

RESEARCH NOTE

DIARRHOEA IN PIGS INDUCED BY ROTAVIRUS

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ABSTRACT

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Electron microscope examination of the faeces of scouring pigs revealed virus particles which were morphologically indistinguishable from rotavirus (reo-like), a virus associated with diarrhoea in neonatal pigs (Lecce, King & Mock, 1976). This is the first record of this virus in the Republic of South Africa.

Résumé

DIARRHÉE A ROTAVIRUS CHEZ DES PORCS

L'examen au microscope électronique de matières fécales de porcs atteints de diarrhée a révélé des particules virales morphologiquement indiscernables de celles du rotavirus ("reo-like"), un virus associé à la diarrhée chez les porcelets nouveau-nés (Lecce, King & Mock, 1976). C'est la première fois que ce virus est signalé dans la République sud-africaine.

INTRODUCTION

Rotavirus (reo-like) has recently been associated with diarrhoea in many species, including calves (White, Mebus & Twiehaus, 1970), foals (Flewett, Bryden & Davies, 1975), lambs (Snodgrass, Smith, Gray & Herring, 1976), infants (Kapikian, Kim, Wyatt, Rodrigues, Cline, Parrott & Chanock, 1974), mice (Woode, Bridger, Jones, Flewett, Bryden, Davies & White, 1976) and pigs (Hall, Bridger, Chandler & Woode, 1976; Lecce, King & Mock, 1976).

Biochemical studies have revealed that the rotavirus, associated with diarrhoea in pigs, is a double-stranded RNA virus (Todd, 1976). This viral condition is clinically and histologically indistinguishable from a coronavirus-associated diarrhoea (transmissible gastroenteritis). Clinically both conditions are accompanied by diarrhoea, vomiting and dehydration (Lecce *et al.*, 1976). An opportunity recently presented itself for investigating the aetiological role of viruses in diarrhoea of young pigs in South Africa.

HISTORY AND CLINICAL SIGNS

A farmer from the Warmbaths area of the Northern Transvaal reported diarrhoea in his 6-week-old pigs. Two of the scouring pigs, which had had no treatment, were submitted to the Veterinary Research Institute, Onderstepoort for examination.

MATERIALS AND METHODS

The 2 pigs were autopsied and suitable specimens of various organs were taken for routine bacteriological examination. Faeces from both pigs were collected for examination with the electron microscope.

Preparation of faecal samples for electron microscopy

The technique described by Petric, Szymanski & Middleton (1975) for the preparation of faecal material for electron microscopy was followed. Carbon-coated formvar grids were placed for 30 seconds on the surface of a mixture of 1 drop of 2% aqueous phosphotungstic acid (PTA) solution pH 6.5 and 1 drop of faecal material. After the excess fluid had been drawn off with filter paper, the grid was allowed to dry. The specimens were examined with a Siemens Elmiskop 102 EM, operating at an acceleration

potential of 80 kV at instrumental magnification of 30 000 \times . The magnification calibration described by Els & Verwoerd (1969) was used.

RESULTS

Gross pathology

Congestion and slight oedema of the small intestine, with dehydration of the carcass, were the only changes seen.

Bacteriological examination

No pathogenic bacteria could be isolated from any of the organs submitted for examination.

Electron microscopic examination of faecal material

The morphology and size of particles seen in negatively stained specimens enabled one to identify 2 distinct forms of virus particles, namely, double- and single-shelled (Fig. 1). The double-shelled particles ranged in size from 70.3-74.6 nm (an average of 72.2 nm), while the single-shelled particles averaged 61.6 nm. In both the single- and double-shelled particles the hexagonal central core was sometimes penetrated by the negative stain (Fig. 1). The outline of the double-shelled virions was completely smooth (Fig. 1 & 2), while that of the single-shelled virions had a spiked appearance (Fig. 1). The subunits (spikes) were approximately 8.4 nm long and had a hole in the centre. In the same material numerous pleomorphic virus-like structures were observed (Fig. 2). Bizarre forms made it difficult to measure the size of the virions which ranged from 60-224 nm and some virions were even larger. The envelope consisted of a membrane studded with projections (10 nm long) while in some of the particles the membrane (12 nm thick) beneath the spikes was less dense than the nucleocapsid.

DISCUSSION

Two distinct viruses were identified from the morphology and size of particles seen in negatively stained specimens. The circular particles were indistinguishable from the rotaviruses observed in the faeces of scouring pigs, infants, foals, calves and mice (Woode *et al.*, 1976) and were classified as rotaviruses on the basis of their morphology and size. Morphologically, the well-defined circular outline differentiated these viruses from reoviruses and bluetongue virus (Browne, 1970, cited by Flewett, Bryden, Davies, Woode, Bridger & Derrick, 1974).

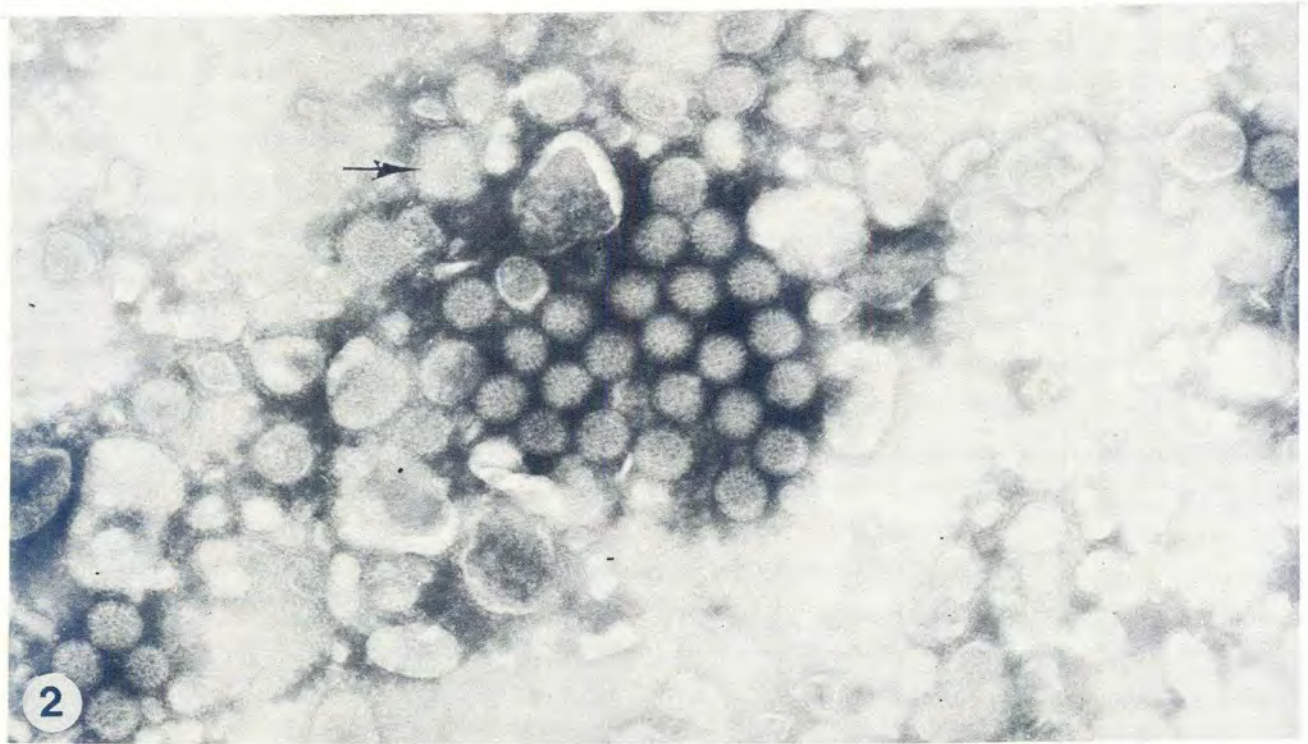
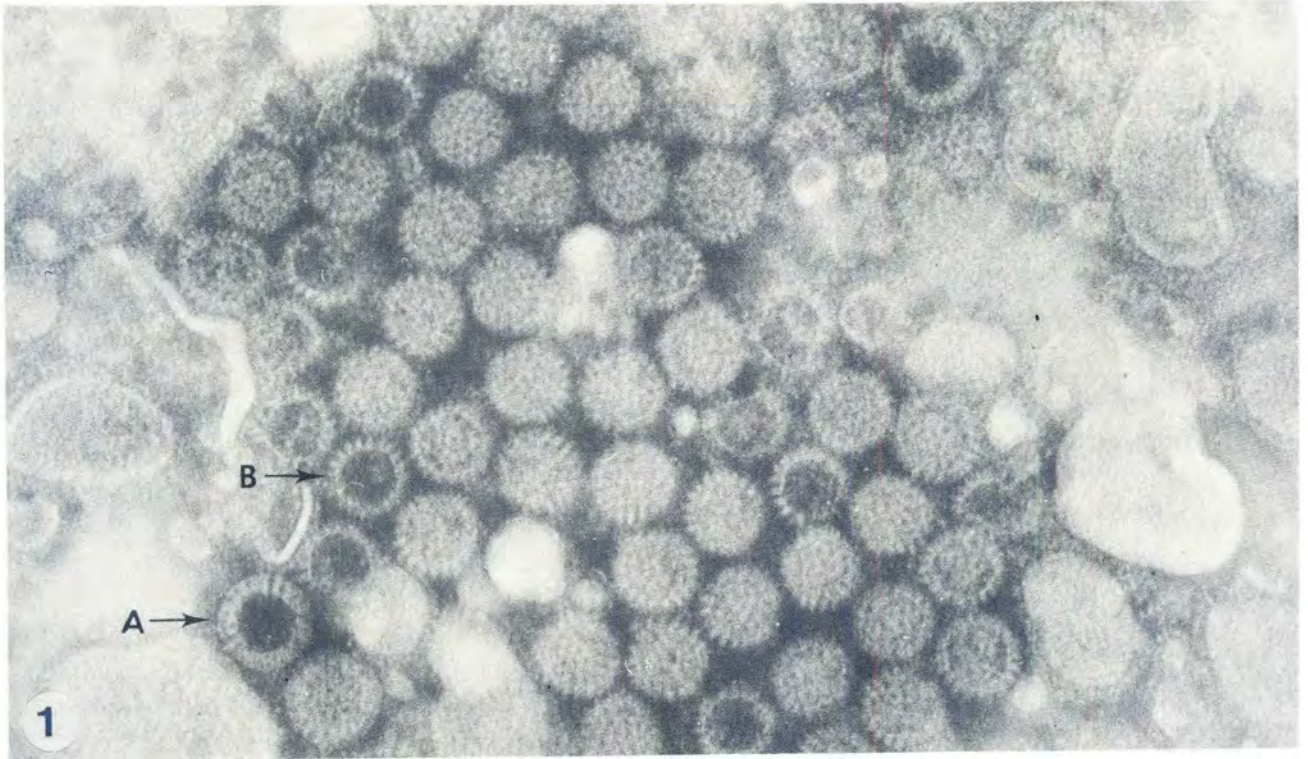


FIG. 1 Double- and single-shelled particles. Note smooth outline of double-shelled particle (A) and spiked appearance of single-shelled particle (B). $\times 180\ 000$
FIG. 2 Myxovirus (arrow) with numerous double-shelled virus particles. $\times 105\ 000$

The pleomorphic viral particles morphologically resembled myxo-, arena- and coronaviruses. However, coronaviruses, which vary in size from 80–160 nm, would appear to be smaller. The surface projections of the pleomorphic particles also differ from those observed in micrographs of coronaviruses in that they were less prominent, closer spaced and lacked the petal shape characteristic of the latter (Dalton & Haguenu, 1973).

Arenaviruses vary in size from 50–300 nm. Small particles are circular, while larger particles are irregularly shaped. The presence of one or more internal electron-dense granules (20–30 nm) which mimic ribosomes is a prominent feature of these viruses. The outer surface is studded with small regularly spaced projections (Dalton & Haguenu, 1973). The absence of internal electron-dense granules in the virions in the present study excluded them from this group.

The myxoviruses have recently been divided in 2 distinct groups, the orthomyxo and paramyxo groups. The virions of the orthomyxo group vary in size from 80–100 nm while those of the paramyxo group vary from 120–450 nm. Both groups are characterized by an envelope consisting of an outer membrane studded with small, closely-spaced, surface projections underlying which is a lipid-containing membrane (Dalton & Haguenu, 1973). From their morphology and size, the virus particles in the present investigation were assumed to be myxoviruses. An interesting observation was the abundance of myxovirus particles in the micrographs.

Woode *et al.* (1976) suggested that, because of the morphological and antigenic relationship between the reovirus-like particles in the faeces of pigs and foals with diarrhoea and the virus causing epizootic diarrhoea of infant mice, they should be included in the group of viruses for which the name "rotavirus" has been suggested (for example, the rotaviruses of children and calves).

During an investigation into diarrhoea in piggeries by the South Dakota Animal Disease Research and Diagnostic Laboratory, U.S.A., Bergeland & McAdaragh (1976) found rotavirus in the intestines of pigs in 20 (12.2%) affected herds, the ages of the infected pigs ranging from 1 day–14 weeks. In 6 of these herds, rotavirus was the only pathogen present and it was assumed that it was responsible for the diarrhoea. Ours is the first reported case of rotavirus-associated diarrhoea in South Africa.

Myxoviruses, similar in structure to those present in the faeces of the 2 pigs, were observed in faeces of calves with virus diarrhoea, as well as those with diarrhoea from which no virus could be isolated (L. Prozesky & A. Theodoridis, unpublished data). Since rotavirus causes diarrhoea in many species besides pigs (Woode *et al.*, 1976) and diarrhoea is not a feature of infection with myxoviruses (Dunne & Leman, 1975), it is assumed that in the present study the diarrhoea was caused by rotavirus while the myxoviruses probably played a secondary role.

During the period 1971–1976, 406 farmers submitted pigs with various ailments, of which diarrhoea in young pigs was one of the most frequent, to the Pathology Department of the Onderstepoort Research

Institute. Pathogenic *Escherichia coli* was isolated from approximately 43% of these cases, while *Salmonella* and *Treponema* were diagnosed in only a limited number. From these results it is clear that pathogenic organisms could not be isolated from a large percentage of young pigs with scours.

Because of the limited number of specimens hitherto examined for rotavirus, it is not possible to estimate the incidence of infection with this pathogen in South Africa. It is clear, however, that rotavirus occurs in pigs with diarrhoea in South Africa and may therefore play an important aetiological role in this ubiquitous condition.

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