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Excretion of faecal viruses during the first year of life including attendance at a day nursery in Lisbon, Portugal

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SUMMARY

In an attempt to determine the frequency of virus infections of the gastrointestinal tract, the duration of virus shedding in faeces and its relation to outbreaks of illness of any kind, faecal samples were collected from children attending a day nursery at a Lisbon institution.

In this study, ten children were surveyed from their enrolment at the nursery, weekly specimens of faeces being collected over a period of 1 year. A total of 459 samples were obtained. In addition four of these children were also followed-up from their first week of life to their enrolment at the nursery, 79 samples being collected during this period.

Viruses were detected in a high percentage (44·4 %) of these stools, including strains of oral vaccine polioviruses together with viruses isolated in routine cell cultures and by electron microscopy (EM). These viruses were detected in both healthy and ill babies.

The possible association between viruses isolated in cell cultures or detected by EM and illness was examined and the results show that the asymptomatic excretion of viruses is frequent, particularly in children within this age group.

INTRODUCTION

Isolation of viruses from faecal extracts in cell cultures has been a standard procedure for many years, although these cultured viruses have only rarely been implicated as causative agents of gastrointestinal disease.

Since the beginning of the seventies the electron microscope has been used to examine stools from patients with acute diarrhoea and this has revealed previously unrecognized types of viruses which do not routinely grow in tissue culture, making possible a new approach to the study of gastroenteritis. Rotavirus (Steinhoff & Rochester, 1980; Brandt *et al.* 1983), adenoviruses (Richmond *et al.* 1979; Brandt *et al.* 1983; Chiba *et al.* 1983), coronaviruses (Caul, Paver & Clarke, 1975; Peigue *et al.* 1978) and astroviruses (Madeley *et al.* 1977; Ashley, Caul & Paver, 1978; Peigue *et al.* 1978; Madeley, 1979) are among the viruses which electron microscopy (EM) showed to be candidate causes of diarrhoea. Nevertheless, with the exception of rotavirus, the etiological significance of all the other viruses is still controversial.

Faecal viruses can be divided into two groups. The first group includes viruses isolated in cell cultures or following inoculation into newborn mice but rarely associated with typical gastrointestinal illness. The second includes viruses which generally do not replicate in cell cultures or newborn mice and are frequently associated with cases of diarrhoea (Madeley, 1979).

In childhood diarrhoea there is still much to be done before the relation between virus and illness and the pathogenic role of these viruses is entirely understood.

This report presents the data from a virological study of faecal samples collected from a small number of children at a day-care centre in Lisbon to determine the frequency of virus infections of the gastrointestinal tract, the duration of virus shedding, the associated clinical symptoms and to detect outbreaks of viral origin.

MATERIALS AND METHODS

The group under study consisted of 10 children, 4 of whom had been followed since their first week of life, and the remaining 6 since their enrolment at the nursery. The children were aged between 3 and 6 months at their enrolment at the nursery.

The nursery is a new building, adequately ventilated and with suitable sanitary facilities. It is divided into three rooms, one of which has cots for the smaller children. This room has a capacity for ten children.

There were no rules to exclude from the nursery any children who developed symptoms of illness.

Collection of specimens

The cooperation of the nurses and of the children 's mothers was obtained to report all cases of illness (described as any change in the normal health of the child). Each child's history was recorded with the symptoms and their duration. A stool sample was routinely collected every week by the laboratory technician involved in the study.

During the period that the four children, followed from birth, remained at home the weekly sampling of stools was made by their mothers who had been previously instructed how to proceed.

A total of 538 faecal samples were collected of which 459 samples were collected at the nursery between October 1981 and October 1982 and 79 were collected by the mothers prior to nursery admission.

Laboratory procedures

Stool samples were prepared by making a 1:10 suspension of faecal material in phosphate buffered saline (PBS) and centrifuging at 1000 r.p.m. for 30 min at room temperature. The supernatant was divided into two aliquots. The one which was to be inoculated onto cell cultures was frozen twice and further centrifuged at 3000 r.p.m. for 30 min at 4 °C. Antibiotics (1000 u. penicillin, 5000 μg streptomycin and 1000 u. neomycin per ml) were added to this supernatant and left overnight at 4 °C. The extracts were then inoculated onto cell cultures (HEp-2 and Buffalo green monkey, BGM) and incubated at 37 °C for 14 days. Viruses isolated in cell cultures were typed by neutralization as described by Lennette & Schmidt (1979).

Lim Benyesh–Melnick ' pools ' of neutralizing sera were supplied by the World Health Organization and monospecific neutralizing sera were kindly given by the Central Public Health Laboratory, Colindale, London. Other sera, prepared in our laboratory, were also used for typing isolates.

The second aliquot was centrifuged at 20000 r.p.m. for 90 min at 4 °C, in a Beckman L8·55 ultracentrifuge using a SW50 rotor. The supernatant fluid was discarded and the virus-containing sediment resuspended in distilled water and examined after negative staining with 3 % phosphotungstic acid (pH 6·3–6·8) in a Philips EM 301 electron microscope.

RESULTS

The ten children under study were numbered from 1 to 10, starting with the first child born in March 1981 up to the last one to enrol at the nursery.

Subjects 1–4 were followed from birth and 79 faecal samples were collected at home by their mothers during this period. Subjects 5–10 were enrolled at the nursery at various ages (3–6 months), and from both groups 459 specimens were collected between October 1981 and October 1982.

The complete results from the 538 faecal samples are presented in Table 1.

Isolation of viruses in cell cultures

Sixty-nine of the 79 stool specimens obtained in the home were negative while 10 were positive for viruses. During the first 3 months of life no viruses were isolated from subjects 1 and 2, nor from subjects 3 and 4 during the first 2 months. Two specimens from subject 3 yielded adenovirus type 2. From the remaining 8 positive specimens, poliovirus type 1 was isolated from 1 sample, poliovirus type 2 from 6 samples. One specimen from subject 1 yielded a mixture of poliovirus 2 and 3.

Table 2 gives the results of the 538 specimens inoculated onto cell cultures with the relevant data for each case under study, (sex, date of birth and immunization against poliomyelitis).

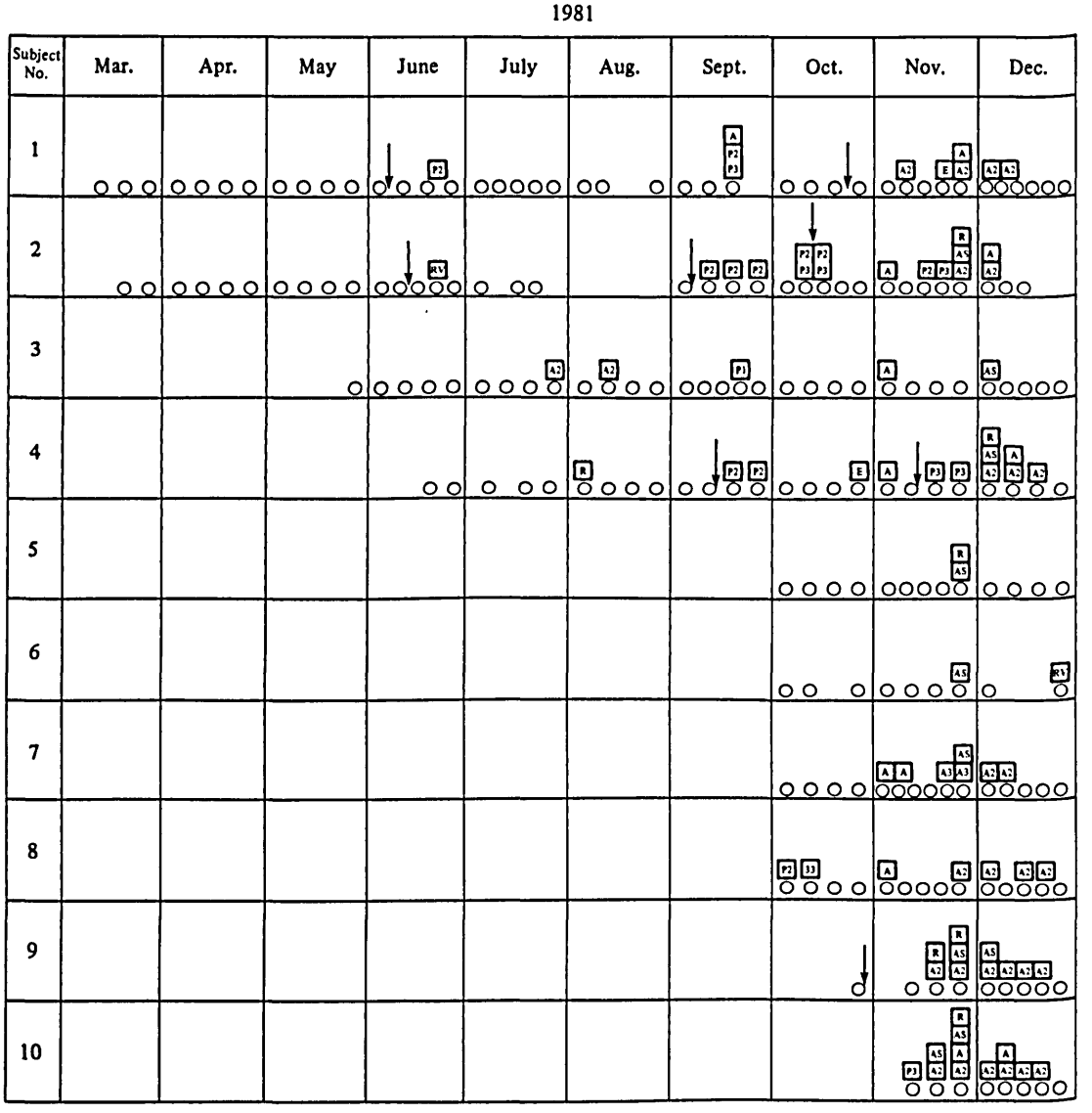
We obtained 172 isolates, corresponding to 32 % of all samples inoculated. These included 46 isolates (26·7 %) of untypable enteroviruses shown by pH stability, chloroform resistance and EM studies to belong to this group. Untypable enteroviruses were first detected in the community in October 1981 (one isolated), were undetectable in December and January 1982 but were again isolated frequently from February onwards. They accounted for 76 % of all isolates during the month of June 1982, decreasing in July and August, with only one isolate in September.

In one subject (no. 3) adenovirus type 2 was isolated from samples collected at home prior to nursery admission. Prolonged, intermittent excretion of adenovirus type 2 was a feature common to all the subjects under study, with the exception of no 6, and represented 25 % of all isolates. They were first excreted in November, predominated in December, but were isolated throughout the year.

In the third week of August 1982, a small outbreak of echovirus type 6 occured, and most of the children excreted this virus up to the end of September totalling 11·6 % of all isolates.

Between March and May 1982, 7 out of 10 children yielded one or more isolates

Table 1. *Excretion of faecal viruses during the first year of life*



R Rotavirus
C Coronavirus
A Adenovirus
A3 Astrovirus
A3 S.R.V.

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and/or one year attendance at a day nursery

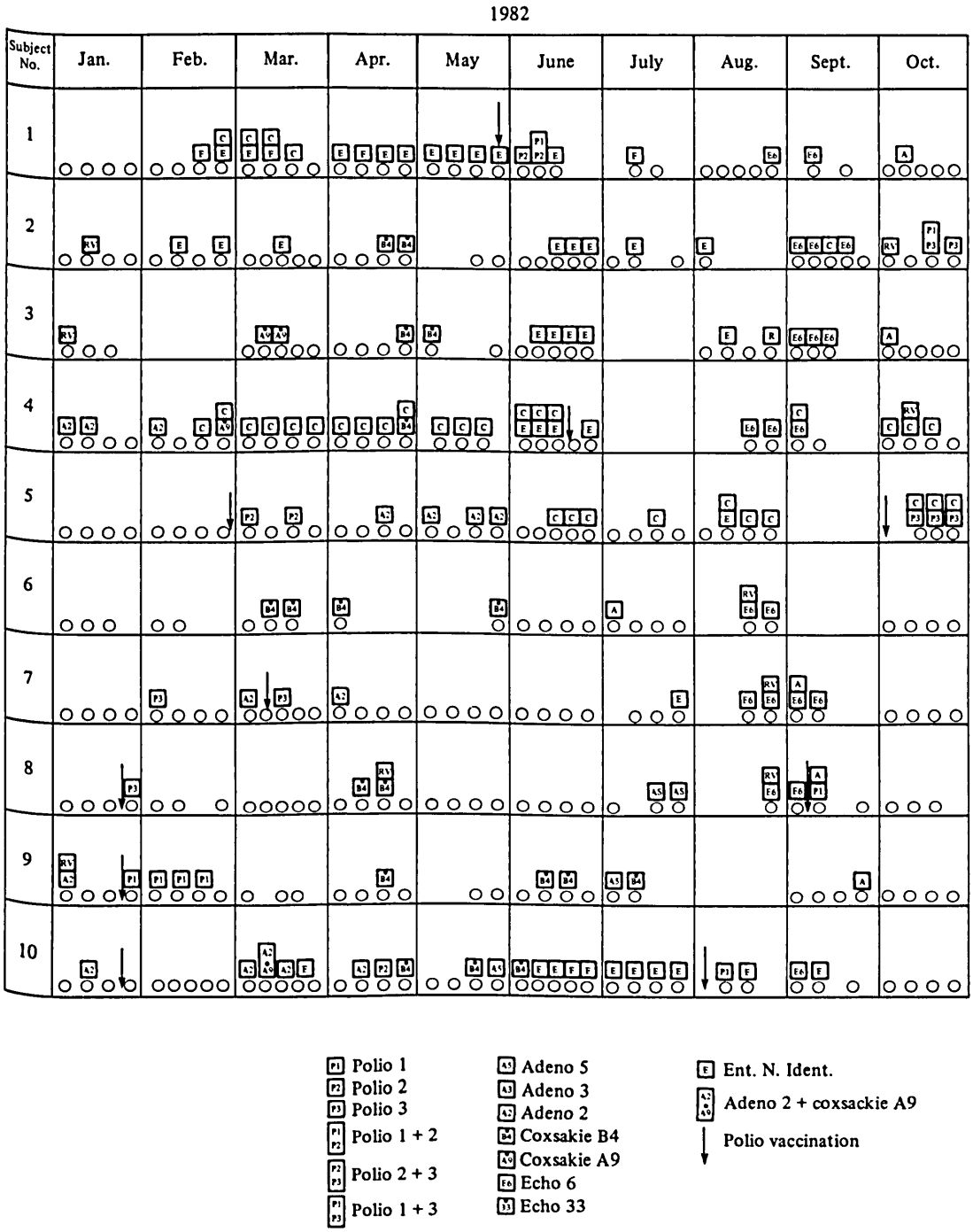

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Table 2. *Summary of subject details, stools obtained, viruses isolated in cell culture and viruses seen by EM*

					Isolation in cell cultures													Electron microscopy								
Subject	Sex	Date of Birth	OPV	Total Samples	P_1*	P_2	P_3	P_1+P_2	P_2+P_3	P_1+P_3	E_6	Cox B4	Ad. 2	Ad. 5	Ent. n. id.	Others	Total	% + Positiv	R	Ad	As	C	SRV	R+As	C+SRV	R+As+Ad	Totl	% + Positiv
1	M	3/81	Yes	78	—	2	—	1	1	—	2	—	4	—	15	—	25	32,05	—	3	—	4	—	—	—	—	7	8,97
2	M	3/81	Yes	72	—	4	2	—	2	1	3	2	2	—	8	—	24	33,33	—	2	—	1	3	1	—	—	7	9,72
3	F	5/81	No	62	1	—	—	—	—	—	3	2	2	—	5	Cox. A_9(2)	15	24,19	1	2	1	—	1	—	—	—	5	8,06
4	F	6/81	Yes	57	—	2	2	—	—	—	3	1	6	—	5	Cox. A_9(1)	20	35,08	1	2	—	19	—	1	1	—	24	42,11
5	M	6/81	Yes	49	—	2	3	—	—	—	—	—	4	—	1	—	10	20,40	—	—	—	10	—	1	—	—	11	22,45
6	M	3/81	Yes	33	—	—	—	—	—	—	2	4	—	—	—	—	6	18,18	—	1	1	—	2	—	—	—	4	12,12
7	F	2/81	Yes	51	—	—	2	—	—	—	4	—	4	—	1	Ad. 3(2)	13	25,49	—	3	1	—	1	—	—	—	5	9,80
8	M	5/81	Yes	48	1	1	1	—	—	—	2	2	4	2	—	E. 33(1)	14	29,16	—	2	—	—	2	—	—	—	4	8,33
9	F	7/81	Yes	40	4	—	—	—	—	—	—	4	7	1	—	—	16	40,00	1	1	1	—	1	1	—	—	5	12,50
10	M	7/81	Yes	48	1	1	1	—	—	—	1	3	11	1	11	Cox. A_9(1)	30	62,50	—	1	1	—	—	—	—	1	3	6,25
Totals				538	7	12	11	1	3	1	20	18	43	4	46		7	172	3	17	5	34	10	4	1	1	75	

* P1, P2, P3, poliovirus types 1, 2, 3. Cox A9,B4 coxsackievirus types A9, B4. E6, E33. echovirus types 6, 33. Ad2, Ad3, Ad5. adenovirus types 2, 3, 5. Ent. n. id. enterovirus not identified. R. rotavirus. As: astrovirus. Ad adenovirus detected by EM, untyped. C coronavirus. SRV: small round virus.

of coxsackievirus type B4, mainly at the end of April but excretion lasted until the second week of July.

Polioviruses, probably OPV derived, were isolated throughout the year.

Mixed and multiple virus isolations were common in all the children under study, with coxsackievirus type A9 being isolated four times, once associated with adenovirus type 2. The remaining positive samples were adenovirus type 3 (1 isolate), echovirus type 33 (1 isolate) and adenovirus type 5 (5 isolates).

Electron microscopy (EM)

Viruses were detected in 75 specimens (16·3 %) out of 459 faecal samples collected at the nursery and examined by electron microscopy (Tables 1 and 2).

The examination of these specimens showed the presence of adenoviruses in 17 (3·7 %), of astroviruses in 10 specimens (2·2 %), coronavirus-like particles in 35 (7·6 %) and rotavirus in 8 (1·5 %). Small round viruses (SRVs) with diameters between 20 and 30 nm were detected in 11 faecal specimens (2·2 %).

Except in 6 specimens all the particles in a stool were of the same morphology. The exceptions were 5 specimens each with 2 morphological types of viral particles (4 specimens with rotavirus and astrovirus; 1 specimen with coronavirus-like particles and SRVs), and 1 specimen containing viral particles of 3 morphologies – adenovirus, rotavirus and astrovirus.

Three of the four children followed from birth excreted viruses from an early age. In subject 4, rotaviruses were present at 2 months of age, while in subjects 1 and 2, adenoviruses and SRVs were detected before admission to the nursery.

The highest incidence of virus infections occurred in November/December 1981, during an outbreak which affected every child at the nursery. During this outbreak the faecal samples yielded adenoviruses, astroviruses and rotaviruses.

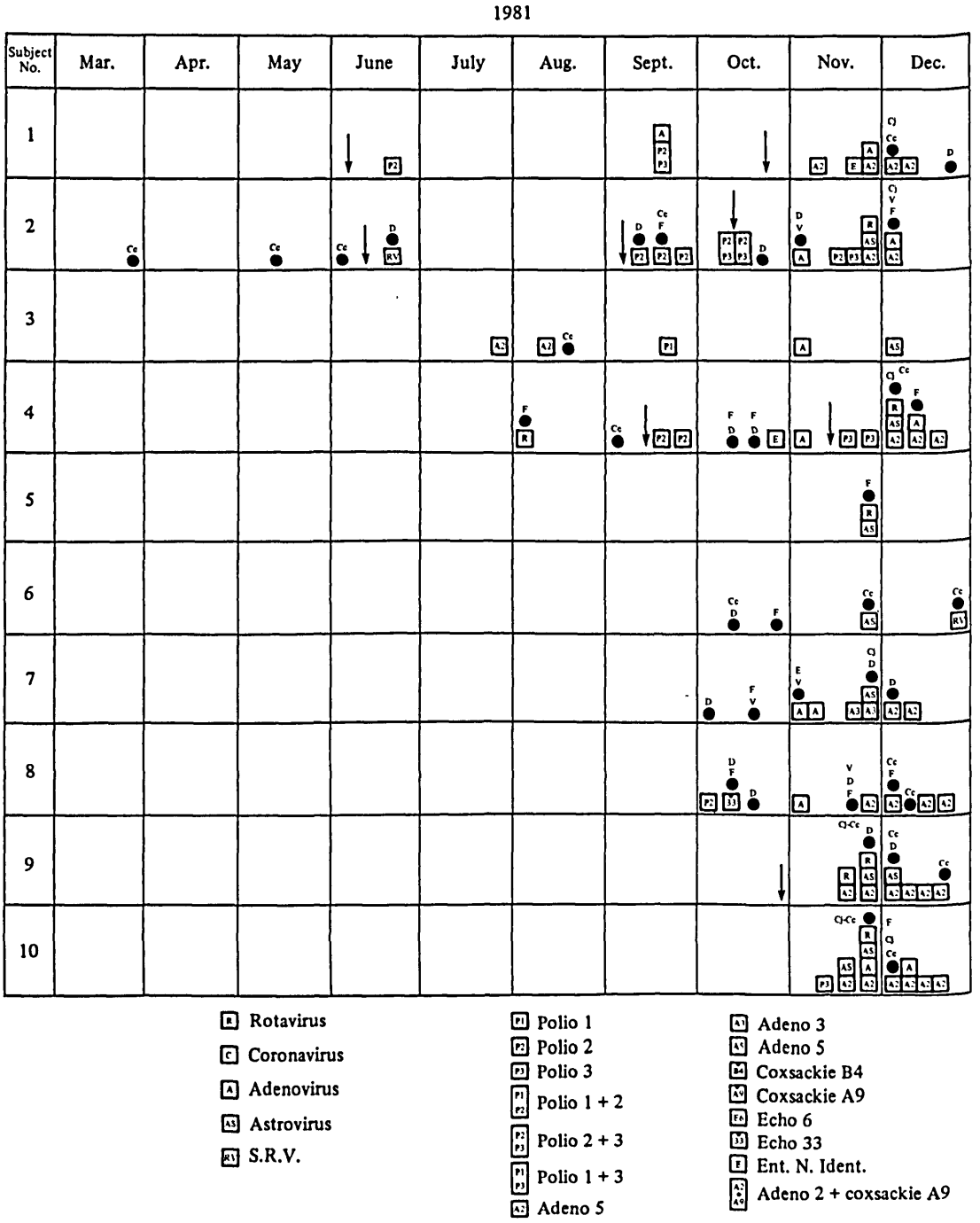
Coronavirus-like particles were detected in large numbers in 7·6 % of all samples collected at the nursery and excreted by subjects 1, 2, 4 and 5. Subject 1 excreted these coronavirus-like particles continually for 1 month; similarly in patients 4 and 5 but intermittently for more than 8 and 5 months respectively. These particles are characterized by a pleomorphic morphology and variable size, with regularly placed surface ' spikes ', and more similar to coronavirus than to any other type of virus.

Clinical symptoms and vaccines

Viruses of several types were found throughout the study but, in two well defined outbreaks, the clinical symptoms were consistent with the laboratory findings (Table 3). In November/December 1981 a viral outbreak was reponsible for fever, diarrhoea, vomiting, conjunctivitis and respiratory symptoms. During the summer of the same year (August/September) there was another outbreak affecting most children, described by the mothers as a ' common cold ' with cough, low grade fever and, in just one case, diarrhoea. In none of these outbreaks was hospitalization required, although the more severe cases were treated by their paediatricians.

During this study, the symptoms most frequently reported by the children's mothers and the nursery staff were diarrhoea, fever, vomiting, respiratory symptoms, otitis, conjunctivitis and rashes. However, most samples were collected without the mothers reporting any noticeable change in the normal health of the child.

Table 3. *Excretion of faecal viruses*



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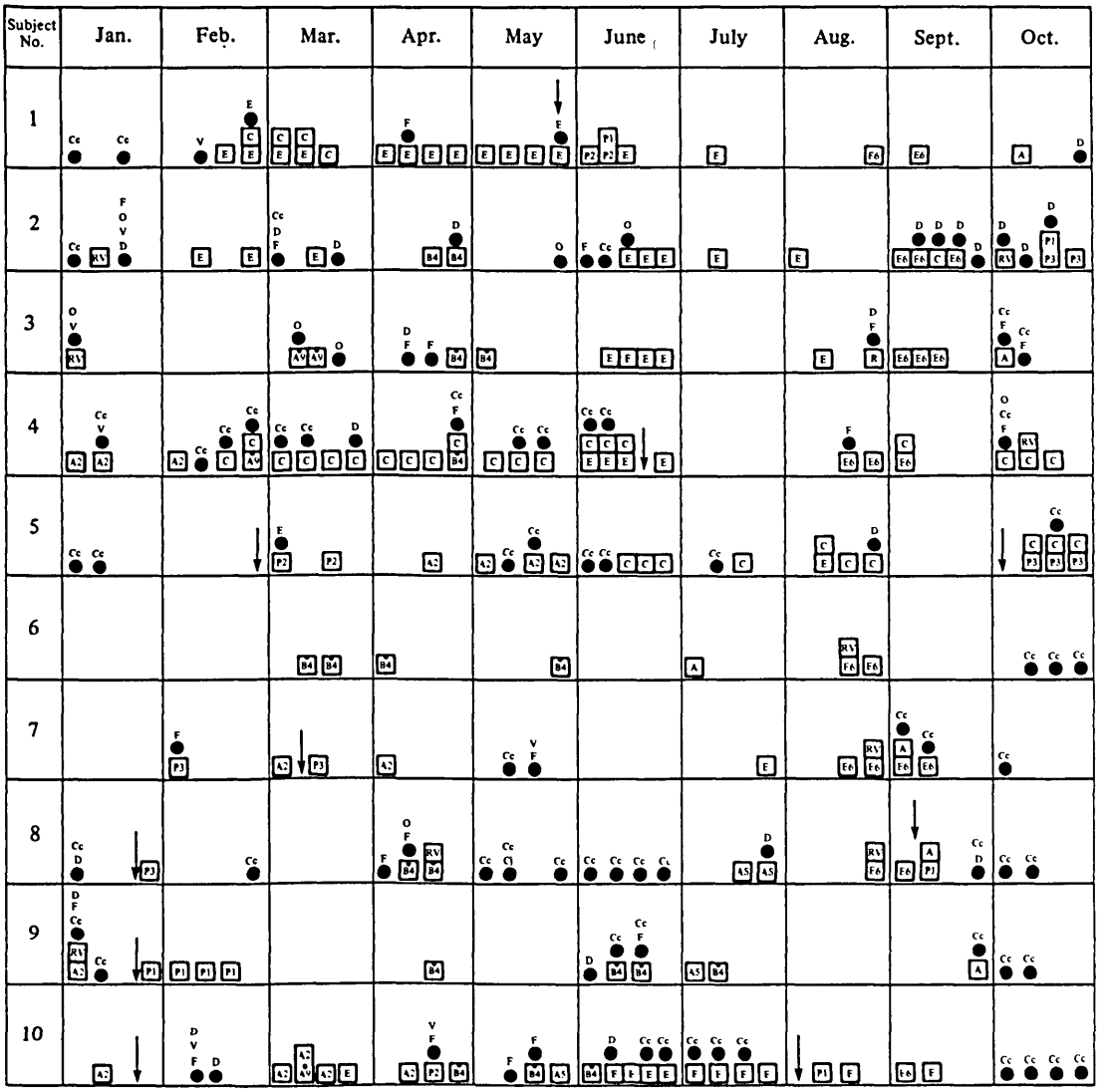

D – Diarrhoea
E – Exanthem
F – Fever
V – Vomiting
Cj – Conjunctivitis
Cc – 'Common cold'
O – Otitis
↓ – Polio vaccination

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Immunization against poliomyelitis was carried out with oral polio vaccine at the recomended ages (3, 5 and 12/15 months) with the exception of subject 3, who was never immunized because the parents refused, and subject 6 who for health reasons received only 2 of the 3 recommended doses.

DISCUSSION

For an epidemiological study such as this, the number of samples collected were thought to be adequate with a mean of 47 specimens per child during their stay at the nursery.

Immunization against poliomyelitis (OPV), naturally-occurring infections by enteroviruses as well as viruses detected by EM resulted in a high percentage of faecal excretion (44·4 %), both in healthy children and in those showing some form of illness. This faecal excretion of viruses showed seasonal variations although our conclusions were limited by the study being confined to 1 year (see, for comparison, Bell, Huebner & Rosen, 1961; Fox *et al.* 1966; Kogon *et al.* 1969).

More than one type of virus was often found in the same specimen making it difficult to establish which virus, if any, was responsible for the illness reported by the mothers. For this reason, the relation between adenoviruses and illness is complex and self-contradictory. Adenoviruses may be excreted in faeces, especially in infants, for long periods without any evidence of illness (Elveback *et al.* 1966; Spigland *et al.* 1966) although they may be involved occasionally in outbreaks of diarrhoea more often than in outbreaks of respiratory illness.

Most adenoviruses detected by EM were not isolated in cell culture and vice-versa. Adenoviruses were isolated in cell cultures on 49 occasions, and detected by EM 17 times. Only in four samples were adenoviruses detected by both methods and because more than one type of adenovirus may be excreted at a time we cannot be sure if the virus seen by EM was the same type as that isolated in cell cultures.

A small outbreak of adenovirus type 2 occurred in December 1981, with 7 out of 10 children excreting this virus. It lasted for about 1 week, progressively smaller numbers being isolated in January and February. It was excreted by all the children (except no. 6) at intervals throughout this study. Further studies are still necessary to clarify the role of adenoviruses excreted in faeces as potential causes of diarrhoea.

As mentioned in previous studies (Bell, Huebner & Rosen 1961; Yow *et al.* 1963; Fox *et al.* 1966; Kogon *et al.* 1969) enteroviruses are prevalent in the community all year long, but with a higher incidence during the summer months. In a second outbreak occurring in August and September, echovirus type 6 was isolated from eight of the children. Their mothers reported symptoms such as 'common cold', cough, low-grade fever and, in one case only, diarrhoea.

Between March and July, coxsackievirus B4 was isolated from specimens taken from 7 children, but only 3 were associated with symptoms like 'common cold', conjunctivitis, low-grade fever or cough (1 case). The child with a cough excreted adenovirus type 5 as well. All other episodes were asymptomatic. However, the lack of association between illness and infection does not mean that these viruses were not pathogenic or are incapable of inducing illness under different conditions.

In some cases, the viruses detected by EM could have been responsible for an illness. With the exception of the viruses detected during the November/December

outbreak, some of the adenoviruses appeared to be associated with diarrhoea and vomiting and, occasionally, with respiratory symptoms. Small round viruses were often associated with diarrhoea, vomiting, fever and otitis. In this study we considered that a virus isolate was responsible for clinical symptoms of illness if a virus was found within the period 1 week before to 1 week after these.

Coronavirus-like particles were detected in large numbers although in 13 specimens they were associated with other viral particles. The occurrence of the coronavirus-like particles in asymptomatic cases could be explained by the existence of non-virulent types or by host tolerance to a chronic infection. Another hypothesis (Dourmashkin *et al.* 1980) is that these coronavirus-like particles could be ' agents ' that infect intestinal parasites or artefacts representing membrane-like structures from a yeast known to exist in the gut. The successful cultivation of this agent would help to answer these questions. Alternatively a specific serological test could provide useful data.

Small round viruses (SRVs) were detected with diameters varying between 20 and 30 nm but it is difficult to be sure about the true nature of these particles. On only four occasions were they associated with diarrhoea and/or vomiting.

Rotaviruses were only detected before admission to the nursery in one specimen, from subject 4. This is in contrast to other situations in which rotaviruses have been detected in healthy newborn babies in hospital (Madeley, Cosgrove & Bell, 1978). The same child had a second rotavirus infection at the beginning of December, possibly due to another serotype.

Although many of the children excreted enteroviruses, mostly strains of OPV, none of these specimens contained particles in sufficiently high number to be detected by EM. This confirms previous reports that viruses isolated in cell cultures are seldom detected by EM and that those observed by this method rarely replicate in cells.

Of all faecal samples collected at the nursery 44·4 % were virus positive. These results encourage a cautious interpretation of finding a virus in the stool of a child in this age group with or without symptons (Harbour *et al.* 1980).

The frequent occurence of asymptomatic infections shows that the exclusion from the nursery of children with symptons of illness would have a limited effect in controlling the spread of infection.

Further studies are still necessary to establish the association of some of these viruses with gastroenteritis, as well as their transmissibility, before they can be accepted as true agents of gastroenteritis.

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