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A study of human respiratory tract chlamydial infections in Cambridgeshire 1986–88

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SUMMARY

Human respiratory tract chlamydial infections have been studied in Cambridgeshire for many years, but until recently we have been unable to distinguish between infection with *Chlamydia psittaci* or *Chlamydia pneumoniae* (TWAR). In this study, we have employed the micro-immunofluorescence (micro-IF) test for this purpose and to look for the relative incidence of *C. psittaci* and *C. pneumoniae* infections in Cambridgeshire. Among 50 patients with community-acquired respiratory tract symptoms whose serum samples had Chlamydia complement fixation test titres $\geqslant 64$, 25 had evidence of recent *C. psittaci* or *C. pneumoniae* infection. Nineteen (76%) of the 25 patients had evidence of recent *C. psittaci* infection and of these 16 (84%) had recently had contact with birds. Six patients (24%) had evidence of recent *C. pneumoniae* infection, and of these, only two (33% had recently had contact with birds. While *C. psittaci* was grown from several of the birds associated with human *C. psittaci* infection, it was not cultured from any of the birds in contact with the two human *C. pneumoniae* cases.

INTRODUCTION

The steady increase in the number of psittacine birds imported into Great Britain in the years 1982–6 has correlated with a proportional increase in the number of cases of human respiratory tract chlamydial infections in Cambridgeshire [1, 2]. In 1987, however, we noted an unusually large increase in the number of cases, out of proportion with the modest increase in the number of psittacine birds imported into Great Britain. This, we believe, was largely the result of increased local awareness of these infections, as a result of publicity when psittacosis was designated as a notifiable disease in Cambridge on 1 January 1987.

We have always assumed that the majority of our cases were a result of *Chlamydia psittaci* infection, since many patients were found to have had recent contact with birds [2]. However, this supposition has been challenged [3–5] by those who suspect that most of our cases were caused by *Chlamydia pneumoniae* [6] (TWAR) which was first described by Grayston and colleagues [7]. Infection with

Chlamydia pneumoniae is not associated with bird or animal contact but mainly with human–human contact. The methods we have employed so far – the complement fixation test (CFT) [8] and μ-capture ELISA for detecting Chlamydia-specific IgM [9] are unable to differentiate between infection with *C. psittaci*, *C. pneumoniae* or *C. trachomatis*, but when employed in conjunction with a complete clinical history, we believe that they can be used reliably to diagnose recent human respiratory tract chlamydial infection.

In this study, we have used the microimmunofluorescence (micro-IF) test employing *C. psittaci*, *C. pneumoniae* and *C. trachomatis* antigens [10] in order to look for the relative incidence of infections with these three organisms in patients with respiratory tract symptoms. This test when correctly carried out and interpreted is capable of distinguishing species-specific chlamydial antibodies and thus determining which *Chlamydia* species is the cause of the infection. We have tested serum samples from 50 patients with respiratory tract infections in Cambridgeshire between May 1986 and November 1988 who had complement fixation test (CFT) antibody titres $\geqslant 64$ and who were available for interview at the time of their illness with regard to evidence to recent bird or animal contact.

MATERIALS AND METHODS

Patients

Between May 1986 and November 1988, all patients with community-acquired respiratory disease whose physician sought assistance in diagnosis by examination of a blood sample were screened by the complement fixation test (CFT) for antibodies to Chlamydia, *Mycoplasma pneumoniae*, influenza viruses A and B, *Coxiella burnetii* and adenovirus. During this period one of us (R. B. B.) attempted to make contact with the patient or his relatives to identify epidemiological features of the cases including details of any bird or animal contact or contact with other people with similar symptoms. Where a bird contact was identified, samples of the birds' faeces were collected for examination by immunofluorescence for chlamydia elementary bodies or chlamydia culture.

Serum samples from 50 patients with respiratory symptoms and either a high ($\geqslant 64$) or fourfold or greater rise in chlamydial CFT antibody titre were further studied by means of the microimmunofluorescence test employing *C. psittaci*, *C. pneumoniae* and *C. trachomatis* antigens. Patients were included in the study only if they were available for detailed questioning with regard to the epidemiological features of their recent disease.

Serological tests

Complement fixation test

The CFT was performed as described by Nagington [11]. The chlamydial group-specific CFT antigen was supplied by DMRQC, Central PHL, 61 Colindale Avenue, London NW9 5HT.

Microimmunofluorescence test

The micro-IF test was performed as described by Treharne and colleagues [10] except that additional *C. psittaci* and *C. pneumoniae* representative antigens were

included as well as all 15 *C. trachomatis* serovars. The two *C. psittaci* strains were 10L–395 (human isolate) and A-10 (guinea-pig conjunctivitis isolate). The two representative strains of *C. pneumoniae* were 10L-207 [12] and TW-183 [7]. All sera were tested for the presence of IgG and IgM chlamydial antibodies. Positive readings in the micro-IF test were identified as those elementary bodies (EBs) showing a bright, apple-green, homogenous and circular fluorescence. Other irregular or dull elementary body patterns of fluorescence were considered negative [13].

RESULTS

We studied 50 patients with community-acquired respiratory tract symptoms who were available for interview with regard to evidence of recent bird or animal contact and whose serum samples had chlamydia CFT antibody titres $\geqslant 64$. Evidence of recent respiratory chlamydial infection was assumed in 25 patients, 17 of whom submitted paired serum samples and had a four-fold or greater rise in micro-IF IgG antibody titre to $\geqslant 32$, or stable micro-IF IgG antibody titres of $\geqslant 512$ to *C. pneumoniae* or *C. psittaci*. Similarly eight patients who submitted a single serum sample and had *C. pneumoniae* or *C. psittaci* micro–IF IgG titres of $\geqslant 512$ were regarded as having recent infection. Chlamydia-specific IgM, when present, was regarded as evidence of recent infection. Where patients had stable raised titres to both *C. pneumoniae* and *C. psittaci*, we assumed that the species of *Chlamydia* to which the greatest antibody titre was found and/or for which specific IgM was present was the most likely current infecting organism.

Of the 17 patients from whom paired serum samples were available, 14 (82%) had evidence of recent *C. psittaci* infection (Table 1) and 3 (18%) had evidence of recent *C. pneumoniae* infection (Table 2). Of the patients with evidence of recent *C. psittaci* infection (Table 1), only one (patient 8) had an antibody rise to *C. trachomatis*, and he had much higher *C. psittaci* antibody titres. Patient 4 was included in Table 1 as a case of *C. psittaci* infection despite the fact that his serum samples had identical rises in titre to *C. pneumoniae* and *C. psittaci*, because of the strong association with a sick parrot and the fact that his father had serological evidence of *C. psittaci* infection. Twelve (86%) of these 14 patients had recently been in contact with birds, one had recently been in contact with sheep and only one had not had recent contact with either. For 11 (92%) of the 12 patients, recent bird contact had been with psittacine birds. Of the three patients with evidence of recent *C. pneumoniae* infection (Table 2) only one, who was a pet shop owner, had recently had contact with psittacine birds, but Chlamydiae were not isolated from these birds' faeces.

Of the 8 patients from whom only a single serum sample was available, 5 (63%) had serological evidence of recent *C. psittaci* infection (Table 3) whilst only 3 (37%) had evidence of recent *C. pneumoniae* infection (Table 4). None of these patients had antibody to *C. trachomatis*. Of the 5 patients with evidence of recent *C. psittaci* infection, 4 (80%) had recently had contact with birds; 3 of these involved psittacine birds and 1 had contact with pigeons. Of the 3 patients with evidence of recent *C. pneumoniae* infection (Table 4), only 1 had evidence of recent bird contact, and Chlamydiae were not isolated from any of these birds.

Overall, 19 (76%) of the 25 patients had evidence of recent *C. psittaci* infection

Table 1. *Details of 14 patients with* C. psittaci *infection from whom paired serum samples were available*

Patient	Age	Days after onset of symptoms	CFT	MICRO-IF							Epidemiology
				C. trachomatis			*C. pneumoniae*		*C. psittaci*		
				A–C	D–K	L1–L3	207	TW183	395	A10	
1	52	36	512	< 8	< 8	< 8	64	64	1024	1024	Contact with 20 budgies and 6 cockatiels; *C. psittaci* grown from birds
		66	1024	< 8	< 8	< 8	64	64	2048	2048	
2	39	4	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	No documented bird contact
		16	256	< 8	< 8	< 8	< 8	< 8	256	512	
		46	128	< 8	< 8	< 8	< 8	< 8	32	32	
		79	64	< 8	< 8	< 8	< 8	< 8	32	32	
3	42	3	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	Bird owner. In contact with sick parrot in a commercial aviary
		9	64	< 8	< 8	< 8	< 8	< 8	32	32	
4	57	2	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	Father of patient 3
		8	8	< 8	< 8	< 8	16	16	16	32	
		19	256	< 8	< 8	< 8	16	64	64	64	
5	12	28	> 256	< 8	< 8	< 8	32	32	128	128*	In contact with sheep at lambing time. Grandma ill 1 month later!
		36	> 256	< 8	< 8	< 8	32	32	128	128*	
6	35	4	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	Bird shop owner with pneumonia and flu-like illness
		10	128	< 8	< 8	< 8	< 8	< 8	64	256	
		13	> 256	< 8	< 8	< 8	< 8	< 8	32	256	
7	63	12	16	< 8	< 8	< 8	16	16	< 8	32	Keeps tropical birds (parakeets and waxbills)
		14	64	< 8	< 8	< 8	16	16	16	128	

8	22	4	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	Pneumonia. Mother had similar illness. In contact with recently acquired parrot. *C. psittaci* cultured from parrot
		11	128	256	512	512	1024	512	4096	2048	
		18	> 256	128	1024	1024	512	256	4096	4096	
9	58	20	8	< 8	< 8	< 8	< 8	< 8	< 8	16*	Pneumonia. Poultry Inspector
		46	64	< 8	< 8	< 8	< 8	< 8	< 8	64	
10	69	7	128	< 8	< 8	< 8	64	64	64	256	Handled sick parrot who subsequently died
		21	> 256	< 8	< 8	< 8	64	32	32	512	
		47	256	< 8	< 8	< 8	32	32	128	128	
11	47	2	< 8	< 8	< 8	< 8	16	16	16	< 8	In contact with father's sick parrot
		16	64	< 8	< 8	< 8	16	16	16	16*	
		24	64	< 8	< 8	< 8	16	16	32	16*	
12	40	NS†	32	< 8	< 8	< 8	128	128	< 8	< 8	Newly-acquired cockatiel
			128	< 8	< 8	< 8	128	128	512	512	
13	45	14	> 256	< 8	< 8	< 8	64	64	128	512	Husband of patient 12
		33	> 256	< 8	< 8	< 8	256	256	1024	1024	
14		9	512	< 8	< 8	< 8	32	32	64	128*	Social worker. Recently visited family with sick parakeets
		25	512	< 8	< 8	< 8	64	64	1024	4096*	

* IgM positive; † No symptoms.

Table 2. *Details of three patients with* C. pneumoniae *infection from whom paired samples were available*

Patient	Age	Days after onset of Symptoms	CFT	MICRO-IF							Epidemiology
				C. trachomatis			*C. pneumoniae*		*C. psittaci*		
				A–C	D–K	L1–L3	207	TW183	395	A10	
15	44	6	< 8	< 8	< 8	< 8	16	16	16	< 8	Pet shop owner
		16	128	< 8	< 8	< 8	32	64	16	< 8	*C. psittaci* not isolated from birds
16	22	3	< 8	< 8	< 8	< 8	16	16	< 8	< 8	'Viral illness'
		12	64	< 8	< 8	< 8	32	64	< 8	< 8	No documented bird contact
17	36	1	< 8	< 8	< 8	< 8	< 8	< 8	< 8	< 8	Pleuritic chest pain.
		30	64	< 8	< 8	< 8	64	64	< 8	32	No documented bird contact

Table 3. *Details of five patients with* C. psittaci *infection from whom a single specimen of serum was available*

Patient	Age	Days after onset of Symptoms	CFT	MICRO-IF *C. trachomatis* A–C	*C. trachomatis* D–K	*C. trachomatis* L1–L3	*C. pneumoniae* 207	*C. pneumoniae* TW183	*C. psittaci* 395	*C. psittaci* A10	Epidemiology
18	39	14	> 256	< 8	< 8	< 8	16	16	64*	64*	Friend had psittacosis. In contact with his birds
19	12	10	512	< 8	< 8	< 8	< 8	< 8	64	16*	In contact with budgie. Bird culture negative
20	38	16	256	< 8	< 8	< 8	< 8	< 8	128*	256	No known bird contact
21	24	18	256	< 8	< 8	< 8	< 8	< 8	2048	1024	Works in pet shop with culture positive parrot
22	35	25	4096	< 8	< 8	< 8	< 8	< 8	16*	16*	Contact with dead pigeon. Husband had similar illness

* IgM positive.

Table 4. *Details of three patients with* C. pneumoniae *infection from whom a single specimen of serum was available*

Patient	Age	Days after onset of symptoms	CFT	MICRO-IF *C. trachomatis* A–C	*C. trachomatis* D–K	*C. trachomatis* L1–L3	*C. pneumoniae* 207	*C. pneumoniae* TW183	*C. psittaci* 395	*C. psittaci* A10	Epidemiology
23	50	180	128	< 8	< 8	< 8	512	512	16	64	Keeps parakeets and canaries. Negative on culture.
24	35	29	> 256	< 8	< 8	< 8	16*	16	> 8	< 8	No known bird contact
25	58	90	64	< 8	< 8	< 8	2048	2048	64	64	No known bird contact

* IgM positive.

and of these 16 (84%) had recently had contact with birds. Six patients (24%) had evidence of recent *C. pneumoniae* infection and of these only 2 (33%) had recently had contact with birds.

DISCUSSION

In recent years we have noted an association between the number of cases of human respiratory chlamydial infections in Cambridgeshire and the number of psittacine birds imported into Great Britain [1, 2]. However, since these studies involved serological methods which could only detect genus-specific antibodies and could not differentiate between infection with *C. psittaci*, *C. pneumoniae* and *C. trachomatis*, we were unable to determine which *Chlamydia* species was responsible for each of these infections. With the availability of serological tests for evidence of *C. pneumoniae* infection, it has been possible to perform a study in which evidence of infection with *C. psittaci*, *C. pneumoniae* or C. trachomatis could be investigated in patients with acute respiratory tract symptoms, and to assess the association with recent birds or animal contact.

Our initial impression [1, 2] was that most of our patients with evidence of recent respiratory tract chlamydial infection had also had recent contact with birds, and in particular, psittacine birds. It was for this reason that we recently made arrangements for psittacosis to be designated as a notifiable disease in Cambridge. As a result, the annual numbers of reported cases of human respiratory tract chlamydial infections almost tripled [1, 2]. This rise in incidence has been sustained in subsequent years, perhaps because many cases of human respiratory tract chlamydial infection in the community were previously undiagnosed.

There are, however, difficulties in establishing which species of *Chlamydia* is responsible for each case of respiratory tract chlamydial infection. Grayston and colleagues [14] have indicated that *C. pneumoniae* micro-IF IgG antibody titres are only significant in single serum samples at a titre of $\geqslant 512$. We have adopted this convention for both *C. pneumoniae* and *C. psittaci* micro-IF antibody titres, but we believe this has resulted in an under-estimate of the number of cases of these infections in our study. For example, four of our patients had CFT titres $\geqslant 256$ but micro-IF IgG titres < 512; *C. psittaci* was cultured from birds associated with these cases. These patients were excluded from the study along with 21 other patients who had micro-IF titres < 512 and/or no specific IgM in other serum samples.

However, our aim was to establish the relative proportion of *C. psittaci* and *C. pneumoniae* infection in Cambridge over the study period. Although the absence of specific IgM in some of these single samples may indicate past infection, it may also be absent in cases of reinfection. The absence of specific IgM in paired serum samples probably indicates reinfection. However, in the case of *C. psittaci*, the antigenic heterogeneity within the species may result in an IgG response to the strain included in the test, but no IgM. Serum samples from several of our patients had antibodies to both *C. psittaci* and *C. pneumoniae*. In the absence of species-specific IgM antibody, we assumed that the highest titre was due to the most recent infection. Whilst this may not always be a completely reliable method, it may be sufficient to establish the approximate prevalence of *C. pneumoniae* and

C. psittaci infections over a given period. There were several patients who had stable micro-IF antibody titres to one *Chlamydia* species, while having rising antibody titres to another. Stable antibody titres may reflect a previous infection with that *Chlamydia* species. For those patients with rising antibody titres to both *C. psittaci* and *C. pneumoniae*, it is clear that infection with either of these may provoke an anamnestic antibody response to the common antigenic determinants shared by the other. This, however, we found to be an infrequent occurrence.

The genus-specific lipopolysaccharide (LPS) antigen appears to be less immunoaccessible on the treated EBs used in the micro-IF test [13, 15], and cross-reactivity to this antigen appears as a dull, irregular and patchy fluorescence. Species-specific, sub-species-specific and serovar-specific epitopes are present on the major outer membrane protein of the EBs used in the micro-IF test [16] and by scoring as positive only those chlamydial EBs which fluoresce with a bright apple-green, regular and round appearance, we are eliminating most cross-reactive antibody to LPS [17] and only measuring species-, sub-species- or serovar-specific antibodies.

Whilst the use of the micro-IF test merits further evaluation for the differential serodiagnosis of respiratory chlamydial infections, this study and others [18] have indicated that certain selected *C. psittaci* strains may be useful in this context.

It is likely that the *C. psittaci* species is a very divergent group of chlamydial organisms which have a wide antigenic heterogeneity. The micro-IF results clearly indicate this antigenic diversity [19] and whilst we believe this test can roughly speciate the Chlamydia genus into *C. trachomatis*, *C. pneumoniae* or *C. psittaci*, until we understand more about the complex interrelationships of the *C. psittaci* species, we cannot assign serovar (-type) patterns of cross-reactivity to the antibody responses found in these patients.

Reports suggest that CFT antibodies are often absent in chronic or reinfections with *C. pneumoniae* [14]. Since we selected patients on the basis of CFT titres $\geqslant 64$, we may have slightly underestimated the number of cases of *C. pneumoniae* infection.

our most significant finding was that in 84% of patients with evidence of recent *C. psittaci* infection there was evidence of recent bird contact. There was a lower association (33%) in those with *C. pneumoniae* infection although the number of cases was relatively small. In addition, we failed to isolate Chlamydia from any of the birds associated with patients with *C. pneumoniae* infections.

The majority (76%) of the patients in this study had evidence of recent *C. psittaci* infection. *C. pneumoniae* infections may arise as part of sporadic epidemics associated with high and low incidence years, similar to those noted with *M. pneumoniae* [14]. In years with epidemics of *C. pneumoniae*, the relative proportions of infection with this species would increase. In contrast with Finland [5], however, it is likely that in Britain a significant proportion of cases of human respiratory tract chlamydial infection will always be associated with *C. psittaci*. The majority of these will be as a result of psittacine bird contact, unless their importation is restricted.

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