGTCCCATGTA-3', nt 375-395, of the NADL sequence; Collett et al., 1988) and with 2.5 U/μl Moloney murine leukemia virus (MMLV) reverse transcriptase at 37°C for at least 1 hr.

Another method was the synthesis of random-hexamer primed cDNA. A mixture consisting of 4 μl of extracted RNA, 1 μl (0.02 A_{260} units) of random hexamers (Pharmacia, Uppsala, Sweden) and 5 μl of water was de-natured at 65°C for 5 min and immediately chilled on ice for 5 min. The final reaction mixture of 25 μl contained the following components: 1 μl (24 U) of RNase inhibitor (RNAguard, Pharmacia), 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 200 μM of each dNTP (Pharmacia), and 8 U/μl MMLV reverse transcriptase (Gibco BRL, Life Technologies Inc., Paisley, Scotland, UK). The mixture was incubated at 37°C for 90 min. The enzyme was inactivated at 95°C for 5 min.

Subsequently, 5 μl of this cDNA were used for PCR amplification of a 156 bp fragment in a 50 μl mixture consisting of 20 mM Tris-HCl, pH 8.3, 60 mM KCl, 2 mM MgCl₂, 200 μM of each dNTP, 2.5 U Goldstar Taq Polymerase (Eurogentec, Seraing, Belgium), and 200 nM of each primer: BVD3 (5'-CTGGAGCA-GGGCATGCACCA-3', nt 237-255, of the NADL sequence; Collett et al., 1988) and PEST2. Cycling conditions (30-36 repeats) were as follows: denaturation at 92°C for 1 min, primer annealing at 59-61°C for 30 sec, elongation at 72°C for 1 min and final elongation at 72°C for 3 min.

A 288 bp fragment was amplified from isolate SH9 by RT-PCR using primer 324 (nt 108-128, of the CSFV-Alfort sequence; Meyers et al., 1989) and primer 326 (identical with PEST2 (see above); Vilcek et al., 1994). The 50 μl reaction mixture consisted of 3-4 μl cDNA, 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1 mg/ml bovine serum albumine, 2.5 mM MgCl₂, 200 μM of each dNTP (Pharmacia), 30 mM of each primer and 0.02 U/μl Taq DNA polymerase (Perkin Elmer), overlaid with 2 drops of mineral oil (Sigma, St. Louis, Missouri, USA). Amplification was carried out using the following thermal profile: 94°C for 1 min, 56°C for 1 min, 72°C for 1 min. After 35 cycles, the last extension step was prolonged to 7 min.

PCR products were separated by electrophoresis in a 2% agarose gel in 0.5× TAE (50× TAE: 2 M Tris, 1 M acetic acid, 0.1 M EDTA, pH 8.3) and in 0.5× TBE (20× TBE: 1 M Tris, 1 M borate acid, 20 mM EDTA), respectively. The products were visualized with ethidium bromide, cut from the gel and purified using the QIAex gel extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing (Sanger et al., 1977) of the purified PCR products was carried out using primers PEST2 and BVD3 and a dye terminator cycle sequencing ready reaction (Applied Biosystems Inc., Weiterstadt, Germany) with an ABI 373A sequencer and with P-33(α)dTTP-labelled primers 324 and 326 (=PEST2) and the final cycle sequencing kit (Promega), respectively. Alignment of the sequences was performed visually. Suitability of the 241 bp fragment chosen for phylogenetic analysis (nt 130-370 of the NADL sequence; Collett et al., 1988) and the number of strains for phylogenetic investigations was estimated according to Hillis and Huelsenbeck (1992) by statistical analysis of the length of 1,000 arbitrarily constructed cladograms using the computer program PAUP (version 3.1.1; Swofford, 1993). Phylogenetic analysis was performed with the Fitch-Margoliash method (Fitch and Margoliash, 1967) of the Phylogeny Interference Package (PHYLIP 3.5c; Felsenstein, 1985; J. Felsenstein, Seattle, Washington, USA) using a distance matrix calculated by the K2 method of Kimura (1980), and with the
maximum parsimony method of PAUP. The CSFV strain Brescia was used as outgroup. Stability of tree topology was tested by bootstrapping of 100 trees each (Felsenstein, 1985). Consensus trees were constructed by majority-rule and by strict consensus tree program version 3.572c with PHYLP.

Table 1 lists the monoclonal antibodies (mAbs) used to characterize the roe deer isolate SH9. Twenty-three monoclonal antibodies directed against CSFV proteins were described previously (Kosmidou, 1995; Kosmidou et al., 1995). The mAb C16 was raised against p125/p80 of BVDV-NADL and reacted with all pestiviruses tested so far (Moennig et al., 1987). The monoclonal antibody N2B12, directed against E2 of BVDV, was obtained from A. D. Shannon, Elizabeth McArthur Agricultural Institute (EMAI, Menangle, New South Wales, Australia) and recognized all BVDV isolates tested hitherto (Shannon et al., 1991).

Immunoperoxidase assays were performed in 96-well plates according to recently reported studies (Weiland et al., 1989, Kosmidou, 1995, Kosmidou et al., 1995). Three different dilutions of viruses were mixed with cells of a MDBK clone established in our laboratory, with the aim to obtain isolated antigenic areas surrounded by non-infected cells in a way so that antigenic reactions could easily be detected microscopically. Infected cells were fixed with 96% ethanol for 20 min at 4°C two days after infection. After incubation with mAbs (diluted 1:10) for 60 min at room temperature, cells were washed with phosphate buffered saline and incubated with anti-mouse peroxidase conjugate (Biorad, München, Germany) for 45 min, washed again and stained with aminoethyl carbazole (20 μg/ml) and H2O2 in sodium acetate buffer (0.05 M, pH 5.0) for 30 min. Excess substrate was removed and 100 μl per well of water was added before the plates were read microscopically. Assays were considered positive, when typical reddish-brown staining of the whole monolayer (after infection with a high virus concentration) or of isolated areas (after infection with a low virus concentration) was observed. In the same manner using identical monoclonal antibodies, 9 isolates of bovine or sheep origin were tested in a study to determine pestivirus diversity.

RESULTS

The amplified 5′ UTR fragments were 156 bp (both isolates SH9 and SH11) and 288 bp (isolate SH9 only) in length. The shorter (156 bp) sequences obtained from both isolates SH9 and SH11 were identical in accordance to findings of Hofmann et al. (1994), who investigated a 120 bp fragment of the 5′ UTR of both isolates. Therefore, both isolates were regarded as belonging to the same pestivirus strain named SH9/11 henceforth. The 120 bp 5′ UTR sequence analyzed by Hofmann et al. (1994) was elongated upstream for another 127 nucleotides, so that in total 245 bp of the 5′ UTR were sequenced (Fig. 1). A cytosin at position 180 (nt 309 of the NADL sequence) was identified in every sequence tested, whereas Hofmann et al. (1994) found a gap at that position after aligning the sequences. At position 131, the SH9/11 5′ UTR has a thymidin, whereas all other pestivirus sequences have a cytosin (Fig. 1).

Further alignment of 241 bp from the 5′ UTR (Fig. 1) with corresponding sequences of one BDV, 25 BVDV and two CSFV strains (Table 2) shows that the roe deer isolates are more similar to BVDV strains described by Pellerin et al. (1994) as group Ia and Ib pestiviruses, whereas their homology to group II pestiviruses is much lower. Homologies to BDV and CSFV 5′ UTRs were even lower (Table 2). Moreover, the 5′ UTR of SH9/11 differed from those of all other BVDV strains at position 7. At positions 3, 78, 88, 186, 188 and 190, SH9/11 differed from all other group I BVDV strains. The nucleotide sequences of the three variable region loci (V1, V2 and V3, Fig. 1) of the 5′ UTR from the roe deer isolates were compared with those of viruses belonging to BVDV subgroups Ia, Ib and group II (Harasawa, 1996). Loci V1 and V2 of SH9/11 were most similar to those of BVDV Ib strains and V3 was almost identical to that of BVDV Ia strains (data not shown).

The suitability of the 241 bp fragment (nt 130–370 of the NADL sequence; Collett et al., 1988) and the number of strains evaluated for the phylogenetic investigations were assessed by statistical analysis of the length of 1,000 arbitrarily constructed cladograms (PAUP 3.1.1) and revealed a g1 value of −0.47, indicating that even this
short sequence fragment contains enough information for reliable construction of a cladogram.

Both methods of phylogenetic analysis of 5' UTR sequences of all pestivirus strains resulted in subgrouping of BVDV strains into three clusters according to the groups Ia, Ib and II (Pellerin et al., 1994; Fig. 2). SH9/11 is related closer to subgroups Ia and Ib than to group II or other Pestivirus sp. Bootstrap values for points of divergence among the documented Pestivirus sp., within the BVDV, and between the BVDV subgroups themselves ranged from 72.9 to 100. Within the subgroups, bootstrap values were lower (between 42.5 and 100) (Fig. 2).

In mAb-typing studies, the roe deer isolate SH9 was recognized by mAbs N2B12, C16, f48 and D5 (Table 1). Except for mAb f48, none of the mAbs raised against CSFV reacted with this pestivirus. Thus, none of the anti CSFV-E2-mAbs with a broader range of specificity, which also recognized several BVDV and BDV strains (mAbs 4b5, 4a11, 4c6 and 24/23), reacted with SH9. Of the four mAbs raised against the NADL-Tübingen strain, only mAb D5 recognized BVDV isolate SH9.

**DISCUSSION**

The number of pestiviruses identified by molecular techniques is steadily increasing (Becher et al., 1995). Some of these strains have been isolated from captive wild ruminants (Doyle and Heuschele, 1983). A cytopathogenic BVDV has been isolated from a giraffe (Giraffa camelopardalis) in Kenya by Plowright in 1969 (Hamblin and Hedger, 1979). The isolates investigated here are the first known cases of cytopathogenic pestiviruses isolated from free-ranging roe deer. From both animals only one virus type has been isolated. Due to the fact that both isolates are identical as shown by sequence analysis of their 5' UTR sequence and because of the close geographic proximity (15 km) in which both animals have been found, these roe deer might have been involved in the same BVDV epidemic. This suggests a reisolation of the same virus. If the pathogenesis in deer is similar to cattle, the two isolates from roe deer probably were taken from...
individuals which were in the early stage of mucosal disease or had an acute transient form of BVD (Frölich and Hofmann, 1995). However, it is not known whether persistently infected carriers are responsible for the maintenance of BVDV in free-ranging deer populations (Van Campen and Williams, 1996). Thus, the role of

FIGURE 2. Phylogenetic tree of pestvirus strains based on the method of Maximum Parsimony. Bootstrap values (100 repeats) are given.
Table 1. List of monoclonal antibodies (mAb) used in this study and in mAb-typing results.

<table>
<thead>
<tr>
<th>mAb</th>
<th>Raised against</th>
<th>Strain</th>
<th>Protein specificity</th>
<th>Reaction with SH9</th>
<th>Crossreactivity with</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16</td>
<td>BVDV</td>
<td>NADL</td>
<td>pl25/80</td>
<td>yes</td>
<td>13 BVDV, 4 CSFV</td>
<td>Moennig et al. (1987)</td>
</tr>
<tr>
<td>N2B12</td>
<td>BVDV</td>
<td>5 different isolates from Australia</td>
<td>E2</td>
<td>yes</td>
<td>BVDV</td>
<td>Shannon et al. (1991)</td>
</tr>
<tr>
<td>D5</td>
<td>BVDV</td>
<td>NADL-Tübingen</td>
<td>E2</td>
<td>yes</td>
<td>6 BVDV</td>
<td>Weiland et al. (1989)</td>
</tr>
<tr>
<td>2b10</td>
<td>BVDV</td>
<td>NADL-Tübingen</td>
<td>E2</td>
<td>no</td>
<td>4 BVDV</td>
<td>Weiland et al. (1989)</td>
</tr>
<tr>
<td>3b2</td>
<td>BVDV</td>
<td>NADL-Tübingen</td>
<td>E2</td>
<td>no</td>
<td>5 BVDV</td>
<td>Weiland et al. (1989)</td>
</tr>
<tr>
<td>lb8</td>
<td>BVDV</td>
<td>NADL-Tübingen</td>
<td>E2</td>
<td>no</td>
<td>6 BVDV</td>
<td>Weiland et al. (1989)</td>
</tr>
<tr>
<td>4a11</td>
<td>CSFV</td>
<td>Alfort-Tübingen</td>
<td>E2</td>
<td>no</td>
<td>1 BVD, 6 BVDV, 18 CSFV</td>
<td>Kosmidou et al. (1995)</td>
</tr>
<tr>
<td>f48</td>
<td>CSFV</td>
<td>Alfort-Tübingen</td>
<td>E2</td>
<td>yes</td>
<td>1 BVD, 6 BVDV, 19 CSFV</td>
<td>Kosmidou et al. (1995)</td>
</tr>
<tr>
<td>4b5</td>
<td>CSFV</td>
<td>Alfort-Tübingen</td>
<td>E2</td>
<td>no</td>
<td>2 BVD, 4 BVDV, 19 CSFV</td>
<td>Kosmidou et al. (1995)</td>
</tr>
<tr>
<td>24/23</td>
<td>CSFV</td>
<td>Alfort-Tübingen</td>
<td>E2</td>
<td>no</td>
<td>1 BVD, 21 CSFV</td>
<td>Kosmidou et al. (1995)</td>
</tr>
<tr>
<td>4c6</td>
<td>CSFV</td>
<td>Alfort-Tübingen</td>
<td>E2</td>
<td>no</td>
<td>1 BVD, 4 BVDV, 19 CSFV</td>
<td>Kosmidou et al. (1995)</td>
</tr>
<tr>
<td>24/16</td>
<td>CSFV</td>
<td>Alfort-Tübingen</td>
<td>E0</td>
<td>no</td>
<td>0 BVD, 13 CSFV</td>
<td>Kosmidou et al. (1995)</td>
</tr>
<tr>
<td>24/10</td>
<td>CSFV</td>
<td>Alfort-Tübingen</td>
<td>E2</td>
<td>no</td>
<td>0 BVD, 21 CSFV</td>
<td>Kosmidou et al. (1995)</td>
</tr>
</tbody>
</table>

* Out of a total of 13 bovine diarrhea virus (BVDV) and 4 classical swine fever virus (CSFV) strains tested.

b Out of a total of 6 BVDV strains tested.

c Out of a total of 7 BVDV, 21 CSFV, and 2 border disease virus (BDV) strains tested.

Free-ranging ruminants in the epidemiology of pestivirus infections or in virus transmission to domestic livestock (Aguirre et al., 1995; Frölich, 1995) should be investigated. In addition to information regarding the general occurrence of pestiviruses in wild animals by serologic survey, it is of particular interest to determine whether viruses infecting different taxonomic groups have corresponding differences within their genomes.

With respect to our sequencing results, more information is required to determine whether the “T” at position 259 in BVDV strain SH9/11 is associated with infecting potential in roe deer. All other BVDV strains from cattle have a “C” at this position (Fig. 1). Analysis of the variable (V) domains within the 5’ UTR revealed that V1 and V2 of roe deer strain SH9/11 is highly similar to BVDV Ib strains, whereas V3 is almost identical to BVDV Ia strains. In addition to strains recently isolated from BVD outbreaks in Canada the BVDV group Ia and Ib include “classical” BVDV strains, whereas group II represents newly recognized BVDV strains with high pathogenicity (Pellerin et al., 1994). It is not known if 5’ UTR sequence differences have an influence on the topology of the secondary structure and, therefore, might directly affect the transcription and translation of viral RNA in different hosts, as suggested recently (Harasawa, 1996).

The nearly identical topology of consensus trees constructed by two different methods supports the inclusion of SH9/11 into BVDV group I. However, it seems to belong neither to subgroup Ia nor Ib. The formation of the distinct subgroups and the outside position of the roe deer strain is clearly supported by bootstrap values ranging between 72.9 and 100 (Fig. 2). However, the absolute positions for all members within the subgroups could not be defined (bootstrap values near 45).

Roe deer isolate SH9 reacted positively with four out of 13 mAbs raised against CSFV and BVDV. From these, one mAb (C16) is known to recognize all pestivirus-
Table 2. Identity (%) of pestivirus 5 UTR sequences with that of bovine virus diarrhea virus (BVDV) strain SH9/S111 within the 241 bp fragment (nt 130–370 of NADL sequence) analyzed in this study. BVDV strains are grouped into Ia, Ib and II according to Pellerin et al. (1994).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Identity (%)</th>
<th>Reference</th>
<th>Strain</th>
<th>Identity (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BVDV-Ia</td>
<td></td>
<td></td>
<td>BVDV-II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cp NADL</td>
<td>93</td>
<td>Collett et al. (1988)</td>
<td>890</td>
<td>76</td>
<td>Ridpath and Bolin (1995)</td>
</tr>
<tr>
<td>cp SINGER</td>
<td>88</td>
<td>DeMoerlooze et al. (1993)</td>
<td>CD37</td>
<td>78</td>
<td>Pellerin et al. (1994)</td>
</tr>
<tr>
<td>ncp SD-1</td>
<td>88</td>
<td>Deng and Brock (1992)</td>
<td>Q1111</td>
<td>77</td>
<td>Pellerin et al. (1994)</td>
</tr>
<tr>
<td>OREGON</td>
<td>92</td>
<td>Pellerin et al. (1994)</td>
<td>Q149</td>
<td>77</td>
<td>Pellerin et al. (1994)</td>
</tr>
<tr>
<td>C1</td>
<td>89</td>
<td>Pellerin et al. (1994)</td>
<td>Q126</td>
<td>77</td>
<td>Pellerin et al. (1994)</td>
</tr>
<tr>
<td>C3</td>
<td>89</td>
<td>Pellerin et al. (1994)</td>
<td>Q2101</td>
<td>77</td>
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<td>12</td>
<td>90</td>
<td>Harasawa (1994)</td>
<td>Q4812</td>
<td>76</td>
<td>Pellerin et al. (1994)</td>
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<td></td>
<td></td>
<td>WATERS</td>
<td></td>
<td>77</td>
<td>Pellerin et al. (1994)</td>
</tr>
<tr>
<td>BVDV-Ib</td>
<td></td>
<td></td>
<td></td>
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<td>NY-1</td>
<td>89</td>
<td>Pellerin et al. (1994)</td>
<td>1494</td>
<td>76</td>
<td>Pellerin et al. (1994)</td>
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<tr>
<td>DRAPER</td>
<td>90</td>
<td>Pellerin et al. (1994)</td>
<td>BULL</td>
<td>76</td>
<td>Pellerin et al. (1994)</td>
</tr>
<tr>
<td>cp OSLOSS</td>
<td>89</td>
<td>DeMoerlooze et al. (1993)</td>
<td>BDV</td>
<td>MOREDUN</td>
<td>Becher et al. (1995)</td>
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<tr>
<td>Q47</td>
<td>88</td>
<td>Pellerin et al. (1994)</td>
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<td>Q69</td>
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<td>Q1808</td>
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<td>1854</td>
<td>90</td>
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es tested so far. MAb N2B12 reacts with all BVDV isolates and mAb D5 recognizes many BVDV strains (Weiland et al., 1989), hence it was not surprising that both mAbs recognized the roe deer isolate strain SH9. Five of seven mAbs raised against CSFV and tested in this study showed cross-reactivity with many BVDV and BDV strains (Kosmidou et al., 1995). However, only one of these mAbs, f48, reacted with isolate SH9. This indicates that the antigenic structure of SH9/11 might differ from that of other BVDV strains. The unique position of strain SH9/11 within the pestiviruses is also supported by the finding that all mAb f48-positive BVDV and BDV strains tested so far showed crossreactivity with at least one and up to four other anti-E2-mAbs used here (Kosmidou, 1995).

Both mAb-typing and sequence analysis of 5' UTR showed a unique position of SH9/11 within the taxon BVDV. Taken together, these results support the idea that distinct BVDV strains might exist among the free-ranging roe deer population we investigated. This hypothesis also is supported by the results of former serological surveys in Germany; Frölich (1995) found no significant differences in antibody prevalence among deer in habitats with high, intermediate and very low density of cattle, indicating that free-ranging deer can become infected with BVDV without having contact with cattle. In conclusion, this study indicates that distinct BVDV strains might circulate in free-ranging roe deer in Germany.

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