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Chlamydial antibodies in farmers in north-west England

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SUMMARY

Because of recent reports of abortion in farmers' wives following infection with ovine strains of *Chlamydia psittaci* during pregnancy, the distribution of chlamydial antibodies was studied in rural populations in north-west England, where endemic chlamydial infection with abortion is common in sheep. Immunoperoxidase assays with *C. trachomatis* and ovine *C. psittaci* showed no significant differences in either the frequency or titres of antibodies between sheep farmers and other types of farmer or non-farming adults living in the same areas. The frequency and titres of antibodies in farmers' wives were no greater than in farmers, and were unrelated to their previous obstetric history or type of farming. Overall, 62/255 (24%) of this rural population had antibody detected by *C. trachomatis* antigen and only 30/255 (13%) detected by *C. psittaci* antigen. The possible significance of these findings is discussed. This survey does not suggest that the risk of infection with *C. psittaci* is especially high in people working with sheep, but the complications following infection during pregnancy deserve the specific instructions that have been given to pregnant women to avoid exposure, especially during lambing, in farming and veterinary work.

INTRODUCTION

It has recently been confirmed (Johnson *et al.* 1985; Buxton, 1986; Herring *et al.* 1987) that farmers' wives and female veterinary surgeons who are infected with ovine strains of *Chlamydia psittaci* during pregnancy may develop severe illness resulting in abortion. *C. psittaci* infection is widely prevalent in flocks of sheep in Britain and elsewhere in Europe, and is the most common cause of endemic ovine abortion (Aitken, 1983). Hence, the Department of Health has recently issued a warning (personal communication, Communicable Disease Surveillance Centre, Public Health Laboratory Service, Colindale, London) that pregnant women should avoid working with sheep, especially during lambing. This seems a sensible interim measure but there is as yet insufficient information on the incidence of human infection with ovine *C. psittaci* in farm-workers from which to assess the particular degree of risk to pregnant women, or on which to base effective control measures.

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Ovine strains differ in biotype and in DNA constitution from avian strains of *C. psittaci* (Johnson, 1984; McClenaghan, Herring & Aitken, 1984; Herring *et al.* 1987); they are spread from sheep to sheep mainly during the lambing season, from heavily infected placentas, and may be maintained between epizootics by persistent but inapparent intestinal infection of surviving lambs and ewes (Johnson, 1984). All farm workers handling sheep may thus be exposed frequently to these organisms, but hitherto there have been no reports of major respiratory illness of the type which can follow exposure to avian strains of *C. psittaci* and it is not known whether milder or subclinical infection occurs. Hence the recent cases of abortion in pregnant farm workers may be a dramatic complication highlighting a widespread but hitherto unrecognized zoonosis. Alternatively, human infection may be rare except in women whose susceptibility may be enhanced by pregnancy and by their special tasks in the lambing season.

Accordingly, it was decided to investigate the frequency and titre of antichlamydial antibodies in sera from farmers, farmers' wives and non-farming country dwellers in north-west England, as an indirect measure of the infective risk in areas in which endemic abortion in sheep flocks is known to be common.

The ovine strains of *C. psittaci* isolated from flocks of sheep in which endemic abortion has occurred have shown considerable antigenic heterogeneity (Johnson & Clarkson, 1986). Hence it was decided to test the human sera in this preliminary survey by procedures which would detect an immune response to any prior chlamydial infection, i.e. responses against the lipo-polysaccharide (LPS) antigens common to the whole chlamydia genus as well as against the more type-specific protein antigens. It is known (Richmond & Caul, 1975) that infected tissue culture cells containing mature chlamydial inclusions have such broad reactivity. Thus, all sera were first titrated in an immuno-peroxidase assay (IPA) using cells infected with a lymphogranuloma-venereum (LGV) strain of *C. trachomatis* (Cevenini *et al.* 1983). All sera were further titrated in IPA with a recent ovine strain of *C. psittaci* (ZC26) as a possible means of differentiating immune responses against the two species of *Chlamydia* by differences in the incidence or titre of antibodies shown by the two preparations.

MATERIALS AND METHODS

Patients and sera

Sera were collected by family doctors in rural areas of Cumbria, Northumberland, N. Yorkshire and Lancashire. Appropriate ethical committee approval had been given and all patients gave informed consent. They were farmers or farm workers, farmers' wives or members of a farming family. Each completed a questionnaire on age, sex, occupation and commercial farming interests, e.g. whether a lowland or hill farm; whether sheep were kept, and if so whether indoor lambing or tending sick lambs in the home was practiced. Details of other animals kept on the farm or home, and veterinary problems of an infective nature in the last 2 years were requested. An obstetric history was obtained from all female patients. The same doctors obtained sera from 'control' individuals living in the same rural areas but with no contact with farm animals. Details of age, sex, obstetric history if female, and contact with animals was obtained. In

addition, sera were obtained in the Royal Liverpool Hospital from 14 patients recently admitted with psittacosis and from 10 with known chlamydial infection of the genital tract. These sera, and a mouse monoclonal antibody against the group LPS antigen of chlamydia (Boots Celltech, Slough, England) were used to measure the reactivity of each batch of antigens.

Serological procedures

All sera were tested according to the manufacturers' recommendations with a commercial slide kit (IPAZYME, Biological Industries Ltd, Glasgow) containing LGV antigen; antibody was detected by an immunoperoxidase-labelled goat antihuman IgG serum. Sera were spot-tested at a 1 in 32 dilution, and those positive were retested at dilutions of 1 in 32, 128, and 512. Sera with titres $\geqslant 128$ were retitrated with IPA-labelled goat antihuman IgA serum. A random selection of the sera negative at 1 in 32 dilution was retested at a 1 in 8 dilution; only occasional weak positive reactions were seen, but these were difficult to read because at this low dilution the whole tissue culture monolayer developed a grey stain which tended to obscure any staining of the chlamydial inclusions. It was presumed that this was due to heterophile (anti-species) antibodies in the human sera reacting directly with the tissue cells; this view was supported by IPA titrations of sera from five patients with suspected glandular fever who had shown titres from 32 to 128 in conventional Paul Bunnell tests. In IPA tests, all showed marked staining of the cell monolayer in titres corresponding to their titre in the Paul Bunnell test.

All sera were tested subsequently by a similar IPA technique against inclusions of the ZC26 ovine strain of *C. psittaci* in McCoy cell cultures by the procedure described for the IPAZYME kits (Cevenini *et al.* 1983). In each batch of tests, the reactivity of the antigen slides was tested by titration with mouse monoclonal antichlamydial antibody by standard immunofluorescence techniques recommended by the manufacturer (Boots Celltech, Slough, England), and against the human sera from patients with psittacosis or genital tract chlamydial infections (described above) using the IPA technique.

Statistical evaluation

The χ^2 calculation with Yates's correction for continuity was applied, wherever relevant, to the results shown below.

RESULTS

Epidemiological data

Sera were obtained from 180 farmers. There were 114 men and 66 women, with 17 married couples. The mean age for males was 46 years (range 17–94 years), and for females 45 years (range 13–78 years). Fifty-nine females had been pregnant and had had 143 live births (2·4 per woman); 17 (29%) had suffered miscarriages and two had had a termination of pregnancy. One hundred and sixty-nine farmers kept sheep, 111 kept beef cattle and 96 kept dairy cattle for commercial purposes. Only six farms kept solely sheep. Most kept other animals on the farm, primarily dogs, cats and chickens. Of the 169 sheep farmers, 95 worked on a hill farm, 61 on

a lowland farm and 12 a mixed lowland/hill farm (1, no data). One farmer milked sheep commercially and 35 milked sheep for personal use; 64 lambed indoors and 116 took sickly lambs into their home. Ovine enzootic abortion had been diagnosed in the flocks of six farms in the last 2 years.

Seventy-five sera were obtained from non-farming controls, with one pair from a married couple. There were 38 men (mean age 50 years, range 19–81 years) and 37 women (mean age 48 years, range 15–83 years); 33 women had been pregnant and had had 73 live births (2·2 per woman); nine (27%) had had a miscarriage and one a termination of pregnancy. Most control patients kept family pets, mainly dogs and cats, but none kept sheep or cattle.

Serological tests with hospital patients and laboratory standard sera

Titration of the monoclonal antichlamydial (antiLPS) antibody showed positive immunofluorescence against both LGV and EA antigen; there were no observable differences between the two antigens in the titre (2048) or in the quality or degree of fluorescence at any dilution.

Sera from all 14 patients with psittacosis were positive (titres 64 to > 256) in routine complement fixation tests with standard commercial *C. psittaci* antigen: 13 were IPA-positive with LGV antigen (titres 32 to > 512) and 9 (all positive in the LGV test) were positive (titres 32–256) in IPA tests with EA antigen. Sera from the 10 patients with *C. trachomatis* infections were all IPA-positive with LGV antigen, (titres 32 to > 512) and in 7 cases antibody was detected, but in lower titre, (32–128), with EA antigen. Sera with titres < 32 against EA antigen were retested at dilutions of 1 in 8 and 1 in 16. Three sera from the patients with psittacosis and one from the patients with *C. trachomatis* infections showed weak staining of the chlamydial inclusions, but because this was accompanied by heterophilic staining of the tissue cells (as described above) they were not accepted as specific positive results.

Tests with sera from farmers and controls

The distribution of antibody titres against LGV and EA antigens is shown in Table 1. Forty–four (24%) of the farming group were antibody-positive in IPA tests with LGV antigen, comprising 27/114 (24%) of the males, and 17/66 (26%) of the females. The mean age of positive males was 50·2 years (range 27–74 years) and of the females 47·6 years (range 17–74 years). In 23 (52%) of these 44 patients, antibody was also demonstrated by *C. psittaci* antigen, but in no case was there antibody against *C. psittaci* alone.

There were no significant differences in incidence or titre of antibodies between the sexes. There had been previous pregnancies in 59/66 women; 3/17 (18%) with previous miscarriages were antibody positive, whilst 12/42 (28%) of women with no miscarriage also had antibody. These differences are not significant, nor were differences in antibody titre between those with or without past miscarriage.

In the non-farming control group 18/75 (24%) showed antibody against LGV antigen, comprising 7/38 (18%) males and 11/37 (30%) females. The mean age of positive males was 53·6 years (range 36–79 years) and of the females 46·8 years (range 15–82 years). Seven (39%) of the 18 patients with antibody detected by LGV antigen also showed antibody against *C. psittaci* antigen. None had antibody against *C. psittaci* alone. There were no significant differences in the incidence or

Table 1. *Distribution of antichlamydial antibodies in 255 sera from farmers and controls*

	C. trachomatis LGV 2			*C. psittaci* EA ZC26		
Group	< 32	32	> 128	< 32	32	> 128
Farmers ($N = 180$)	136 (76%)	26 (14%)	18 (10%)	157 (87%)	7 (4%)	16 (9%)
Controls ($N = 75$)	57 (76%)	15 (20%)	3 (4%)	68 (91%)	1 (1%)	6 (8%)

Table 2. *Distribution of antichlamydial antibodies in farmers*

Risk factor	Total number	Number positive (%)
Sheep	169	42 (25)
No sheep	11	2 (18)
Beef cattle	111	29 (26)
No beef cattle	69	15 (22)
Dairy cattle	96	19 (20)
No dairy cattle	25	84 (30)

Table 3. *Distribution of antichlamydial antibodies in sheep farmers*

Risk factor	Total number	Number positive (%)
Milk sheep*	35	6 (17)
Do not milk sheep	133	36 (27)
Hill farm*	95	25 (26)
Lowland farm	61	12 (20)
Mixed	12	5 (42)
Lamb indoors	64	17 (27)
Do not lamb indoors	105	25 (24)
Lambs in house	116	28 (24)
No lambs in house	53	14 (26)

* One, no data.

titre of antibodies or in the mean age or the sex ratio of positive results between the farming and non-farming groups. The mean age of the antibody positive patients did not differ significantly from those without antibody.

In none of the positive sera described above was antibody of the IgA class demonstrated at titres of 32 or greater whereas an IgA component (titre = 32) was found in 8 of the 14 positive sera from hospital patients with recent psittacosis and 5 of the 10 cases with current *C. trachomatis* infection.

The incidence of antibody in relation to the type of farm and the possible risk factors involved are shown in Table 2. There were no significant differences in either the incidence or the titres of antibody between sheep farmers and other types of farmer. Within the sheep farmers, there were no significant differences in antibody related to the various locations or practices (Table 3).

DISCUSSION

In this survey the LGV chlamydial antigen was chosen deliberately to detect a broad immune response to any prior exposure to chlamydiae, and further testing with ovine *C. psittaci* antigen was done to look for more specific responses.

In any of the presently available immunological procedures for diagnosis of chlamydial infections the probability of cross-reactivity between *C. psittaci* and *C. trachomatis* is high. Even in microimmunofluorescence (MIF) tests with purified elementary body suspensions of *C. psittaci* and *C. trachomatis* antibodies against antigens common to both species may be detected although in recent or current infection the titre may be 2–4 times higher with the homologous than with the heterologous antigen (Eb *et al.* 1982). Similarly, enzyme-linked immunosorbent assays with purified LGV antigens detected antibody in patients with *C. psittaci* infections as well as in those with *C. trachomatis* infections (Levy, Muñoz & McCormack, 1983).

Thus in the present series it is not possible to conclude categorically that the antibody responses were due uniquely to *C. psittaci* rather than to *C. trachomatis* infections, nor that they result from infection with ovine strains rather than avian strains of *C. psittaci*. On general epidemiological and sociological grounds it seems unlikely that all the positive findings were the result of unnoticed genital tract infections with *C. trachomatis*, although in fact there are no reports on the incidence of antibody to *C. trachomatis* to be expected in 'normal' (i.e. non-STD clinic) populations. More to the point, there is very little information available on the incidence of antibody to *C. psittaci* to be expected in rural communities. In blood-donor sera from Cambridgeshire, Nagington (1984) found complement-fixing (CF) antibody in 29/288 (10%), and in only 506/10381 (5%) of sera from patients with various illnesses seen in hospital. In poultry-workers in E. Anglia antibody was found in 61% of those working with ducks where chlamydial infection is common and in 23% of people working with hens or turkeys (Andrews, Major & Palmer, 1981). Nagington (1984) believed that some of the complement-fixing antibodies against *C. psittaci* found in the general population might represent anamnestic responses to other non-chlamydial infections, but the possibility of human-to-human infection with the recently identified TWAR strains of *C. psittaci* (Kuo *et al.* 1986) which have no apparent animal reservoir, may also need to be taken into account.

Recent unpublished observations (Johnson, personal communication) suggest that titres of antibody against EA may be higher than against LGV antigens in IPA tests on sera from sheep with recent infection with ovine *C. psittaci* and also in early convalescent sera from three pregnant women who aborted following infection. However, in the present survey, the overall results suggest that both EA and LGV antigens were detecting antibody mainly against common group antigens of chlamydiae, and that LGV was somewhat more sensitive in this respect. There was no evidence that antibodies directed against antigens peculiar to or predominant in ovine strains of *C. psittaci* had been induced or had persisted in this population. Indeed these results suggest that previous chlamydial infection, or whatever nature, had not been common.

Only 24% of the population showed anti-chlamydial antibody which was in

general of low titre, suggesting that the exposure had not been recent. The absence of the IgA class of antibody in any of the positive sera also supports the view that none was the result of a recent or current infection (Cevenini *et al.* 1983). It is apparent that neither sheep farmers nor especially their wives have had an undue frequency of antibody-inducing chlamydial infections in comparison with other types of farmer or with non-farming members of the same community. Chlamydial abortion is known to be widespread in sheep throughout this area, so it would appear that the infectivity of ovine strains of *C. psittaci* for humans, or the risk of exposure to farm workers, is not high. However, even though the risk of infection may be low, recent reports make it clear that the apparent virulence of these strains is enhanced in pregnant women. Thus, as recommended in several recent publications (e.g. Johnson *et al.* 1985), a chlamydial etiology for threatened abortion or septicaemic illness in any pregnant women in contact with sheep should be considered, diagnosed quickly, and treated promptly with tetracyclines. It is reassuring to note, however, that we did not find an increased history of abortion in the farming population we tested.

For the future, the development of simple and reliable serological tests that can be used on a wide scale especially in rural populations to differentiate the various types of chlamydial infection is urgently needed. No such test is currently available, but the fundamental immunochemical studies of Caldwell & Judd (1982) on fractionation and purification of the many protein components of chlamydiae have promise for the resolution of these problems.

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