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- 1 A common neutralizing epitope on envelope glycoprotein E2 of different pestiviruses:
- 2 Implications for improvement of vaccines and diagnostics for Classical swine fever (CSF)?
- 3
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1 <u>Abstract</u>

2	The Pestivirus genus within the family of Flaviviridae consists of at least three species; Classical
3	swine fever virus (CSFV) found in swine and wild boar, Bovine viral diarrhoea virus type 1 and
4	type 2 (BVDV-I and BVDV-II) mainly isolated from cattle, and Border disease virus (BDV)
5	preferably replicating in ovine species. Many features demonstrate differences between CSFV
6	and other pestiviruses, BVDV-I, BVDV-II, and BDV, here defined as nonCSFV, whereas other
7	features show similarities between all different species of pestiviruses. Focussing on the major
8	envelope glycoprotein E2, the immunodominant protein of pestiviruses, CSFV seems to be a
9	more distinct species within the pestivirus genus. Here we confirm on one hand the more
10	separated grouping of CSFV by isolation of monoclonal antibodies (MAbs) raised against E2 of
11	BVDV-I and BVDV-II. None of these MAbs recognize E2 of CSFV strains. On the other hand,
12	only one MAb, MAb 912, was isolated against E2 of BDV. MAb 912 binds to E2 of CSFV
13	strains and partly neutralizes CSFV. The epitope of MAb 912 is mapped in antigenic domain B
14	of CSFV-E2. This common epitope of CSFV strains and nonCSFV strains could have
15	implications for development of DIVA vaccines and serological diagnostics for CSF.
16	
17	Key words:

18 pestivirus; envelope glycoprotein; E2; epitopes; antigenic structure

1 <u>Introduction</u>

2	The Pestivirus genus within the family of Flaviviridae consists of at least three species;
3	Classical swine fever virus (CSFV) found in swine and wild boar, Bovine viral diarrhoea virus
4	type 1 and type 2 (BVDV-I and BVDV-II) mainly isolated from cattle, and Border disease virus
5	(BDV) preferably replicating in ovine species (Thiel et al., 2005). Pestiviruses are
6	immunologically and genetically related. Similar genome organization, high percentage of
7	homology and cross-reacting sera and monoclonal antibodies (MAbs) demonstrate the
8	relationship between pestiviruses. However, many features demonstrate differences between
9	CSFV and other pestiviruses, BVDV-I, BVDV-II, and BDV, here defined as nonCSFV. CSFV is
10	restricted to swine and wild boar, whereas nonCSFV infect many different ruminant species as
11	well as swine and wild boar. Many MAbs raised against nonCSFV-strains neutralize strains of
12	nonCSFV species (cross-neutralization), but not CSFV strains. In contrast MAbs against CSFV
13	do not neutralize strains of nonCSFV-species. A key question in this is: Is CSFV a more distinct
14	species within the pestivirus genus?
15	Pestiviruses can be subdivided into four to six neutralization groups (Paton et al., 1995),
16	Dekker et al., 1995). Phylogenetic studies based on different genetic regions resulted in a very
17	similar subdivision (Pellerin et al., 1994, van Rijn et al., 1997, Becher et al., 1999). A correlation
18	between six proposed neutralization groups and six genetic groups has been noticed for envelope
19	glycoprotein E2 (Dekker et al., 1995, van Rijn et al., 1997). Recently, more pestivirus isolates
20	are studied and extensive phylogenetic studies divide these pestivirus group in subgroups
21	(Becher et al., 2003). Although these data are very supportive for (molecular) epidemiological
22	studies, the significance of these detailed subgroupings for cross-neutralization or cross-
23	protection has not been addressed.



Envelope glycoprotein E2 is the immunodominant protein of pestiviruses. For vaccine

1	development against CSF, E2 (E2-CSFV) is a major target in all kind of (experimental) vaccines
2	(van Zijl et al., 1991, Hulst et al., 1993, Konig et al., 1995, van Rijn et al., 1996, Widjojoatmodjo
3	et al., 2000, van Gennip et al., 2000, van Gennip et al., 2002, Reimann et al., 2004), as well as
4	for diagnostics (Wensvoort et al., 1988, van Rijn et al., 1999, Clavijo et al., 2001). A proposed
5	model of the antigenic structure of CSFV-E2 describes four antigenic domains on two structural
6	units in the N-terminal half of E2 (van Rijn et al., 1994). All studied epitopes on this part of
7	CSFV-E2 are: 1) conformational, 2) specific for CSFV, 3) and highly (domains B and C) or even
8	completely (domain A) conserved among CSFV (Wensvoort et al., 1989, van Rijn et al., 1994).
9	In addition, studies with peptides showed a linear epitope on domain A (Lin et al., 2000).
10	A comparable model for E2 of BVDV-I, BVDV-II, and BDV (nonCSFV) is not available
11	yet. Many panels of MAbs have been raised against different nonCSFV strains (Paton et al.,
12	1991, Paton et al., 1994, Weiland et al., 1989, Deregt et al., 1998b, Kreutz et al., 2000). Epitopes
13	on E2 of nonCSFV are never completely conserved, but many of these are present on E2 of
14	strains belonging to nonCSFV species, and not present on CSFV-E2 (Paton et al., 1992, van Rijn
15	et al., 1997). Concerning vaccine development, E2 has been targeted as many MAbs against E2
16	of nonCSFV are neutralizing (Paton et al., 1992, Deregt et al., 1998a). However, vaccination
17	with E2 subunit vaccines of BVDV-I and BVDV-II is not completely protective against vertical
18	transmission in sheep (Bruschke et al., 1997, Bruschke et al., 1999). For BDV, such experiments
19	are not performed yet.
20	All Cys-residues in CSFV-E2 are completely conserved among the entire pestivirus
21	genus (van Rijn et al., 1997). Several amino acid changes in MAb-resistant- or escape mutants of
22	CSFV and nonCSFV are located on similar positions in E2 (van Rijn et al., 1994, Paton et al.,
23	1992, Deregt et al., 1998b). Up to three immunodominant antigenic domains with neutralizing
24	epitopes have been found on E2s of CSFV and BVDV-II (Wensvoort, 1989, Deregt et al., 1998b,

1	and a linear epitope in the C-terminal half of E2 is highly conserved among the pestivirus genus
2	(Yu et al., 1996). These data suggest a similar overall antigenic structure and viral function of E2
3	among all pestivirus species. However, the overall percentage of homology for the N-terminal
4	half of E2 can be as low as 40%. Amino acids crucial for completely conserved epitopes on
5	CSFV-E2, such as Pro ⁸³³ (van Rijn et al., 1994), are not present in nonCSFV (van Rijn et al.,
6	1997). Remarkably, nonCSFV-E2s contain two additional Cys-residues in the N-terminal
7	antigenic half compared to CSFV-E2. This could implicate an extra sulphur bridge, or a
8	completely different pair-wise formation of disulphide bonds. Both could result in significant
9	differences in the E2 antigenic structure. Indeed, previous reports indicate that the antigenic
10	structure of nonCSFV-E2 is different from CSFV-E2 (Paton et al., 1997). Summarizing, on the
11	one hand data indicate major differences between E2s of CSFV and nonCSFV, and on the other
12	hand data indicate similarities between all E2s of different pestivirus species.
13	
14	Materials and methods
15	To study the E2 antigenic structure of different pestivirus species in more detail, we
16	expressed different E2 genes in insect cells. Therefore, the CSFV-E2 gene in the baculovirus
17	transfervector (van Rijn et al., 1996) was exchanged by the E2-containing SpeI-AfIII fragment of
18	expression plasmids pPRKbvd42, 43, 65, 80, 85, 96 (van Rijn et al., 1997). Regeneration of
19	recombinant viruses (RBVs) resulted in RBV2 (BVDV-Ia strain 150022), RBV3 (BVDV-II
20	strain 178003), RBV5 (BVDV-Ib strain 4800), RBV7 (BDV strain F), RBV8 (pestivirus strain
21	Deer), and RBV9 (pestivirus Giraffe). E2-expression was confirmed with a selected panel of

MAbs by immunoperoxidase monolayer assay (IPMA) as described (van Rijn et al., 1996, van
Rijn et al., 1997).

24

In order to determine common epitopes on E2 of CSFV and nonCSFV, we produced E2

1	of BDV strain F (RBV7), BVDV-I strain 4800 (RBV5), and BVDV-II strain 178003 (RBV3),
2	for immunization of mice according standard procedures. Ab-producing hybridomas were
3	selected, and cloned by standard procedures. Screening was carried out on EBTr- monolayers
4	infected with the respective strain by IPMA. Hybridomas (24 directed to each of BVDV-I and
5	BVDV-II) were cloned, and MAbs were studied. Despite many attempts, only one MAb-
6	producing hybridoma was found for E2 of BDV strain F, designated MAb 912. Remarkably,
7	EBTr-monolayers infected with nonCSFV strains were all negative in IPMA with MAb 912,
8	except for BDV strain F. The uniqueness of this MAb was further investigated in the next
9	described experiments.
10	
11	Results
12	A: MAbs, 24 raised against E2 of BVDV-I and 24 raised against E2 of BVDV-II,
13	recognize many nonCSFV strains from different pestivirus groups, including BVDV-Ia, BVDV-
14	Ib, BVDV-II, Deer and Giraffe, but none of these MAbs immunostained SK6 cells infected with
15	CSFV strains C or Brescia c1.1.1 (not shown). These findings confirmed the difference between
16	E2 of CSFV and nonCSFV strains.
17	B: Surprisingly, the only MAb raised against E2 of BDV, MAb 912, immunostained SK6
18	cells infected with CSFV strain C. Further, staining of cells infected with Brescia c1.1.1 was
19	very weak (not shown). These results suggested that CSFV and BDV could have a common
20	epitope on E2.
21	C: Immunostaining of Sf21 cells infected by the generated RBVs, including bac.cE2 (E2
22	of strain C) and bac.bE2 (E2 of Brescia c.1.1.1) (van Rijn et al., 1996) was performed with MAb
23	912 and a selected panel of MAbs as controls, since E2-expression is more sensitive in the RBV
24	expression system (van Rijn et al., 1996). Immunostaining of nonCSFV-E2 expressed by RBVs

1	was negative with CSFV-specific MAbs. Staining with MAb 912 was positive for several of
2	these RBVs as well as for bac.bE2 and bac.cE2. (Table 1). We conclude that the epitope of MAb
3	912 is a common epitope present on CSFV-E2 and different but not all nonCSFV-E2s.
4	D: We roughly mapped the epitope by use of deletion CSFV-E2 genes according to van
5	Rijn et al., 1993 (van Rijn et al., 1993) (Table 1). As staining is associated with the presence of
6	the BC unit of CSFV-E2, we suggest that the epitope of MAb 912 is located in the region of
7	BDV-E2 corresponding to the BC-unit of CSFV-E2.
8	E: A detailed epitope mapping was performed with mutant E2 genes of strain Brescia
9	c1.1.1 containing single mutations in regions encoding domain B or domain C as described (van
10	Rijn et al., 1994). Preliminary results demonstrated that the epitope of MAb 912 is located at
11	domain B (not shown). Three amino acids at position 710, His, Gln, and Leu in E2 originating
12	from strain Brescia c.1.1.1, E2 of CSFV strain C (pPRc34), and E2 of BDV strain F
13	(pPRKbvdv80) were studied with a selected panel of MAbs, b3, b6 and c6, and MAb 912 (Table
14	2). E2s with Leu ⁷¹⁰ (pPK12, pPRc34, and pPRKbvd80) were bound by MAb 912 (van Rijn et al.,
15	1997), whereas E2s with His or Gln on position 710 were not recognized. We conclude that
16	Leu ⁷¹⁰ is essential for binding of MAb 912. Binding of MAb c6 also depends on Leu ⁷¹⁰ ,
17	however, additional amino acids appears to play a role in binding as shown by the negative
18	staining of E2 of pPRKbvdv80, and the weak staining of pPK12 (Table 2). Finally, binding of
19	MAb b6 depends on the presence of His ⁷¹⁰ . In conclusion, position 710 in domain B is very
20	important for the epitopes of MAb 912, c6 and b6. Apparently, the N-terminal part of E2 of
21	BDV corresponding with domain B is similar to that of CSFV-E2.
22	F: Neutralization of CSFV strains Brescia c1.1.1, CobrB, C, and BDV strain F with MAb
23	912 and polyvalent sera was studied (Table 3). CSFV strain CobrB is an independent virulent
24	variant of Brescia, strain CoBrB, containing Leu on position 710 (Van Gennip et al., 2004).

1	BDV strain F and CSFV strain CobrB were neutralized by MAb 912, very strongly and weakly,
2	respectively, whereas both Brescia c.1.1.1, and CSFV strain C were not neutralized by MAb 912.
3	Within domain B, CSFV strains Brescia c.1.1.1 and C contain only one difference between
4	position 706 and 719 compared to BDV strain F, Leu^{710} -> His ⁷¹⁰ , and Glu ⁷¹³ -> Gly ⁷¹³ ,
5	respectively (Figure 1) (van Rijn et al., 1997). Again, Leu ⁷¹⁰ is very important for the epitope of
6	MAb 912, as was observed by immunostaining of transiently expressed E2 with different amino
7	acids on position 710 (Table 2). In contrast, as the amino acid on position 713 also appeared to
8	be important for neutralization by MAb 912, no difference in immunostaining with MAb 912
9	was observed between these transiently expressed E2s from pPRc34 and pPRKbvdv80 (Table 2).
10	CobrB contains no differences in domain B from position 706 to 719 compared to BDV strain F
11	(Figure 1). The difference in level of neutralization of CSFV strain CobrB and BDV strain F by
12	MAb 912 was also not detected by immunostaining. These results suggest that amino acid
13	residues outside the region 706-719 contribute to neutralization by MAb 912, which is in
14	agreement with the finding that these epitopes are conformational.
15	
16	Discussion
17	This is the first report of a MAb directed against E2 that recognizes and neutralizes
18	CSFV as well as BDV. Taken all presented data together, we conclude that pestiviruses from
19	different pestivirus species could contain common neutralizing epitopes on E2. Most likely, local
20	variability by one or more amino acids results in differences in affinity/avidity and consequently
21	results in differences in (cross)-neutralization. Nevertheless, our findings implicate a comparable
22	antigenic structure of the BC unit for all pestiviruses. Based on the BC unit of E2, CSFV is
23	closely related to nonCSFV species. It remains to be investigated whether the same is true for

24 unit A, the other immunodominant structural unit of E2 of CSFV.

1	We speculate that seroconversion by previous BDV- or BVDV infections in pigs could
2	influence the efficacy of vaccination for CSF by the common epitopes on the BC unit. On one
3	hand, a booster response against the BC unit could be expected; on the other hand, a
4	neutralization of the CSF vaccine strain could be expected reducing the efficacy of vaccination.
5	We also speculate that humoral responses by previous infections with BDV or BVDV will
6	contain Abs against the common BC unit in nonvaccinated pigs. Consequently, serological
7	diagnostics (partly) based on the BC unit could be less specific in populations with a high BDV-
8	and/or BVDV-seroprevalence. Indeed, prior to the CSFV-outbreak in 1997 in The Netherlands,
9	the seroprevalence by BVDV and BDV hampered the diagnostics for CSF. In particular, the
10	ELISA (Colijn et al., 1997) showed high percentages of false positive results (Eble et al., 2000).
11	Here, it is shown that cross-reacting Abs raised against the BC unit could contribute to this
12	serological problem. We suggest taking this cross-reaction into account for improvement of both
13	vaccines and serological diagnostics for CSF.
14	
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1 <u>References</u>

2

3	Becher, P., Avalos Ramirez, R., Orlich, M., Cedillo Rosales, S., Konig, M., Schweizer, M.,
4	Stalder, H., Schirrmeier, H., Thiel, H.J., 2003, Genetic and antigenic characterization of
5	novel pestivirus genotypes: implications for classification. Virology 311, 96-104.
6	Becher, P., Orlich, M., Kosmidou, A., Konig, M., Baroth, M., Thiel, H.J., 1999, Genetic
7	diversity of pestiviruses: identification of novel groups and implications for
8	classification. Virology 262, 64-71.
9	Bruschke, C.J., Moormann, R.J., van Oirschot, J.T., van Rijn, P.A., 1997, A subunit vaccine
10	based on glycoprotein E2 of bovine virus diarrhea virus induces fetal protection in sheep
11	against homologous challenge. Vaccine 15, 1940-1945.
12	Bruschke, C.J., van Oirschot, J.T., van Rijn, P.A., 1999, An experimental multivalent bovine
13	virus diarrhea virus E2 subunit vaccine and two experimental conventionally inactivated
14	vaccines induce partial fetal protection in sheep. Vaccine 17, 1983-1991.
15	Clavijo, A., Lin, M., Riva, J., Mallory, M., Lin, F., Zhou, E.M., 2001, Development of a
16	competitive ELISA using a truncated E2 recombinant protein as antigen for detection of
17	antibodies to classical swine fever virus. Res Vet Sci 70, 1-7.
18	Colijn, E.O., Bloemraad, M., Wensvoort, G., 1997, An improved ELISA for the detection of
19	serum antibodies directed against classical swine fever virus. Vet Microbiol 59, 15-25.
20	Dekker, A., Wensvoort, G., Terpstra, C., 1995, Six antigenic groups within the genus pestivirus
21	as identified by cross neutralization assays. Vet Microbiol 47, 317-329.
22	Deregt, D., Bolin, S.R., van den Hurk, J., Ridpath, J.F., Gilbert, S.A., 1998a, Mapping of a type
23	1-specific and a type-common epitope on the E2 (gp53) protein of bovine viral diarrhea

1	virus with neutralization escape mutants. Virus Res 53, 81-90.
2	Deregt, D., van Rijn, P.A., Wiens, T.Y., van den Hurk, J., 1998b, Monoclonal antibodies to the
3	E2 protein of a new genotype (type 2) of bovine viral diarrhea virus define three
4	antigenic domains involved in neutralization. Virus Res 57, 171-181.
5	Eble, P.L., de Kluijver, E.P., Schrijver, R.S., de Smit, A.J., 2000, [Laboratory findings during the
6	classic swine fever epidemic of 1997- 1998]. Tijdschr Diergeneeskd 125, 108-112.
7	Hulst, M.M., Westra, D.F., Wensvoort, G., Moormann, R.J., 1993, Glycoprotein E1 of hog
8	cholera virus expressed in insect cells protects swine from hog cholera. J Virol 67, 5435-
9	5442.
10	Konig, M., Lengsfeld, T., Pauly, T., Stark, R., Thiel, H.J., 1995, Classical swine fever virus:
11	independent induction of protective immunity by two structural glycoproteins. J Virol 69,
12	6479-6486.
13	Kreutz, L.C., Donis, R., Gil, L.H., Lima, M., Hoffman, A.N., Garcez, D.C., Flores, E.F.,
14	Weiblen, R., 2000, Production and characterization of monoclonal antibodies to Brazilian
15	isolates of bovine viral diarrhea virus. Braz J Med Biol Res 33, 1459-1466.
16	Lin, M., Lin, F., Mallory, M., Clavijo, A., 2000, Deletions of structural glycoprotein E2 of
17	classical swine fever virus strain alfort/187 resolve a linear epitope of monoclonal
18	antibody WH303 and the minimal N-terminal domain essential for binding
19	immunoglobulin G antibodies of a pig hyperimmune serum. J Virol 74, 11619-11625.
20	Paton, D., McGoldrick, A., Lowings, J.P., Drew, T., Yapp, F., 1997, Transient expression and
21	mutagenesis of the E2 protein of BVDV, In: Edwards, J., Paton, D., Wensvoort, G. (Eds.)
22	Proceedings of the third ESVV Symposium on pestivirus infections, Lelystad. CVL,
23	Weybridge UK, pp. 28-32.

1	Paton, D.J., Lowings, J.P., Barrett, A.D., 1992, Epitope mapping of the gp53 envelope protein of
2	bovine viral diarrhea virus. Virology 190, 763-772.
3	Paton, D.J., Sands, J.J., Edwards, S., 1994, Border disease virus: delineation by monoclonal
4	antibodies. Arch Virol 135, 241-252.
5	Paton, D.J., Sands, J.J., Lowings, J.P., Smith, J.E., Ibata, G., Edwards, S., 1995, A proposed
6	division of the pestivirus genus using monoclonal antibodies, supported by cross-
7	neutralisation assays and genetic sequencing. Vet Res 26, 92-109.
8	Paton, D.J., Sands, J.J., Roehe, P.M., 1991, BVD monoclonal antibodies: relationship between
9	viral protein specificity and viral strain specificity. Arch Virol Suppl 3, 47-54.
10	Pellerin, C., van den Hurk, J., Lecomte, J., Tussen, P., 1994, Identification of a new group of
11	bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities.
12	Virology 203, 260-268.
13	Reimann, I., Depner, K., Trapp, S., Beer, M., 2004, An avirulent chimeric Pestivirus with altered
14	cell tropism protects pigs against lethal infection with classical swine fever virus.
15	Virology 322, 143-157.
16	Thiel, H.J., Collett, M.S., Gould, E.A., Heinz, F.X., Houghton, M., Meyers, G., Purcell, R.H.,
17	Rice, C.M., 2005, Family Flaviviridae in : Virus Taxonomy, 8th ed. Edition. Elsevier
18	Academic Press, San Diego, pp. 981–998 p.
19	van Gennip, H.G., Bouma, A., van Rijn, P.A., Widjojoatmodjo, M.N., Moormann, R.J., 2002,
20	Experimental non-transmissible marker vaccines for classical swine fever (CSF) by trans-
21	complementation of E(rns) or E2 of CSFV. Vaccine 20, 1544-1556.
22	van Gennip, H.G., van Rijn, P.A., Widjojoatmodjo, M.N., de Smit, A.J., Moormann, R.J., 2000,
23	Chimeric classical swine fever viruses containing envelope protein E(RNS) or E2 of

1	bovine viral diarrhoea virus protect pigs against challenge with CSFV and induce a
2	distinguishable antibody response. Vaccine 19, 447-459.
3	van Gennip, H.G., Vlot, A.C., Hulst, M.M., De Smit, A.J., Moormann, R.J., 2004, Determinants
4	of virulence of classical swine fever virus strain Brescia. J Virol 78, 8812-8823.
5	van Rijn, P.A., Bossers, A., Wensvoort, G., Moormann, R.J., 1996, Classical swine fever virus
6	(CSFV) envelope glycoprotein E2 containing one structural antigenic unit protects pigs
7	from lethal CSFV challenge. J Gen Virol 77, 2737-2745.
8	van Rijn, P.A., Miedema, G.K., Wensvoort, G., van Gennip, H.G., Moormann, R.J., 1994,
9	Antigenic structure of envelope glycoprotein E1 of hog cholera virus. J Virol 68, 3934-
10	3942.
11	van Rijn, P.A., van Gennip, H.G., de Meijer, E.J., Moormann, R.J., 1993, Epitope mapping of
12	envelope glycoprotein E1 of hog cholera virus strain Brescia. J Gen Virol 74, 2053-2060.
13	van Rijn, P.A., van Gennip, H.G., Leendertse, C.H., Bruschke, C.J., Paton, D.J., Moormann,
14	R.J., van Oirschot, J.T., 1997, Subdivision of the pestivirus genus based on envelope
15	glycoprotein E2. Virology 237, 337-348.
16	van Rijn, P.A., van Gennip, H.G., Moormann, R.J., 1999, An experimental marker vaccine and
17	accompanying serological diagnostic test both based on envelope glycoprotein E2 of
18	classical swine fever virus (CSFV). Vaccine 17, 433-440.
19	van Zijl, M., Wensvoort, G., de Kluyver, E., Hulst, M., van der Gulden, H., Gielkens, A., Berns,
20	A., Moormann, R., 1991, Live attenuated pseudorabies virus expressing envelope
21	glycoprotein E1 of hog cholera virus protects swine against both pseudorabies and hog
22	cholera. J Virol 65, 2761-2765.
23	Weiland, E., Thiel, H.J., Hess, G., Weiland, F., 1989, Development of monoclonal neutralizing

1	antibodies against bovine viral diarrhea virus after pretreatment of mice with normal
2	bovine cells and cyclophosphamide. J Virol Methods 24, 237-243.
3	Wensvoort, G., 1989, Topographical and functional mapping of epitopes on hog cholera virus
4	with monoclonal antibodies. J Gen Virol 70, 2865-2876.
5	Wensvoort, G., Bloemraad, M., Terpstra, C., 1988, An enzyme immunoassay employing
6	monoclonal antibodies and detecting specifically antibodies to classical swine fever virus.
7	Vet Microbiol 17, 129-140.
8	Wensvoort, G., Terpstra, C., de Kluijver, E.P., Kragten, C., Warnaar, J.C., 1989, Antigenic
9	differentiation of pestivirus strains with monoclonal antibodies against hog cholera virus.
10	Vet Microbiol 21, 9-20.
11	Widjojoatmodjo, M.N., van Gennip, H.G., Bouma, A., van Rijn, P.A., Moormann, R.J., 2000,
12	Classical swine fever virus E(rns) deletion mutants: trans- complementation and potential
13	use as nontransmissible, modified, live- attenuated marker vaccines. J Virol 74, 2973-
14	2980.
15	Yu, M., Wang, L.F., Shiell, B.J., Morrissy, C.J., Westbury, H.A., 1996, Fine mapping of a C-
16	terminal linear epitope highly conserved among the major envelope glycoprotein E2
17	(gp51 to gp54) of different pestiviruses. Virology 222, 289-292.

1	Table 1. Results of IPMAs of Sf21 monolayers infected with different RBVs using a selected
2	panel of MAbs.
3	Immunostaining was interpreted as negative (white) or positive (black).
4	
5	
6	Table 2. Results of IPMAs of SK6 monolayers transfected with expression plasmids.
7	Expression plasmids harbour (mutant) E2 genes of different pestiviruses. Viruses corresponding
8	to the respective E2 genes are indicated. Immunostaining with a selected panel of MAbs was
9	interpreted as negative (white), weak (grey) or positive (black).
10	
11	
12	Table 3. Results of VNTs with different pestiviruses.
13	Viruses (start virus titres are given) were neutralized with equal amounts of MAb 912, or
14	polyvalent serum WIS (weak positive serum, serum of a C-strain vaccinated pig), or HIS
15	(hyperimmune serum, serum of vaccinated pig, repeatedly challenged with CSFV strains of
16	increasing virulence). Reduction in virus titres by neutralization is expressed in 50% tissue
17	culture infective dose per ml (TCID ₅₀ /ml).
18	
19	
20	Figure 1. Comparison of deduced amino acid sequences of the N-terminal part of E2
21	proteins from position 690 to 722 of pestivirus strains relevant in this study.
22	Amino acid differences in CSFV strains with respect to BDV strain F are indicated in bold and
23	are underlined.
24	

BDV strain F				QIGCREDFRY	ALAKTKEIGL	LGAEGLTTTW	TDY
CSFV	strain	CobrB	:	rla C k ED h RY	A ist t n eigl	LGAEGLTTTW	KEY
CSFV	strain	c.1.1.	:	rla C k ED h RY	$\mathtt{A}\underline{\mathtt{IST}}\mathtt{T}\underline{\mathtt{N}}\mathtt{EIGL}$	H GAEGLTTTW	KE Y
CSFV	strain	С	:	$\underline{\textbf{RLA}}\textbf{C}\underline{\textbf{K}}\textbf{E}\textbf{D}\underline{\textbf{Y}}\textbf{R}\textbf{Y}$	$\mathtt{A}\underline{\mathtt{IST}}\mathtt{T}\underline{\mathtt{D}}\mathtt{EIGL}$	LGA G GLTTTW	KE Y
				•	•		•
				690	700	710	720

Figure 1.

MAb					Recom	binant b	baculo	/irus (RE	3V)			
	cell control Sf21	virus control empty	RBV2 178003	RBV3 150022	RBV5 4800	RBV7 F	RBV8 Deer	RBV9 Giraffe	bac.cE2 C	bac.bE2 c1.1.1	bac.bE2 (dA) BC unit	bac.bE2 (dBC) A unit
b3 b6	-											
08 c6 912												
Table 1												

-		transient expression plasmid							
	MAb	neg	pPRb2	pPK04	pPK12	pPRc34	pPRKbvd80		
		cells	c1.1.1	c1.1.1	CobrB	strain C	strain F		
-	1.0		His710	His710->Gln	His710->Leu				
	D3								
	00 60								
	912								
Т	able 2								
10									

virus	start	redu	s titer	
	virus titer	HIS	WIS	Mab 912
strain C	3.80	<u>></u> 2.00	1.75	0.00
strain F	5.05	<u>></u> 3.25	<u>></u> 3.25	<u>></u> 3.25
c1.1.1	5.50	<u>></u> 3.75	1.75	0.00
CobrB	7.80	<u>></u> 6.00	2.25	1.75

Table 3