Onderstepoort Journal of Veterinary Research, 62:241–244 (1995)

Detection of bovine-virus-diarrhoea-virus antibodies in cattle with an enzyme-linked immunosorbent assay

P. MUVAVARIRWA, D. MUDENGE, D. MOYO and S. JAVANGWE

Department of Paraclinical Veterinary Studies, Faculty of Veterinary Science
University of Zimbabwe, P.O. Box MP167, Mt Pleasant, Harare, Zimbabwe

ABSTRACT

MUVAVARIRWA, P., MUDENGA, D., MOYO, D. & JAVANGWE, S. 1995. Detection of bovine-virus-diarrhoea-virus antibodies in cattle with an enzyme-linked immunosorbent assay. *Onderstepoort Journal of Veterinary Research*, 62:241–244

The serum-neutralization (SN) and the indirect-immunofluorescence (IIF) assays have invariably been used for detecting antibodies against bovine virus diarrhoea virus (BVDV) in cattle sera. An enzyme-linked immunosorbent assay (ELISA) was applied which has a sensitivity comparable with the SN and IIF in detecting antibody to BVDV. A total of 472 bovine sera were assayed and a high prevalence of 79,2% was recorded. Positive correlations between the ELISA and the SN were found when certain sera were assayed, implying that the former test could then be used for routine diagnosis of BVDV.

Keywords: Antibodies, bovine-virus-diarrhoea-virus, BVDV, cattle, detection, enzyme linked, immunosorbent assay

INTRODUCTION

Bovine-virus-diarrhoea mucosal disease (BVD-MD) is a disorder of cattle and is characterized by abortion, mucosal disease (MD), teratogenic defects and clinically inapparent infections (Roeder & Drew 1984; Van Oirshot 1983). The aetiological agent, BVDV, is related serologically to other pestiviruses, namely border disease virus (BDV) of sheep and hog cholera virus (HCV) of pigs (Edwards, Sands & Harkness 1988). The most common feature of BVDV is its ability to cross the placenta and infect the foetus during early pregnancy. Subsequently, offspring are born that are persistently infected and immunotolerant to the infecting noncytopathogenic strains (Malmquist 1968; Roeder & Drew 1984; McClurkin, LittleDike, Cutlip, Frank, Coria & Bolin 1984). Such calves may develop MD (Malmquist 1968; Roeder & Drew 1984) when superinfected with a homologous cytopathogenic form of the virus (Brownlie, Clarke & Howard 1984).

Serological evidence indicates that infection with BVDV is very common in cattle (Harkness, Sands & Richards 1978). The SN, immunodiffussion and complement-fixation tests were used to detect antibodies to BVDV (Harkness *et al.* 1978; Hopkinson, Hart, Seger, Larson & Fulton 1979). Other tests such as the IIF (Giangaspero, Alders, Boer, Blondeel & Morgan 1991; Muvavarirwa & Munjeri 1992) and the ELISA (Howard, Clarke & Brownlie 1985; Chu, Zee, Ardans & Dai 1985) have since been used.

The aim of the present study was to screen various cattle sera for antibodies to BVDV with the ELISA. The ELISA was further compared with the SN and the IIF tests.

Accepted for publication 5 September 1995—Editor

MATERIALS AND METHODS

Bovine sera

A total of 472 bovine sera, including 306 samples obtained from the Central Veterinary Laboratory (Harare) were tested. About 50% of the farms where the sera were collected are located in the Matabeleland, Midlands and Masvingo provinces.

The rest were collected from farms located in the Mashonaland province of Zimbabwe. A minimum of ten sera were randomly collected from each farm and stored at –20°C. Sixty-two samples (3 weeks later, second serum samples were collected from 15 of these animals and were not included in data analysis) were collected from a herd (farm 19, Table 2) which was experiencing problems of infertility, abortion and the birth of weak, stunted calves, resulting in a loss of 15,5% (farmer's record).

Virus and cells

The cytopathic Oregon strain of BVDV was obtained from the Central Veterinary Laboratory (Harare) and was propagated in Madin-Darby-bovine-kidney (MDBK) cell lines. Monolayers, 1–2 d old, of MDBK cells were grown in 12 x 150 cm^2 tissue-culture flasks (Nunclon, Delta) with minimum essential medium (MEM) (Gibco), antibiotics, sodium bicarbonate in Hepes buffer and 10% (growth) or 5% (maintenance) horse serum. The cells were incubated under 7,5% carbon dioxide at 37°C.

Virus adsorption to the cells was carried out at 37°C for 2 h, after which 50 mℓ of MEM, supplemented with 5% horse serum, was added to each flask. Six flasks were inoculated with the virus while the remaining six were not infected (controls). The cells were incubated at 37°C for an additional 48 h. The media was tipped off and the cells were washed with 10 mℓ of phosphate-buffered saline (PBS) (pH 7,2). An additional 10 mℓ of PBS was added and a silicone-rubber policeman band was used to scrape off the cells from the flasks. The infected cells were sedimented by centrifugation at 500 *g* for 15 min and 2 mℓ of 1% Nonidet P-40 (BHD Chemicals Ltd) was added to the pellet to lyse and release the virus from the cells. After the cell suspension had been mixed, it was incubated for 60 min at 37°C and the aliquots were stored at –70°C as the stock BVDV antigen for ELISA. As required, samples were thawed, centrifuged for 15 min at 500 *g*, and the clear supernatant was removed and stored at 4°C for use as antigen. The non-infected cells were treated in a similar manner.

Enzyme conjugate

Horseradish peroxidase-conjugated immunoglobulin G (IgG) fraction of rabbit anti-bovine IgG (Nordic Immunological Laboratories Ltd) was divided into small aliquots and stored at –20°C until use.

Substrate

The colour reagent, 3´3´5´tetramethylbenzidine (TMB) (ICN Immunobiologicals) was prepared in a solution consisting of the following:

- 100 µℓ of stock TMB (100 mg of TMB powder + 10 mℓ of DMSO)
- 10 mℓ of TMB buffer [0,1M sodium-acetate buffer (pH 6) with 0,1M citric acid]
- 2 µℓ of 30% hydrogen peroxide

Controls

The positive control serum (BVD serum produced in gnotobiotic calf) was included in all assays. The number of units of antibody (5000) in this standard serum was taken as being equal to the titre. Both the positive and negative controls (serum from a gnotobiotic calf) were obtained from the Institute of Animal Health, Compton, UK.

ELISA procedure

Preliminary checkerboard titrations established the optimum concentration of antigen, positive control serum and enzyme conjugate. The ELISA method described by Howard *et al.* (1985) was followed, with minor modifications. For the assay, alternate rows of wells in the microtitre plate (Nunc-immuno-Plate Polysorp) were sensitized with 50 µℓ of diluted (1/100) extract of either BVDV-infected or non-infected cells. Plates were then incubated overnight at 37°C to dry the extract. One hundred microlitres of PBS containing 0,05% Tween 20 and normal pig serum (diluent) that was free from antibody to BVDV by the virus neutralization test, was used for blocking all wells for 30 min. All subsequent incubations were at room temperature (about 20°C), for 90 min, and all plates were washed five times with PBS containing 0,05% Tween 20. All dilutions of test and control (positive and negative) sera (1/500) and enzyme-coupled rabbit anti-bovine Ig (1/1000) were done by means of the diluent. The positive (standard curve) serum was further diluted to 1/5000. The diluted sera were later added to duplicate wells sensitized with BVDV or control antigen. The enzyme-coupled rabbit anti-bovine Ig was added, followed by the substrate, and the optical density (OD) was read at 492 nm after the reaction had been stopped with 50 µℓ of 1 M HCL, against a substrate blank on a Titerek Multiscan (Flow Laboratories). The corrected OD (average OD in wells with BVDV antigen minus average OD in wells with control antigen) was calculated, and the units of antibody read off the standard curve.

Standard curve

The standard-curve serum (same as positive control) was titrated for each plate from 1/100 (50 units); 1/300 (16,67 units); 1/900 (5,56 units), etc. The logarithms (log) of the units were determined. The corrected OD (OD in wells with BVDV antigen minus OD in wells with control antigen) was calculated (Table 1). These were plotted against the corresponding log units to produce the standard curve.

Determining cut-off points

Units of antibody in test sera ≤ negative control = true negative

Units of antibody in test sera ≤ 1/5000 dilution of positive sera = true positive

Units of antibody in test sera > negative control, but < 1/5000 dilution of positive sera = doubtful

Neutralization assay for antibody to BVDV

A standard microtitre assay (Frey & Leiss 1971) was used to measure antibody titres to the cytopathogenic BVDV (Oregon) strain in paired sera from farm 19. The titres (50% end points) were recorded as the reciprocal of the highest dilution that inhibited cytopathic effects due to 10 TCD 50 of strain Oregon.

Indirect immunofluorescence assay

The standard procedure for the IIF, described by Muvavarirwa and Munjeri (1992), was applied in the current study.

RESULTS

The BVDV antibody titres for each farm are illustrated in Table 2.

Altogether 472 sera were tested for antibodies to BVDV, and of these, 374 (79,2%) proved positive. Of these sera, 143/472 (30,2%) had very high antibody levels which were greater than 20 000 units.

No BVDV antibodies were detected in 60/472 (12,7 %) of the sera tested. Thirty-eight sera (8%) were considered to be doubtful cases.

Comparison of the ELISA and the SN

Table 3 illustrates a comparison of the levels of BVDV antibodies detected by the ELISA and SN in 15 serum samples that were collected on the first day from farm 19. The SN titres for the second serum sample are also illustrated.

Fifteen paired sera were tested, and a rise in antibody titres was clearly demonstrated in ten of the animals. Positive correlations were found between the SN titre and the ELISA titre. The correlation coefficients (*r*) were 0,455 (for all 15 of the paired sera tested); 0,576 (when sera with an SN titre ≤ 1 in 320 were excluded) or 0,824 (when sera with an SN titre ≤ 1 in 1 000 were excluded).

The *r* values were higher among the sera with relatively high SN titres.

Comparison of the ELISA and the IIF

The results of the ELISA and IIF tests (Table 4) were similar. Eight sera were positive for BVDV antibodies by both tests. One serum that was negative by IIF was considered doubtful by the ELISA.

TABLE 1 Log units and OD differences for one of the standard curves

Units	Log units (x)	OD = Ag(OD) – C(OD)(Y)
50,00	1,70	0,210
16,67	1,20	0,097
5,56	0,75	0,071
1,85	0,27	0,068
0,62	–0,21	0,049
0,21	–0,68	0,068*
0,07	–1,15	0,072*
0,02	–1,70	0,060*

* These OD values were ignored since they fell outside the standard curve

TABLE 2 Bovine-virus-diarrhoea-virus-antibody detection by the ELISA

Farm area	No. of animals	Positive	Negative	Doubtful
Beatrice	10	9	1	0
Nyamandlovu	10	8	2	0
Masvingo	10	8	1	1
Mvurwi 1	10	8	0	2
Bulawayo 1	10	8	0	2
Bulawayo 2	10	5	5	0
Bulawayo 3	10	7	2	1
Msinje 1	10	10	0	0
Msinje 2	10	10	0	0
Bulawayo 4	10	10	0	0
Mwenezi	20	14	4	2
Gweru	10	0	3	7
Mvurwi 2	20	13	3	4
Chivhu	20	20	0	0
Bulawayo 5	15	13	2	0
ABC	34	20	14	0
Chinamora	40	33	1	6
Chinhoyi	11	8	1	2
Norton	62	55	6	1
Mazoe	27	25	1	1
Harare	11	8	2	1
Shamva	74	62	10	2
Nharira	15	8	2	5
Marirangwe	13	12	0	1
Total	472	374	60	38

TABLE 3 SN antibodies on paired samples; a comparison of BVD antibody titres per units detected by the SN and the ELISA in serum samples collected on day 1

Animal	SN titre		ELISA units
No.	Day 1	3 weeks	Day 1
1	5 120	2 560	63 556
2	640	160	53 797
3	1 280	2 560	9 051
4	320	5 120	48 500
5	640	2 560	9 456
6	5 120	5 120	18 236
7	40	2 560	9 051
8	40	1 280	3 221
9	1 280	640	3 454
10	2 560	5 120	18 385
11	1 280	2 560	2 571
12	1 280	5 120	2 714
13	640	5 120	5 000
14	5 120	5 120	40 106
15	640	2 560	12 166

TABLE 4 Bovine-virus-diarrhoea-virus-antibody detection by the IIF and the ELISA

Test	Positive	Negative	Doubtful	Total
IIF	8	3	0	11
ELISA	8	2	1	11

DISCUSSION

Of the serum samples tested by the ELISA, 79,2% were positive for antibodies to BVDV. The current findings and those of Muvavarirwa & Munjeri (1992) and Bryant & Norval (1985) clearly indicate that BVDV infection is widespread in Zimbabwean cattle. The rise in antibody titres observed in paired sera collected from farm 19 was indicative of an active BVDV infection in that herd. However, the ELISA could be 100 times more sensitive than the SN in detecting certain levels of BVDV antibodies. For example, serum no. 4 had an SN titre of 1/320 and an ELISA titre of 1/48 500 (Table 3). The correlation between the SN and the ELISA tests was greater among sera with relatively high SN titres.

Howard *et al.* (1985) used the NADL strain of BVDV grown in calf testis cells and 2% foetal bovine serum. Apparently the ELISA remained sensitive despite the use of the Oregon strain of BVDV as antigen which was grown in MDBK cell lines supplemented with horse serum.

Since there is a difference in the antigenic specificities of the antibodies being measured by ELISA and SN (Bolton, Chu, Ardans, Kelly & Zee 1981), it is expected that ELISA, which detects antibodies against all viral components, is more sensitive than SN, which detects antibodies against viral neutralizing antigens only. The superiority of ELISA in detecting BVDV antibodies, as revealed here, has made it a suitable replacement routine diagnostic method for rapidly identifying immunologically compromised cattle in danger of developing clinical MD.

ACKNOWLEDGEMENTS

The University of Zimbabwe Research Board and the European Union are acknowledged for funding this project. The Central Veterinary Laboratory and the farming community are thanked for their assistance in collecting serum samples. Lastly, the Director of the Institute of Animal Health (Compton, UK) is thanked for the technical assistance provided by Prof. Brownlie's team and also for the provision of some of the reagents.

REFERENCES

BOLTON, D.C., CHU, H.J., ARDANS, A.A., KELLY, B. & ZEE, Y.C. 1981. *Veterinary Microbiology,* 6:265–279.

BROWNLIE, J., CLARKE, M.C. & HOWARD, C.J. 1984. *Veterinary Record:* 535–536.

BRYANT, B.A. & NORVAL, R.A.I. 1985. *Zimbabwe Veterinary Journal,* 16:9–17.

CHU, H.J., ZEE, Y.C., ARDANS, A.A. & DAI, K. 1985. *Veterinary Microbiology,* 10:325–333.

EDWARDS, S., SANDS, J.J. & HARKNESS, J.W. 1988. *Archives of Virolology,* 102:197–206.

FREY, H.R. & LIESS, B. 1971. *Zentralblatt für Veterinär Medizine,* Reihe B., 18:61–71.

GIANGASPERO, M., ALDERS, R., BOER, J., BLONDEEL, H. & MORGAN, D. 1991. *Zimbabwe Veterinary Journal,* 22:57–61.

HARKNESS, J.W., SANDS, J.J. & RICHARDS, M.S. 1978. *Research in Veterinary Science,* 24:98–103.

HOPKINSON, M.F., HART, L.T., SEGER, C.L., LARSON, A.O. & FULTON, R.W. 1979. *American Journal of Veterinary Research,* 40:1189–1191.

HOWARD, C.J., CLARKE, M.C. & BROWNLIE, J. 1985. *Veterinary Microbiology,* 10:359–369.

MALMQUIST, W.A. 1968. *Journal of American Veterinary Medical Association,* 152:763–770.

McCLURKIN, A.W., LITTLEDIKE, E.T., CUTLIP, R.C., FRANK, G.A., CORIA, M.F. & BOLIN, S.R. 1984. *Canadian Journal of Comparitive Medicine,* 48:156–161.

MUVAVARIRWA, P. & MUNJERI, N. 1992. *Zimbabwe Veterinary Journal,* 23:137–142.

ROEDER, P.L. & DREW, T.W. 1984. *Veterinary Record,* 114:309–313.

VAN-OIRSHOT, J.T. 1983. *Veterinary Microbiology,* 8:321–361.