The Iraqi J. Vet. Med. 19 & 20 , No. (2) , 1995-1996

ISOLATION AND IDENTIFICATION OF ROTAVIRUS FROM NEONATAL CALVES

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SUMMARY

Bovine rotavirus was successfully isolated in vero cell line and secondary calf kidney cell culture from faecal samples of newly born calves affected with diarrhoea. The isolated viruses were cultivated from cell culture produced cytopathogenic effect which consisted of cytoplasmic granulation degeneration and detachment of infected cells from monolayer cell culture. The rotavirus was identified by indirect immunofluorecent test (IFT) and neutralization test (NT) by using reference hyperimmunser.

INTRODUCTION

Rotavirus have been associated with neonatal diarrhoea in a variety of animal species and human (1). These viruses are difficult to isolate in vitro, and few reports describe the isolation and propagation of rotavirus from calves (2,3), birds (4), pig (5) and human (6). The propagation of the viruses could only seen be after adaptation in cell culture in the presence of trypsin (7,8). And calcium in the media(9,10).

The purpose of the present report was to isolate and cultivate bovine rotavirus from faecal samples of calves affected with sever watery diarrhoea after testing positive faecal samples by reference commercial rotavirus kits of enzyme- linked immunosorbent assay (ELISA) and latex agglutination test (LAT).

MATERIALS AND METHODS

Specimens for Virus Isolation:

Faecal samples were collected from newly born calves age 4-10 days old affected with sever watery diarrhoea at AL- Nasar dairy farm station. Faecal samples were transport in ice box and processed immediately for virus identification by ELUISA and latex agglutination test.

Those virus positive faecal samples (1: 10) supplemented with 0.5% Lactalbumine hydrolysate,500i.u.penicillin, 500 µg treptomycin and 500 µg Mycostatin per ml. These suspension were clarified by centrifugation at 3000 r. p.m for 30 min. , the supernatant were collected and exposed once for freezing and thawing and used for virus isolation in cell culture.

Cell Culture:

Secondary culture of calf kidney were prepared as described (8) and grown in Hank's salt solution supplemented with 0.5 percent lactalbumin hydrolysate and 10 percent faetal calf serum.

A continuous cell line of monkey kidney (vero) cell were grown using MEM media containing 10% fetal calf serum, and the mixture of antibiotic were added to all cell culture media at a concentration of 100 i.u. penicillin and 100 mg streptomycin per ml.

Virus Isolation:

Complete monolayer cell culture grown in 25 cm^2 tissue culture flask were inoculated with 0.5 ml of positive faecal suspension and adsorbed for 1 hr at 37°C after washing the culture with phosphate buffer saline (PBS), the maintenance media without serum and 5 mg trypsin per ml was added.

The cell culture was examined daily for development of cytopathic effect (CPE), CPE positive cell culture were harvested at different intervals after inoculation depending on the degree of development of CPE.

Serological Test:

1. Indirect Immunofluorecent test:

Cell culture of calf kidney and vero cell line prepared in lighten tube, were inoculated with isolated viruses, at 72 hr post inoculation. The cell were fixed with old acetone (4°C) and stained with reference antibovine rotavirus serum (supplied by Dr. Gabery national animal disease center Ames. Iowa and goat antibovine gammaglobuline FITC conjugated (Nordic immunological laboratories). Cytoplasmic fluorescence was checked to characterize the infected cells in comparison with non fluorescent control cell.

2. Neutralization Test:

Micro neutralization test was applied by using serial doubling dilution of reference bovine rotavirus serum with 100 TCD 50 of the isolated virus (4).

RESULTS

The CPE of rotavirus appeared 48 hr. post inoculation in calf kidney cells. Some patches of the cells showed granulation this effect increased during subsequent passages and in addition to granulation spindle- shaped cells many cells adhering to plastic by single process were seen. Some small rounded cells which had detached from the monolayer were floating in the fluid media.

At 72 hr. post inoculation the number of detached cells had increased leaving large empty spaces in surface monolayer cells. Such types of changes were not evident in unfected trypsin treated control culture.

The type and shape of CPE in vero cell line appeared similar to that in calf kidney in the first 3 passages which became unclear in the following passages.

IFT results showed detection of rotavirus antigen in the cytoplasm of infected cells at 72 hr. post infection. The number of fluorescing rotavirus antigen increased with successive passages of

the virus in calf kidney cells but is markedly decreased with each passage of the virus in vero cells (Fig.1).

The results of NT reveled that the reference anti rotavirus serum was able to neutralize the infectivity of 100 TCID 50 of isolated virus with a titer of 8. The isolated virus was not inhibited with normal control serum.

DISCUSSION

Rotavirus has been difficult to grow in vitro but consequently there are some literature of their growth characteristics in cell culture (14). Some investigator growth Nebraska calf rotavirus in calf embryonic kidney cell culture (12), and also growth the Northern Ireland isolated of calf rotavirus in calf kidney and MDBK cell line (3).

Our study is considered the first in the country to isolate calf rotavirus in primary calf cells during an out break of diarrhoea in newly born calves.

The development of CPE which is granulation and detachment of cells from monolayer cell culture was a feature common to our isolated virus and similar to those reported by other (15).

The use of trypsin in maintenance media of cells was very successful for virus growth and enhancement of CPE development (7,8).

Several passage of the virus isolated was successful in calf kidney cells than in vero cell line. The same conclusion was reach by other study (12).

The development of the specific characteristics viral CPE in infected calf kidney cells beside detection of specific rotavirus antigen in IFT and neutralization of infectivity of the isolated virus by using reference antisera indicated very clearly that the isolated virus is calf rotavirus.

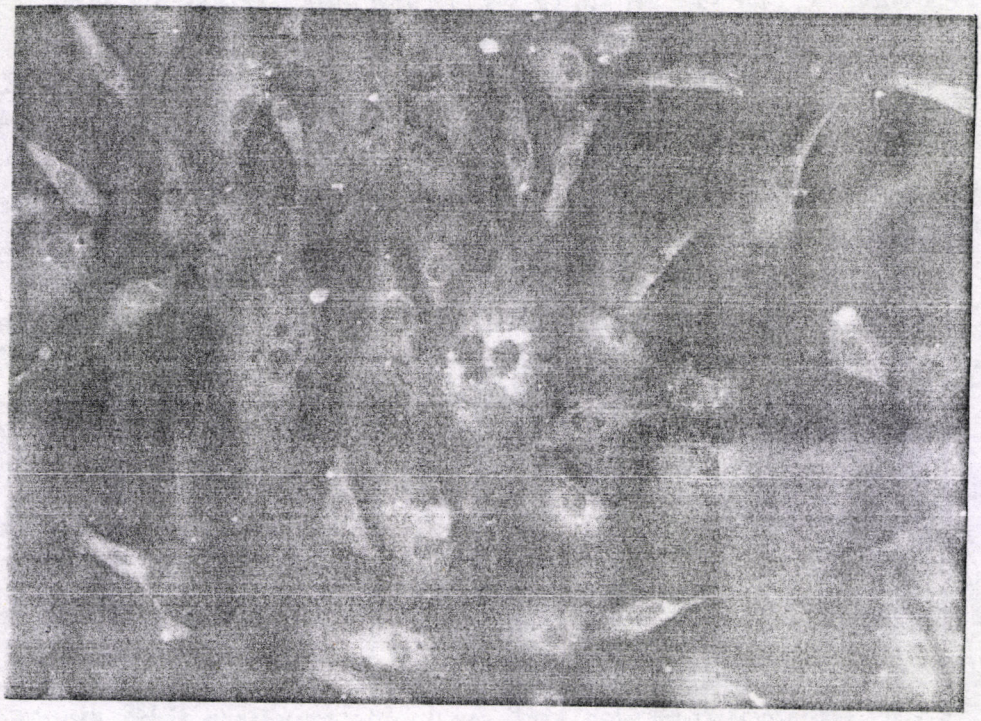

Figure. 1 :Immunofluorescent reaction in infected cells at 72 hr. post infection

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عزل وتشخيص فايروس الروتا من عجول حديثة الولادة

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الخلاصة

عزل فايروس الروتا البقري في خلايا كلية العجول وخلايا الخط الزرعي المستمر (vero) من عينات براز لعجول حديثة الولادة مصابة بالاسهال ، احدث الفايروس المعزول المزروع خلايا الزرع الخلوي احدث تاثير مرضي تمثل بحدوث تحبب وتنكس في السايتوبلازم مع انسلاخ الخلايا من طبقة الخلايا الواحدة. وتم تشخيص الفايروس باختباري التعادل المصلي والتألف المناعي غير المباشر باستخدام مصل ممنع مرجعي.