Veterinary Microbiology, 23 (1990) 193–201
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

ELISA detection of bovine viral diarrhoea virus specific antibodies using recombinant antigen and monoclonal antibodies

C. Lecomte[1], J.J. Pin[2], L. De Moerlooze[1], D. Vandenbergh[1], A.F. Lambert[1], P.P. Pastoret[3] and G. Chappuis[2]

[1]*Eurogentec, Campus du Sart Tilman, Allée du Six Août, B6, B-4000 Liège (Belgium)*
[2]*Rhône-Mérieux, Laboratoires IFFA, Rue Marcel Mérieux, 254, B.P. 7009, 69742 Lyon (France)*
[3]*Department of Virology, Faculty of Veterinary Medicine, University of Liège, Rue des Vétérinaires, 45, B-1070 Brussels (Belgium)*

ABSTRACT

Lecomte, C., Pin, J.J., De Moerlooze, L., Vandenbergh, D., Lambert, A.F., Pastoret, P.P. and Chappuis, G., 1990. ELISA detection of bovine viral diarrhoea virus specific antibodies using recombinant antigen and monoclonal antibodies. *Vet. Microbiol.*, 23: 193–201.

A panel of monoclonal antibodies was prepared by immunization of BALB/c mice with Moredun (BD) virus strains. These antibodies were characterized by immunofluorescence and seroneutralization against BD, BVD and hog cholera (HC) virus strains, and radioimmunoprecipitation of BVD-infected cells extracts. The MAbs reacting with the majority of the Pestivirus strains recognize the 80 kDa antigen of the BVD cytophathic strains. The 80 kDa antigen of the BVD/Osloss virus strain has been cloned and expressed in *E. coli* as a fusion protein with β-galactosidase. The fusion protein has been purified from inclusion bodies and used successfully as an antigen for ELISA detection of BVDV specific antibodies in bovine sera. A competitive ELISA using MAbs is more specific than a direct assay. These results compare well with the ones obtained with antigen extracted from BVDV-infected cells.

INTRODUCTION

Bovine viral diarrhoea virus (BVDV) is one of the most important viral pathogens of cattle, causing a wide range of clinical syndromes like abortion, teratogenic defects, stillbirths or weak calves and a variety of illnesses in adult cattle (Brownlie, 1985). BVDV is currently classified in the *Pestivirus* genus of the Togaviridae family, which also includes hog cholera (HC) virus and border disease virus (BDV) of sheep (Horzinek, 1981; Westaway, 1985). However, doubt on this classification has been raised recently (Renard et al., 1985; Collett et al., 1988).

Field isolates of BVD virus can be divided into two biotypes according to

0378-1135/90/$03.50

their ability to induce cytophathology in bovine cell cultures: cytopathic or non-cytopathic. Infection during pregnancy with a non-cytopathic BVDV strain can lead to the birth of persistently infected calves that present a highly specific immunotolerance (McClurkin et al., 1984). Superinfection of persistently viraemic animals with an antigenically related cytopathic strain usually results in the invariably fatal mucosal disease (Brownlie et al., 1984). To date, the molecular determinants of the pathogenic mechanisms remain largely unknown.

Several types of assays have been described for BVD virus serology, the most widely used being the serum neutralization test (Rossi and Kiesel, 1971; Ruckerbauer et al., 1971). Enzyme-linked immunosorbent assays (ELISA) have been described for the detection of BVDV antibodies in cattle sera, using antigens isolated from bovine infected cells and test sera from hyperimmunized calves (Chu et al., 1985; Howard et al., 1985; Straver et al., 1985; Katz and Hanson, 1987).

Here we report on the preparation of a panel of monoclonal antibodies (MAbs) that were characterized by serum neutralization (SN), immunofluorescence (IF) and radioimmunoprecipitation. We describe the purification of a recombinant protein expressed in bacteria as a fusion protein with β-galactosidase and its use for ELISA detection of BVD specific antibodies in bovine sera. An indirect ELISA is compared with a competitive assay using MAbs, and recombinant antigen is compared with antigen extracted from BVDV-infected cells.

MATERIAL AND METHODS

Preparation and characterization of monoclonal antibodies

The Aveyron (AV) and Moredun (BD) isolates of BDV were used to immunize BALB/c mice as a source of lymphocytes for hybridoma production (Köhler et al., 1976; Fazekas de St. Groth and Scheidegger, 1980). Screening of the hybrids was carried out by testing supernatants in an indirect immunofluorescence assay (IFA; Fernelius, 1964).

Characterization and classification of MAbs were performed by IFA, by virus neutralization (Rossi and Kiesel, 1971; Ruckerbauer et al., 1971) and by radioimmunoprecipitation of BVD-infected cell extracts (Donis and Dubovi, 1987).

Virus strains and cells

All virus strains were propagated in fetal ovine kidney cells (OCK). The virus strains used in characterizing MAbs are listed in Table 1. Cytophatic BVD and BD virus strains were plaque-purified. Some of the initial isolates (Osloss, Singer and Moredun) yielded both cytopathic and non cytophatic biotypes (c/nc).

Preparation of ELISA antigens

The recombinant antigen is produced as a fusion protein with β-galactosidase (Renard et al., 1985). Inclusion bodies were purified as described (Klempnauer et al., 1983), then denatured in 7 M urea, 0.25 M Tris-HCl, pH 8, 10 mM dithiothreitol; the renaturation process consisted of dialysis against 0.25 M Tris-HCl, pH 8, 1 mM dithiothreitol. β-galactosidase was produced and purified in the same way to provide control recombinant antigen.

The Singer strain of BVD virus was grown in OCK and used to prepare the cellular antigen. Infected cells were harvested before cytophatic effects became apparent, and frozen at −70°C. Cells that had not been infected with BVDV were extracted in the same way as control antigen.

ELISA procedure

For the assay, microtitre plates were sensitized with recombinant or cellular antigens and their respective negative controls, in the usual carbonate buffer, pH 9.6. Plates were incubated overnight at 4°C, washed twice with

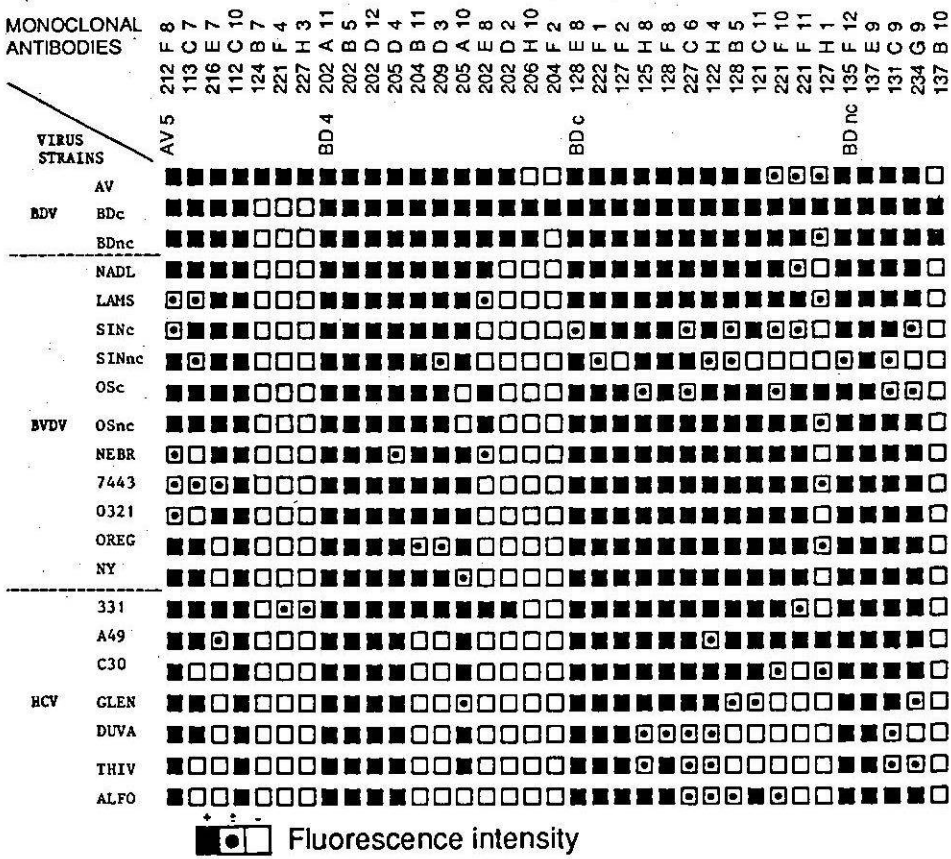
TABLE 1

Origin and biotype of virus strains

Strain	Origin	Biotype
BVDV		
NADL	Gutekunst and Malmquist (1963)	Cytopathic
Osloss (c/nc)	Liess (1967)	Cytopathic
Singer (c/nc)	McClurkin and Coria (1978)	Cytopathic
New-York	ATCC	Non-cytopathic
Lamspringe	Liess (1967)	Cytopathic
Nebraska	–	Non-cytopathic
Oregon	Gillespie et al. (1960)	Cytopathic
0321	Liess et al. (1983)	Non-cytopathic
7443	–	Non-cytopathic
BDV		
AV-Aveyron	Rhône-Mérieux (1984)	Non-cytopathic
BD (c/nc)	Moredun (1983)	Cytopathic
HCV		
Alfort	Aynaud (1968)	Non-cytopathic
A49	Rhône-Mérieux (1965)	Non-cytopathic
C30-chinoise	Rhône-Mérieux (1970)	Non-cytopathic
Duvaxin	Duphar-RM (1984)	Non-cytopathic
Glentorf	Pittler et al. (1968)	Non-cytopathic
Thiverval	Launais et al. (1972)	Non-cytopathic
331	Mengeling and Cheville (1968)	Non-cytopathic

TABLE 2

Specificity of MAbs determined by IFA



PBS containing 0.1% Tween 20 and incubated at 37°C with dilutions of bovine sera and MAbs.

Bovine sera

Groups of sera from several lots of animals were examined. The first group was collected from 400 calves from the "Centre de sélection de Ciney". Calves were 6 months old when first bled and were bled again 4 weeks later.

The second group of sera was from 80 cattle on six farms, in which some animals had shown clinical symptoms of BVDV.

RESULTS

From ten fusions of SP2/O myeloma cells with spleen cells of BALB/c mice immunized with BD (c/nc) and AV virus strains, more than 100 hybridoma

colonies secreting specific antibodies were identified by IFA. Out of these, 40 cell lines were selected for subcloning and ascitic fluid production.

MAbs were characterized by IFA using OCK cells infected with 3 BD, 11 BVD and 7 HC virus strains. The results are summarized in Table 2. Only three of these MAbs were found to neutralize viral infectivity in seroneutralization (SN) assays performed using 19 Pestivirus strains (Table 3). When the MAbs were used in radioimmunoprecipitation (RIP) assays on BVDV-infected cell extracts, we mainly detected two distinct classes of antibodies (Fig. 1): class 1 MAbs reacted with the 80 kDa protein exclusively present in the BVD cytophatic virus strains (Donis and Dubovi, 1987), whereas class 2 MAbs reacted with glycosylated polypeptides, as shown by the endoglycosidase F treatment of the NY immunoprecipitated material.

The RNA genome of the cytopathic Osloss isolate of BVDV has been previously cloned and the nucleotide sequence determined (Renard et al., 1985). The pUR290 plasmid (Rüther and Müller-Hill, 1983) was used to express the 80 kDa protein-coding region as a fusion protein with the β-galactosidase (Renard et al., 1985); the fusion protein is easily purified as inclusion bodies, that can be denatured in urea and renatured by successive dialysis steps.

The purified recombinant fusion protein was tested by indirect ELISA for the detection of BVDV antibodies in bovine sera, and the results were compared with the virus neutralizing activity of these samples. Fig. 2 illustrates an indirect ELISA performed on several cattle sera that were previously iden-

TABLE 3

Neutralization titres of MAbs. SN titres are recorded as the logarithm of the highest dilution inhibiting 50% initial plaque formation

MAbs \ VIRUS STRAINS	AV	BDc	BDnc	8875	331	NADL	LAMSPRINGE	OSnc	NY	OREGON	NEBRASKA	0321	7443	OSc	SINC	SINnc	A49	ALFORT	THIVERVAL
SS AV5 216 E7	5.2	5.1	5.7	5.6	5.4	3.7	3.8	4.8	-	2.3	3.7	5.8	3.6	4.3	5	-	2.3	-	-
SS BD4 204 B11	4.1	4.3	4.5	4.4	3	1.8	3.4	3.4	1.8	3.3	2.2	3.7	3.4	2	3.9	2.7	-	-	-
SS BD4 209 D3	3.6	3.3	4.5	3.5	2.6	2	2.4	3.4	-	3.3	1.3	3.5	2.5	2.2	4	-	-	-	-
SS BDnc 137 E9	-	-	-	ND	-	1.7	2.4	-	-	-	1.3	-	2.3	-	-	-	2.3	-	-
SS AV5 113 C7	-	1.7	-	ND	-	-	2.1	-	-	2.3	-	-	-	-	2.5	-	-	-	-
SS BDnc 134 G11	-	1.8	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	2.3	2	2.3
SS BDc 127 F2	-	1.7	-	ND	-	1.5	-	-	-	-	1.4	-	-	-	-	-	-	-	-
SS AV5 212 F8	2.4	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SS BD4 202 D12	2.3	1.9	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SS BD4 202 E8	-	-	-	ND	-	-	-	-	-	-	-	-	-	-	2.5	-	-	-	-
SS BDnc 135 F12	-	-	-	ND	-	1.7	-	-	-	-	1.3	-	-	-	-	-	-	-	-
SS BDnc 131 C9	-	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SS BD4 101 F8	ND	3	4	ND	4.1	3.3	ND	3.2	-	-	ND	ND	ND	2.3	ND	ND	ND	2	ND

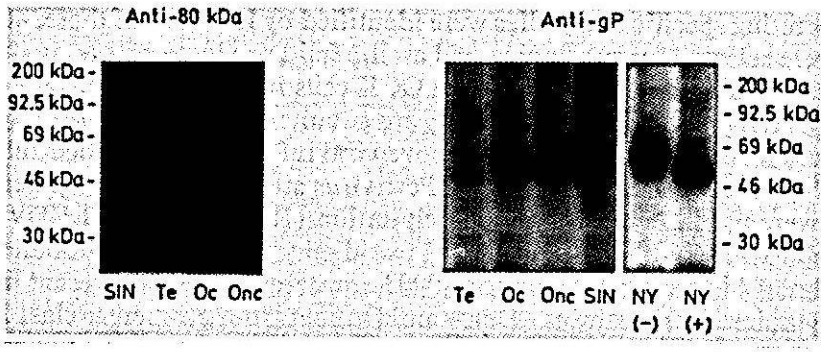


Fig. 1. Use of MAbs in RIP assays. Te: non infected OCK cells; SIN: Singer BVDV isolate; Oc: cytopathic Osloss BVDV isolate; Onc: non-cytopathic Osloss BVDV isolate; NY: New-York BVDV isolate treated with endoglycosidase F (+) or left untreated (−).

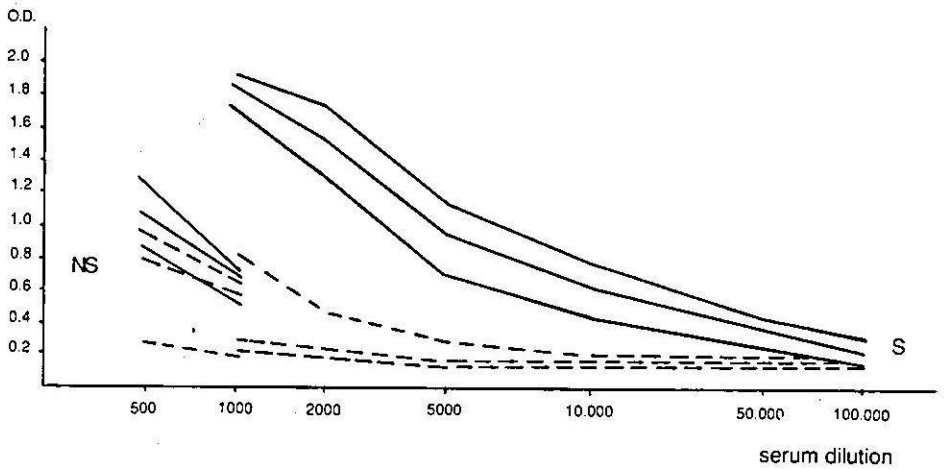


Fig. 2. Detection of BVDV antibodies by indirect ELISA, using recombinant antigen. S: neutralizing sera; NS: non-neutralizing sera. Continuous lines correspond with the fusion protein and dotted lines with the control antigen (β-galactosidase).

tified as positive (S) or negative (NS) in a SN assay carried out against the Singer BVDV isolate. The results of this ELISA show that the recombinant antigen induces a non-specific answer with non-neutralizing bovine sera, probably due to the presence of the β-galactosidase in the fusion protein.

Nevertheless, this problem can be circumvented by performing a competitive ELISA with the class 1 MAbs (Fig. 1). Fig. 3 illustrates such an ELISA on several bovine sera. In this competitive ELISA using recombinant antigen and class 1 MAbs, we observe that the results obtained for a number of cattle sera are quite similar to those obtained from a competitive ELISA using the same MAbs and an antigen extracted from BVDV-infected cells (Fig. 4).

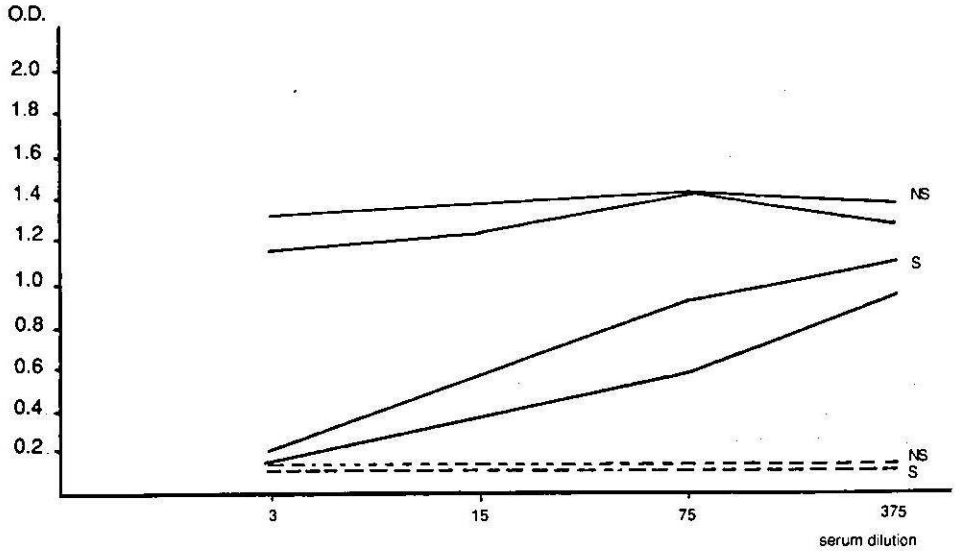


Fig. 3. Competitive ELISA using class 1 MAbs and recombinant antigen. S: neutralizing sera; NS: non-neutralizing sera. Continuous lines correspond with the fusion protein and dotted lines with the control antigen (β-galactosidase).

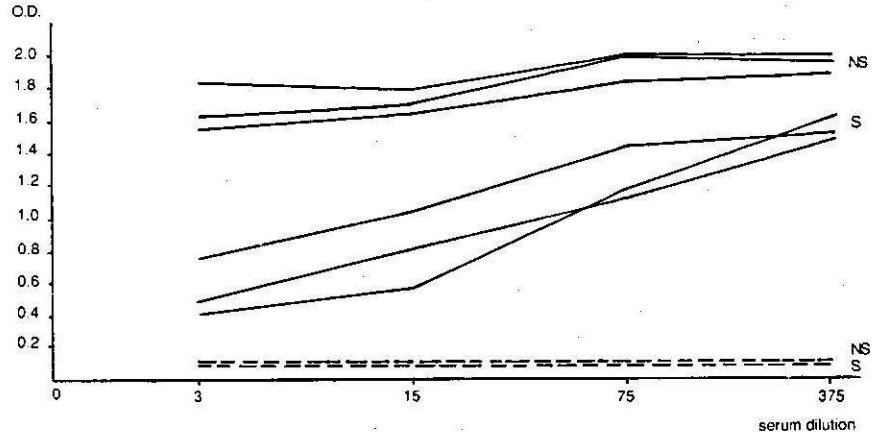


Fig. 4. Competitive ELISA using class 1 MAbs and antigen extracted from BVDV-infected cells. S: neutralizing sera; NS: non-neutralizing sera. Continuous lines correspond with the fusion protein and dotted lines with the control antigen extracted from non-infected cells.

CONCLUSIONS

We have prepared a panel of monoclonal antibodies that were characterized and classified by IF, SN and RIP assays. Most of these recognize the 80

kDa protein that is exclusively present in the BVD cytopathic virus strains (Donis and Dubovi, 1987). These MAbs were used in a competitive ELISA for the detection of BVDV antibodies in cattle sera: the ELISA antigen being a recombinant protein expressed in *E. coli* as a fusion protein with the β-galactosidase. This assay is highly specific and compares well with a similar ELISA that uses an antigen extracted from BVDV-infected cells.

REFERENCES

Aynaud, J.M., 1968. Etude de la multiplication en cycle unique d'un clone du virus de la peste porcine classique au moyen de l'immunofluorescence. Ann. Rech. Vet., 1:25–36.

Bronlie, J., 1985. Clinical aspects of the bovine virus diarrhea/mucosal disease complex in cattle. In Practice, 7: 195–202

Brownlie, J., Clarke, M.C. and Howard, C.J., 1984. Experimental production of fatal mucosal disease in cattle. Vet. Rec., 114: 535–537.

Chu, H.-J., Zee, Y.C., Ardans, A.A. and Dai, K., 1985. Enzyme linked immunosorbent assay for the detection of antibodies to bovine viral diarrhea virus in bovine sera. Vet. Microbiol., 10: 325–333.

Collett, M.S., Anderson, D.K. and Retzel, E., 1988. Comparisons of the Pestivirus Bovine Viral Diarrhea Virus with members of the Faviviridae. J. Gen. Virol, 69: 2637–2643.

Donis, R.O. and Dubovi, E.J., 1987. Differences in virus-induced polypeptides in cells infected by cytopathic and noncytopathic biotypes of bovine virus diarrhea-mucosal disease virus. Virology, 158: 168–173.

Fazekas de St. Groth, F. and Scheidegger, D., 1980. Production of monoclonal antibodies: strategy and tactics. J. Immunol. Methods, 35: 1–21.

Fernelius, A., 1964. Noncytopathic bovine viral diarrhea viruses detected and titrated by immunofluorescence. Can. J. Comp. Med. Vet. Sci., 28: 121–126.

Gillespie, J.H., Baker, J.A. and McEntee, K., 1960. A cytopathic strain of virus diarrhea virus. Cornell Vet., 50: 73–79.

Gutekunst, D.E. and Malmquist, W.A., 1963. Separation of a soluble antigen and infectious particles of Bovine Virus Diarrhea virus and their relationship to Hog Cholera. Can. J. Comp. Med. Vet. Sci., 27 no. 5.

Horzinek, M.C., 1981. In: T.W. Tinsley and F. Brown (Editors), Non-Arthropod-Borne Togaviruses. Academic Press, London.

Howard, C.J., Clarke, M.C. and Brownlie, J., 1985. An enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to bovine viral diarrhea virus (BVDV) in cattle sera. Vet. Microbiol., 10: 359–369.

Katz, J.B. and Hanson, S.K., 1987. Competitive and blocking enzyme-linked immunoassay for detection of fetal bovine serum antibodies to bovine viral diarrhea virus. J. Virol. Methods, 15: 167–175.

Klempnauer, K.-H., Ramsay, G., Bishop, J.M., Moscovici, M.G., Moscovici, C., McGrath, J.P. and Levinson, A.D., 1983. The product of the retroviral transforming gene v-myb is a truncated version of the protein encoded by the cellular oncogen c-myb. Cell, 33: 345–355.

Köhler, G., Howe, S.C. and Milstein, C., 1976. Fusion between immunoglobulin secreting and non-secreting myeloma cell lines. Eur. J. Immunol., 6: 292–295.

Liess, B., 1967. Die atiologische Abgrenzung selbständiger Virusinfecktionen, insbesondere der virusdiarrhoe-mucosal Disease im sogenannten "Mucosal-Disease-Komplex" bei Rindern. Dtsch. Tierärztl. Wochenschr., 74: 46–49.

Liess, B., Frey, H.-R., Orban, S. and Hafez, S.M., 1983. "Bovine virusdiarrhea (BVD)-mucosal disease". Persistente BVD Feldvirusinfektionen bei serologisch selektierten Rindern. Dtsch. Tierärztl. Wochenschr., 90: 261–266.

Launais, M., Aynaud, J.M. and Corthier, G., 1972. Swine fever virus: properties of a clone (Thiverval strain) isolated in cell culture at low temperature. Use in vaccination. Rev. Med. Vet., 123: 1537–1554.

McClurkin, A.W. and Coria, M.F., 1978. Selected isolates of bovine viral diarrhea (BVD) virus propagated on bovine turbinate cells: virus titre and soluble antigen production as factors in immunogenicity of killed BVD virus. Arch. Virol., 58: 119–128.

McClurkin, A.W., Littledike, E.T., Cutlip, R.C., Frank, G.H., Coria, M.F. and Bolin, S.R., 1984. Production of cattle immunotolerant to bovine viral diarrhea virus. Can. J. Comp. Med., 48: 156–161.

Mengeling, W.L. and Cheville, N.F., 1968. Host response to persistent infection with hog cholera virus. Proc. U.S. Livestock. San. Assoc. 72nd Annu. Meet., October 1968, pp. 283–296.

Pittler, H., Brack, M., Schulz, L.-Cl., Rohde, G., Witte, U. and Liess, B., 1968. Untersuchungen über die europäische Schweinepest. I. 1. Mitteilung: Ermittlungen zur gegenwärtigen Seuchensituation in Norddeutschland. Dtsch. Tierärztl. Wochenschr., 75: 537–542.

Renard, A., Brown-Shimmer, S., Schmetz, D., Guiot, C., Dagenais, L., Pastoret, P.P., Dina, D. and Martial, J.A., 1985. Molecular cloning, sequencing and expression of BVDV RNA. Seminar in the CEC programme of coordination of research on animal husbandry. J.W. Harkiness (Editor). Brussels, 10–11 September 1985.

Rossi, C.R. and Kiesel, G.K., 1971. Microtiter tests for detecting antibody in bovine serum to parainfluenza 3 virus, infectious bovine rhinotracheitis virus, and bovine diarrhea virus. Appl. Microbiol., 22: 32–36.

Ruckerbauer, G.M., Girard, A., Bannister, G.L. and Boulanger, P., 1971. Studies on bovine virus diarrhea: serum neutralization, complement-fixation and immunofluorescence. Can. J. Comp. Med., 35: 230–238.

Rüther, U. and Müller-Hill, B., 1983. Easy identification of cDNA clones. EMBO J., 2: 1791–1794.

Straver, P.J., Middel, W.G.J., Westenbrink, F. and de Leeuw, P.W., 1985. An ELISA for BVD virus serology. J.W. Harkness (Editor). CEC seminar, September 1985.

Westaway, E.G., Brinton, M.A., Gaidamovitch, S.Ya., Horzinek, M.C., Igarashi, A., Kääriäinen, L., Lvov, D.K., Porterfield, J.S., Russell, P.K. and Trent, D.W., 1985. Togaviridae. Intervirology, 24: 125–139.