PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST GROUP-SPECIFIC BLUETONGUE VIRUS ANTIGEN

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Summary. **Monoclonal antibodies (MAbs) against the group-specific antigen of Bluetongue virus were developed using Bluetongue virus serotype 4. The hybridomas were obtained from immunized BALB/c spleen cells fused with myeloma cells line Sp2/O-Ag14 using polyethylenglycol 1500, and cloned by limiting dilution. The supernatant from eight MAbs was tested against group-specific antigens used in agar gel immunodiffusion tests by different laboratories. The eight monoclonal antibodies presented good reactions to the group-specific antigen. Therefore, these MAbs have great potential for application in the identification of group-specific antibodies by competitive ELISA tests.**

Bluetongue (BT) is a viral disease that affects mainly sheep and other domestic and wild ruminants. It is widely distributed throughout the African, American, and Australian continents, the Middle East and Southeast Asia. It is transmitted by hematophagous insects, and mosquitoes of the genus Cullicoides constitute its main vectors *(14)*.

The Bluetongue virus (BTV) belongs to the *Reoviridae* family, genus Orbivirus. Based on the virus-neutralization tests, 24 BTV serotypes are recognized *(4)*. The viral genome consists of 10 segments of double-stranded RNA, each one of them coding for at least one viral polypeptide. Five proteins (P1, P3, P4, P6, and P7), out of seven composing the double layered viral particle form the core. The core is surrounded by a diffuse, outer capsid layer composed of P2 and P5 *(15)*.

To date, the functions of the nonstructural proteins NS1, NS1a, NS2, NS3, and NS3a, identified in infected cells as BTV have not been clarified *(13)*.

Polypeptide P2, the main serotype-specific antigen of BTV is responsible for viral neutralization. Together with P5, they are the proteins with the largest variation amongst BTV serotypes *(13, 15)*.

One of the major group-specific proteins of BTV is the P7 polypeptide. It is the most conserved, highly hydrophobic, may be presented in polymeric forms and has at least one epitope in the surface of the viral particle *(4, 9, 10, 11)*.

The serological diagnosis of BTV is carried out mainly by immunodifusion in agar gel (IDAG) test. This test, despite being simple and practical, presents a low sensitivity, cross reactions with other orbiviruses and its interpretation is subjective *(1)*. On the other hand, the competitive enzyme-linked immunosorbent assay (ELISA), using MAbs presents a higher sensitivity and specificity in the detection of antibodies against BTV and does not reveal cross-reactions with other orbiviruses *(3)*. On this basis, FAO/IAEA suggested the International Office of Epizootics (OIE), the international standardization of this ELISA test for the detection of group-specific antibodies against BTV *(5)*.

The present paper describes the production of MAbs against group-specific antigens (GSA),

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using BTV serotype 4, and its partial characterization against GSA used in the serological diagnosis of BTV by IDAG.

BALB/c mice (6-8 weeks old) and BTV serotype 4 were used *(8)*. The inoculum consisted of $10^{4.09}$ LD_{50}/ml of a second suckling mouse brain passage in 0.3ml, inoculated intraperitoneally at 10 day-intervals. The first inoculation consisted of equal parts of a viral suspension and Freund's complete adjuvant. A viral suspension plus Freund's incomplete adjuvant, also in equal parts, was used in the second and third inoculations. For the fourth and fifth inoculations, only viral suspension was used.

Three days after the last inoculation the spleens from two mice were removed aseptically. They were washed and macerated in modified Dulbecco's medium (DMEM). Subsequently, the macerate was treated with ACK buffer (NH4CL, 0.15M; KHCO3 0.01M, Na2 EDTA 2 H2O 0.01M) for a minute in cold water and then neutralized with 40ml of DMEM containing 5% of fetal calf serum and 5 IU/ml of heparin. The suspension was centrifuged at 1000 g for 12 minutes at 4°C. The sediment was resuspended in DMEM medium to carry out lymphocyte counts.

In parallel, myeloma cells Sp2/O-Ag14 *(16)* at the logarithmic growth stage were washed with DMEM medium at a myeloma-lymphocyte ratio of 1:4. The myeloma cells and the lymphocytes were fused by adding 1 ml of polyethylenglycol (PEG) 1500 for every 180 million cells according to the method described by Galfre y Milstein *(6,7)*, modified by the Laboratory of Monoclonal Antibodies of PANAFTOSA.

After the fusion, the cells were cultivated in selective growth medium (DMEM, 4 mM of L-glutamine, 1 mM of sodium pyruvate) with 2% of HAT and 15% of fetal calf serum. The selection of positive hybridomas was carried out through the indirect ELISA test, in which the solid phase contained the GSA of BTV. This GSA was obtained from the supernatants of monolayer cultures of BHK-21 cells inoculated with BTV, according to the method described by Allende *et al.* *(2)*.

The positive hybridomes selected were cloned by limited dilution and cultivated in growth medium with 2% of HT and 15% of fetal calf serum.

Table 1. Monoclonal antibody isotype determination by immunodiffusion in agar gel.

Monoclonal antibody	K	-	IgG1	IgG1a	IgG2b	IgG3	IgM
38Ah11	+	-	-	+	-	-	-
38BC3	+	-	-	+	-	-	-
38BD11	+	-	-	+	-	-	-
38EC8	+	-	-	-	+	-	-
38ED6	+	-	-	+	-	-	-
38BE10	+	-	-	+	-	-	-
40FD8	+	-	-	+	-	-	-
40HD5	+	-	-	-	+	-	-

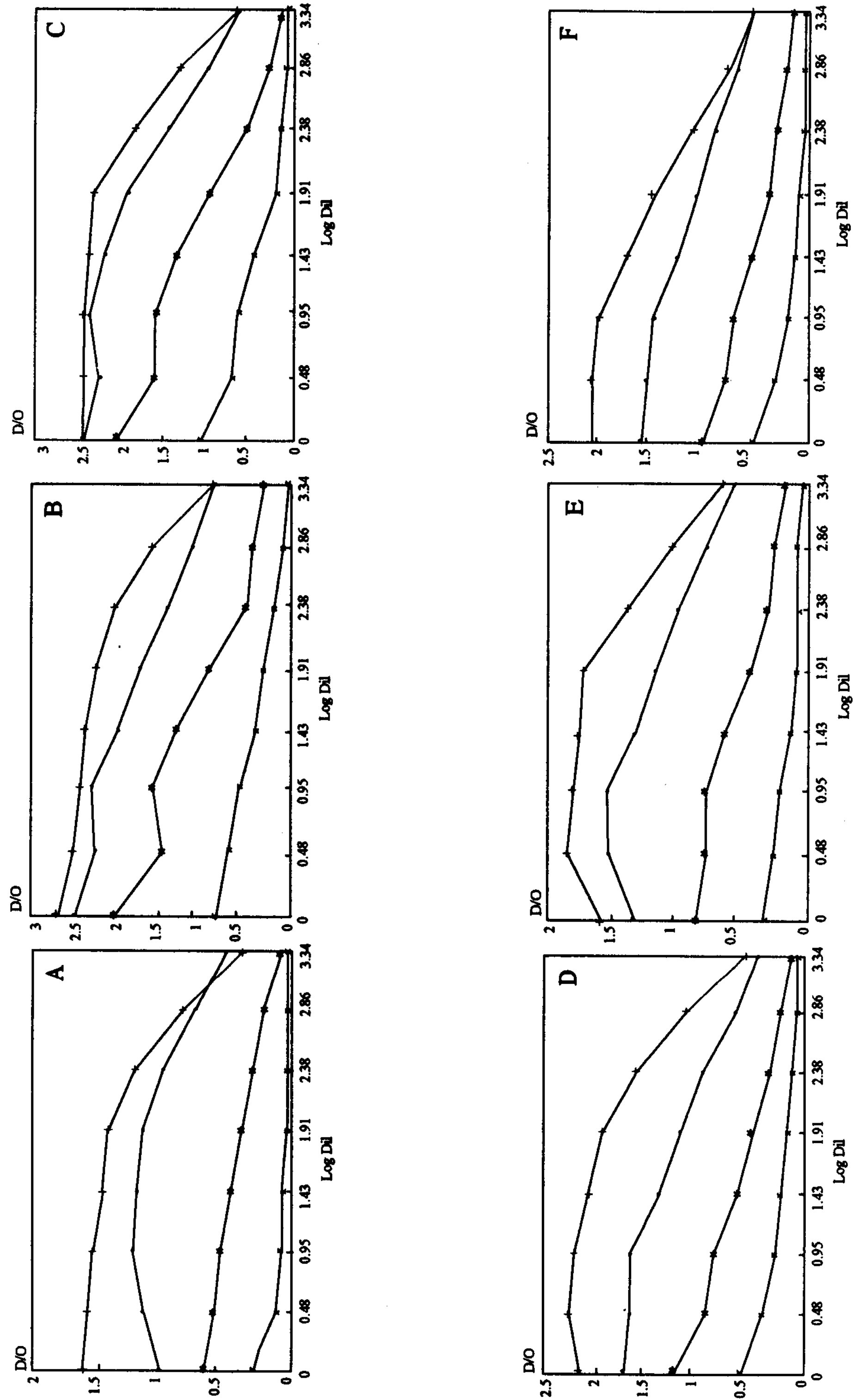
Indirect ELISA tests were used to assess the stability of cultures throughout all stages of cloning and amplification of hybridomes.

Eight MAbs were obtained from these fusions (Figure 1). In each case, the respective isotypes were determined by IDAG using specific antisera for each isotype (Table 1).

The eight MAbs were evaluated on the basis of their reactivity against four GSA produced and used routinely for the detection of BTV antibodies by IDAG in different laboratories: PANAFTOSA, Veterinary Diagnostic Technology, Inc., USA (VDT), LARA-Campinas, Sao Paulo (LARA) and National Veterinary Service Laboratory, USA, Ames, Iowa (NVSL). Each GSA embedded in the solid phase of an indirect ELISA test was cheeked against the eight MAbs (Figure 1). A hyperimmune mouse serum against BTV (Figure 1J) and a MAb 3-17-A3 (Figure 1I) specific for the group-specific protein (P7) of BTV were used as positive controls and a foot-and-mouth disease C_3 Indaial MAb as negative control *(3,5)* (Figure 1K).

The MAbs presented a higher reactivity to PANAFTOSA GSA due to the use of the homologous strain of BTV in the production of GSA at PANAFTOSA and at the LARA Laboratory.

The high reactivity of MAbs to GSA from VDT Laboratory is possibly accounted for, due to the use of more than one strain in the production of this antigen. The lower reactivity observed with the antigen from the NVSL may be explained by the use of a strain of BTV that presents small variations in


A
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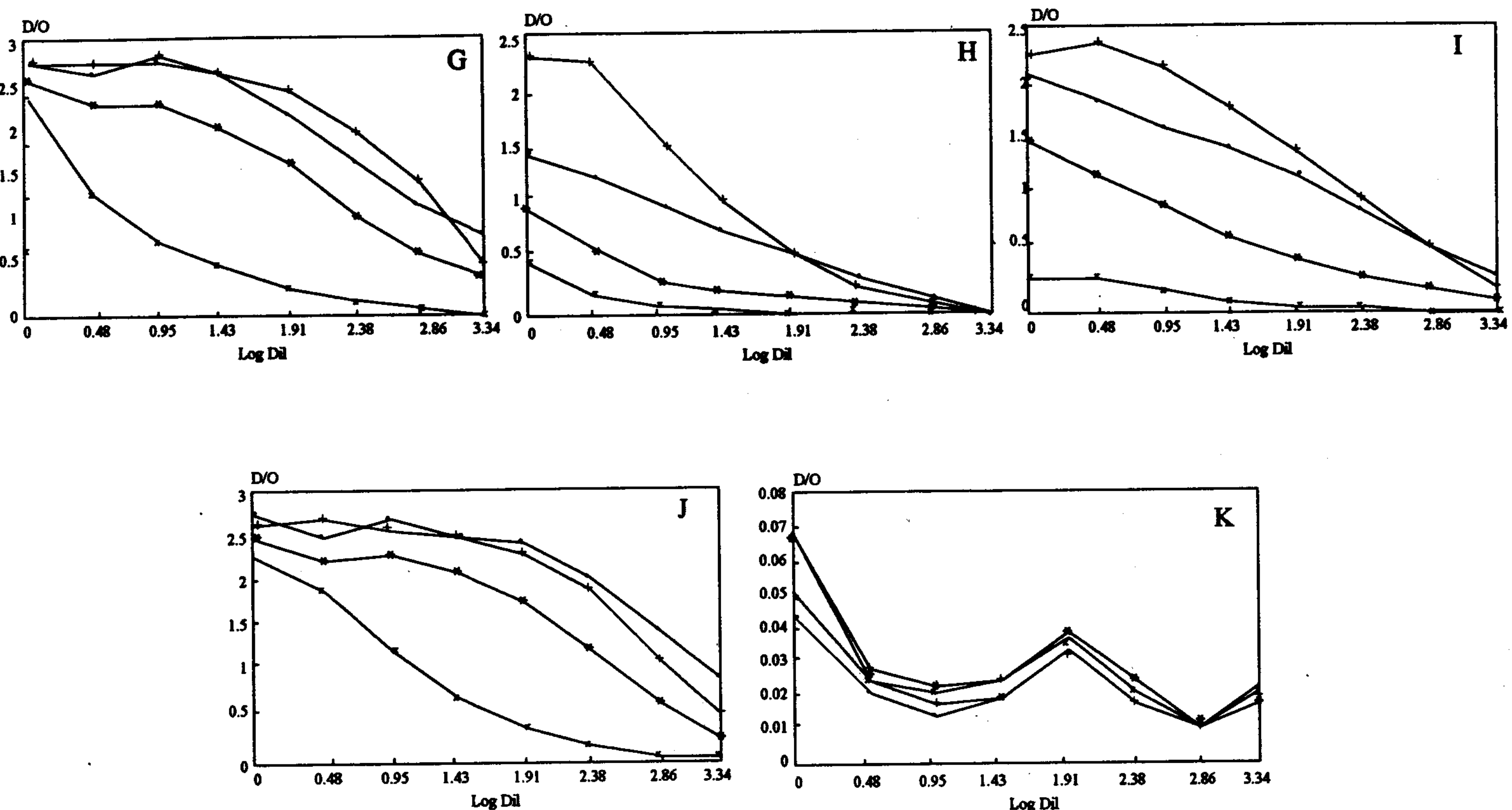

FIGURE 1. Reactivity of monoclonal antibodies to group specific antigens (PANAFTOSA, VDT, LARA, NVSL) by ELISA test. A 38ED6, B 38BC3, C 38BD11, D 38AH11, E 38EC6, F 39BE10, G 40FD8, H 40HD5, I 3-17-A3, J Positive Serum, K monoclonal antibody against foot-and-mouth disease virus.

+ PANAFTOSA • VDT * LARA X NVSL

the viral RNA segment that codes for the group-specific protein amongst the various BT serotypes, observed in strains isolated in different geographical regions *(12)*.

According to FAO/IAEA (5) in the competitive ELISA test, using MAbs is the most indicated to replace the IDAG, complement fixation, and immunofluorescence tests, in the serologic diagnosis of BT. The MAbs developed in the present study suggest a great potential for their use in competitive ELISA tests and in epidemiological studies of BT.

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