Detection of Bluetongue Virus by Using Bovine Endothelial Cells and Embryonated Chicken Eggs

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Received 19 June 1990/Accepted 23 October 1990

Two systems, inoculation of bovine endothelial cells and of embryonated chicken eggs, were compared for detection of bluetongue virus (BTV) in blood specimens from experimentally inoculated sheep. For all BTV serotypes tested, embryonated chicken eggs detected longer periods of viremia than did bovine endothelial cells, primarily by detecting BTV in samples containing lower virus concentrations.

Bluetongue virus (BTV) is an orbivirus that causes economic losses to domestic livestock producers (14). BTV can be difficult to isolate from animal specimens; intravascular inoculation of embryonated chicken eggs (ECE) is the recommended procedure (5, 9). ECE inoculation is labor intensive for trained personnel, requires large numbers of chicken embryos that can be difficult to obtain, and utilizes equipment not used in cell culture. Since cell culture assays are used routinely in diagnostic laboratories, different cell lines have been tested for the ability to detect BTV (2, 4, 7, 8, 13, 15). Recently, a cattle pulmonary artery endothelial (CPAE) cell line was reported to be more sensitive to BTV infection than other cell lines (17). The objective of the present experiment was to compare CPAE cells and ECE for detection of BTV in blood samples from experimentally infected sheep.

Sheep were inoculated with one of four U.S. serotypes. Three serotypes, BTV-10 (California BT-8), BTV-11 (Texas Station strain), and BTV-13 (72-1411), were utilized as whole sheep blood. BTV-2 (AL85B7) was used as a Vero cell suspension. For three of the four serotypes (BTV-10, BTV-11, BTV-13), two sheep were inoculated intradermally and subcutaneously with 200,000 median chicken embryo intravascular lethal doses (CEIVLD₅₀). For BTV-2, two sheep were inoculated with 10,000,000 CEIVLD₅₀. Heparinized blood samples were collected daily for 14 days after inoculation and then on days 17, 21, and 28 after inoculation. Blood samples taken after 10 days after inoculation were washed twice with phosphate-buffered saline (PBS; pH 7.2) to eliminate specific antibody.

Titrations of blood samples were conducted with serial 10-fold dilutions. For samples inoculated into ECE, infected blood cells were diluted in PBS and inoculated intravascularly into 11-day-old ECE (0.1 ml per egg; six egg-per-dilution) as previously described (11, 12), except titers were calculated by the Spearman-Karber method (3). Isolates were confirmed as BTV by specific immunofluorescence (6, 11).

For samples passaged in CPAE cells, serial 10-fold dilutions of blood samples were used in the first passage; the first dilution was made in distilled water to lyse erythrocytes, and subsequent dilutions were made in minimum essential medium (Flow Laboratories, McLean, Va.) with 10% fetal bovine serum (free of BTV antibody). Each dilution (0.05 ml) was placed into 6 replicate wells of 96-well plates to which cells (30,000 per well) were added. Plates were incubated for 7 days at 36°C with 5% CO₂ in humidified air. The number of wells with cytopathic effect after 7 days of incubation was recorded, and virus titer was calculated in 50% tissue culture infective doses (TCID₅₀) per milliliter by the Spearman-Karber method (3). For the next passage, contents of three replicate wells of the first 1:10 dilution of each sample were combined, inoculated into six replicate wells with fresh cells, and incubated. Any samples that were negative during the first passage but became positive during the second passage were assigned a titer of 0.2 log₁₀ (10-fold less than the lowest titer detectable on the first passage) to distinguish them from negative samples. Blood samples negative after two passages were retested by inoculating a freshly made 1:10 sample dilution onto 12 replicate wells. The presence or absence of BTV in all samples passed in CPAE cells was confirmed by immunoperoxidase staining (16). Immunoperoxidase detection of BTV in chicken embryo samples was not useful because of high background activity (data not shown).

CPAE cells detected the presence of BTV of each serotype during peak viremia (Fig. 1). However, CPAE cells were less sensitive than ECE to BTV in blood samples collected early and late in the infection period. BTV titers measured in CEIVLD₅₀ per milliliter and TCID₅₀ per milliliter of samples were significantly different (P ≤ 0.01) by the paired-difference t test (Abstat; Anderson-Bell, Parker, Colo.). Generally, BTV titers were at least 10-fold higher when expressed in CEIVLD₅₀ than when measured in TCID₅₀. A similar difference in BTV titer as determined by assay type was reported between infectious doses and PFU (16).

In diagnostic situations, only the success or failure of viral isolation, not the titer, is important. The difference in the sensitivities of CPAE cells and ECE to BTV in experimental sheep blood samples was especially obvious in blood samples with low BTV concentrations; for titers of BTV of ≤ 1, 2, 3, and ≥ 4 log₁₀ CEIVLD₅₀/ml, 17, 38, 74, and 100%, respectively, of samples positive for BTV in ECE were positive in CPAE cells. CPAE cells, however, did detect BTV in three samples that were negative for BTV in ECE. A similar variation in BTV detection among assay types occurs frequently (10). Since the majority of BTV in the blood is associated with erythrocytes (1, 12), it is unlikely that the difference in blood sample processing (sonication versus
distilled water lysis) caused the difference in the sensitivities of ECE and CPAE cells. Two procedural modifications improved BTV detection in CPAE cells. Ninety-four percent of all samples that were positive for BTV in CPAE cells were detected in the first passage; the rest were detected in the second passage. A third blind passage did not increase detection. Increasing the number of replicate wells from 6 to 12 detected 33\% more BTV-positive samples. Other attempts to improve BTV detection in CPAE cells, including low-speed centrifugation or addition of polybrene to medium, were unsuccessful.

The advantages of CPAE cells over ECE are as follows: incubation and handling conditions are those used for other cell lines, less labor is necessary, and fewer supplies and space are required. The significant disadvantage is that CPAE cells detected fewer positive blood samples than ECE, although in a few instances, the CPAE cells detected virus in samples not positive in ECE. Variation in BTV isolation among assay systems has been observed previously and led to the suggestion that several detection systems be used (10). Although CPAE cells are not as sensitive as ECE to BTV, these cells have been shown to be more sensitive than other cell lines (17) and can be useful for BTV detection.

We thank H. A. Rhodes for technical assistance.

FIG. 1. BTV titers in blood from experimental sheep inoculated with the indicated BTV serotype. Symbols: ○, mean BTV titer in log_{10} TCID_{50} per milliliter in CPAE cells (n = 2); ●, mean BTV titer in log_{10} CEIVLD_{50} per milliliter (n = 2). (A) Serotype 2; (B) serotype 10; (C) serotype 11; (D) serotype 13.

REFERENCES


