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DIARRHEA IN CALVES
DIAGNOSIS AND INCIDENCE AROUND TEHRAN

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SUMMARY

Between March 1980 through September 1980 feoes from 63 newborne calves were examined by the electron microscope. About 40 % of the samples contained virus particles. These included mainly Rota virus (RV) (80%) and a few cases small round particles (SRV).

Bacteriological tests were also performed on each sample. The bacteria isolated were mainly E. Coli (60%) and two cases of Salmonella.

INTRODUCTION

Calf scours have been traditionally called the « Calf killer » and neonatal diarrhea is considered to be a major cause of economic loss for the cattle industry. Adequate prophylaxis and therapy of this disease depend on a clear definition of its cause (s) and how this cause (s) produces lesions in the calf. A great deal of knowledge has been gained in the last ten years, regarding the proper identification of the causes (Mebus, G. A. et al 1971) & (Mebus, G. A. et al 1973) & (Mebus, G. A. et al 1976). Laboratory assistance has proven to be a necessary tool in determining the possible role which infectious agents play in the genesis of calf scours. This presentation deals with routine examinations performed at the Razi Institute on feces of diarrheic calves and summerized the results for a six months period (March 1980 - Sept. 1980).

MATERIALS & METHODS

Samples collection:

Specimens from diarrheic claves collected from a few large cattle farm around Tehran, were submitted by the practicing Veterinarians. In some cases fecal material were collected directly from various parts of intestine from the carcases of dead calves.

Bacteriologic Examination :

Feces were cultured into Selenit F. broth, and incubated at 37°C. After 24-48 hours, they were sub-cultured on Mac Conkey agar, SS agar, desoxycholate, violet red bile agar (V. R. B. A.) Bacterial entropathogenes were isolated and identified by standard laboratory procedures (Myers, L. L. 1975) .

Antimicrobial sensitivity results were determined by the kirby Bauer methods (Bauer, A. W. et al 1966).

Virologic Examination:

a) Concentration. Two to five grams of feces were suspended in 15 ml. of distilled water, centrifuged 15 minutes at 3500 r. p. m. in a Sorval RC2P centrifuge and decanted supernatant fluid was recentrifuged 30 - 45 minutes at 30000 r.p.m. in a Sorval OTD65 Ultracentrifuge using T865 fixed angle rotor. The pellet was then resuspended in 0.3 ml. of distilled water and kept frozen until used.

b) Electron microscopy. Negative stain preparations were made by the method of Brenner, A. W. et al (1959) modified by Ritchie, A. E. et al (1968). One drop of the resuspended pellet with 16 drops of distilled water, twodrops 3% phosphotangstic acid and oen drop 0.1 % bovine serum albumin. This mixture was sprayed with a glass nebulizer onto a formwar coated grid and examined in a Philips EM 400 electron microscope.

RESULT

Bacteriologic findings:

In total of 63 samples cultured E. Coli was detected in 31 cases. In 17 cases proteus was also detected in association with E. Coli (Table 1). Salmonella typhimurium was isolated only from two samples. The majority of isolated strains of E. Coli were non hemolytic. however one strain of E. Coli frequently produced hemolysis. This was recognized by the ability to lyse rabbit red blood

cells on blood agar plates. Pathogenicity of the E. Coli isolates was determined by serotyping of these strains. Serotypes were 0-78,0-2aH8 and 0-111.

Virological findings:

Electron microscopic examination of the samples revealed presence of viral particles in the feces of some of the diarrheic calves. In each electron microscope grid at least 20 meshes were carefully examined and in case of presence of viral particles photographs were taken on 35 mm. negatives. The viruses found were indentified morphologically and were detected to belong to the Reoviridoe family.

These reovirus like particles which are known to be rotavirus were present in 40% of cases (Derbyshir, J.B. & Woode, G.N. 1978).

The virus particles were about 70 nm. in diameter with two distinct capsid layers (Fig.1).Both the full and empty particles were present in most of the samples (Fig. 2). Some particles lacked the outer capsid layer and were smaller in diameter than the full particles with distinct capsomers (Fig. 3).

In three cases some small virus like particles (SRV) measuring about 25 nm. in size were observed whose identity could not be determined (Fig. 4). Presence of virus particles in association with various bacteria is shown in Table 2.

DISCUSSION

The results of this study indicated that in the cattle farms around Tehran, neonatal calf diarrhea was associated with bacterial and viral enteropathogens. About 40% of the samples contained virus particles. Eighty per cent of these viruses were reo like particles. These particles have been designated as rotavirus which differ antigenically from other reo viruses. Although 3 samples contained (SRV), but the role of these particles in causing diarrhea is in doubt. Attempts/ are being made to culture the virus isolates in tissue cultures and to characterized them both antigenically and biochemically. It appears that EM techniques was quite sensitive in detecting virus particles. Undoubtedly the quality of the specimens examined and the age of the infectious process may influence the outcome of these tests.

However EM tests tend to be selective for detecting (RV) in feces, isolation of E. coli from the specimens of diarrheic calves was not conclusive, because many isolates were found to be non pathogenic. Serotyping of the E. Coli isolates revealed that many of them belonged to the 0-78,0-2aH8, and 0-111 serptypes (Moon, H.W. et al 1978).In the cattle farms around Tehran most calf operations are managed in such a manner that the majority of calving takes place during late winter and early spring. The dramatic increase in number of scouring calves

during this time of the year appears to be associated primarily with the sudden increase of the population. Undoubtedly environmental factors (e. g. crowding, regorous climate etc.) may accelerate or compound the problem.

The results of these examinations emphesize that is only a clinical sign of a complex of disease which may affect young calves, and that numerous causes may be associated with it (Acres et al 1975), (Acres et al 1977, McNulty et al 1976, Woode et al 1975). while the accurate detection of infectious enteropathogens is essential, factors such as managerial skills of the herdsman; health and mothering ability of the dam, healths, vigor nad immune status of the neonetal calf, diet of the neonate, environmental stress, housing facilities, and sanitation can not be over looked in an effort to determine the cause (or causes) of diarrhea. The value of an etiologic diagnosis must be weighted against numerous other non infectious factors which may affect the new borne calf. In addition, it can not be over emphesized that it is nearly impossible to make an etiologic diagnosis based only on clinical sign and results of necropsy examination. It is therefore, imparative the diagnosis of calf diarrhea be based on:

1 - Through clinical investigation .
2 - Accurate laboratory examination, and
3 - Judicious interpretation of all findings.

Table 1. The bacteria isolated from 63 feces of the diarrheic calves.

Name	Number of cases	% of incidence
E. Coli	31	50
Proteus sp.	17	27
Salmonella Sp.	2	3
Total	50	80

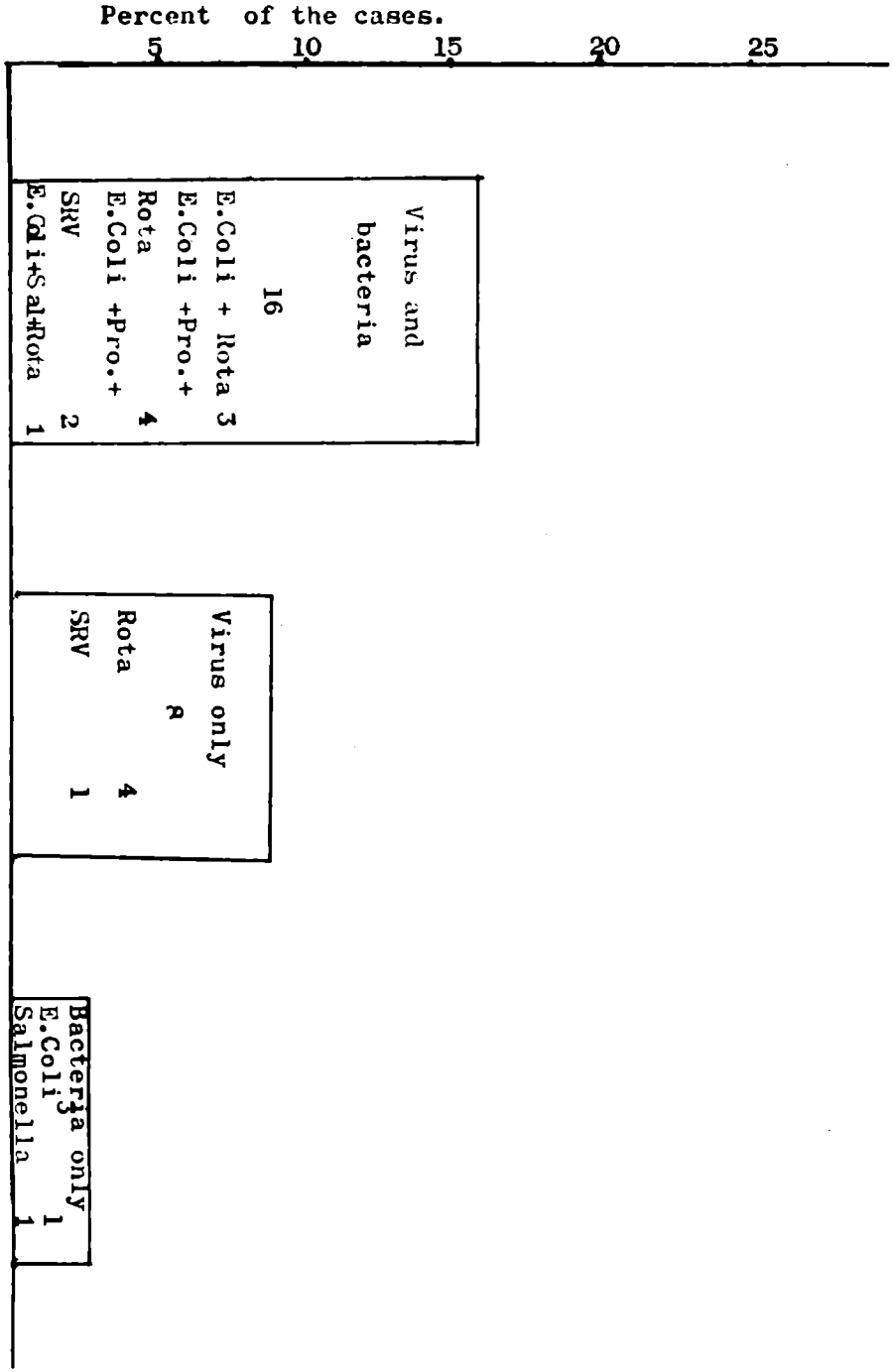

Table 2. Incidence of infectious agents detected in feces from 63 calves with diarrhea

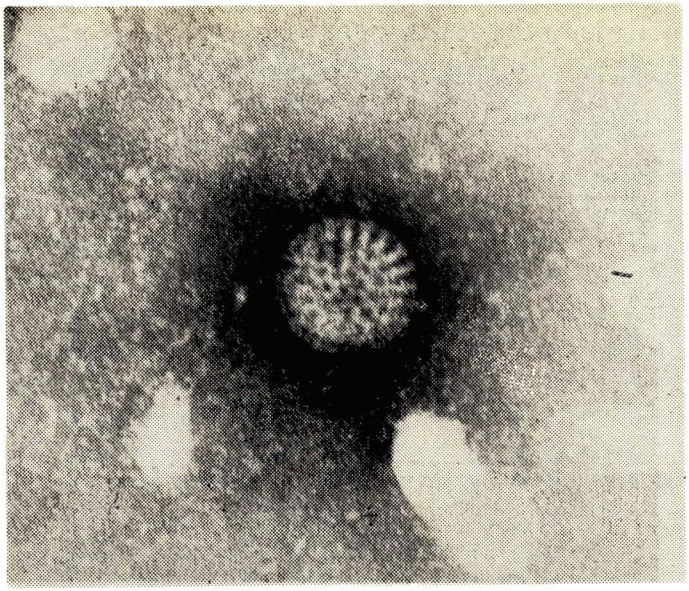

Fig. 1. Electron micrograph of a complete Rota virus particle with two capsid layers. X 280, 000.

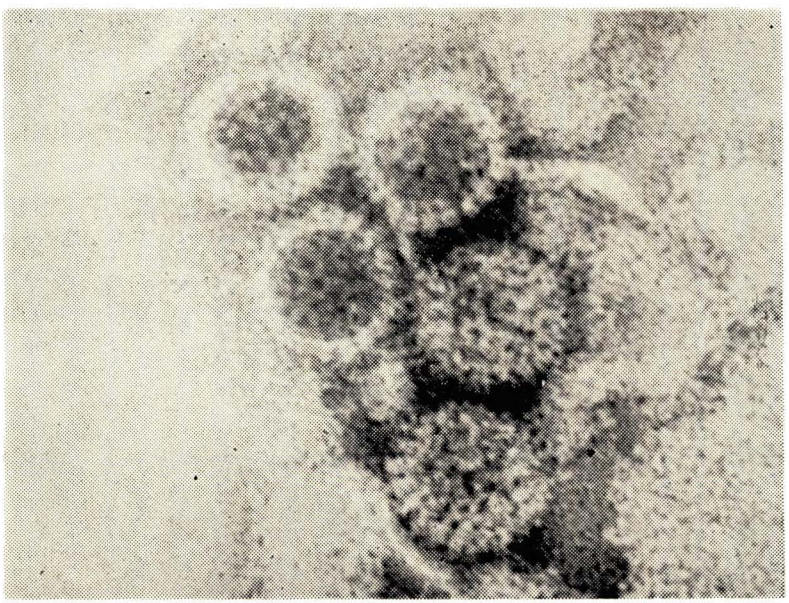

Fig. 2. E'ectron micrograph of Rotavirus showing both full and empty particles, X 280, 000.

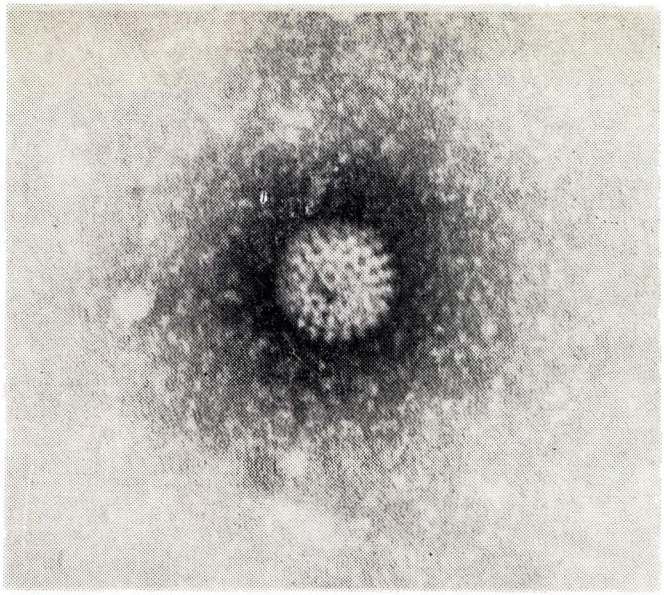

Fig. 3. Electron micrograph of an incomplete Rotavirus particles. The outer capsid layer is not present. X 280, 000.

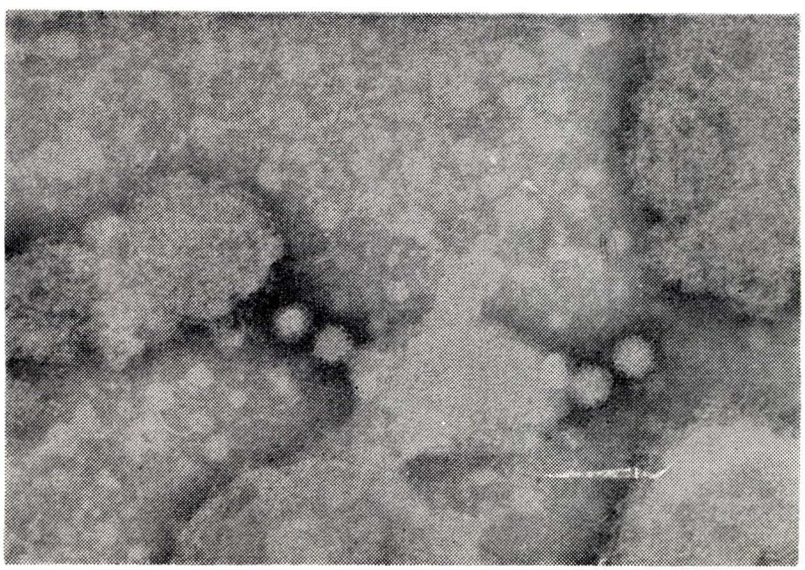

Fig. 4. Electron micrograph of virus like particles (SRV). X 200, 000.

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