J. Hyg., Camb. (1973), **71**, 139
Printed in Great Britain

On the aetiology of whooping cough

By F. A. LEWIS, I. D. GUST and N. McK. BENNETT
Virus Laboratory, Fairfield Hospital for Communicable Diseases, Yarra Bend Road, Fairfield, Victoria 3078, *Australia*

(*Received* 28 *June* 1972)

SUMMARY

The results of routine bacterial and viral studies on 483 patients with whooping cough, investigated at one hospital over a five year period are presented. The possible role of respiratory viruses in the aetiology of the disease is discussed.

INTRODUCTION

A number of studies have suggested that viruses may cause whooping cough.

In 1934 McCordock & Smith described inclusion bearing cells in the epithelium of the upper respiratory tract of children who died of pneumonia following whooping cough. The inclusions which they described resemble those produced by some viruses.

Following the introduction of tissue culture techniques, Olson, Miller & Hanshaw (1964) isolated adenovirus type 12 from the throats of four children with whooping cough. Infection was confirmed by subsequent rises in neutralizing antibodies, and in each patient cultures for *Bordetella pertussis* were negative. In a prospective study conducted by Edinburgh City Hospital, Urquhart, Moffat, Calder & Cruickshank (1965) recovered viruses, a third of which were adenoviruses, from 15 of 49 children with clinical whooping cough. *B. pertussis* was isolated from only two of the 49 patients. In 1966 Collier, Connor & Irving isolated adenovirus type 5 from the liver, lungs and kidney of a 4-year-old child who died following a whooping cough-like illness complicated by pneumonia. Connor (1970) isolated adenoviruses (type 1, 2, 3 and 5) from 11 of 13 sporadic cases of whooping cough in which there was no evidence of infection with *B. pertussis* by culture or serology.

Pereira & Candeias (1971) isolated 37 strains of virus, almost a third of which were adenoviruses, from 136 Brazilian children with whooping cough. Although *B. pertussis* was recovered from 29 children, the virus isolation rate was twice as high in the group from whom *B. pertussis* was not cultured.

About the same time, Sturdy, Court & Gardner (1971) reported on 34 children with whooping cough admitted to hospitals in the Newcastle area. *B. pertussis* was not isolated from any of these children, whereas viruses, mainly respiratory syncytial virus and adenovirus, were obtained from 20. These authors suggested that, in view of the doubt existing about the aetiology of whooping cough, a large-scale prospective study should be undertaken.

The results presented in this paper review 5 years experience of routine bacterial and viral studies performed on patients with whooping cough at Fairfield Hospital for Communicable Diseases, Melbourne.

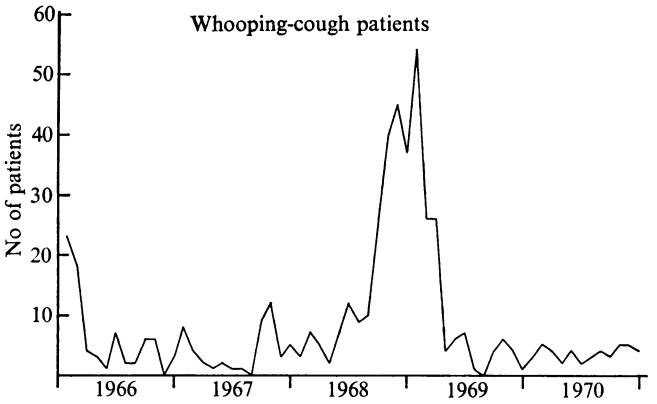

Fig. 1. The number of patients admitted to Fairfield Hospital for Communicable Diseases with whooping cough from 1 January 1966 to 31 December 1970.

PATIENTS AND METHODS

Epidemics of whooping cough occur in Melbourne every three or four years, particularly during the spring and summer months (see Fig. 1) and are reflected by the admission of large numbers of patients with this disease to Fairfield Hospital.

All patients with respiratory illnesses admitted to this Hospital are nursed in single rooms until a diagnosis is made. Two throat swabs, one for bacterial, the other for viral culture, are taken from each patient by the admitting doctor.

During this study throat swabs for viral studies were collected and processed as previously described (Kennett, Ellis, Lewis & Gust, 1972). The resulting material was inoculated into duplicate tubes of primary cynomolgus monkey kidney, HeLa and human embryonic fibroblast cell cultures. Standard procedures were used for the identification of strains isolated. In patients with suspected whooping cough an additional specimen was obtained for bacterial study by swabbing the nasopharynx with a cotton-wool swab introduced through the nose on a long flexible wire. These swabs were transported to the laboratory promptly and immediately plated out on horse blood agar and a second medium chosen to facilitate the growth of *B. pertussis*. Before June, 1968, meat extract medium (Nicholson & Turner, 1954) with 20% citrated sheep's blood was used, but subsequently this has been replaced by Bordet-Gengou (Oxoid) containing 20% defibrinated sheep's blood,* penicillin 0·35 units/ml. and no glycerol. All media were incubated aerobically at 37° C. and held for 5–7 days.

RESULTS

The results obtained over the 5-year period from 1 January 1966 to 31 December 1970, are presented. This period included a large whooping cough epidemic (see Fig. 1).

During the five years 502 patients with clinical whooping cough were admitted to Fairfield Hospital for Communicable Diseases, Melbourne. Throat swabs for

* Commonwealth Serum Laboratories, Melbourne.

Table 1. *The number of patients with whooping cough, admitted to Fairfield Hospital over a 5-year period, from whom* Bordetella pertussis *and virus strains were isolated*

Year	No. of patients	No. from whom *B. pertussis* isolated	No. from whom a virus or viruses isolated
1966	70	13 (18·6 %)	9 (12·8 %)
1967	36	11 (30·6 %)	12 (33·3 %)
1968	199	60 (30·2 %)	46 (23·1 %)
1969	134	70 (52·2 %)	34 (25·4 %)
1970	44	21 (47·7 %)	15 (34·0 %)
Total	483	175 (36·2 %)	116 (24·0 %)

Table 2. *The isolation rate of* Bordetella pertussis *from patients with whooping cough, related to the patient's age*

Age	No. of patients *B. pertussis* Isolated	Not isolated	Isolation rate (%)
< 1 year	94	144	39·5
1–2 years	36	30	54·5
> 2–3 years	17	32	34·7
> 3–4 years	13	24	35·1
> 4–5 years	6	24	20·0
> 5-10 years	7	47	13·0
> 10 years	2	7	22·2
Total	175	308	36·2

viral culture and nasopharyngeal swabs for bacterial culture were obtained from 483 patients. The nineteen patients from whom both types of swab were not received have been excluded from the study.

The isolation rate of *B. pertussis* was low in 1966–68 but in the last 2 years has been approximately 50 % (see Table 1).

The isolation rate was higher in babies and younger children (see Table 2) who are usually admitted to hospital earlier in their illness (N. McK. Bennett, in preparation).

A total of 132 strains, representing 22 different viruses were recovered from 116 of the 483 patients (see Tables 3 and 4).

The virus isolation rate was similar whether *B. pertussis* was also isolated or not (see Table 5).

Adenoviruses (types 1, 2, 5, 6, 7 and 9) were the most commonly encountered group, and were isolated from 49 (42 %) of the 116 patients from whom virus was recovered.

As viral studies are routinely performed on all patients with respiratory disease admitted to this hospital, the relative isolation rate provides an index of their occurrence in the community. During the 11-year period, 1 January 1960 to 31 December 1970, 513 strains of adenovirus (types 1–7, 9, 10 and 15) were isolated from 7267 patients with respiratory disease (including whooping cough). The monthly isolations are compared with the number of admissions of patients with whooping cough during the same period (see Fig. 2).

Table 3. *The strains of virus recovered from patients with whooping cough*

Virus	*B. pertussis* Isolated	*B. pertussis* Not isolated	Total
Single isolation			
Adeno. (1, 2, 5, 6, 7)	18	20	38
Influenza (A and B)	1	1	2
Para-influenza (1, 2, 3)	3	7	10
Rhino.	5	8	13
Respiratory syncytial	1	8	9
Coxsackie (A 9, B 1, B 4)	1	4	5
Herpes hominus	4	5	9
Cytomegalo.	3	7	10
Miscellaneous enteroviruses (ECHO 6, 22, Polio. 2, ENT.*)	3	2	5
Multiple isolations (see Table 4)	10	21	31
Totals	49	83	132

* Enterovirus not typed.

Table 4. *Viruses involved in multiple isolations*

B. pertussis isolated	*B. pertussis* not isolated	
Adeno. 1:H. hominus	Adeno. 2:Paraflu. 1	Adeno. 9:ENT.*
Adeno. 1:Paraflu. 3	Adeno. 2:Rhino.	Influenza A 2:R.S.V.†
Adeno. 2:Rhino.	Adeno. 2:H. hominus	Influenza B:Echo 11
Adeno. 5:C.M.V.	Adeno. 2:Polio. 2	H. hominus:polio. 2
Adeno. 5:Polio. 2	Adeno. 7:Paraflu. 2	Paraflu. 3:R.S.V.:H. hominus

* ENT. = enterovirus, not typed.
† R.S.V. = Respiratory syncytial virus.

Table 5. *The isolation rate of viruses from patients with whooping cough from whom* Bordetella pertussis *was and was not isolated*

	No. of patients	No. from whom a virus or viruses isolated	No. from whom an adenovirus isolated
B. pertussis isolated	175	44 (25·1 %)	23 (13·1 %)
B. pertussis not isolated	308	72 (23·4 %)	26 (8·4 %)
Total	483	116 (24·0 %)	49 (10·1 %)

DISCUSSION

In recent years there has been a tendency to emphasize the importance of viruses in the aetiology of whooping cough and to diminish the role of *B. pertussis*. The reason for this altered emphasis is not hard to find. The isolation rate of *B. pertussis* from patients with whooping cough has often been disappointingly low and this had led to a search for other possible agents, in particular viruses.

In this study the results of routine bacterial and viral studies on 483 patients with whooping cough investigated at Fairfield Hospital over a 5-year period are analysed.

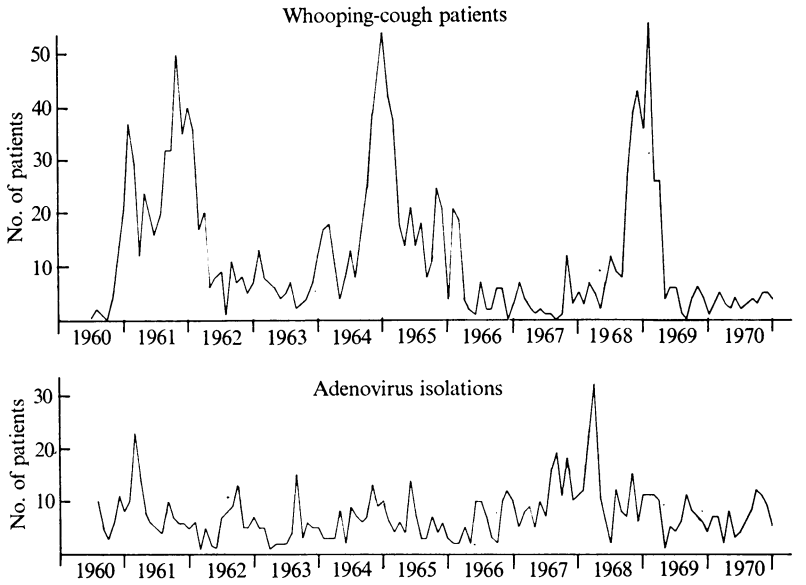

Fig. 2. The monthly admissions of patients with whooping cough to Fairfield Hospital for Communicable Diseases from January 1960 to December 1970, compared with the monthly isolations of adenoviruses from all patients with respiratory disease seen at the same hospital over this period.

The isolation rate of *B. pertussis*, 36·2 %, compares favourably with other series (A combined Scottish study, 1970; Pereira & Candeias, 1971), and after the introduction of Bordet–Gengou medium in mid-1968 it increased to approximately 50 %. This figure probably underestimates the proportion of infections due to *B. pertussis* as usually only one swab was taken from each patient and no serological investigations were performed.

As *B. pertussis* is only capable of surviving for a few hours *in vitro* in nasal secretions, isolation attempts require considerable co-operation between clinicians and laboratory staff. The different isolation rates found in this series probably represent variations in efficiency and frequency of swabbing, delays in plating out and differences in the type and age of culture media used.

In this study one or more respiratory viruses were recovered from the throat of 24 % of patients. The techniques used were effective for the isolation of all the common respiratory viruses, except coronaviruses, and over the 5-year period a wide range of strains was obtained. In common with other series the group most commonly encountered were adenoviruses.

If, as is postulated, respiratory viruses have an important role in the aetiology of whooping cough, it would be expected that they would be isolated more frequently from patients from whom *B. pertussis* was not grown. In our series there was no significant difference between the viral isolation rate in the two groups ($P > 0{\cdot}05$).

Table 6. *A statistical analysis of the relationship between the isolation of virus and of* Bordetella pertussis *from patients with whooping cough investigated over a 5-year period*

	Virus isolated	Virus not isolated	Total
B. pertussis isolated:			
Observed	44	131	175
Expected	42	133	
B. pertussis not isolated:			
Observed	72	236	308
Expected	74	234	
Total	116	367	483

$\chi^2 = 0{\cdot}11$; d.f. $= 1$; $P > 0{\cdot}05$.

Adenoviruses are often proposed as aetiological agents in whooping cough. In this series there was little difference in the isolation rate of adenoviruses from patients in whom *B. pertussis* was, or was not, isolated. In addition inspection of Fig. 2 fails to reveal a correlation between the number of patients admitted to hospital with whooping cough and the prevalence of adenovirus infections in the community.

Although viruses may be implicated in occasional cases of whooping cough, there is no evidence to suggest that they are responsible for a large proportion of cases.

The authors wish to acknowledge the help of Miss J. Weeks in preparation of the manuscript.

REFERENCES

A COMBINED SCOTTISH STUDY (1970). Diagnosis of whooping cough: Comparison of serological tests with isolation of *Bordetella pertussis*. *British Medical Journal* iv, 637.

COLLIER, A. M., CONNOR, J. D. & IRVING, W. R. (Jr.) (1966). Generalized type 5 adenovirus infection associated with the pertussis syndrome. *Journal of Pediatrics* **69**, 1073.

CONNOR, J. D. (1970). Evidence of an aetiological role of adenoviral infection in pertussis syndrome. *New England Journal of Medicine* **283**, 390.

KENNETT, MARGERY L., ELLIS, A. W., LEWIS, F. A. & GUST, I. D. (1972). An epidemic associated with echovirus type 18. *Journal of Hygiene* **70**, 325.

MCCORDOCK, H. A. & SMITH, M. G. (1934). Intra-nuclear inclusions: Incidence and possible significance in whooping cough and in a variety of other conditions. *American Journal of Diseases of Children* **47**, 771.

NICHOLSON, D. E. & TURNER, G. C. (1954). A simple selective medium for the primary isolation of haemophilus pertussis and parapertussis. *The Journal of General Microbiology* **10**, appendix i.

OLSON, L. C., MILLER, G. & HANSHAW, J. B. (1964). Acute infectious lymphocytosis presenting as a pertussis-like illness: Its association with adenovirus type 12. *Lancet* i, 200.

PEREIRA, M. S. & CANDEIAS, J. W. (1971). The association of viruses with clinical pertussis. *Journal of Hygiene* **69**, 399.

STURDY, P. M., COURT, S. D. M. & GARDNER, P. S. (1971). Viruses and whooping cough. *Lancet* ii, 978.

URQUHART, G. E. D., MOFFAT, M. A. J., CALDER, M. A. & CRUICKSHANK, G. M. (1965). An aetiological study of respiratory infection in children, Edinburgh City Hospital 1961–1963. *Journal of Hygiene* **63**, 187.