

SEROLOGIC INVESTIGATION OF THE PREVALENCE OF *CHLAMYDOPHILA PSITTACI* IN OCCUPATIONALLY-EXPOSED SUBJECTS IN EASTERN SICILY

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Abstract: The aim of the study was to investigate the occurrence of serum antibodies to *C. psittaci* in workers at 8 stock farms located in a rural area of eastern Sicily. Serum samples from 188 workers and 160 controls were tested for immunoglobulin IgA, IgG and IgM direct against *C. psittaci* by microimmunofluorescent test (MIF). Seropositive subjects were defined as those with IgG titres of 1:16-1:256, and seronegative subjects as those whose titre was <1:16. To rule out the presence of cross reactive antibodies, testing was also performed for *C. trachomatis* and *C. pneumoniae*. A total of 28 (14.9%) subjects studied met the criteria for seropositivity to *C. psittaci*. Two of the 188 (1.06%) had an IgG titre of 1:32, 16 (8.51%) an IgG titre of 1: 64, 6 (3.19%) an IgG titre of 1:128 and 4 subjects (2.12 %) demonstrated an antibody titre of 1:256. The high prevalence rate of *C. psittaci* antibodies among farmers suggests that this infection is spread in those subjects living in areas with an high percentage employed in rearing activities. The authors stress the importance of carrying out health surveillance in subjects working in close contact with animals receptive to infection, and confirm the need to adopt a serological test, such as MIF as a preventive measure for activities at risk.

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INTRODUCTION

Chlamydiae are obligate intracellular pathogens that can infect epithelial cells and monocyte/macrophages of a wide host range, resulting in a broad spectrum of diseases [21, 13].

Within the genus *Chlamydia* there are 4 species: *C. trachomatis*, *C. psittaci*, *C. pneumoniae* and *pecorum*, which have been classified according to their antigenic, pathological and molecular properties [18].

Following reclassification in 1999, the order *Chlamydiales* currently comprises four families, namely the

Chlamydiaceae, Parachlamydiaceae, Simkaniaceae and Waddiaceae, that are divided into 2 genera, *Chlamydia* and *Chlamydomphila*, and 9 species. This classification is based on limited phenotypic, morphological and genetic criteria [6, 4, 11].

Chlamydomphila psittaci (formerly known as *Chlamydia psittaci*) is a member of the family Chlamydiaceae. It is a bacterium that can be transmitted from pet birds to humans. In humans, the resulting infection is referred to as psittacosis (also known as parrot fever and ornithosis). Psittacosis typically causes flu-like symptoms and can lead to severe pneumonia and non-respiratory health

problems. With appropriate treatment, the disease is rarely fatal. From 1988-2003, Centres for Disease Control (CDC) received reports of 935 cases of psittacosis [3], which is an underrepresentation of the actual number of cases. Most human cases were associated with exposure to pet birds. Other persons at risk include pigeon fanciers and person in specific occupations (e.g. employees in poultry slaughtering and processing plants, veterinarians, veterinary technicians, laboratory workers, workers in avian quarantine stations, farmers, wildlife rehabilitators and zoo workers). Because human infection can result from brief, passing exposure to infected birds or their contaminated excretions or secretions, persons with no identified leisure time or occupational risk can also become infected.

Infection with *C. psittaci* usually occurs when a person inhales organisms that have been aerosolised from dried faces or respiratory tract secretions or infected birds. Other means of exposure include mouth-to-beak contact and handling plumage tissues of infected birds. Even brief exposures can lead to symptomatic infection; therefore, certain patients with psittacosis might not recall or report having any contact with birds.

Mammals occasionally transmit the Chlamydiaceae organism to humans. Certain chlamydial species infect sheep, goats and cattle, causing chronic infection of the reproductive tract, placental insufficiency and abortion. Those species are transmitted to humans when humans are exposed to the birth fluids and placentas of infected animals. Infection person-to-person transmission has been suggested but not proven [8].

Onset of illness typically follows a period of incubation from 5-14 days, but longer periods have been reported. The severity of the disease ranges from inapparent illness to systemic illness with severe pneumonia. Before antimicrobial agents were available, 15-20% of human with *C. psittaci* infection died. However, only <1% of properly treated humans now die as a result of the infection. Humans with symptomatic infection typically have an abrupt onset of fever, chills, headache, malaise and myalgia. They usually develop a non-productive cough that can be accompanied by breathing difficulty and chest tightness [10].

In our country, both the unapparent illnesses and the moderate illness are under-reported therefore, in Italy the real prevalence of *C. psittaci* infections is underestimated. Moreover, in an occupational setting, the serologic and epidemiologic investigations are important to evaluate the prevalence of *C. psittaci* among the occupational categories at risk of infection [17, 20].

The objective of the present survey is to evaluate the prevalence of antibodies to *C. psittaci* in a group of workers employed at 8 stock farms.

MATERIALS AND METHODS

The present study was conducted in 2005 in 8 cattle and pig farms located in eastern Sicily.

A group of 188 male farmers, caucasian origin, age range from 27-62 years, mean (SD) 36.5 (\pm 8.2) years; length of employment mean (DS) 28.5 (\pm 9.8) years, were recruited as study subjects. None used any respiratory protection devices.

The control group consisted of 160 age matched men, in a good health who were employed in 3 public offices of Messina. All subjects gave informed consent before inclusion in the study.

All participants were interviewed by well-trained occupational physicians, and information about sociodemographic characteristics, occupational and disease history, with a particular regard to flu-like symptoms during the last 6 months, were gathered.

Sera from workers and controls were stored to -20°C until processed at the Department of Social and Environmental Medicine Laboratory, University of Messina.

All serum samples from 188 workers and 160 controls were tested for immunoglobulin IgA, IgG and IgM direct against *C. psittaci* by microimmunofluorescent (MIF) test kit (Servibio – France).

Serum specimens were screened at 1:16 dilution for IgA, IgG and IgM and titrated to end point; an IgG titre of $\geq 1:512$ or an IgA and IgM antibody titre of $\geq 1:16$ were indicative of possible acute *C. psittaci* infection; seropositive subjects were defined as those with IgG titres of 1:16-1:256 and seronegative subjects as those whose titre was $< 1:16$.

To rule out the presence of cross-reactive antibody, testing was also performed for *C. trachomatis* and *C. pneumoniae*. Serum samples were considered positive if they reacted only with *C. psittaci* antigen or if they reacted with the other chlamydial types, but showed the highest grade of reaction with *C. psittaci* antigen.

RESULTS

The results concerning the sociodemographic characteristics, occupational and disease history are shown in Table 1; none both of the subjects under studying and control had suffered from flu-like symptoms during the last 6 months.

A total of 28 subjects under study met the criteria for seropositivity to *C. psittaci*. A seroprevalence of IgG at a titer of at least 1:16 was evidenced in 28 (14.9%) stock-farmers. Two of the 188 (1.06%) had an IgG titre of $> 1:32$, 16 (8.51%) an IgG titre of $> 1:64$, 6 (3.19%) an IgG titre of 1:128 and 4 (2.12%) demonstrated an antibody titre of 1:256. No antibodies IgA and IgM against *C. psittaci* were present in all of the serum tested (Tab. 2).

No subjects of the control group resulted positive for IgG, IgA and IgM to *C. psittaci*.

DISCUSSION

In the present study, the authors explored *C. psittaci* seroprevalence by MIF test in 188 farmers by assessing

Table 1. Demographic characteristics of study subjects and control group.

| Sociodemographic characteristics | Study subjects (188) | Controls (160) |
|---|---------------------------------|---------------------|
| Age (M ± SD) | 36.5 ± 8.2 | 38.5 ± 7.1 |
| Gender | Male 100% | Male (100%) |
| Length of employment at farms (years) (M ± SD) | 28.5 ± 9.8 | 28.2 ± 9.4 |
| Residence | Rural areas in Messina province | Cities near Messina |
| Hobbies in contact with animals | None | None |
| Occupational history | 120 (63.8%) | 68 (42.5%) |
| • rearing and agriculture | 68 (36.2%) | 92 (57.5%) |
| • craftsmanship | | |
| • other | | |
| Disease history | nonenone | nonenone |
| • systemic pathologies | | |
| • flu-like symptoms in the last 6 months (fever, chills, headache, malaise myalgia) | | |

Table 2. *C. psittaci* seropositivity in study subjects and controls.

| | Study subjects (188) | Controls (160) |
|------------------------------|----------------------|----------------|
| <i>C. psittaci</i> IgG titre | | |
| No | 160 (85.1%) | 160 (100%) |
| Yes (≥1:16) | 28 (14.9%) | |
| 1:16 | 0 | |
| 1:32 | 2 (1.1 %) | |
| 1:64 | 16 (8.5 %) | |
| 1:128 | 6 (3.2 %) | |
| 1:256 | 4 (2.1 %) | |

the occurrence of IgA, IgM and IgG antibodies. The results showed that IgG seroprevalence to *C. psittaci* was present in 28 (14.9%) farmers.

Although *C. psittaci* infection is common in birds and domestic animals such as ruminants and pigs, humans are also susceptible to infection because *C. psittaci* has a high anthrozoönotic potential so that anyone in contact with an infected animal is at risk. Human cases occur both sporadically and as outbreaks, that are frequent in rural regions, both also in the general population and in some occupational categories [23].

Under field conditions, cattle may be infected with different chlamydial species (*C. psittaci*, *C. pecorum*), that include strains showing only mild pathogenicity [17]. The habitat of chlamydia is in the intestinal tract, where the microorganism establishes a kind of “chronic latent infection” that can lead to clinical disease if the host-agent balance is altered by endogenous or exogenous causes. The presence of chlamydia in the intestinal tract elicits an antibody response; detection of seropositivity is considered an indication of chlamydial infection, but not necessarily of chlamydial disease [2].

Several seroprevalence investigations were performed by MIF test, even if it is limited by high cross-reactivity

among different chlamydial species; in fact lipopolysaccharides, the antigen used in available serology tests, is a common molecule found among several chlamydial species [9]. In the present study, to rule out the presence of cross-reactivity antibodies, the test was also performed for *C. pneumoniae* and *C. trachomatis*. Therefore, the results definitively excluded other chlamydial infections. The authors stated that an IgG titre of ≥ 1:16 be interpreted as presumed past infection [16], according the Consensus Recommendations on Standardized Testing [5] for *C. psittaci* by MIF test. Some studies have also used the same criterion [16, 7], while others have set it as ≥ 1:32 [11].

In European countries, *C. psittaci* infection is a rare condition. Although data for the prevalence of *C. psittaci* DNA detection in blood samples from the general population are lacking, serology data indicate that only 3% of elderly individuals in Northern Europe are *C. psittaci* IgG seropositive [19, 12].

In Italy, many authors have performed surveys, both in the general population and in occupational settings, examining group specific antibodies to chlamydia [1, 14, 15]; nevertheless, few studies have so far addressed the occurrence of the prevalence of type-specific antibodies against *C. psittaci* in a selected population such as stock farm workers.

Musso *et al.* (1991), in a preliminary survey conducted in children in northern Italy, indicated a percentage of over 8% of antibodies against *C. psittaci*. Moreover, this study confirmed the high risk in parrot-owning households where anti-Chlamydia antibodies were found in 37.5% of children.

Maffei *et al.* (1987) carried out a seroepidemiological survey in order to define the epidemiological features of psittacosis in a region of the mid-Italy. The results showed that 51 out of 143 subjects examined were exposed to birds (35.7%), but only 7 out of 96 (7.3%) urban adult blood donors were positive for chlamydial antibodies using the MIF test.

In Sicily, little is known about the epidemiology of the *C. psittaci* infection. The spread of this infection in cattle has prompted an epidemiological study on the subjects who perform work in close contact with domestic animals at some stock-farms located in a rural areas of eastern Sicily, where a high percentage of people is employed in agriculture and rearing. In the same region, several serological investigations performed on bovine, sheep, goats, pig, buffalo and horses detected a seroprevalence for *C. psittaci* ranging from 90.41-28.05%, while a serologic investigation conducted in 348 farm workers, showed a seroprevalence of 18.96% [17, 20].

According to this result, the high prevalence of *C. psittaci* (14.9%) in the present study might reflect the occurrence of a subclinical of *C. psittaci* infection that is spread to farmers who perform work in close contact with animals. Moreover, this finding suggest that psittacosis is not limited to direct bird contact and supports the hypothesis

that rural environments may increase risk for psittacosis [22]. The answers to the questionnaire evidenced that the subjects (under study) were living in a rural environment and performing rearing activities in direct contact with animals, such as bovine, pigs, goats and sheep, because the stock-farms were not organised according to modern standards and the farmers did not use any personal protection device. In addition, no subjects kept birds or other animals; therefore the infection had probably occurred as a result of exposure to the birth fluids and placentas of infected animals at workplaces.

In an occupational setting, control measures should be carried out to prevent transmission of *C. psittaci*. The primary prevention should focus both on disinfection of the farms and identifying and treating sick animals, according to the AVMA recommendations [23]. Within health surveillance programmes, the occupational physician should evaluate the presence of signs and symptoms of *C. psittaci* infection among workers; moreover, the MIF might represent an adequate screening method for early diagnosing the infection both in animals and humans. In addition, in order to limit exposure, the workers must be informed about the nature of the disease and use gloves and masks during all activities at risk of exposure [23].

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