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Research Note

A VIRUS WITH BI-SEGMENTED DOUBLE-STRANDED RNA GENOME IN GUINEA PIG INTESTINES

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Examination of faeces or intestinal contents of 102 guinea pigs by polyacrylamide gel electrophoresis (PAGE) as described by Holmes (Holmes, I. H., 1985. In: S. Tzipori et al. *Infectious diarrohoea in the young.* Elsevier Science Publications B. V.) for the detection of rotavirus RNA revealed, in one sample, two sharp bands stainable by ethidium bromide or silver nitrate. The positive sample was from an apparently normal adult from the colony maintained at this Institute. The two bands stained with approximately equal intensity, one migrating to a position between genome segments 3 and 4 and the other a little above segment 5 of rotavirus SA 11.

The molecules forming these bands were shown not to be digested by proteinase K (Sigma type XI) in concentrations up to 1 mg/ml, by DNAse I (Enzo Biochem. Inc.) at 100 U/ml, by RNAse T1 (BRL) at 100 U in 10 μl in appropriate buffers but were completely digested by pancreatic RNAse 1 (Sigma, 5X crystallised) at 20 μg/ml in 10 mM NaCl, 10 mM Tris pH 7.4, 2 mM EDTA (STE), indicating that they are formed by double-stranded (ds) RNA. Comparison of their electrophoretic migration distances with those of rotavirus genome segments allowed the sizes of the slow- and fast-moving bands to be estimated as being of about 1,600 and 2,600 base pairs respectively on the basis of values calculated by Holmes for rotavirus SA11 (quoted by Both et al., 1982, *Nucleic Acids Research, 10:* 7075-7088).

Centrifugation experiments revealed the two bands to be sedimented at 100.000 g for 1 h and to co-sediment at a density of 1.39 to 1.40 g/ml in isopycnic CsCl gradients. Furthermore, they were shown to be inaccessible to

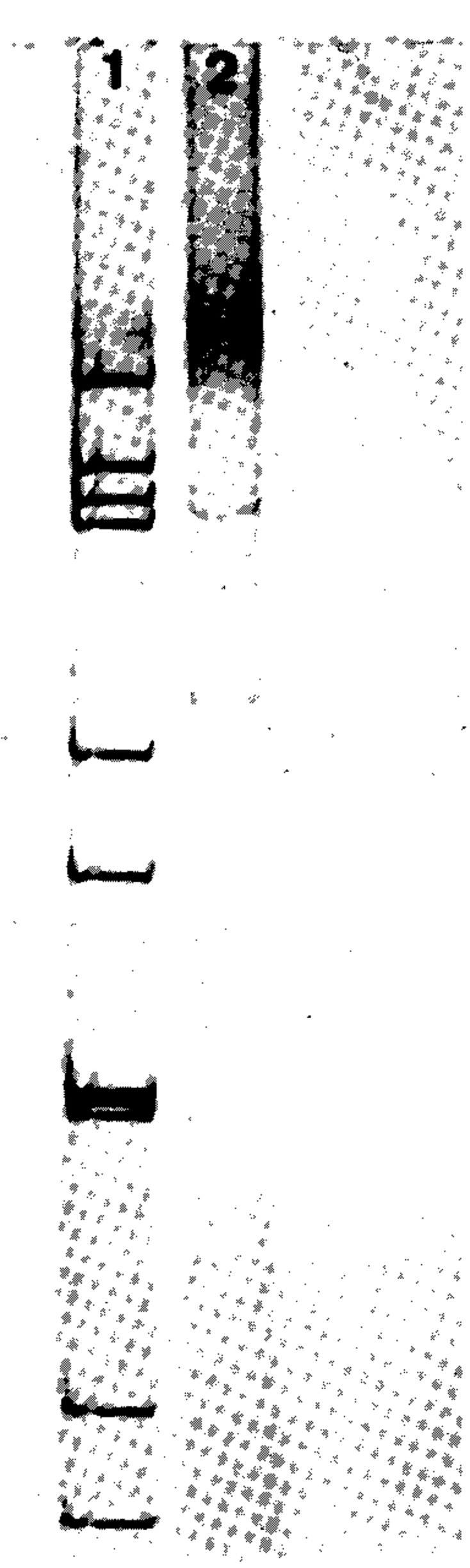

Fig. 1: electrophoresis of alcohol precipitated phenol extracts in 7.5% polyacrylamide gels stained with silver nitrate. Lane 1: simian rotavirus SA11. Lane 2: guinea pig faecal suspension with the two band profile.

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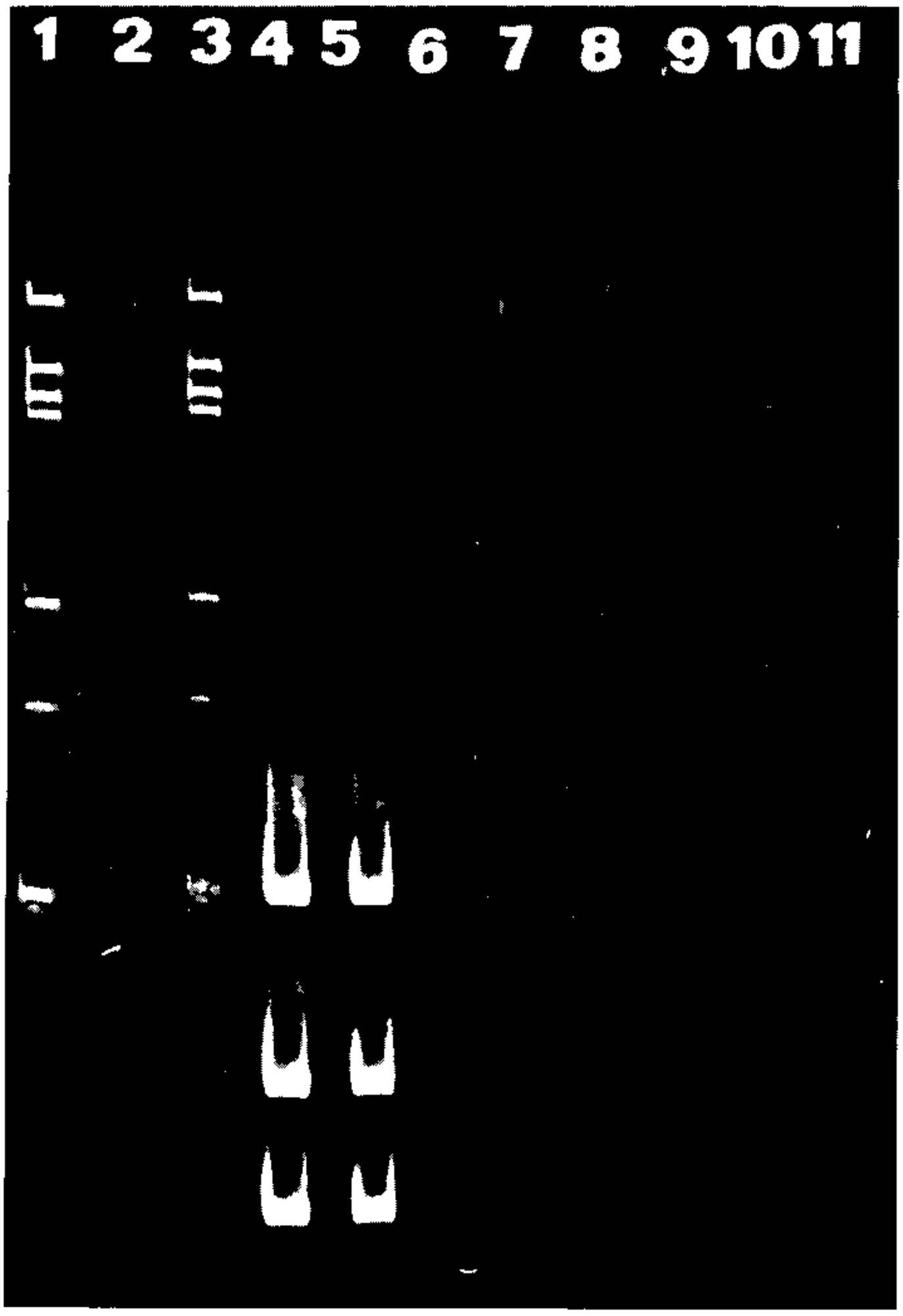

Fig. 3: action of pancreatic RNAse 1A (20 μg/ml) and of DNAse I (10 μg/ml) on gradient-purified guinea pig sample. PAGE on 7.5% gel stained with ethidium bromide. Lanes 1 to 3: aliquots of purified rotavirus SA11 heated at 80 °C for 2 min and incubated at 37 °C for 30 min without enzymes (lane 1), with RNAse 1A (lane 2) and with DNAse I (lane 3). Lanes 4 to 6: aliquots of Phi-X replicative DNA forms predigested with HaeIII, incubated at 37 °C for 30 min without enzymes (lane 4), with RNAse 1A (lane 5) and with DNAse I (lane 6). Lanes 7 to 11: aliquots of gradient-purified guinea pig sample unheated and incubated at 37 °C without enzymes (lane 7), with RNAse 1A (lane 9) or with DNAse I (lane 11) in parallel with heated (80 °C for 2 min) aliquots digested with RNAse 1A (lane 8) or with DNAse (lane 10).

molluscs (Dobos et al., 1979, *J. Virology, 32:* 593-605), some cryptoviruses of plants (Boccardo et al., 1983, *Adv. Virus Res., 32:* 171-214) and several fungal viruses with separately encapsidated genome segments (Matthews, 1982, *Intervirology, 17:* 1-199), all of which have virions with larger diameters (60 to 65 nm as opposed to about 35 nm) with clearly defined rather than indistinct surface subunit structure, lower buoyant density (1.32 to 1.35 g/ml as opposed to about 1.39 g/ml), containing genome segments more than 3000 (as opposed to about 1600 and 2600) base pairs in length.

Preliminary attempts to infect suckling guinea pigs per os with the faecal sample containing the virus resulted in the detection of the two typical electrophoretic bands in the faeces collected 6 days after inoculation of one out of 10 newborns. This result may be due to poor infectivity of the virus or to transfer of maternal immunity since all the animals used

were from the colony where the virus had been detected.

Attempts to propagate the virus in a variety of cell systems resulted in the detection of the characteristic two bands on first but not on subsequent passages in cultures of guinea pig embryo cells, human diploid fibroblasts (MRC 5), primary or secondary monkey kidney and in HEp2 human cells. In contrast with these, parallel experiments with permanent lines of hamster (BHK-21), simian (VERO, MA 104), porcine (IBRS-2) or *Aedes albopictus* (C6/36) cells gave uniformly negative results. These results suggest that the guinea pig virus undergoes a cycle of abortive replication in some of the cell culture systems tested, resulting in the synthesis of at least viral nucleic acid. The fact that this occurs in some but not in other cell types inoculated and maintained under identical conditions indicates that the electrophoretic bands detected on first passages are not derived from residual virus in the inoculum.

Our present results do no warrant conclusions regarding the origin or the pathogenic potential of the virus here described. The possibility that it may have been ingested with food and passively excreted in stools must be considered but is unlikely in view of its detection in a suckling guinea pig. Another possibility that cannot be excluded is that the two bands originate from as yet undescribed viruses of enteric bacteria or other organisms. This would also seem unlikely if our evidence that it can replicate, even if abortively, in mammalian cell cultures can be confirmed.

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