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Chlamydia pneumoniae, strain TWAR, as the cause of an outbreak in a boys' school previously called psittacosis

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SUMMARY

Sera from an outbreak of acute respiratory illness in a boys boarding school originally thought to have been due to psittacosis (1) have been re-examined and evidence is presented that suggests that the outbreak was caused by *Chlamydia pneumoniae*, strain TWAR (2).

INTRODUCTION

An outbreak of acute respiratory illness considered to have been psittacosis on the basis of chlamydia complement fixation serology occurred in a boarding school for boys in a rural area of Somerset, England in 1980 (1). Between May and July 1980, 20 boys, 2 teachers and a kitchen worker were diagnosed as having psittacosis. In addition, one of the investigators also contracted the infection. The illness was characterized by a sore throat usually progressing to a bad 'cold'. Headache and fever were frequent; some patients had catarrh, abdominal pain, vomiting or diarrhoea. Half the cases gave a history of cough. The illness was mild and bed rest was usually unnecessary. The epidemic curve was consistent with case to case spread or a continuing single source outbreak with low exposure or low infectivity rates. No psittacine origin for the outbreak could be identified.

The summary of the original report stated 'it is suggested either that sources of chlamydia other than avian exist, which may produce a milder illness than the avian type, or that human-to-human spread of a mild form of chlamydiosis occurs'(1). The authors of the report thought that if *Chlamydia psittaci* strains responsible for outbreaks similar to the one in the boarding school could be isolated and characterized then there was a possibility of identifying a pathogen that was mainly, if not solely, passaged between humans.

Subsequently, a series of isolates of a new chlamydia organism called TWAR were made from persons who had an illness that was clinically indistinguishable from mild psittacosis (3). The cases developed complement fixing antibody to

chlamydia antigen but no avian source was identified. The epidemiology of TWAR infection suggested human-to-human spread. The name TWAR was derived from the laboratory designation of the first two isolates; TW-183 and AR-39. These organisms have now been designated as *Chlamydia pneumoniae* (2). They were identified as a new third species of chlamydia by the unique morphology of their elementary bodies (4) and by DNA analysis which showed less than 10% homology with *Chlamydia trachomatis* and *Chlamydia psittaci* (5). This paper presents results of a re-investigation of the sera from the 1980 outbreak in Somerset.

MATERIALS AND METHODS

Two sets of sera were available to test for TWAR antibody. The first consisted of all but two serum specimens from the 24 ill persons previously reported. The missing sera were the first of a pair from a 16-year-old student and the only serum from the investigator. An additional set of 17 single serum specimens considered controls were available from schoolboys and other school personnel of the same school who had not been ill. All of the sera had been stored at −20 °C since 1980. The sera were sent to the University of Washington for testing without being identified as cases or controls.

The sera were examined in the microimmunofluorescence test (6) with elementary body antigens of the TWAR strain (3), of all *C. trachomatis* serovars. and of 8 *C. psittaci* strains, including 3 of human origin. The serum fraction containing chlamydial antibody was determined by the use of fluorescein-isothiocyanate conjugated goat anti-human IgM and anti-human IgG (Hyland Laboratory, Los Angeles, California). The following antibody titres in the microimmunofluorescence test were considered evidence of acute (current or recent) infection: fourfold or greater rise in either serum fraction; IgM antibody $\geqslant 16$; and IgG antibody $\geqslant 512$.

RESULTS

When the microimmunofluorescence test results were sent to England to correlate with the clinical, epidemiological, and previous serologic results, it became clear that the outbreak had been due to *Chlamydia pneumoniae*, strain TWAR. Paired sera were available from 8 of the patients and 7 showed levels of antibody consistent with an acute infection (Table 1). The other patient had a fourfold antibody titre fall in the IgG serum fraction from 64 to 16. The possibility that the two serum specimens had been mislabelled could not be ruled out. A fourfold rise in IgM or IgG titre was demonstrated in 5 of the 7 pairs. One pair of sera taken only 1 week apart had IgM antibody at dilutions of 32 and 16 and one pair had a high IgG antibody in both samples (512 and 1024).

Among the 15 patients from whom only a single serum specimen was available, none showed significant TWAR antibody in the IgM serum fraction. Eight patients (53%) had some TWAR IgG antibody, two at a titre of 512 and one at a titre of 256. Single serum specimens from the 17 controls were tested and none had significant acute antibody (Table 2). Five (29%) had low TWAR IgG antibody with titres of 16 to 64.

Table 1. *Original antibody levels (1980) of 24 persons in the outbreak*

(Also recent (1988) testing of the same sera to demonstrate IgM and IgG to *Chlamydia pneumoniae* strain TWAR)

			Serum 1					Serum 2				
				1980 (GB)		1988 (USA)		Tested	1980 (GB)		1988 (USA)	
Case	Age	Date of illness	Date tested	CF	IF	IgM	IgG	Date tested	CF	IF	IgM	IgG
1	15	15 May	1 July	160	128	< 8	32					
2	16	28 May	10 June	< 20	8	< 8	8	9 July	40	32	< 8	512
3	16	30 May	10 June	80	256	Insufficient		1 July	> 160	512	< 8	8
4	18	31 May	24 June	160	256	32	8	1 July	160	256	16	< 8
5	16	1 June	10 June	80	64	< 8	64	1 July	160	64	< 8	16
6	17	7 June	12 June	< 20	8	< 8	16	9 July	80	256	< 8	64
7	16	7 June	9 June	160	256	< 8	64					
8	17	11 June	16 June	< 20	8	< 8	< 8	9 July	80	256	16	< 8
9	16	11 June	1 July	> 160	128	< 8	< 8					
10	15	14 June	1 July	160	128	8	< 8					
11	14	15 June	17 July	< 20	8	< 8	< 8	1 July	> 160	64	16	< 8
12	15	15 June	2 July	> 160	512	< 8	256					
13	15	16 June	1 July	160	512	< 8	< 8					
14	25	17 June	2 July	160	64	< 8	< 8					
15	14	23 June	7 July	80	128	< 8	512					
16	15	25 June	7 July	320	1024	< 8	< 8					
17	14	28 June	2 July	80	256	< 8	64					
18	15	3 July	7 July	40	128	< 8	512					
19	16	5 July	9 July	80	96	< 8	64					
20	17	5 July	9 July	40	64	< 8	64					
21	52	5 July	10 July	160	128	< 8	< 8					
22	21	7 July	10 July	40	16	< 8	< 8	22 July	40	ND	< 8	128
23	15	19 July	21 July	160	256	< 8	512	30 July	80	256	< 8	1024
24	46	15 July	September	160	256	Insufficient						

Table 2. *Original (1980) antibody levels (CF only) of 17 persons with no illness*

(Also recent (1988) testing of the same sera to demonstrate antibody IgM and IgG to *Chlamydia pneumoniae* strain TWAR)

				1988 (USA)	
Case	Age	Date of testing of sample	1988 (GB) CF	IgM	IgG
1	16	3 June	< 20	< 8	< 8
2	17	12 June	< 20	< 8	< 8
3	19	16 June	< 20	< 8	< 8
4	13	1 July	20	< 8	32
5	14	1 July	< 20	< 8	16
6	14	1 July	< 20	< 8	64
7	16	1 July	< 20	< 8	< 8
8	27	3 July	< 20	< 8	< 8
9	16	3 July	< 20	< 8	< 8
10	16	3 July	< 20	< 8	< 8
11	55	3 July	< 20	< 8	< 8
12	17	7 July	< 20	< 8	16
13	17	9 July	< 20	< 8	32
14	18	10 July	< 20	< 8	< 8
15	44	10 July	< 20	< 8	< 8
16	18	10 July	< 20	< 8	< 8
17	17	10 July	< 20	< 8	< 8

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No evidence of acute infection with any *C. trachomatis* serovar, or any of the eight *C. psittaci* strains tested, was found. No antibody in either the IgM or IgG serum fraction $\geqslant 16$ was found with any *C. trachomatis* or *C. psittaci* strain tested.

DISCUSSION

While retrospective serology is limited in its ability to assign causality, the results obtained were quite definitive, with 7 of 8 ill persons from whom paired sera were available having acute TWAR antibody, and the eight having a fourfold antibody titre fall. TWAR antibody in the IgM serum fraction has been helpful in identifying current TWAR infection in patients whose single or paired serum specimens are obtained early in the course of illness (7, 8). The authors attribute the failure to find IgM antibody in the ill persons with single serum and many of those with paired serum specimens to loss of antibody in this serum fraction due to long storage with several freeze and thaw cycles. Although many of the single serum specimens were obtained early in the course of illness before antibody could be expected to occur, more frequent and higher titre TWAR IgG antibody was found in the ill persons than in the control persons from the same school who had not been ill.

There is long-standing evidence that the microimmunofluorescence test of Wang and Grayston has the specificity to differentiate among *C. trachomatis* serovars, and when utilized to measure TWAR antibody does not show significant cross reaction with *C. trachomatis* species (9). Because of the relatively low TWAR antibody titres, especially in the IgM serum fraction observed in some of these patients, all of the sera were also tested against *C. trachomatis* serovars and eight strains of *C. psittaci*. The failure to find antibody against these other chlamydial antigens rules out *C. trachomatis* as a potential cause of this epidemic. Because all *C. psittaci* strains that may potentially cause human illness have not been classified and no species-specific *C. psittaci* antigen is available, it is not possible to test sera against all potential *C. psittaci* strains. However, the failure to find any antibody against eight *C. psittaci* strains makes it very unlikely that the antibody found against the TWAR strain would be a cross-reaction from an unknown *C. psittaci* strain.

The first report of this outbreak included results from chlamydia CF tests from pupils at another school and in sera from blood donors in two adjacent towns (1). While there was little evidence of recent infection producing chlamydia CF antibodies in the other school, there were sera showing widespread low and high titre CF antibody among the blood donors. The authors suggest that the boys' school outbreak could have been part of a more widespread *C. pneumoniae* epidemic. There is indirect evidence that England and Wales underwent a TWAR epidemic that began in 1980 and lasted for at least 3 years. There is some evidence (10) of a sharp increase in the numbers of patients with chlamydia CF antibody both in Cambridge, and in all England and Wales beginning in 1980. The yearly frequency of such patients more than doubled from the preceding 5 years compared to 1980–3. This finding is similar to that which occurred in Norway, Sweden and Denmark where an even sharper increase in the numbers of notified cases of ornithosis (and laboratory identified chlamydia CF positives) occurred in

1981–3. Country-wide epidemics in Scandinavia were shown to be associated with the development of TWAR antibody (11). In addition to the results from this epidemic, an indirect suggestion that the increased incidence of chlamydia CF positive patients in England may have been due to a widespread TWAR epidemic comes from serologic studies at the Institute of Ophthalmology in London. Extensive serologic studies have been carried out with the IOL-207 organism, which has been stated to resemble closely the TWAR organisms. While Forsey, Darougar and Treharne (12) did not study respiratory disease, they did find an increasing frequency of antibody in eye clinic patients from 1981–4 (from 10 to 25%). The authors suggest that this increase in frequency of antibody is consistent with continuing widespread TWAR infections in England beginning in 1980.

The mildness of the illness in the schoolboys reported in this outbreak is reminiscent of the first reported TWAR outbreak in teenagers and young adults in a northern Finnish community (7), where many people who developed TWAR antibody and had pneumonitis demonstrated during a chest radiographic survey had little or no clinical illness. A tendency for TWAR infection to cause milder illness in the young and more severe illness in older persons has been reported (13).

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