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PAPULAR STOMATITIS IN CATTLE IN IRAN ISOLATION AND CHARACTERIZATION OF THE AGENTS.

by

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Abstract

An incidence of papular stomatitis accured in young calves in Iran.

The disease was characterized by the appearance of lesions which appeared in and around oral cavity. A virus was isolated from the specimen taken from the lesions and morphologicaly resembeled paravaccinia virus. The virus produced cytoplasmic inclusions in bovine kidney cells and these inclusions contained virus particles.

INTRODUCTION

Bovine papular stomatitis is a viral disease of calves which has been observed in many parts of the world (Griesemer and Cole. 1960, Blood et al. 1957, Snowdon & French 1961).

The disease is characterized by proliferative lesions in oral cavity and around the muzzle which sometimes resemble the lesions at the early stages of foot and mouth disease.

The disease is relatively mild with no systemic involvement but several cases of severe form associated with fever, excessive salivation, diarrhea and death have been reported (Gibbons, W. J. 1963, Carson and Kerr 1967).

The causative agent of the disease is a virus belonging to the paravaccinia subgroup (Negington, J. et al 1962).

The virus has similar biophysical properties to pseudo cowpox virus which belongs to the same subgroup and produce similar lesions in cattle under experimental conditions (Plowright and Forris 1959).

In naturally affected animals with pseudopox virus usually the papules appear on the skin of the teats and in human causes milker nodule (Carson, C. A. and K. M. Kerr 1967).

Although presence of papular stomatitis in calves in Iran has been suspected but occurance of the disease has not been reported and its causative agent has not been isolated.

In this study we report isolation and characterization of a virus causing papular stomatitis in young calves in few cattle raising farms around Teheran.

MATERIALS AND METHODS.

FIELD:

The main field observation was made during 1980 on 20-90 days old Hulstin calves. The calves were kept on a milk cattle raising farm near Tehran. clinical observation were made on calves suffering from the severe case of the disease.

Biopsy specimens were obtained from the lesions in and around oral cavity. In some cases necropsy specimens were also obtained from the lesions during autopsy of dead calves.

The specimen were placed in a strile jars kept in ice and were delivered to the lab processing.

Tissue culture:

Bovine embryonic kidney cell culture was used for viral growth.

The cells were grown in monolayers using Eagles Minimal Essential Medium with 7% fetal calf serum.

The cells were infected with virus in the first passage and sometimes in second passage.

The cells in 3 OZ bottles were infected with 0.5 ml of tissue extract and incubated at 37° C. After 2–3 days cytopathic effect appeared. The infected cells were disrupted by 3 times freezing and thawing and then used for inoculation of cell monolayers.

After 3 passages of the virus a stock was prepared and used for the subsequent experiments.

PREPARATION OF VIRUS INOCULOM:

Samples taken from lesions were homogenized in concentration of 10% in phosphate buffer saline containing antibiotics (P. B. S.) in Omni Mixer. The homogenized was centrifuged at 4000 rpm for 30 min. in a sorval Rc2-B centrifuge.

The pellet was discarded and the supernatant was used for virus inoculom. In some cases the supernatant fluid was centrifuged at 25000 rpm for I hr. in a sorval T–65 ultracentrifuge The pellet was suspended in 0.5 ml of P. B. S. and used for electron microscopy.

The pellet obtained after ultracentrifugation was resuspended as mentioned above, ten drop dialysed on a collodion membrane against 0.5 M ammunium acetate. A sample was negatively stained with phosphotungstic acid on formvar coated cupper grids.

Histopathology.

Primary bovine embryonic cells were grown on coverslips.

The cells were infected with 0. I ml of virus inoculom.

After I hr adsorption time the unadsorbed virus was removed and the infected cells were incubated in MEM at 37° C.

At 24, 48, 72, and 96 hr postinfection, coverslips were removed, fixed in carnoy's fixative and stained with hematoxylin eosin stains.

For electron microscopy, cells in 3 0Z bottles were infected with 2 ml of virus Inoculom.

RESULTS

Clinical Features. During the summer 1980 a disease infecting young calves with oral lesions occured in a herd consisting of more than 2000 head. More than 70% of calves 2-6 weeks old showed the symptoms of papular stomatitis. The disease was characterized by the appearance of thick grayish white strands of exudate in areas and development of 3-4 mm hyperemic foci on the ventral margins of the nostrils. In some calves there was protrusion of the tongue, excessive salivation anorexia and reluctance to move. In the mouth cavity lesions were found on the mucosa of the lower lip and gum especially around the roots or sites of eruption of the teeth and the opssing lip surface (fig 1, 2). On the tongue, lesions in various sizes were found on the lower surface and on the lateral margins and ventral tip. Lesions persisted for a few days then regressed and disappeared. The presence of the lesions caused discomfort in some calves.

They did not continue to eat normally and lost weight. A few calves apparently died of starvation but majority recovered without treatment.

At different time intervals samples were removed, and fixed in 3% glutaraldehyde in 0.I M phosphate buffer PH 7. 2. After 2 hr the cells were washed in phosphate buffer and post fixed in 1% osmium tetroxide, then embeded in epon 812. Section were cut, stained with uranyl acetate followed by lead citrate and examined in a Philips EM 400 electron microscope.

Virus isolation, Pieces of tissue taken from the lesions were homogenized and examined in the electron microscope as described Virus particles could be observed in negative stained preparation of the specimen. The Virus had typical appearance of papular stomatitis virus with the size of 250-300 nm. on the surface had tubular like structures and it seemed that the virus had a thin membrane on the outer surface (Fig. 4).

Both types, the mature and the immature particles could be found (Fig 5).

Growth in cell culture.

Primary bovine embryonic kindney cell (BK) cultures were inoculated with material from the nasal and oral cavity.

Cytopathic effects (CPE) appeared after 4-6 days. On subsepuent passage CPE was more evident after 48-72 hr after infection.

The virus did not grow in other cells such as Hela, BHK, and Vero lines

and produced no CPE after 10 days. Cultures inoculated with the virus and stained with H and E exhibited cytoplasmic inclusions. The cells showing inclusions. The cells showing inclusions were less frepuent after 24-48 hr and inclusions were small bodies inside the cytoplasm. As the time of infection proceeded many cells showed large eosinophilic inclusions inside the cytoplasm (Fig 6).

Thin section of both infected and non infected BK cells was prepared and examined in the electron microscope. At 48 hrs after infection very few cells contained some granular material inside the cytoplasm with in which virus particles started to appear (Fig 7).

At 72 hr to 96 hr the number of virus particles increased and many cells showed accumulation of granular material inside their cytoplasm with many virus particle at different stage of maturation (Fig 8).

DISCUSSION

Papular stomatitis is relatively a mild disease which affect cattle without systemic illness, but there are some reports that describes the disease with sings of reduced appetite, diarrhea, depression and temperature elevation (Fraser & Savan 1962).

The virus causing the disease has been isolated from adult cattle and it has been suggested that adult cattle may serve as a reserver of infection (Plowright & Ferris 1959). In our study the disease was observed in young calves and the lesions were typical of papular stomatitis. Although there is a resembrance between the signs of papular stomatitis and the oral lesions such as Muzzle disease (Hollister et al 1956) and pseudo cow pox (Nagington et al 1966), but they can be differenciated on the basis of virus isolation and histological examination.

The virus isolated from the lesions of infected calves had morphological properties of paravaccinia virus. On the basis of clinical signs, the site of lesions, and morphological appearance of the agent, the disease was diagnosed as papular stomatitis .

The virus had thick, tubular, parallel fibrils which were crossed longitulinally on the surface and resembeled the structure of paravaccinia viruses (Buttner et al 1946; Nogintan et al 1962).

The virus grew slowly in BK cells but after a few passage in BK cells it was adapted to these cells and produced CPE after 48 hr.

The eosinophilic inclusions produced inside the cytoplasm were characteristic of Pox Virus. At the level of electron microscope these inclusions contained numerous virus particles and probably were the site of virus assembly. Studies are being carried out to determine the level of antibody against papular stomatitis virus in both young calves and adult cattle. In addition serological relationship between this virus and other viruses from the pox family will be determined.

Fig. 1- A calf with lesions on the upper lip close to the muzzle.

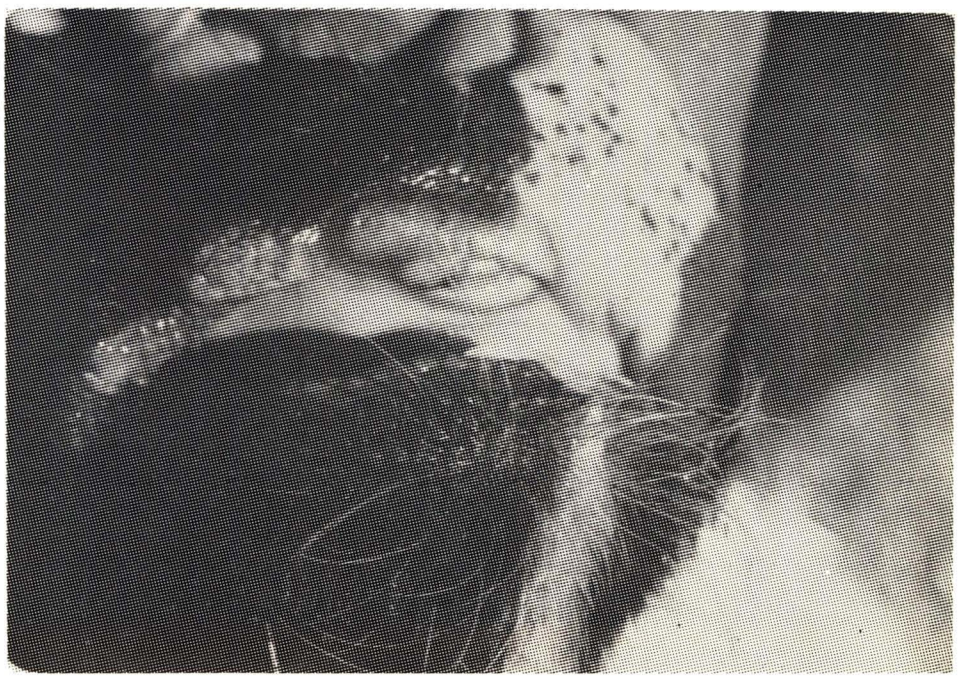


Fig. 2- Lesions on the gum at the junction with the inciser teeth.

Fig. 3- Lesions on the lower surface of the tongue.

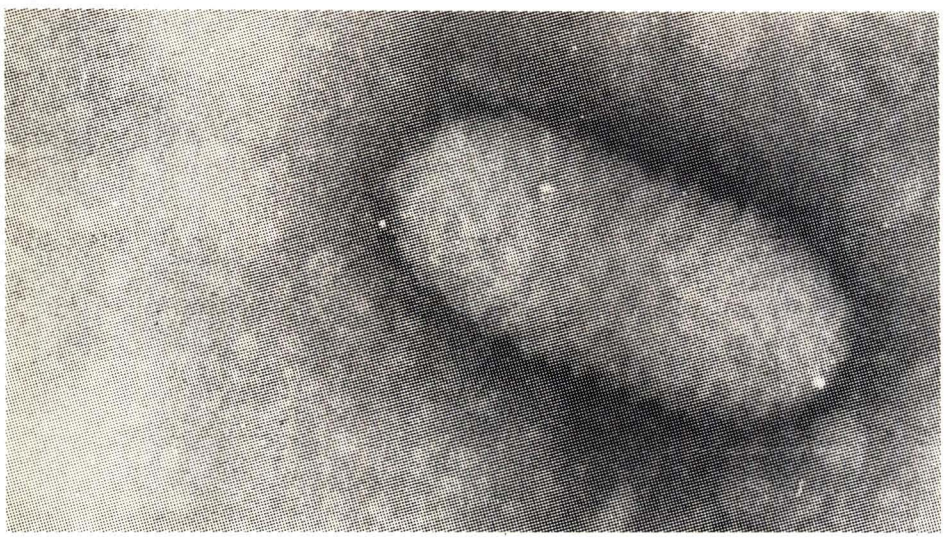


Fig. 4-X 200 000- Electron micrograph of a papular stomatitis virus particle negatively stained with phosphtungstic.

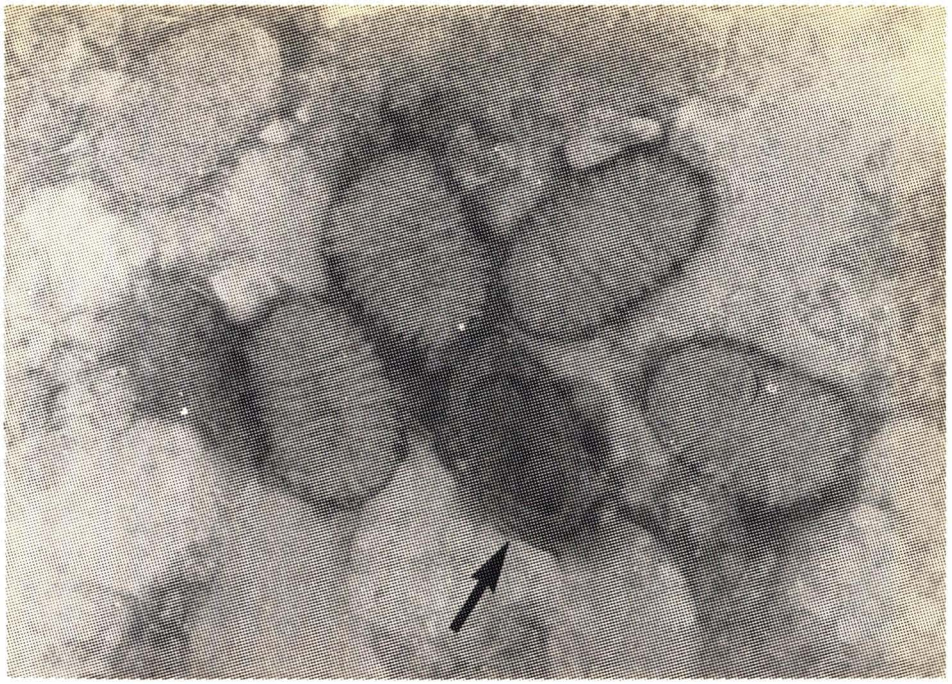


Fig. 5-X 120 000- The same virus particles as in fig. 4 both mature and immature particles are seen (arrow).

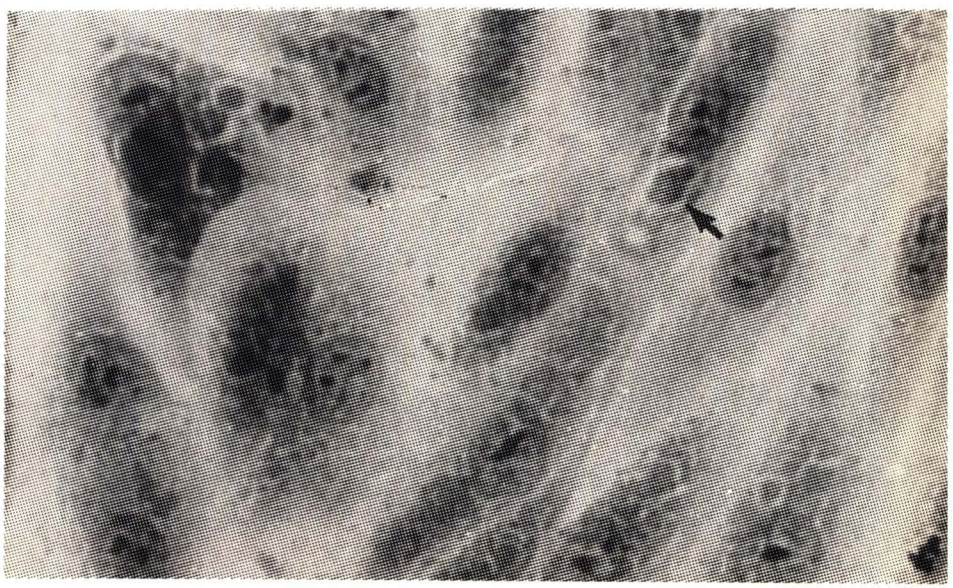
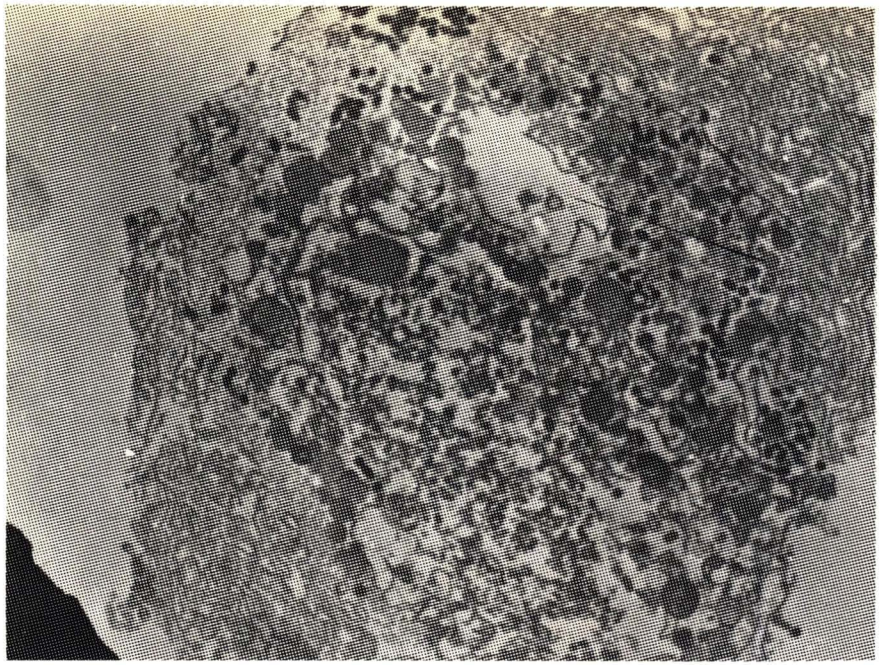


Fig. 6- Cytoplasmic eosinophilic inclusions in BK cells infected with the virus (arrow)



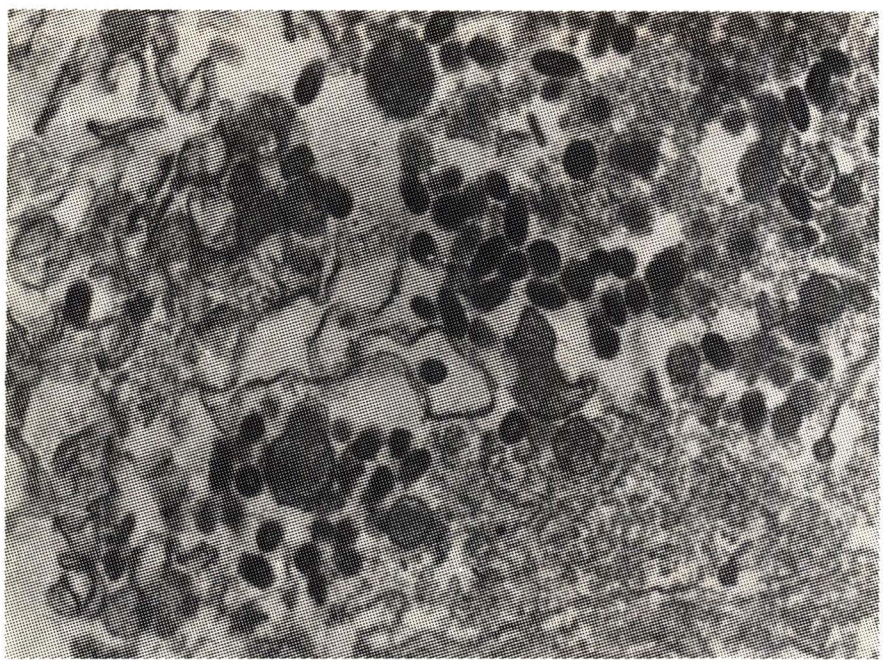


Fig. 7-X 10 000- Thin section of an infected cell showing a cytoplasmic inclusions with some virus particles.

Fig. 8-X 30 000- Virus particles inside the cytoplasm in a higher magnification.

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