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ISOLATION OF VIRUS LIKE PARTICLES FROM FECAL MATERIAL OBTAINED FROM DIARRHEAL CALVES

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Many factors have been reported as causative agents of neonatal calf diarrhea (1). Among these, viruses have been reported to be the most important etiologic factors for the disease (2.3). Viruses such as rotavirus, adenovirus, enterovirus and inectious bovine rhinotracheitis virus have been isolated from fecal material of diarrheal calves (4,5,6). In recent years this disease has been common among the cattle industry centers in suberb Tehran causing severe outbreaks with some mortality. We report here the results of a preliminary study on the isolation, purification and morphological characteriztion of two viruses isolated from the fecal material of diarrheal calves.

Fecal samples were collected from 3 to 5 days old calves. Samples were diluted 1:1 with phosphate buffer saline (PBS) and centerifuged 4000 rpm for 10 min. The pellet was discarded and the supernatant was further centrifuged at 8000 rpm for 20 min. The supernatant was centrifuged in Spinco ultracentrifuge at 30000 rpm for 2 hours using SW 39 swinging bucket. The supernatant was discarded and the pellet was resuspended in 0.5ml. of 0.5 M. ammonium acetate and used for electron microscopy. A small drop was stained with sodium phosphotungstate on formvar coated grids and examined in a phillips EM 400 electron microscope. From a total of 18 samples examined 8 contained corona virus like particles with distinct projections and spikes. Attempts were made to grow these virus particles in cell culture but was unsuccessful. It was decided to purify the virus using concentrated virus from fecal material. Crude virus suspension was layered over 20–50% sucrose in 0.1 M. Tris buffer PH 7.2 and centrifuged at 25000 rpm for 2 hr using SW 39 rotor. The virus appeard as a faint graish band and was collected by piercing the side of the tube with a needle connected to a syring. The virus suspension was dialysed against phosphate buffer and examined in the electron microscope Fig **1** shows a few virus particles wich morphologically resemble corona viruses with a diameter 100–140nm. In 4 samples from

the 18 samples examined small virus like particles were observed (Fig 2). These particles were hexagonal with an average size of 28 nm.

These virus particles were partially purified as above then were grown on primary calf kidney cells. They caused a rapid CPE and cell death (less than 24 hr).

The supernatant medium from the infected cell monolayers was first centrifuged at 3000 rpm for 20 min. The supernatant was then ultracentrifuged at 30000 rpm for 2 hr. The virus pellet was suspended in 1 ml of 0.1 M. phosphate buffer PH 7.5 and layered over the top of Cscl gradient with density 1.4–1.2 mg/ml. The tubes were centrifuged at 30000 rpm for 4 hr. The virus which peared as a sharp band was collected as above. The density of virus was determined which was 1.38 gm/ml. Fig 3 shows a sample after Cscl cntrifugation. One sample from the 18 samples examined contained both types of viruses. Corona type viruses have been described as causative agents for calf diarrhea and transmissible gastroenteritis of pigs (2,7). The small hexagonal particles resemble picornavirus and their role in causing diarrhea remains to be determined. Studies are in progress to further characterize these particles and determine their importance in outbreaks of calf diarrhea in cattle industry centers around Tehran.

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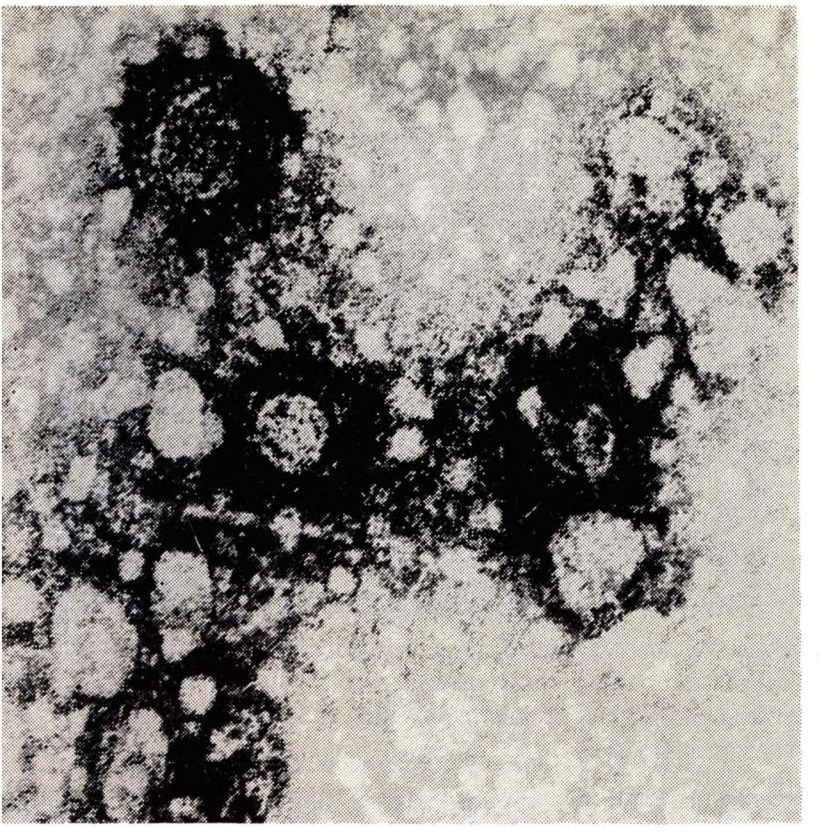

1. Electron micrograph of Corona - like virus particles negatively stainded with sodium phoshotungstate X 120000

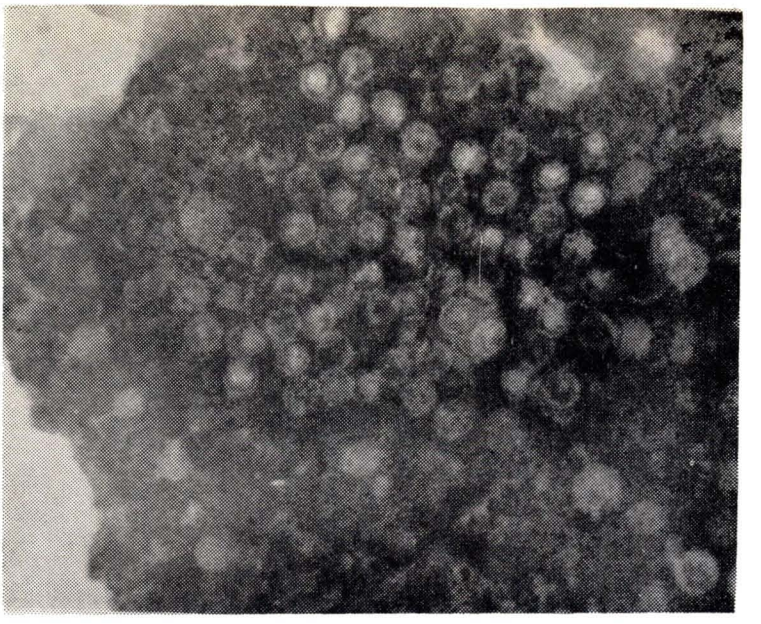

Fig. 2. Electron micrograph of small hexagonal virus particles isolated from fecal material. The preparation was negative stained as in Fig. l. Both full particales (light color particles) and empty particles (dark color particles) are present X. 120000

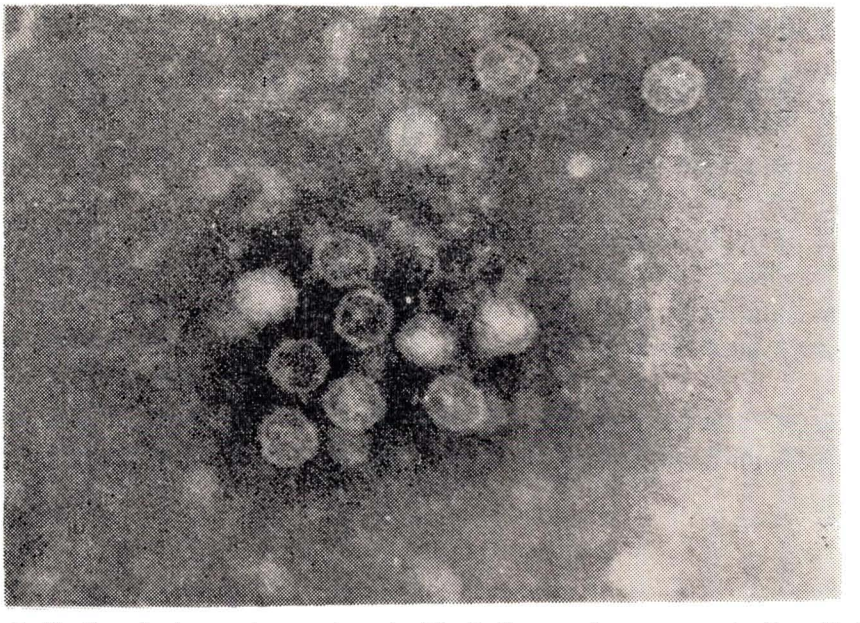

Fig 3. Similar electron micrograph as in Fig 2. Preparation was madeafter Cscl density gradient centrifugation. Both full and empty particles are shown. X 220000.