APPLICATION OF ELISA TO DETECT BLUETONGUE VIRUS GROUP INFECTION

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ABSTRACT

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A competitive enzyme-linked immunosorbent assay (C-ELISA) using a group specific monoclonal antibody (MAb) against the bluetongue viruses (BTV) was established to detect antibodies against BTV. Sera from sentinel cattle in West Java were tested. The results of the C-ELISA were compared with the classical method for detection of antibodies to the bluetongue group, the agar gel immunodiffusion test. Increased specificity was observed using the Mab in the BTV C-ELISA. Not all sera that reacted in the AGID test reacted in the C-ELISA, and this was interpreted as the elimination of cross-reactions to other orbiviruses, including epizootic haemorrhagic disease of deer viruses, that are a problem in the agar gel immunodiffusion (AGID) test. Increased sensitivity with this test was also observed as seroconversions to BTV were detected earlier by C-ELISA than with the AGID. The C-ELISA not only has increased sensitivity and specificity, but also cost less, and can be used for sera from all species, and should replace the AGID test for bluetongue serology.

Kay words: Elisa, bluetongue virus

ABSTRAK

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Kompetitif enzyme-linked immunosorbent assay (C-ELISA) dengan menggunakan antibodi monoklonal (Mab) terhadap virus bluetongue diterapkan untuk mendeteksi antibodi virus bluetongue terhadap serum berasal dari sentinel sapi di Jawa Barat. Hasil C-ELISA dibandingkan dengan uji agar immunodifusi. Hasil menunjukkan bahwa uji C-ELISA selain lebih sensitif dari uji agar immunodifusi, C-ELISA juga lebih spesifik. Tidak semua serum yang bereaksi dengan uji AGID juga akan bereaksi dengan uji C-ELISA. Hal ini merupakan reaksi silang diantara orbivirus seperti Epizootic haemmorhagic disease of deer, dimana reaksi silang tersebut merupakan sebuah masalah pada uji AGID. Pada uji C-ELISA reaksi silang di antara orbivirus itu sendiri dapat dieliminir. Peningkatan sensitrifitas uji C-ELISA diamati dengan terdeteksinya serokonversi terhadap BTV lebih awal dari pada dengan uji AGID. Selain uji C-ELISA lebih sensitip dan spesifik, uji ini juga relatip lebih murah dan dapat digunakan untuk mendeteksi BTV antibodi dari berbagai spesies. Sehingga uji ini dapat menggantikan uji AGID untuk uji serologi BTV.

Kata kunci: Elisa, virus bluetongue

INTRODUCTION

The serological systems used to detect antibodies to bluetongue viruses (BTV) are firstly group specific tests, which detect group specific antibodies such as the agar gel immunodiffusion (AGID), complement fixation (CF), enzyme linked immunosorbent assay (ELISA) or fluorescence antibody technique (FAT) tests (Della-Porta *et al.* 1985); and secondly, type specific tests, that are used to serotype the antibodies. The latter include serum neutralisation (SN) and plaque reduction (PR) tests (De Villiers, 1974). Twenty four BTV serotypes have been distinguished by SN or PR tests (Knudson, 1986). However, cross-reactivity between BTV serotypes and other orbiviruses is found in group reactive serological tests (Della-Porta *et al.* 1985, Campbell *et al.* 1985). At present the CF and AGID tests are widely used to detect BTV group antibodies (Jochim, 1985).

To overcome poor specificity and sensitivity, an indirect ELISA was investigated (Manning & Chan, 1980). However, this test gave similar results to the AGID test, in that it did not eliminate cross-reactions between orbiviruses (Anderson, 1984). A further problem was purification of antigen, complicated by the highly cell-associated nature of the virus (Anderson, 1985). Monoclonal antibodies (MAb) have been produced to a wide array of antigens, including MAbs with predefined specificity for group-specific BTV target antigenic epitopes (Anderson, 1984; Lunt *et al.*, 1988). Sensitive and specific tests have hence been developed using such MAbs in competition with naturally occurring BTV antibodies for binding to BTV antigens in competitive ELISAs (C-ELISA). This paper will describe the application of such a C-ELISA using a group specific MAb to BTV in detection of BTV antibodies in Indonesian cattle in West Java, and will discuss briefly

some advantages of using a C-ELISA in place of the AGID test.

MATERIALS AND METHODS

Test sera

Sentinel cattle were established at Cisarua, high altitude area (1300 m), and Depok, low altitude area (70 m) in West Java as described by Sendow *et al.* (1989). The cattle were bled at monthly intervals.

AGID test

The AGID test was based on that described by Della-Porta *et al.* (1983). BTV 20 (CSIRO19) antigen and positive reference cattle serum were supplied by the New South Wales Department Agriculture, Elizabeth MacArthur Agricultural Institute, Camden, New South Wales, Australia.

C-ELISA

Sera were tested by C-ELISA using BTV-specific, group-reactive MAb 3-17-A3 and BTV antigen supplied by the International Atomic Energy Agency - FAO (Vienna). The procedure used was that described by Anderson (1984) with some small modifications.

In summary, polystyrene 96 well ELISA grade microtitre plates (Nunc) were coated with antigen in phosphate buffered saline (PBS, pH 7.2) at 37$C for two hours and one hour at room temperature with shaking. Plates were washed between steps with PBS containing 0.05% Tween 20. Sera was tested at 1:4 dilution in blocking buffer (PBS containing 1% skim milk, Difco). Control positive and negative (foetal calf serum, FCS) samples were included in the test. Diluted MAb was added to each well within 10 minutes of adding the test sera. The plates were incubated at 37$C for one hour, with occasional shaking. The plates were washed and bound MAbs detected using on a goat anti-mouse IgG (H-L) conjugated with horseradish-peroxidase (Bio-Rad). Following an hour incubation at 37$C, the conjugate was detected using 3,3',5,5' tetra methyl benzidine (TMB) and the plate read at 450 nm. Results were expressed as percentage inhibition which was calculated as follows:

$$\% \text{ inhibition} = \{1-(OD_{\text{test serum}}/OD_{FCS})\} \times 100\%$$

If the percentage inhibition of the tested serum was 40% it was considered positive.

RESULTS

At the beginning of observation at Depok, low altitude, (Table 1), all of the animals had antibodies in the AGID test, and all of these animals were less than 3 months of age. Two animals (D16 and D19) remained reactive for 6 months while the titre of the others declined below detectable limits. Seroconversions occurred starting in October (D04) and November (D13), but only with low titres in the AGID test. However, in February and March, all the cattle showed seroconversion with higher titres which persisted for several months. Using C-ELISA, seroconversion started in November (D13) and January (D12), but most animals seroconverted in March.

At the high altitude site at Cisarua (Table 2) none of the sera reacted in the AGID test at the beginning of observation period. These animals were 3 - 5 months old on the first bleed. However, 6 animals were positive in the C-ELISA and titre of antibodies declined to 40% inhibition and increased again in June (C24). However, using the AGID test, three animals seroconverted in May and June.

Sentinel goats (5) and sheep (7) held at Cikidang, high altitude (700 m) area did not seroconvert when tested by both the C-ELISA and AGID test during the 12 month period.

Table 3. shows the comparison of the C-ELISA and AGID test results derived from data in Table 1 and Table 2. Forty six sera were positive in the C-ELISA, but negative in the AGID test, whereas only four sera were AGID positive but negative in the C-ELISA.

DISCUSSION

The results presented in Table 3 indicated that the C-ELISA was more sensitive in detecting lower titre of antibodies than in the AGID. This conclusion is supported by data in Tables 1 and 2. Animals loosing their maternal antibodies were positive longer in the C-ELISA than the AGID test, for example D04, D07, D12, D15 and D45 (Table 1), and became C-ELISA positive sooner than they became AGID positive following seroconversion (Table 1, D12 and D45).

As shown in Table 1, calves D07, D15 and D45 gave positive reactions with the AGID test for only 3 months, while they were positive until 6 months using the C-ELISA. These animals were 2-3 weeks old when first bled and were presumed to have maternal antibodies.

The data in Table 2 indicates that calves C24, C29, C30 and C43 were reactors in the C-ELISA, but not in the AGID test. These animals were more than 3 months old on the first bleed. These results also indicate that C-ELISA detected maternal antibodies longer than the AGID test.

In this study it was not determined whether SN-antibodies persisted longer than those detected by AGID as a full panel of all BTV serotypes was not available in this laboratory. However, the results of the sentinel calves were consistent with those reported in experimental infection by Afshar *et al.* (1988). They found that C-ELISA can detect maternal antibody up to 6 months while in the AGID test they were detected for only 3 months.

Animal D12 and D45 in Table 1 also showed that C-ELISA detected BTV antibodies earlier than the AGID test. This result is consistent with that observed by Afshar *et al.* (1988) with experimental infection.

Table 3 also indicated that 4 serum samples were positive in the AGID, but negative in the C-ELISA. It is possible that the C-ELISA may detect antibodies to other orbiviruses. Antibodies to related orbiviruses including EHDV, Palyam or Eubenangee viruses will react with BTV antigen in the AGID test (Della-Porta *et al.*, 1983; 1985). Anderson (1984) demonstrated that a C-ELISA using MAb 3-17-A3 in competition with antibodies to 22 serotypes of BTV resulted in high levels of inhibition of the monoclonal reactivity, whereas antisera to EHD viruses gave little or no inhibition even at low serum dilutions. Anderson (1985) also demonstrated that, unlike the AGID test and indirect ELISA, there were no problems due to antibodies to cellular proteins reacting with the antigen used in the C-ELISA.

Table 1. Comparison of C-ELISA and AGID test for detecting antibody in sentinel cattle at Depok, West Java

Animal No.	Jun		Jul		Aug		Sep		Oct		Nov		Dec		Jan		Feb		Mar	
	A[1]	E[2]	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E
D04	1+	+	1+	+	–	+	–	+	1+	+	2+	+	2+	+	1+	+	–	+	3+	+
D07	3+	+	2+	+	1+	+	–	+	–	+	–	+	–	–	–	–	–	–	3+	+
D12	2+	+	1+	+	–	+	–	+	–	+	–	–	–	–	–	+	–	+	2+	+
D13	2+	+	1+	+	–	+	–	+	–	–	1+	+	1+	+	–	–	–	–	3+	+
D15	3+	+	2+	+	2+	+	–	+	–	+	–	+	–	–	–	–	–	–	1+	+
D16	3+	+	3+	+	3+	+	3+	+	3+	+	2+	+	2+	+	1+	+	–	+	2+	+
D17	1+	+	2+	+	1+	+	1+	+	1+	+	2+	+	–	+	–	+	1+	+	3+	+
D18	3+	+	1+	+	1+	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–
D19	1+	–	2+	+	3+	+	1+	+	1+	+	2+	+	2+	+	1+	+	2+	+	1+	+
D45	NA[3]		3+	+	1+	+	–	+	–	+	–	+	–	+	–	–	–	+	3+	+
D46	NA		1+	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	3+	+
D47	NA		–	+	1+	+	2+	+	1+	+	2+	+	1+	+	–	+	2+	+	3+	+

1. AGID
2. + C-ELISA ≥ 40% inhibition; – C-ELISA < 40% inhibition
3. Not available

Table 2. Comparison of C-ELISA and AGID test for detecting antibody in sentinel cattle at Cisarua, West Java

No. Animal	Jun		Jul		Aug		Sep		Oct		Nov		Dec		Jan		Feb		Mar		Apr		May		Jun	
	A[1]	E[2]	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E	A	E
C23	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C24	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1+	–	2+	+
C25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C29	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C30	–	+	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C31	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C34	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3+	–
C37	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3+	–
C39	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C43	–	+	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

1. AGID
2. + C-ELISA ≥ 40% inhibition; – C-ELISA < 40% inhibition.

Table 3. Comparison of BTV reactivity in the C-ELISA and AGID test

		ELISA	
	Status	+	-
AGID	+	65	4
	-	46	171
	Total	111	175

Compilation of data for cattle in Tables 1 and 2

The four sera that reacted in the AGID test gave 40% inhibition in the C-ELISA. Monoclonal antibodies react more specifically in immunoassays than do polyclonal antibodies due to the fact that MAbs are specific for a single epitope on the target antigen. On the other hand, a polyclonal serum reacts with several different epitopes on the target antigen. This is the reason why an indirect ELISA using polyclonal antibodies cannot eliminate cross reactions between orbiviruses, and thus high levels of non-specific reactions may be observed. Similarly the properties of the MAb means that crude antigenic preparations can be used with the C-ELISA without affecting the specificity of the system (Anderson 1985). Also the use of anti-mouse enzyme conjugate for detection of bound murine MAb in the final step of the C-ELISA makes it possible to test sera from different species, including wild ruminants for which no anti-species conjugates are commercially available.

In comparison with the SN test, the C-ELISA can also detect antibodies in sera contaminated lightly with bacteria or fungi, or containing material which is toxic to cell cultures. This test is also a rapid test compared to the AGID and SN tests, taking less than 8 hours.

In conclusion, the C-ELISA can detect BTV group antibody from different species and can be used to replace the AGID test which is more expensive. However, the sensitivity and specificity of this test must be further evaluated using animals of known infection status.

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