Membrane Concentration of Infectious Bovine Rhinotracheitis Virus from Water

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A membrane adsorption procedure was used to concentrate infectious bovine rhinotracheitis virus from 1-liter quantities of distilled water. Cellulose nitrate membrane filters (0.45-μm pore size) efficiently adsorbed this herpesvirus from water, and virus was recovered from the membrane by elution with 10 ml of fetal calf serum during sonic treatment. The average recovery rate was 70%.

Infectious bovine rhinotracheitis (IBR) is a disease of cattle that produces upper respiratory inflammation as well as several other syndromes, the most economically important of which being abortion (6). Diagnostic investigations for a 4-month period in 1971 indicated that 67.8% of the 370 fetuses submitted to the North Dakota State Veterinary Diagnostic Laboratory were aborted due to IBR (3).

The purpose of this investigation was to develop an IBR virus concentration procedure which could possibly be used to determine if this virus is present in livestock drinking water. The virus concentration methodology developed for this purpose was modified after numerous reported virus concentration techniques (1, 4, 5, 8, 9). (This study represents a portion of a thesis submitted by S. R. T. in partial fulfillment of the requirements for the M. S. degree in bacteriology at North Dakota State University, Fargo, 1974.)

MATERIALS AND METHODS

Virus. The Cooper strain of IBR virus used throughout this study was originally obtained from T. L. Chow, Colorado State University, Fort Collins. The virus was passed 17 times in bovine tissue culture and stored in sealed glass ampoules at −70°C.

Tissue culture. The MDBK cell line (ATCC-CCL 22) was used to detect virus plaque formation. Cell cultures were maintained in Blake bottles with Eagle minimal essential medium prepared in Hanks salt solution containing 0.5% lactalbumin hydrolysate, 10% inactivated (56°C for 30 min) calf serum, 100 U of sodium penicillin per ml, and 100 μg of streptomycin sulfate per ml.

Determination of PFU. Plaque-forming units (PFU) were determined by the procedures of Dulbecco (2) with the following modifications. Monolayers of MDBK cells, grown in Blake bottles, were dispersed with 100 ml of trypsin (0.25%) containing 0.2% ethylenediaminetetraacetate, and the cell suspensions were centrifuged for 10 min at 500 × g to pellet the cells. The cells were then suspended in minimal essential medium containing 10% calf serum and antibiotics. The cells were cultured in disposable tissue culture dishes (60 by 15 mm) at 37°C in a CO₂ incubator until confluent monolayers were formed. The minimal essential medium was then removed, and the monolayers were inoculated with 0.5-ml volumes of virus suspensions and the agar was overlaid (minimal essential medium with 1% ion agar). Upon development, plaques were resolved by staining the cells with a 1:1,500 neutral red solution.

Membrane concentration procedure. One-milliliter volumes of an IBR virus suspension (4 × 10⁴ PFU/ml) were diluted 1:1,000 in acetate-buffered (0.1 M, pH 6.5) water samples containing MgCl₂ (0.5 M). The addition of MgCl₂ was necessary to prevent virus loss during filtration (9). The samples were then filtered through type HA membrane filters (47-mm diameter, 0.45-μm pore size; Millipore Corp., Bedford, Mass.) to adsorb the virus. Virus was released by suspending the filters in a 10-ml volume of fetal calf serum which was then sonically treated in an ice bath with the standard probe of a Biosonic IV sonic oscillator (Bronwill Scientific, Rochester, N.Y.) at maximum output.

RESULTS AND DISCUSSION

No IBR virus was detected in any of the type HA membrane filtrates, indicating complete virus retention. An increase in adsorption pH from 3.0, a recommended pH for the adsorption of enteroviruses (5), to 6.5 maintained the viability of this herpesvirus yet did not impede virus adsorption. Recovery of the adsorbed viruses was accomplished by sonically treating the membrane filters in 10 ml of fetal calf serum. To determine the amount of sonic treatment required for optimum IBR virus release, membranes were sonically treated for 2- to 5-min intervals. Maximum virus PFU were
obtained after 3 min of sonic treatment (Table 1). The inactivation of IBR virus was determined by sonically treating virus in fetal calf serum alone; virus was added to 10-ml samples and sonically treated for various times. The recovery rates were combined with the virus inactivation curve (Fig. 1) to indicate that, although 100% of the virus was released after 3 min of sonic treatment, 30% of the virus was inactivated.

Although an average virus PFU recovery rate of 70% was obtained, the standard error of the mean was 9.4%. This variation within replicates indicates why there were no significant differences among mean recovery rates listed in Table 1, as determined by the one-way analysis of variance ($F_s = 0.342$). Based on the average of the recovery rates, however, 3 min of sonic treatment was found most acceptable.

The membrane concentration technique was found more effective in recovering the IBR virus than were three other procedures (S. R. Tschider, M. S. thesis, North Dakota State Univ., Fargo, 1974). Polyelectrolyte 60 (11) and aluminum hydroxide (10) adsorption procedures were found to effectively remove virus from water samples, but the inability to recover IBR virus from these adsorbents restricted their usefulness. The aqueous polymer two-phase procedure that was utilized, a modification of the procedures of Shuval et al. (7), was found to be unacceptable. The two-phase virus concentrates were cytotoxic, and therefore the detection of virus PFU was not possible. All methods examined were modified from the originally reported procedures. Differences in virus characteristics (e.g., pH stability) between the enterovirus group and the herpesvirus necessitated these modifications.

In summary, the membrane procedure was found to be an efficient method for obtaining 100-fold concentration of the IBR virus from water, with a recovery efficiency of 70%. Not only is this technique applicable to the concentration of IBR virus, but it also provides a procedure which could be used to concentrate other herpesviruses. Presently, this technique is being investigated for use in evaluating supplies of North Dakota livestock drinking water.

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