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The association of viruses with clinical pertussis*

By MARGUERITE S. PEREIRA
Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London, NW9 5HT

and J. A. N. CANDEIAS
Department of Microbiology and Immunology, Institute of Biomedical Sciences, University of São Paulo, Brazil

SUMMARY

This study describes the results of attempts to grow viruses from per-nasal swabs taken from 136 children with clinical pertussis.

Altogether 37 strains of a variety of different viruses were isolated. Adenovirus was the most frequent, making up 30% of the total. Besides these, herpes simplex, measles, influenza A2, influenza B, mumps, poliovirus and respiratory syncytial virus were detected.

Bordetella pertussis was isolated from 22% of the cases.

It appears that a pertussis-like syndrome can be caused by many agents besides *Bord. pertussis* and an accurate diagnosis requires laboratory confirmation.

INTRODUCTION

The clinical diagnosis of pertussis is based on a respiratory disease syndrome with a characteristic whooping cough. In the past it has been a common and often serious illness of childhood, particularly in those under 1 year of age. The use of a specific vaccine made of *Bordetella pertussis* has considerably modified this picture in many countries. In Britain, for example, the number of cases notified has fallen from 174,000 in 1952 to under 4,000 in 1969. However since notifications had not continued to decline in the last few years doubts have been raised as to the continued efficacy of pertussis vaccine, especially since there were reports of the disease in vaccinated children.

A study to investigate this was undertaken in Britain in 1966 and in a preliminary report (1969) the bacteriological findings described indicated that *Bord. pertussis* was isolated from 792 of 3564 households where a possible case of whooping cough had been reported. The incidence of viruses in these cases has so far not been reported.

Links between viruses and whooping cough have been sought by many workers in the past. In Lapin's monograph on whooping cough published in 1943 he comments on the long history suggesting a virus aetiology for pertussis. Feyrter in 1927 found nuclear inclusions in the lungs of a child who had died of pertussis. He

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noted the striking similarities of the pulmonary lesion with those found in pneumonia after measles. This resemblance was emphasized by McCordock (1932), who saw intranuclear inclusions in the lungs of 35 fatal whooping cough cases, and similar lesions were reported by Goodpasture, Auerbach, Swanson & Cotter in 1939 in five infants who died from what he called a new virus infection following measles and whooping cough. However, filtrates of infectious material from cases did not transmit whooping cough nor did filtrates of *H. pertussis*, so his comment was: 'practically all investigators are now convinced that whooping cough is not due to a virus'.

However, since then there have been several reports of viruses causing a pertussis-like illness. Chany and his colleagues reported in 1958 their results of virus studies with children with pneumonia. They presented evidence associating adenoviruses with whooping cough and pneumonia and compared their histological findings with those reported by Goodpasture *et al.* in 1939. They commented on the difficulty of distinguishing some of their cases of proven adenovirus infection from whooping cough and measles. Farber & Vawter in 1961 reported on a fatal case of 'clinical pertussis' where inclusions typical of adenovirus were found in sections of lung.

In 1964 Olson, Miller & Hanshaw reported on the isolation of adenovirus type 12 from four children with a pertussis-like syndrome, and in 1966 Collier, Connor & Irving reported the isolation of adenovirus type 5 from a child who died after a pertussis-like illness. In this case several siblings had colds at the same time and one younger brother was observed to have a paroxysmal cough with a whoop, typical of pertussis. None of these children had antibody to *Bord. pertussis* but antibody to adenovirus was detected and the lungs in the fatal case showed typical adenovirus inclusions. Finally Connor (1970) found evidence of infection with adenovirus in 11 out of 13 children with a pertussis syndrome in Florida. Thus when a virus has been associated with pertussis it has almost always been adenovirus. The association of other viruses does not seem to have been reported, but it seemed possible that other viruses could be implicated, especially as the first strain of the Hong Kong/68 variant of influenza A2 to be isolated in Britain was from a child diagnosed as having pertussis and only when attempts to grow *Bord. pertussis* failed was the possibility of a virus aetiology investigated.

The results reported here were obtained from a study in São Paulo, Brazil, over a period of 8 months in 1969–70.

MATERIALS AND METHODS

Pernasal swabs were taken from 136 children with clinical pertussis and after the inoculation of media for bacteriology the swabs were inoculated into HeLa cells, rhesus monkey kidney cultures (from Flow Laboratories) and into fertile hens' eggs. Standard procedures for the detection and identification of viruses were followed.

RESULTS

Altogether 37 strains of virus were grown from 37 (27 %) of the children (Table 1). A wide variety of different viruses were represented, nearly all of which are commonly associated with respiratory infection. Suitable systems for isolating rhinovirus were not available and none were detected.

Table 1. *Virus isolations from cases of clinical pertussis, São Paulo* 1969–70

Virus isolated	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Total
Adenovirus	3	3	3	1	—	1	—	—	11
Herpes simplex	1	3	—	1	2	1	1	—	9
Measles	—	1	—	2	1	—	1	1	6
Influenza A 2	—	1	1	1	—	—	—	—	3
Para influenza	—	1	1	—	—	1	—	—	3
Influenza B	—	—	1	—	—	1	—	—	2
Mumps	—	—	1	—	—	—	—	—	1
Poliovirus	—	—	1	—	—	—	—	—	1
Resp. syncytial	—	—	—	1	—	—	—	—	1
Total	4	9	8	6	3	4	2	1	37
No. of specimens examined	6	12	26	14	21	31	16	10	136

Table 2. *Frequency and type of* 37 *viruses isolated*

Virus	Type	Number	Total	%
Adenovirus	1	4	11	30
	2	1		
	3	3		
	5	3		
Herpes simplex	—	—	9	24
Measles	—	—	6	16
Influenza A 2	—	—	3	8
Para influenza	1	1	3	8
	3	2		
Influenza B	—	—	2	5
Mumps	—	—	1	3
Polio	2	—	1	3
Resp. Syncytial	—	—	1	3

The number of viruses isolated was considerably greater during the three winter months, July–September. The average number of cases of suspected pertussis in those months was 17 and a virus was isolated in 44 %.
During the other 5 months the number of cases averaged 17, but in this period the percentage of viruses isolated was only 17 %.

Table 2 shows the frequency with which different viruses were detected. Adenoviruses were the most frequent virus isolated and formed 30 % of the total strains isolated. The serotypes were those commonly associated with infections in childhood. The next most frequent was herpes simplex. Whether these were aetiological

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agents in the disease process is uncertain, but since most of the children were young it is possible that some of the illnesses anyway were primary infections with this virus.

The isolation of measles virus was perhaps surprising. These strains were isolated in rhesus monkey kidney after 2–3 passages and were identified by the characteristic inclusions in stained cover-slip preparations and finally by specific neutralization tests. One cannot exclude the possibility that they had been carried as latent viruses in the monkey kidney cultures as has been shown by Ruckle (1958) to occur in a proportion of rhesus monkeys. Against this is the fact that measles virus was not found in other cultures in the same batch, neither inoculated nor controls.

Table 3. *Isolation of viruses and* Bord. pertussis *in 136 cases of clinical pertussis*

	No. of patients	No. of virus isolations	%
Bord. pertussis isolated	29	5*	17
Bord. pertussis not isolated	107	32	30

* Three adeno-, 1 polio-, 1 herpes virus.

Of the six strains isolated, four were from children who had been ill for 10 days with fever and cough, one from a child with fever, cough and sore throat, and one from a child 11 days after onset of illness with fever, cough and a discrete rash.

The three strains of influenza A 2 were all similar to the Hong Kong/68 variant and were isolated one in each of the three winter months. These strains provided an indication of the prevalence of this virus in the population as no epidemic was recorded. This pattern was not unlike that observed in Britain earlier that year when this variant was isolated during an unusually prolonged period of several months but did not cause any well-defined epidemic.

The isolations of influenza B viruses were also widely separated in time.

The strain of mumps virus was isolated from a child in whom parotitis was not a feature; but it is of course well known that a considerable proportion of mumps infections are similarly without parotitis.

The genetic markers of the strain of poliovirus were not investigated and it is not known whether it was a vaccine or a wild strain.

Table 3 shows the proportion of cases where *Bord. pertussis* was grown, where a virus was isolated and where they were associated. The 21 % isolation rate for *Bord. pertussis* is curiously enough almost identical with that reported in the study done in Britain on a much greater scale where *Bord. pertussis* was isolated from 792 in 3564 households, just over 22 %. The virus isolation rate in cases where *Bord. pertussis* was not isolated was nearly twice as great as in cases where it was isolated.

DISCUSSION

It appears from these results that many viruses besides adenovirus may be associated with a syndrome easily confused with pertussis. Some of the viruses isolated may well have been unconnected with the current infection but it was impossible to determine which of these was merely incidentally detected in the respiratory tract. Even a serological response would not confirm the association since double infections occur frequently and either of the isolated agents could have provoked the clinical syndrome observed.

The large number of different viruses detected suggests that the diagnosis of pertussis cannot be made accurately on clinical grounds and must be confirmed bacteriologically.

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