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Virus isolations from patients in general practice, 1961–71

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SUMMARY

During the period 1961–71 of 1785 viruses isolated from patients in the general population 503 (28%) were rhinoviruses, 465 (26%) influenza viruses, 248 (14%) enteroviruses, 234 (13%) herpes simplex virus, 132 (7%) parainfluenza viruses, 129 (7%) adenoviruses and 49 (3%) respiratory syncytial virus. Also isolated were 18 strains of mumps virus, 7 coronaviruses and 295 streptococci of groups A, C or G.

Fluctuations were observed in the frequency with which respiratory syncytial virus, parainfluenza virus type 2, and the adenoviruses were isolated over the 10-year period.

Influenza viruses types A and B, parainfluenza viruses types 1 and 2, respiratory syncytial virus, adenoviruses types 3, 4, 6, 7 and 21, and many enteroviruses were all associated with outbreaks.

Infections with influenza viruses A and B and parainfluenza viruses types 1 and 2 came during the winter, whereas those with parainfluenza virus type 3, enteroviruses, and rhinoviruses were more frequently seen in the summer and early autumn.

INTRODUCTION

Between 1954 and 1960 several new viruses were described in association with acute respiratory infections. In order to determine the significance of these agents in the general pattern of acute infections a number of surveys were undertaken (e.g. Medical Research Council, 1965; Fox *et al.* 1966), but few were continued for more than 2 or 3 years. By examining specimens submitted by local practitioners to the virus laboratory at Cirencester over a 10-year period, 1961–71, and the Public Health Laboratory at Gloucester from October 1970 to September 1971 it was possible to determine the relative frequency with which certain viruses or groups of viruses could be isolated from patients within a local population and also the variation in these frequencies with time and season.

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MATERIALS AND METHODS

Population sampled

Between October 1961 and September 1971 specimens were received from patients thought to be suffering from acute virus infections. Most of the patients lived in or around Cirencester, but during the last year patients from a wider area of Gloucestershire were included. Many of the specimens were collected during the course of other investigations (Medical Research Council, 1965; Hope-Simpson & Higgins, 1969; Medical Research Council/Public Health Laboratory Service, 1973) when an attempt was made to secure an unselected sample; at other times specimens were examined without a precise knowledge of the relation of the illness sampled to the total amount or type of sickness present in the community at that time.

Laboratory techniques

Specimens consisted mainly of nose and throat swabs in transport medium, but faeces and material from local lesions were also received. Most specimens were delivered to the laboratory on melting ice within a few hours of collection.

The extent to which nose and throat swabs were examined increased with time. Initially only cultures of human embryo kidney and monkey kidney were used, but within a few months the inoculation of cultures of the Bristol line of HeLa cells and a blood plate were introduced. Cultures of diploid fibroblasts (WI 26 or WI 38) were employed when they became available in October 1962 and specimens were examined in suckling mice after the end of 1962.

From September 1965 onwards a limited number of specimens (approximately 700), generally those negative on examination by these methods, were inoculated into organ cultures of human embryonic ciliated respiratory epithelium. For limited periods specimens were examined in fertile hen eggs, the L132 line of human embryo lung (Davis & Bolin, 1960), or on solid media for the isolation of mycoplasmas.

The methods employed have been described in detail elsewhere (Higgins, Ellis & Boston, 1963; Higgins, Boston & Ellis, 1964; Higgins, 1966).

RESULTS

Frequency of isolation of viruses and variation with time

The number of specimens examined and the distribution of the agents isolated are shown, at monthly intervals, in Fig. 1.

Sample

The number of patients sampled each year ranged between 307 in the first year and 599 in 1968–9 with a larger number of specimens being received in the winter than in the summer months of each year. The disproportionately large number of specimens examined in the colder months from 1968 onwards was the result of the particular interest of one practitioner in influenza (Hope-Simpson, 1970). In all, specimens from well over 5000 patients were examined during the 10-year period.

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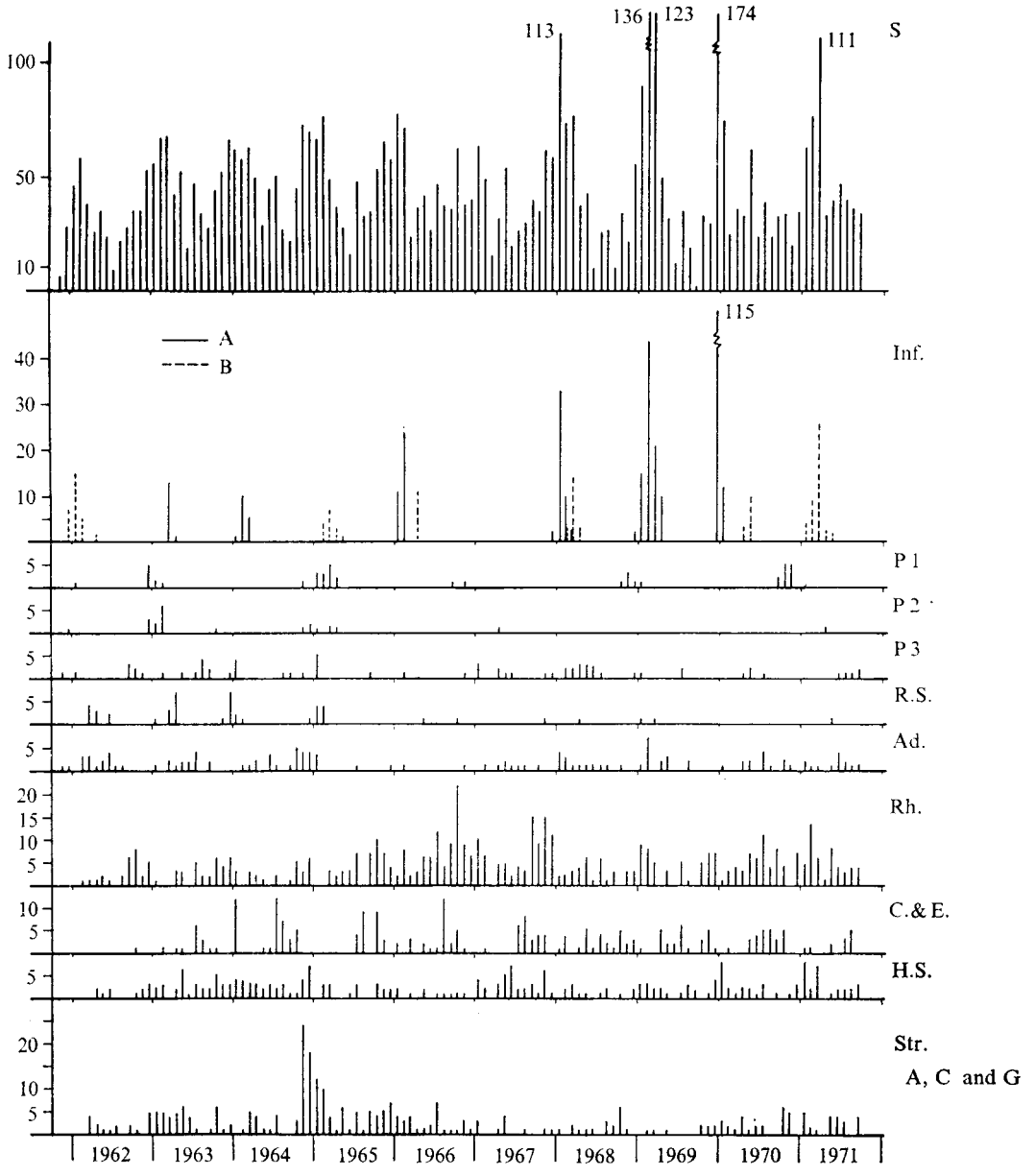

Fig. 1. The number of specimens examined and agents isolated between October 1961 and September 1971. *Key*. S = total samples; Inf. = influenza viruses; P1, P2, P3 = para-influenza 1, 2 and 3 viruses; R.S. = respiratory syncytial virus; Ad. = adenoviruses; Rh. = rhinoviruses; C & E = Coxsackie and echoviruses; H.S. = Herpes simplex virus; Str. = Streptococci of groups A, C or G.

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Influenza virus infections

A total of 465 infections with influenza viruses were diagnosed, 333 were with type A virus and 132 with type B virus. There were six outbreaks of infections with influenza virus A and six with influenza virus B during the 10 years and on three occasions infections with influenza A virus preceded those with influenza B virus in the same winter. Strains of one or both types of virus were isolated between January and December of every year, although there was a period in excess of 12 months in 1966 and 1967 when no influenza virus was detected.

Parainfluenza virus infections

Of the 132 parainfluenza viruses isolated, type 3 (68 strains) was the commonest. Infections with type 3 virus showed clustering in time but not in the pronounced fashion that was observed with types 1 and 2. Forty-three strains of type 1 virus were isolated from outbreaks which happened every second year so that five distinct periods were established when parainfluenza virus type 1 was circulating in the community. Parainfluenza virus type 2 behaved in a similar manner for the first 4 years when 19 strains were isolated, but only two further strains were detected in the 6 years beginning in the autumn of 1965. Type 4 virus was not isolated.

Respiratory syncytial virus infections

During the first 4 years well-defined outbreaks of infections with respiratory syncytial virus were observed and 39 strains of the virus were isolated over this period. However, only seven further strains were detected during the next 6 years.

Adenovirus infections

Adenoviruses were most often encountered during the early part of the study. After 2 years when infections with these viruses were rarely seen, adenoviruses were again commonly isolated in the latter half of the period of observation.

A total of 119 strains were isolated, 74 (62%) of which belonged to type 1, 2, or 3. Infections with types 1 and 2 were diagnosed in all but one of the 10 years; 22 of the 26 strains of type 3 virus were isolated during three outbreaks, one in each of 1964, 1968 and 1971. Similarly, 10 of 13 strains of type 4 virus were isolated during two outbreaks, one in 1970 and another in 1971 and six of nine infections with type 6 were between April 1964 and January 1965. Ten strains each of types 7 and 21 were isolated; each virus appeared only twice, type 7 in 1962 and 1969 and type 21 in 1962 and 1963.

Rhinovirus infections

The most commonly isolated agent was a rhinovirus. Five hundred and three strains were detected, of which only 96 (19%) could be grown in monkey kidney tissue culture (M strains). Rhinovirus infections were less frequently diagnosed in the early part of the study when fewer methods for their detection were employed; even so there was not a time when there was a complete failure to isolate these viruses.

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Enterovirus infections

A total of 248 enterovirus infections were diagnosed.

(i) *Poliovirus infections.* Twenty-one strains of poliovirus were isolated and in most instances a history of recent vaccination with live attenuated virus or close contact with such a person could be established. None of the patients from whom poliovirus was isolated had evidence of involvement of the central nervous system.

(ii) *Coxsackievirus infections.* Almost half, 114, of the enteroviruses isolated were shown to be Coxsackie A viruses and nine serotypes were represented. Type 16 was the commonest type and 36 of the 38 strains isolated were detected in three periods, 1963–4, 1967, 1969–70. Similarly, 13 of 15 strains of type A9 were isolated during the 12 months from August 1966 to July 1967 and five of six strains of type A6 between June and August 1964. Infections with type A10 were diagnosed mainly (12 of 15 strains) during two outbreaks, one in 1966–7, the other in 1969, whereas 15 out of 18 strains of type A4 were detected in 1965–6 and 1968. Type A2 (10 strains), type A3 (2 strains), type A5 (9 strains) and type A8 (2 strains) were isolated too infrequently or were too widely dispersed in time to provide evidence of outbreaks of infections with these viruses in the community.

All types but type 6 were represented among the 57 Coxsackie B viruses isolated. Twenty-three strains of type B5 were detected – 19 of these infections in two outbreaks, one in 1965 and the other in 1971. Type B2 was the next commonest type; 10 of the 13 strains were isolated during the summers of 1964 and 1967. Type B1 (7 strains), type B3 (9 strains) and type B4 (5 strains) made up the rest of this group.

(iii) *Echovirus infections.* Fifty-six echoviruses of 16 different serotypes were isolated but only types 6 and 9 were detected on more than five occasions. Ten of the 15 infections with type 6 virus were diagnosed between July 1968 and July 1969, and 9 of the 13 strains of type 9 virus between July and November 1969. The remaining serotypes isolated were types 3, 4, 7, 11, 13, 15, 17, 19, 21, 22, 23, 24, 25, and 30.

Herpes simplex virus infections

Isolations of herpes simplex virus were almost as common as those of enteroviruses (234 against 248) and isolations were made with approximately the same frequency during each year of the study.

Streptococcal infections

Of the β-haemolytic streptococci isolated only those belonging to groups A, C or G were recorded; 295 such infections were diagnosed. Most of the streptococci were members of group A; few belonged to group C (23 strains) or group G (17 strains). Isolations were made more frequently during the first half of the study and included a large outbreak of infections in the winter of 1964–5.

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Infections with other agents

Mumps virus was isolated on 18 occasions, although infection with this virus was seldom suspected on clinical grounds at the time the patients were swabbed.

No isolations of *Mycoplasma pneumoniae* were made during a search for this agent between September 1963 and October 1966.

Only seven coronavirus infections were proved, but their distribution in time suggests that there were outbreaks of infection with these viruses. One strain of 229E virus was isolated in April 1968 and three further infections with this agent were diagnosed as illnesses in the spring of 1971. Another probable outbreak, in the spring of 1970, was suggested by the isolation of three organ-culture strains from illnesses in March and April of that year. A coronavirus was not isolated from any of the remaining 144 suitably examined specimens collected between 1966 and 1971.

Variation in the frequency of isolation of viruses with season

The frequency with which the various agents or groups of agents were isolated during each of the months of the year is shown in Fig. 2*a–g*. This frequency was calculated by determining the proportion of specimens collected in any one month (e.g. January) for all of the 10 years which yielded a particular virus.

Influenza viruses

The isolation of these viruses was restricted to the winter months and no strain of either influenza A or B virus was isolated between June and November of any year. During the 10 years studied influenza A virus was isolated from a greater proportion of specimens in December, January and February than other months when it was present, whereas influenza B virus showed a tendency to appear later and to be relatively more frequently isolated in March and April. These figures may be biased by the timing of influenza epidemics in the later years as these were more extensively sampled than earlier outbreaks.

Parainfluenza viruses

Although these viruses were never shown to be a dominant cause of illness there was a decided difference in the frequency with which each of the three types was isolated in relation to the time of year. Types 1 and 2 were more frequently isolated during the winter months and no isolations of either type were made from May to August in any year. The reverse was true of type-3 virus which was shown to be a more important cause of illness from April to September than during the remainder of the year.

Respiratory syncytial virus

Respiratory syncytial virus was another virus which was commoner in the cooler months of the year (December–April) with, in this study, the greatest incidence in the spring (April).

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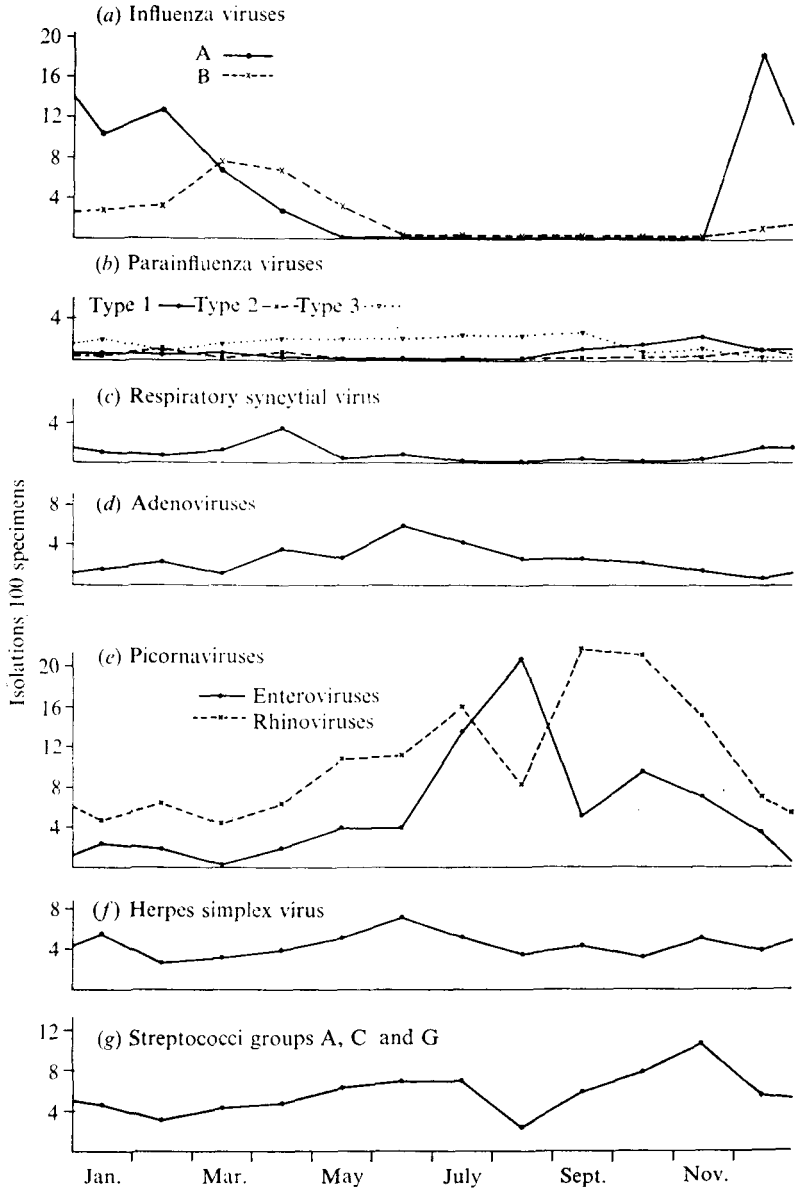

Fig. 2. The monthly variation in the proportion of specimens shown to contain the various agents isolated between October 1961 and September 1971.

Adenoviruses

Although there were a number of periods of a month or more when these viruses were not isolated, these occasions were at different seasons in different years. The isolation rate for adenoviruses was slightly higher in April to July than during other months.

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Picornaviruses

For much of the year the isolation rates for enteroviruses and rhinoviruses ran parallel, with rhinoviruses the more common in every month except August. For the first 4 months of the year the isolation rates were low but rose during the summer to reach a peak in July and August for enteroviruses and in September and October for rhinoviruses.

Herpes simplex virus

Herpes simplex virus was demonstrated on average in 2–8% of the specimens examined each month with no special change in frequency at any season.

Streptococci

β-Haemolytic streptococci belonging to groups A, C or G were isolated on average from between 3 and 7% of specimens during most months. However, isolation rates of little over 2% (August) and as high as 10% (November) were also noted.

DISCUSSION

The epidemiology of the viruses encountered will vary according to the type of population studied and the results described here may be applicable only to small urban and semi-rural communities similar to those sampled in this survey.

Unfortunately the nature of the sample examined does not allow an accurate interpretation of the results in terms of infections in the local community as a whole. However, apart from the intensive sampling of respiratory illnesses during outbreaks of influenza in the last four winters there is no evidence that the sample was biased and it is not unreasonable to accept these findings as, at least, indicative of the prevalence of the various agents in the general population.

A more likely source of error is the known variation in efficiency with which the methods employed were able to isolate the different agents sought. For example, influenza viruses were probably isolated from a high proportion of those who were infected and sampled (Higgins & Ellis, 1972) but, despite the use of more than the average number of techniques, the efficiency of isolation of the rhinoviruses was probably less than half that of the influenza viruses. The incidence of respiratory syncytial virus and the parainfluenza viruses was also probably underestimated.

Although coronaviruses were isolated they were sought in less than 5% of the specimens and their importance in the total illness suffered by the community remains to be determined.

These findings show that certain viruses such as influenza virus types A and B, parainfluenza viruses types 1 and 2, respiratory syncytial virus, many of the enteroviruses and some adenoviruses are responsible for outbreaks of infections within a community. Infections with other agents, rhinoviruses as a group (isolates were not typed), other adenovirus serotypes (e.g. types 1 and 2), herpes simplex virus and β-haemolytic streptococci of groups A, C or G are either constantly present in the population or are associated with sporadic cases only.

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It is apparent from this study that some viruses are of major importance in causing illness only at certain times of the year. The myxoviruses, with the exception of parainfluenza virus type 3, strongly influence morbidity during the winter months, whereas parainfluenza virus type 3 and the enteroviruses are responsible for a greater proportion of sickness during the summer months. A significant proportion of illness throughout the year is caused by rhinoviruses but these viruses have their greatest impact on the health of the general population in the autumn.

The value of this type of long-term study is the ability to observe the changing pattern of infections in a community. Because all isolations were made in one laboratory any change in the observed frequency with which a particular agent was detected probably reflected the true situation in the community. Where a number of different laboratories take part the question of their relative efficiency in isolating the various agents must arise and lead to doubts as to the significance of the findings. Similar doubts may result from the pooling of the findings in different populations. In this study the virtual disappearance of infections with respiratory syncytial and parainfluenza type-2 viruses after outbreaks in the first 4 years would have been more difficult to establish had the timing of the survey been different or the specimens divided among a number of laboratories. Similarly, the variation in the frequency with which adenoviruses, particularly type 21, and streptococcal infections were encountered and the seasonal fluctuations in the relative importance of rhinovirus infections could only be determined with certainty by localizing the community studied and limiting the number of investigating units.

Long-term studies are also of value in focusing attention on the epidemiological problems which still exist concerning many common viral infections. However, until it is possible to diagnose in the laboratory many of the illnesses which at present remain unconfirmed, it will be difficult to define these problems with accuracy and progress towards solving them will be correspondingly slow.

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