

Investigation of an outbreak of adenovirus type 3 infection in a boys' boarding school

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(Received 26 March 1984; accepted 8 May 1984)

SUMMARY

An outbreak of pharyngoconjunctival fever caused by adenovirus type 3 was studied in a boarding school for 800 boys aged 11-18 years. A total of 96 clinical cases were confirmed by laboratory tests. Clinical infection rates were higher in the younger boys but total infection rates did not vary with age. Previous infection provided 88% protection against reinfection.

The techniques of virus isolation, complement fixation and neutralization were compared in the diagnosis of cases. Virus isolation diagnosed 86% of confirmed cases. Where acute sera (collected at onset) and convalescent sera (collected within one month) were available complement fixation and neutralization tests each diagnosed 96% of cases.

INTRODUCTION

Adenovirus type 3 commonly causes pharyngoconjunctival fever (PCF), an acute respiratory disease predominantly of children and adolescents. A number of outbreaks of PCF caused by adenovirus type 3 and the closely related type 7 have been studied in 11- to 19-year-olds in boys' boarding schools (Tyrell, Balducci and Zaiman, 1956; Kendall *et al.* 1957; Munro-Ashman *et al.* 1958). The rate of clinical adenovirus infection was found to decrease with increasing age. Conclusions were however based mainly on clinical diagnosis, and laboratory investigations were limited.

Several workers (Brandt *et al.* 1969; Fox *et al.* 1969) suggested that approximately 50% of all adenovirus infections might be subclinical. Evidence of subclinical infection was obtained by Harris *et al.* (1971) in a study of PCF caused by adenovirus type 7 in a children's home. The clinical infection rate was again found to decrease with increasing age but the total infection rate was similar in all age groups. There was no evidence of reinfection among these children but Kawana *et al.* (1966) detected two reinfections in a school outbreak of PCF.

In this study an outbreak of PCF which occurred in early summer 1981 in a boarding school for 800 boys aged 11-18 years was investigated. Laboratory-confirmed clinical infection rates were compared in different age groups. Total infection rates (clinical and subclinical) were investigated in 12-year-old and 16-year-old boys. For these two groups the effect of previous experience on both

the incidence of infection and its clinical outcome was analysed. Laboratory techniques for the diagnosis of infection were compared.

MATERIALS AND METHODS

All boys who reported symptoms of PCF were examined by the school medical officer (Dr T. W. Hoskins), who was responsible for clinical diagnosis. Throat swabs and, where appropriate, conjunctival swabs were collected from every sick boy and these were examined for pathogenic bacteria and viruses. Acute and convalescent blood samples were collected from approximately one-third of the boys with symptoms. For some boys involved in the outbreak in addition to convalescent sera there were also later sera available which had been collected for other laboratory investigations. Sera which spanned the outbreak were also available from some boys with and without symptoms of PCF. These sera had been collected in October 1980 and October 1981 as part of a long-term study of influenza.

Virus isolation

The swabs were collected in virus transport medium which did not contain antibiotics. 0.1 ml of the fluid was inoculated on to cell cultures of primary baboon kidney, fibroblasts (MRC-5) and human amnion. The cultures were incubated at 33°C on a roller drum for 21 days and examined for cytopathic effect (CPE). Cultures negative in human amnion were passaged to human embryonic lung (HEL) fibroblasts and incubated for a further 14 days. Adenovirus was identified by the complement fixation test using adenovirus antiserum. Strains were typed at the Virus Reference Laboratory, Central Public Health Laboratory, Colindale.

Serology

Acute and convalescent paired sera from cases were examined by the complement fixation (CF) test (Bradstreet & Taylor, 1962) using 0.025 ml unit volumes. Sera were tested against a range of antigens: influenza virus A and B; respiratory syncytial virus; parainfluenza viruses; and *Mycoplasma pneumoniae*. These antigens were supplied by the Division of Microbiological Reagents and Quality Control (DMRQC), Colindale. Complement fixation tests for adenovirus antibody were performed using the strain isolated during the outbreak and the strain supplied by DMRQC, which was an adenovirus type 5. Fourfold or greater differences in the titre were considered evidence of infection.

Sera were also examined for evidence of adenovirus infection by the microneutralization (Nt) test. The test was a modification of that described by Hierholzer & Bingham (1978) and shown by Schrader & Wigand (1981) to detect virus neutralization and not toxin neutralization. Serial twofold dilutions of serum from 1 in 10 to 1 in 640 were prepared in duplicate and incubated with adenovirus at 3 TCD₅₀. After four days incubation at 37°C the results were read using an inverted light microscope (Leitz Diavert). The titre of each serum was the reciprocal of the highest dilution in which there was complete inhibition of CPE. Fourfold or greater differences in titre were considered evidence of infection. Sera were tested for toxicity at the initial 1 in 10 dilution.

RESULTS

The course of the outbreak

The outbreak occurred in the summer term of 1981 between 10 May and 9 July and spanned a one week mid-term break. During the outbreak 174 boys reported sick and infection with adenovirus was confirmed by isolation and/or serology in 96 of them. The distribution of these cases is shown in Fig. 1, where it can be seen that the peak of the outbreak occurred during week 5, two weeks after the mid-term break. The most common symptoms reported among those infected with adenovirus were pyrexia and pharyngitis with or without conjunctivitis and lymphadenopathy (Table 1).

Virus was not isolated from the remaining 78 boys. From 44 of these, pre- and post-outbreak sera were available and none had evidence of adenovirus infection. The distribution of these 78 cases differed from those in which adenovirus infection was confirmed (Fig. 1) – there were fewer such cases at the beginning of the outbreak but more towards the end. The clinical symptoms shown by these 78 boys were similar to those with adenovirus infection (Table 1) but pyrexia with pharyngitis was less common in this group and pharyngitis alone or coryza alone more common. There was evidence of infection with other respiratory tract pathogens. β -haemolytic streptococci (Group A) were isolated from the three boys, and among 12 boys from whom acute and convalescent paired sera were available there were seven infections with parainfluenza virus (58%) and one infection with Epstein-Barr virus. The 78 boys in whom adenovirus infection was not demonstrated have been excluded from further analysis.

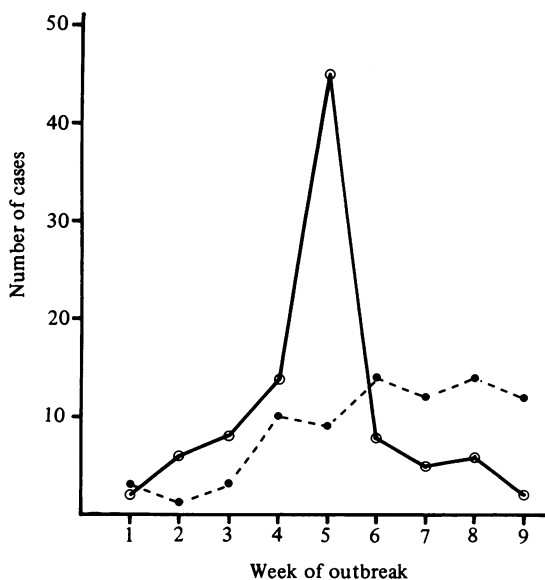


Fig. 1. The course of the outbreak, showing the number of adenovirus cases (○—○) and non-adenovirus cases (●---●) each week.

Table 1. *Presentation of symptoms in adenovirus and non-adenovirus cases*

Symptoms	Adenovirus cases (%)	Non-adenovirus cases (%)
Pyrexia and pharyngitis ± conjunctivitis and lymphadenopathy	60 (63)	15 (19)
Pharyngitis and conjunctivitis	9 (9)	2 (3)
Pharyngitis only	11 (12)	25 (32)
Conjunctivitis only	6 (6)	3 (4)
Pyrexia only	7 (7)	10 (13)
Coryza only	2 (2)	13 (16)
Other*	1 (1)	10 (13)
Total cases	96	78

* Enteric (nausea, vomiting, diarrhoea), otitis media, epistaxis, headache.

Infection with adenovirus

Effect of age on clinical attack rate

The age range of the challenged population was 11–18 years. The case rate for each age group was investigated (Fig. 2) and was shown to decrease with increasing age ($\chi^2 = 28.17$, $v = 6$, $P < 0.001$). The attack rates in the groups of boys under 15 years were similar ($\chi^2 = 1.6$, $v = 3$, $P > 0.1$), but the attack rate of 19% in this group as a whole was significantly higher than that in the 15- to 18-year-olds, where the attack rate was 6% ($\chi^2 = 30.74$, $P < 0.001$).

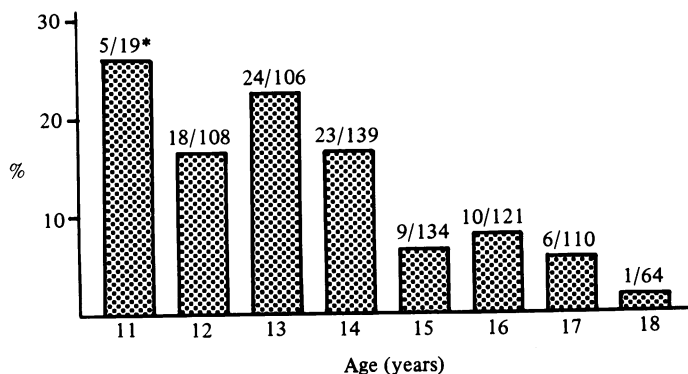


Fig. 2. The effect of age on clinical attack rate, showing the number of boys with symptoms as a percentage of the group. *Figures above the columns indicate number of boys affected/number of boys in age group.

Total infections in 12- and 16-year-olds

For 72 of the 12-year-old boys and 70 of the 16-year-old boys pre- and post-outbreak sera were available. These had been collected for an influenza study and were therefore independent of adenovirus symptoms. In these groups the incidence of subclinical infection was investigated (Table 2).

Table 2. *Clinical and subclinical infections in 12-year-olds and 16-year-olds*

Age (years)	Number of boys	Number of infections		Total infection rate (%)
		Clinical	Subclinical	
12	72	11	12	32
16	70	7	14	30

Although clinical infection was less frequent in the older boys subclinical infection occurred to a greater extent than was observed in the 12-year-olds. The total infection rates for both groups were similar.

Effect of previous experience

The effect of previous experience of adenovirus as determined by the presence of neutralizing antibody to type 3 in pre-outbreak sera was investigated. Sixty-eight per cent of 12-year-olds and 70% of 16-year-olds had neutralizing antibody titres of 10 or more in their pre-outbreak sera. The infection rate was lower in boys with pre-existing antibody than in those without and the protective effect of antibody against reinfection was 88% (Table 3).

Table 3. *Effect of previous experience on infection*

Age (years)	Previous experience (+ or -)	Number of boys	Number of infections		Total infections (%)
			Clinical	Subclinical	
12	-	23	9	9	18 (78)
	+	49	2	3	5 (10)
16	-	21	7	10	17 (81)
	+	49	0	4	4 (8)

Protective effect of antibody is calculated as

$$\frac{(\text{Expected number of infections} - \text{observed number of infections})}{\text{Expected number of infections}} \times 100\%$$

where the expected number of infections in the group with antibody is calculated from the rate in those with no antibody. From the data in the table the expected number in 35/44 × 98, i.e. 78 and the protective effect of antibody is thus

$$\frac{(78 - 9) \times 100}{78} = 88\%$$

There were only nine infections in boys with antibody and two of these were symptomatic (22%). Among 35 primary infections there were 16 boys with symptoms (46%). Although there appeared to be fewer symptomatic infections in those with antibody the difference between the proportions for the two groups was not significant.

Comparison of laboratory techniques for diagnosis

For 29 boys in whom adenovirus infection was demonstrated isolation specimens and paired sera collected at onset of symptoms and again after three weeks were available. The efficiencies of virus isolation, CF and Nt were compared.

Virus was isolated from 25 of the 29 (86%) and all of these were confirmed by serological tests. Both the CF and the Nt tests diagnosed 28 of the 29 cases (96%). The CF test failed to diagnose one case which was confirmed by the Nt test, and the Nt test failed to diagnose one case which was confirmed by both CF and virus isolation. This failure may have been due to the late collection of the acute serum.

The efficiency of the CF test was affected by the CF antigen used. Whilst the infecting strain detected 96% of cases the CF antigen (type 5) supplied by DMRQC detected 82% of cases.

The efficiencies of the serological tests were also investigated in cases where the interval between the collection of the acute and convalescent specimens was greater than three weeks. Where the convalescent serum was collected five months after infection, the CF test diagnosed nine of 14 cases (64%): all 14 were confirmed by the Nt test. Paired sera from eight proven cases where the convalescent serum was collected 10 months after infection were examined. None of the eight was diagnosed by the CF test but all were confirmed by neutralization. By comparison the Nt test proved valuable where convalescent sera were collected late. For 31 proven cases with sera collected up to 2 months post infection and again 10 months post infection, diagnosis would have been confirmed in all 31 using the later serum.

DISCUSSION

Ninety-six infections with adenovirus type 3 were detected among 174 boys with symptoms. The remaining 78 boys were not considered to have been infected with adenovirus. Other respiratory agents were circulating in the school: these included β -haemolytic streptococci, parainfluenza virus and Epstein-Barr virus. Seven infections with parainfluenza type 3 were identified, but in fact the incidence of infection with this virus may well have been higher. Paired sera suitable for diagnosis by the CF test were only available from 12 of the 78 and the infection rate among these 12 was 58%.

Of the adenovirus cases the highest case rate was seen in boys aged 11-14 years: in boys aged 15-18 years the case rate was significantly lower. However, total infection rates were similar for both groups. These findings agree with those of Harris *et al.* (1971). The lower rate of clinical infection in the older boys may, in part at least, be due to the failure of older boys to report symptoms. The phenomenon has been observed on other occasions at this school and by workers in other schools (Tyrell, Balducci & Zaiman, 1956). In this study 26 out of 44 (59%) of the infections were subclinical. This confirms the suggestion made by other workers (Brandt *et al.* 1969; Fox *et al.* 1969).

Eighty per cent of boys with no evidence of previous experience of adenovirus type 3 became infected; previous experience reduced the infection rate to 9%. The protective effect of antibody against reinfection was 88%.

All the technical methods used in the diagnosis of cases where swabs and paired sera were available were efficient. Human amnion cells provided a sensitive culture system and the CF test using the outbreak strain was good where the convalescent serum was collected promptly. However, where the collection of this specimen was delayed the efficiency of the CF test fell and diagnosis was only confirmed by this method in 64% of cases where the convalescent serum was collected five months

after infection. Not one of eight cases where the convalescent serum was collected 10 months after infection was diagnosed by this method. Clearly the antibodies detected by this system wane rapidly. In contrast the Nt test provided a reliable method of serological diagnosis where the convalescent serum was collected up to 10 months after infection. The Nt test thus provides a good index of exposure to adenovirus long after infection.

The authors would like to thank Mr R. C. Hendrie for his assistance and the staff and boys at Christ's Hospital, Horsham for their co-operation.

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