Isolation of Porcine Parvovirus from Commercial Trypsin

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The isolation of porcine parvovirus from a lot of commercial 1:250 trypsin is reported and the method is described.

Trypsin solutions, widely used as cell dispersants, have been suspected of contaminating cell cultures with endogenous viral agents for some time. Definite proof, such as isolation of an agent, has been lacking, although circumstantial evidence has been strongly incriminating (1). Porcine parvovirus (PPV) has been found in a lot of commercially available trypsin (control no. 496516, Difco) preparation by using the method described. In the following procedure, all operations were conducted at 4°C. To test a 5-g sample, a 3.09% trypsin solution was made with cold phosphate-buffered saline at pH 7.2 (5 g = 3.09% × 162.0 ml = the capacity of the 50 TI Spinco centrifuge rotor). The trypsin solution was filtered successively through a clarifying pad and a membrane filter with a 0.45-nm pore size. Pretested rabbit serum was added to a final concentration of 5%, and the material was then centrifuged (in the rotor described above) for 1 h at 80,000 × g.

The supernatant fluid was discarded, and the area where the pellet would normally be found in each tube was scraped with a small sterile metal spatula into a small amount of cold, sterile distilled water. The material was withdrawn by using a sterile syringe fitted with a long 18-gauge cannula. One milliliter of the total material was cultured for mycoplasma.

Samples of the remaining material were inoculated into three plastic flasks, each with an area of 75 cm², containing freshly seeded cells (swine testicle cell line; McClurkin) in F-10 medium with 1% sodium pyruvate, 10% pretested SPF swine serum, and 50 μg of gentamicin per ml. Uninoculated control flasks were maintained in parallel. The flasks were incubated at 35 to 36°C until a confluent monolayer was formed at 5 days.

At this time the cells in the respective flasks were loosened with a rubber policeman, and the harvested material was pooled and further broken apart by repeated aspirations and aspirations through a sterile syringe with a 3-inch (7.62-cm) gauge needle attached. The cells were centrifuged at slow speed and resuspended in a volume of fresh medium equal to the initial amount. Twenty to 25 Leighton tubes containing cover slips and two flasks were seeded with the cell suspension and incubated as before. The control flasks were handled in the same manner.

Starting at the 2nd day after seeding, two inoculated and two control cover slips were removed each day and stained with antiporcine parvovirus fluorescent antibody conjugate as described elsewhere (2). On day 3, one of the two inoculated slides showed typical parvovirus nuclear staining, whereas the controls were negative (3). By day 4, all of the inoculated slides examined were showing nuclear fluorescence, although the number of nuclei infected varied widely (Fig. 1, 2).

At the 7th day after seeding, fluids were harvested from inoculated and control flasks, respectively, and sent to another laboratory for verification of identification by hemagglutination and hemagglutination-inhibition techniques. The cells from the flasks were removed and further subcultured as previously described.

At the third cell passage, the inoculated cells in flasks and on Leighton tube cover slips were replicating so poorly that it was necessary to process cover slips on the 2nd day after seeding. Upon fluorescent antibody examination practically all the cell nuclei showed positive staining whereas the controls remained negative. On the 3rd day after seeding, fluids harvested from inoculated and control flasks were examined in hemagglutination and hemagglutination-inhibition tests, and one inoculated and one control
flask, respectively, were stained with May-Grunwald-Giemsa stain. Again, practically all the cells from the inoculated flasks showed intranuclear inclusion bodies, whereas none was observed in cells from the control flask.

Infected fluids taken at the second cell passage had a hemagglutination titer of 1:32; at the third cell passage the titer was 1:256. The control cell culture fluids at both passage levels were negative for hemagglutination activity at a 1:2 dilution. Hemagglutination-inhibition tests were performed by using both a known PPV virus and the infected cell culture fluids, each diluted to contain four hemagglutination units. The antiserum had a hemagglutination-inhibition titer of 1:1280 against both viruses. A negative control serum tested against both viruses showed no hemagglutination-inhibition activity.

The above procedure had been conducted twice on the same lot of trypsin, but using primary embryonic swine kidney cells that had been frozen and stored rather than swine testicle cells. Cells on Leighton tube cover slips...
which had been inoculated directly with trypsin residue material showed a few nuclei in some of the slides which gave positive staining for PPV, but the swine kidney cells would not replicate after the initial passage. It was therefore decided to switch to a cell line, even at the possible risk of decreased cell susceptibility to the virus.

Initially, two lots of trypsin were tested, but the subsequent work was done only on the contaminated lot to improve the test procedures.

Mycoplasmas were never isolated in this study. This is not unexpected, as information obtained on the processing of trypsin indicated that three precipitations of the enzyme were made with alcohol and, at one stage, a temperature of 65.5°C was maintained for 4 h (E. J. Horton, personal communication). PPV would probably be the only known endogenous virus to survive this treatment. Complete information on thermal resistance of the PPV is lacking, but it has been shown that another parovirus, feline panleucopenia virus, when subjected to 75°C for 3 h, survived, while losing approximately 3 logs of titer (D. L. Croghan, Ph.D. thesis, Cornell University, Ithaca, N. Y., 1968).

It is our opinion that trypsin of bovine origin should also be examined for the bovine parovirus, which is possibly as ubiquitous as the porcine parovirus. This could easily be done by substituting primary bovine lung cells in the described system.

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LITERATURE CITED