

Brief Communication

REO-LIKE NEONATAL CALF DIARRHOEA (NCD)
VIRUS DEMONSTRATED IN DENMARK

A reovirus-like agent has been reported as a cause of neonatal calf diarrhoea in the USA. Isolates of this virus induced diarrhoea when inoculated into gnotobiotic and conventional calves (*Mebus et al.* 1969).

A fluorescent antibody test for the demonstration of this virus in diarrhetic faeces was developed, and the application of this test suggested that the infection was widespread in the USA and associated with enzootic diarrhoea among newborn calves (*White et al.* 1970). Recently the infection was also reported from Great Britain (*Woode et al.* 1974).

In order to see whether the reovirus-like agent was present also in Denmark, fluoresceinisothiocyanate (FITC) conjugates against the prototype strain of this agent (the Lincoln isolate*) were prepared and used for staining of (1) smears from the mucosa of the small intestine of calves that had died of scours in their first week of life, and (2) tissue cultures inoculated with such material.

Two smears of intestinal mucosa and contents were produced from each calf by inserting a cotton-wool swab into the lumen of the intestine and then rolling the swab on a glass slide. The smears were fixed for 10 min. in acetone and 1 stained with FITC-conjugate against the reo-like agent, the other with "normal" control conjugate. Antisera for FITC-conjugates were produced by immunizing 2 rabbits and a 1-year-old calf with the prototype strain of the reovirus-like agent. FITC labelled globulins were produced essentially as described by *Beutner et al.* (1968).

Clarified 10 % suspensions of intestinal tissue and contents were inoculated into primary or secondary bovine kidney cultures grown in Leighton tubes in Hank's solution with 0.5 % lactalbumin hydrolysate, 0.01 % yeast extract, 10 % calf serum, and antibiotics. Eagles minimum essential medium (MEM) was used for maintenance. The cultures were washed 3 times before inco-

* Received from Dr. C. A. Mebus, University of Nebraska, Lincoln, USA.

cultivation with 0.1 ml suspension of intestinal material. After incubation for 1 hr. at 37°C for absorption, the cultures were washed again, whereafter maintenance medium was added.

The fluorescence of cell cultures infected with either the Lincoln strain of the reo-like agent or the field isolates was cytoplasmic and granular. In smears of intestinal mucosa evaluated as positive the cells were often packed with fluorescent material making distinction of cytoplasm and nucleus difficult. No fluorescence was seen in smears stained with the control conjugates. In some cases only 1 or a few cells could be found, in other cases fluorescence lacked intensity. Such cases were recorded as doubtful.

Smears of intestinal mucosa of 98 calves were examined by direct FA-staining, and of these 34 (34.7 %) originating from 33 herds from different parts of the country were found positive. Suspensions of intestinal contents and tissues from 51 of the calves were inoculated into bovine kidney cultures grown in Leighton tubes, which were processed for FA-staining 3—4 days later. Reo-like NCD-agent was demonstrated by this method in 22 calves. Table 1 gives the staining results of intestinal smears from the same calves. It appears that the cultural technique was slightly less sensitive than direct demonstration of the virus in smears, but the interpretation of the FA-reaction was easier in cultures although the number of fluorescent cells in the infected cultures was often low.

Attempts were made to pass and isolate virus strains from the tissue suspensions that seemed to infect Leighton tube cultures most readily. Three such strains have been passed up to 6 times using undiluted inocula and with transfers at 6—7 day intervals. The cultures were checked for virus growth at each

Table 1. FA-demonstration of reo-like NCD-virus in intestinal smears and cell cultures inoculated with intestinal material from calves that had died of diarrhoea.

FA-staining of smears	FA-staining of tissue cultures	
	positive	negative
Positive	25	5
Doubtful	6	5
Negative	20	19
Total	51	29

passage level by immunofluorescence, because the cytopathic effect of the isolates was too slight to be a reliable indicator of growth.

A colostrum-deprived calf was given, orally, 20 ml infective tissue culture fluid and cells from the 4th passage of one of the isolates. This calf shed semifluid faeces 24 hrs. after the inoculation, and fluorescent cells could be detected in the faeces. The calf was killed 72 hrs. p.i., and cryostat sections were cut from the intestines of the calf. Brilliantly fluorescent cells were detected in the contents and on the surface of the villi of the duodenum and jejunum. Infected cells were present also in the colon, caecum, and rectum. The virus was recovered from all tested sites of the intestine (duodenum, jejunum, colon, caecum, and rectum) but could not be cultured from spleen and lung.

The reo-like NCD-virus is considered to be a primary cause of neonatal calf diarrhoea. It is therefore important to note that this infection is prevalent in Denmark. Many of the calves examined originated from herds where antibacterial measures had been without effect. A newly developed vaccine (Mebus *et al.* 1973) against the reo-like agent is likely to be of benefit in the prevention of neonatal calf diarrhoea in such herds.

A. Meyling,
The State Veterinary Serum Laboratory,
Copenhagen, Denmark.

REFERENCES

- Beutner, E. H., Marion R. Sepulveda & E. v. Bernett:* Quantitative studies of immunofluorescent staining. *Bull. Wild Hlth Org.* 1968, **39**, 587—606.
- Mebus, C. A., N. R. Underdahl, H. B. Rhodes & M. J. Twiehaus:* Calf diarrhoea (Scours): Reproduced with a virus from a field outbreak. *Univ. Nebraska, Agric. exp. Sta. Res. Bull.* 1969, **233**.
- Mebus, C. A., R. G. White, E. P. Bass & M. J. Twiehaus:* Immunity to neonatal calf diarrhoea virus. *J. Amer. vet. med. Ass.* 1973, **163**, 880—883.
- White, R. G., C. A. Mebus & M. J. Twiehaus:* Incidence of herds infected with a neonatal calf diarrhoea virus (NCDV). *Vet. Med. Small Anim. Clin.* 1970, **65**, 487—490.
- Woode, H. N., J. C. Bridger, G. Hall & M. J. Dennis:* The isolation of a reovirus-like agent associated with diarrhoea in colostrum-deprived calves in Great Britain. *Res. vet. Sci.* 1974, **16**, 102—105.

(Received August 28, 1974).

Reprints may be requested from: A. Meyling, The State Veterinary Serum Laboratory, Bülowsvej 27, DK-1870 Copenhagen V, Denmark.