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Isolation of Rotavirus from Calves in the Federal Republic of Germany

By

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With one figure

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Introduction

Infectious gastroenteritis viruses as likely causative agents of acute gastroenteritis have been observed in faeces from several animal species including man (ANON, 1975). First isolations were reported from Australia (BISHOP et al., 1974) and the USA (KAPIKIAN et al., 1974). FLEWETT et al. (1974) studied infantile gastroenteritis from cases in Britain and proposed the term *rotavirus* for particles approximately 65 nm in diameter having a morphology similar to reoviruses. We now report the isolation of rotaviruses from calves in the Federal Republic of Germany.

Material and Methods

Faeces samples were obtained from fourteen calves with acute enteritis. It was considered unlikely that the enteritis was caused by bacterial pathogens, because all the animals had received antibiotic supplements in their feed. All samples were of a liquid consistency. Samples were processed within 24 hours of collection as follows: twenty percent (v/v) suspensions were prepared in phosphate-buffered saline (PBS), emulsified on a Vortex mixer, and centrifuged at 1000 xg. for 15 min. The supernatants were then ultracentrifuged at 12,500 xg. for 45 min at 4 °C, the resulting pellets were washed by resuspension in PBS and the ultracentrifugation step was repeated. Pellets from this second run were resuspended in a little distilled water, one drop stained, either with 4 % phosphotungstic acid (PTA) pH 6.0, or with uranyl-acetate pH 4.3, transferred to a 400 mesh pioloform-covered copper-grid and examined in the electron microscope at a screen magnification of 60,000.

Results

Two out of the fourteen samples examined contained large numbers of *rotavirus* particles typically 60 nm. in diameter (Fig. 1). Though two types

Fig. 1 A. Clump of rotavirus particles prepared from faecal extracts as described in the text. Both "full" and "empty" particles may be seen. Stained with 4 % PTA, pH 6.0

Fig. 1 B. Higher power view of 2 "full" and 1 "empty" particles. Stained with 4 % PTA, pH 6.0

Fig. 1 C. Single particle stained with uranyl acetate, which affords a better three dimensional effect

of particle are routinely described (FLEWETT et al., 1974) only particles lacking the outer layer of capsomeres were seen. Larger particles (70—75 nm.) having an extra capsomere layer and a better defined boundary, were not observed. This preponderance of one type of particle may however only be a function of the preparative process.

Discussion

From field observations it has been widely accepted that *rotaviruses* are an important cause of sporadic enteritis in the young of several species and

this has been confirmed by experimental infection of gnotobiotic animals (SNODGRASS et al., 1976). Our studies have shown that the agent is present in calves in the Federal Republic of Germany. It is likely that rotavirus infections have always been present, though, since they are generally transient and cause low mortality they have hitherto been masked by the potentially more serious bacterial diseases; only with the almost ubiquitous addition of antibiotics to feedstuffs have such viruses come into greater prominence. It should not be assumed, however, that the presence of rotavirus particles in faeces can necessarily be directly correlated with acute enteritis; other types of particle are also frequently observed, including parvo-, paramyxo- and coronaviruses, and it is possible that one or more of these could cause symptoms of enteritis. Until further work on the interrelationships of these different types has been performed the picture will not be entirely clear.

Summary

Faeces samples obtained from fourteen calves with acute enteritis were examined in the electron microscope. Two out of fourteen samples contained large numbers of rotavirus particles typically 60 nm. in diameter, lacking the outer layer of capsomeres. Larger particles (70—75 nm.) having an extra capsomere layer were not observed.

Zusammenfassung

Isolierung von Rotavirus aus Kälbern in der Bundesrepublik Deutschland

In Fäzesproben von 2 Kälbern, die unter akuter Enteritis litten, wurden elektronenmikroskopisch Rotavirus-Partikel nachgewiesen. Die Partikel hatten einen Durchmesser von 60 nm; die äußere Kapsomerenschicht fehlte.

References

1. Anon., 1975: Rotaviruses of man and animals. *Lancet* *1*, 257—259.
2. BISHOP, R. F., G. P. DAVIDSON, I. H. HOLMES, and B. J. RUCK, 1974: Detection of a new virus by electron microscopy of fecal extracts from children with acute gastroenteritis. *Lancet* *1*, 149—151.
3. FLEWETT, T. H., A. S. BRYDEN, H. DAVIES, G. N. WOODE, J. C. BRIDGER, and J. M. DERRICK, 1974: Relationship between viruses from acute gastroenteritis from children and newborn calves. *Lancet* *1*, 61—64.
4. KAPIKIAN, A. Z., H. W. KIM, R. G. WYATT, W. J. RODRIGUEZ, S. ROSS, W. L. CLINE, R. H. PARROTT, and R. M. CHANOCK, 1974: Reovirus-like agent in stools: association with infantile diarrhea and development of serological tests. *Science* *185*, 1049—1053.
5. SNODGRASS, D. R., J. A. HERRING, and E. W. GRAY, 1976: Experimental rotavirus infection in lambs. *J. Comp. Path.* *86*, 637—642.

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