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**Epidemiology of *Chlamydia psittaci* in pet birds associated with psittacosis  
cases in humans**

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## RESUMO

FERREIRA, V. L. **Epidemiologia da *Chlamydia psittaci* em aves de companhia associada aos casos de psitacose em humanos.** [Epidemiology of *Chlamydia psittaci* in pet birds associated with psittacosis cases in humans]. 2016. 74 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2016.

As zoonoses representam a maior parte das doenças infecciosas emergentes, as quais tem ocorrência variável de acordo com fatores biológicos, ambientais e sócio-econômico-culturais. No que tange aos fatores sócio-culturais, uma prática crescente no Brasil é a manutenção de espécies silvestres como animais de estimação. Estes podem ter significativo papel na disseminação de agentes patogênicos com potencial zoonótico, tal como *Chlamydia psittaci*, agente etiológico da clamidiose em aves e da psitacose em seres humanos. Os Psittaciformes representam a principal Ordem de aves acometida pela *C. psittaci*, sendo também a mais comumente mantida como pet. A clamidiose aviária é endêmica no Brasil, contudo, são raros os estudos direcionados a avaliação do seu potencial zoonótico. Em humanos a psitacose pode desencadear um quadro severo de pneumonia atípica, no entanto, devido à dificuldade relacionada ao diagnóstico laboratorial e pelo relativo desconhecimento da doença pelos profissionais de saúde, sua prevalência no país é ainda desconhecida. Dentro desse contexto, o presente trabalho teve como objetivo determinar a ocorrência da *C. psittaci* em pacientes suspeitos de psitacose atendidos no Ambulatório de doenças tropicais e zoonoses do Instituto de Infectologia Emílio Ribas (IIER); estabelecer o vínculo epidemiológico com aves realizando o diagnóstico nestas, assim como avaliar os fatores de risco relacionados com essa zoonose. Para tanto, amostras de sangue de pacientes com quadros suspeitos de psitacose foram coletadas para a investigação de anticorpos anti-*C. psittaci* IgA, IgM e IgG. Paralelamente, amostras biológicas de quaisquer espécies de aves relacionadas com os casos suspeitos de psitacose foram coletadas para a pesquisa molecular de *C. psittaci*. Entre os pacientes elegíveis deste estudo, 27% (10/37) foram classificados como casos confirmados de psitacose; 13,5% (5/37) como prováveis e 59,5% (22/37) como descartados. Pneumonia ( $p = 0.004$ ), tosse ( $p = 0.002$ ) e calafrio ( $p = 0.011$ ) foram estatisticamente significantes quando comparado com os pacientes nos quais a psitacose foi descartada. Quanto ao vínculo epidemiológico com aves, 73% (11/15) dos casos prováveis e confirmados de psitacose relataram exposição domiciliar com aves e em 27% (4/15) a exposição foi ocupacional. Adicionalmente, 47% (7/15) dos pacientes tiveram contato com

aves nas quais a infecção por *C. psittaci* foi comprovada laboratorialmente. Em 47% (7/15) dos casos não foi possível obter material biológico das aves relacionadas com os casos e em 6% (1/15) dos casos *C. psittaci* não foi detectada nas aves avaliadas. Ainda, os casos prováveis e confirmados de psitacose relataram manter contato próximo com suas aves, como pega-lás na mão (100%, 15/15), mantê-las no ombro (67%, 10/15), beijá-las (40%, 6/15) e dividir alimento com elas (13%, 2/15). Ressalta-se que essas práticas facilitam a transmissão do patógeno. Profissionais da saúde tanto humana quanto animal têm um papel importante a desempenhar na identificação de fatores que afetam a saúde de seus pacientes e devem, portanto, trabalhar juntos. Esforços mútuos contribuiriam no conhecimento de doenças com potencial zoonótico e certamente contribuiriam para medidas mais eficazes de prevenção e controle.

Palavras chave: Clamidiose aviária. Psitacose. Vigilância epidemiológica. Zoonose.

## ABSTRACT

FERREIRA, V. L. Epidemiology of *Chlamydia psittaci* in pet birds associated with psittacosis cases in humans. [**Epidemiologia da *Chlamydia psittaci* em aves de companhia associada aos casos de psitacose em humanos**]. 2016. 74 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2016.

Zoonosis represent the majority of emerging infectious diseases, which have variable occurrence according to biological, environmental, socio-economic and cultural factors. With respect to socio-cultural factors, a growing practice in Brazil is keeping exotic animals as pets. These can have significant role in the spread of pathogens with zoonotic potencial, such as *Chlamydia psittaci*, etiologic agent of chlamydiosis in birds and psittacosis in humans. The Psittaciformes are the main order of birds affected by *C. psittaci* and is the most commonly kept as a pet. Avian chlamydiosis is endemic in Brazil; however, few studies have been conducted regarding its zoonotic potential. In human, psittacosis can lead to atypical pneumonia, however, due to difficulties related to laboratory diagnosis and the relative lack of knowledge by health professionals about this disease, its prevalence in the country is still unknown. In this context, this study aimed to determine the occurrence of *C. psittaci* in patients with psittacosis symptoms attended at the Ambulatory of Tropical Diseases and Zoonosis of the Infectology Institute Emilio Ribas (IIER). In addition, establishing the epidemiological link with birds and evaluate risk factors related to this zoonosis. Therefore, serum samples from eligible patients were collected in order to be tested for *C. psittaci* IgA, IgM and IgG antibodies. At the same time, biological samples from any species of birds related to suspected cases of psittacosis were collected for molecular analysis of *C. psittaci*. Among the eligible patients in this study, 27% (10/37) were classified as confirmed cases of psittacosis; 13.5% (5/37) as probable and 59.5% (22/37) as discarded. Pneumonia ( $p = 0.004$ ), cough ( $p = 0.002$ ) and chills ( $p = 0.011$ ) were statistically significant when comparing with those patients in which psittacosis was discarded. With reference to the epidemiological link with birds, 73% (11/15) of the confirmed/probable cases had domiciliary contact with birds and 27% (4/15) had occupational contact. In addition, 47% (7/15) patients had contact with infected birds in which *C. psittaci* was laboratorial confirmed; from another 47% (7/15) of the cases, biological samples of the birds related to the patient could not be obtained, and in 6% (1/15) of the cases *C. psittaci* was not detected in the bird hosts evaluated. In addition, several confirmed/probable cases reported having close contact with the birds as handling (100%,

15/15), keeping the bird in the shoulder (67%, 10/15), kissing the bird (40%, 6/15) and sharing the food with the bird (13%, 2/15), practices that facilitate diseases transmission. Human and animal health care providers have an important role to play in identifying specific factors affecting the health of their patients and should work together. Conjointly efforts would increase the understanding on zoonotic disease and would, ultimately, improve prevention and control strategies.

Key words: Avian chlamydiosis. Epidemiological surveillance. Psittacosis. Zoonosis.

## 1 INTRODUCTION

The family Chlamydiaceae includes coccoid, non-motile, obligate intracellular organisms of 0.2 up to 1.5  $\mu\text{m}$  diameter that reside in vacuole-like inclusions of eukaryotic cells, where they parasitize and multiply in a unique developmental cycle. These microorganisms are pathogens of both mammals and birds and infect many hosts, with variable tissue tropism causing a multiplicity of acute and chronic diseases (BEECKMAN; VANROMPAY, 2009; SACHSE et al., 2015). *Chlamydia* is the only currently defined genus in the Chlamydiaceae and is composed of the current species *Chlamydia trachomatis* (humans); *C. pneumoniae* (mainly humans), *C. psittaci* (birds), *C. muridarum* (rodents), *C. pecorum* (mainly cattle and koala), *C. suis* (swine), *C. avium* (birds), *C. gallinacea* (birds), *C. abortus* (sheep, goat, cattle), *C. caviae* (guinea pig), and *C. felis* (cats) (SACHSE et al., 2015). Significant species of the genera representing sources of infection for humans are *C. psittaci*, *C. abortus* and *C. felis*. (RODOLAKIS; MOHAMAND, 2010).

Nonetheless, undoubtedly the most important animal chlamydioses with zoonotic potential is caused by *Chlamydia psittaci*, which is the causative agent of human psittacosis and avian chlamydiosis. Psittacosis occurs mostly after exposure to infected sources. Infection usually happens when a person inhales the aerosolized organism from dried faeces or respiratory secretions, which can occur through mouth-to-beak contact or handling of infected birds. Zoonotic transmission may result in subclinical infection or manifest as ‘flu-like’ illness or a potentially fatal interstitial pneumonia. The onset of the illness usually follows an incubation period of 5–14 days. As the disease is rarely fatal in properly treated patients, early diagnosis is important (NASPH, 2010; SACHSE et al., 2015).

In humans, psittacosis is usually diagnosed using a combination of clinical signs and serology. The most common confirmatory test is a fourfold rising titer (immunoglobulin G) to *C. psittaci* in paired sera with the microimmunofluorescence test. For epidemiological surveillance, the CDC (Center for Diseases Control and Prevention) has established case definitions (NASPH, 2010), nonetheless psittacosis confirmed cases might be difficult to determine. Some causes include cross-reactivity with other *Chlamydia* species infecting humans and empirical therapy for community-acquired-pneumonia, which may blunt antibody response to *C. psittaci*. Therefore, history of bird contact is a valuable clue in disease investigation, since exposure to birds is reported in 85% of the psittacosis cases (JUNG; GRAYSON, 1988; BEECKMAN; VANROMPAY, 2009).

In birds, transmission of *C. psittaci* primarily occurs from one infected bird to another susceptible bird in close proximity. The agent is excreted intermittently in faeces and exudates, and the primary route of infection is through the respiratory tract, followed by the oral route. The most common visible clinical sign of avian chlamydiosis involves the respiratory or gastrointestinal systems of birds. Clinical signs include lack of appetite, weight loss, depression, diarrhea, conjunctivitis, discharge from the eyes or nares, or even death. In other cases, birds actively infected with *C. psittaci* may be only mildly affected or show no signs of illness (VANROMPAY et al., 1995; SACHSE et al., 2015).

All over the world, at least 465 avian species were found to be infected with this zoonotic agent. *C. psittaci* is highly prevalent in Psittacidae, such as macaws, cockatoos, cockatiels, parrots, parakeets and lorries (KALETA; TADAY, 2003); those are also de main bird species kept as pets.

It should be emphasized that birds are among the most popular pets around the world. For example, in Brazil, 37 million birds are kept as companion animals (ABINPET, 2015). In general, the top sources for acquiring a pet are friend/acquaintance, pet shop, recommended breeder, and private advertisement (HALSBY, 2014). Concerning zoonosis pathogens, it is well establish that *Chlamydia psittaci* is endemic among birds from different origins in the country (RASO, 2014). With reference to pet birds, there are also studies examining *C. psittaci* infections in birds from pet shops and breeders, revealing high prevalences (RASO et al., 2011; SANTOS et al., 2014) but there is little exploration of human infections arising from these facilities. In this scenario, how to recognize when birds may be effectively serving as a source of infection of *C. psittaci* to human beings? This query can only be clarified through a collaborative effort between veterinary and human health professionals, with mutual approaches in which the disease or infection can be observed simultaneously in both the human and the animal patient. For this reason, this study aimed continuous epidemiological investigations of *C. psittaci* infection in birds that played a role as a potential source of *C. psittaci* infection for human patients. In order to reach both susceptible hosts (human and animal), this research was developed by our team (Veterinary Faculty of the University of São Paulo), along with a partnership with the medical team of the Ambulatory of Tropical Diseases and Zoonosis of the Infectology Institute Emilio Ribas (IIER). The results of this investigation are pioneering in the national medical literature and can be verified in the chapters below.

## 1.1 CHAPTERS PRESENTATION

This thesis is written in chapters, including this introduction (chapter 1). Each chapter discloses relevant issues on avian chlamydiosis/human psittacosis disease, such as critical points on the interface of the bird's pathogen and the clinically ill person, the gaps in understanding the animal management determinants for the disease occurrence and the lack of awareness among medical health professionals about this underestimated zoonosis.

Chapter 2 entitled *History Aspects and the Relevance of Avian Chlamydiosis in Brazilian Cage Birds* provides past aspects of avian chlamydiosis, on how since the beginning, i.e. the first case description, it has been considered a public health threat. Moreover, discuss the relevance of the disease in Brazilian pet birds, and why we do keep seeing the same failures in disease detection and prevention.

Chapter 3, *Epidemiological Surveillance of Psittacosis: the Usefulness of the Multidisciplinary Approach*, describe a thoroughgoing epidemiological study on human psittacosis at an Infectology Reference Center in the city of São Paulo. In Brazil, authors have performed surveys in occupational settings as well as investigating important psittacosis outbreaks (RASO et al., 2010; RASO et al., 2014); nevertheless, none studies have so far addressed the occurrence of the prevalence of type-specific antibodies (IgA, IgM and IgG) against *Chlamydia psittaci* in a selected hospital population. Furthermore, upon intensive investigation of each psittacosis case, attempts to reach the bird that potentially act as the source of infection was carried out. This is a pioneering work accomplished by veterinarians and physicians conjointly, and highlights the importance of multidisciplinary teams working on zoonotic diseases that can actually lead to the implementation of daily actions and strategies that bring the nexus of human and animal health into better focus to ensure positive and real health impact. This study will be submitted to a Public Health scientific journal with the following co-authors; Dr. Marcos Vinícius da Silva, Sátiro Márcio and Dr. Tânia Freitas Raso.

Chapters 4, *Intersectoral Action for Health: preventing psittacosis spread after one reported case* and Chapter 5, *Psittacosis associated with Pet Bird Ownership: a Concern for Public Health*, accurately describe two case reports scrupulously selected from the epidemiological study described in Chapter 3. Each of the reports attempts to outline different hidden scenarios behind psittacosis cases; to be precise: lack of sanitary practices in pet birds

breeding and trade, the role of pet shops as a connection point for zoonotic disease, unrestricted use of antibiotics by bird owners and very limited public awareness about the risks of contracting zoonotic diseases from pet birds. On the other hand, the difficulties related to human psittacosis diagnosis and surveillance. The lack of consciousness about this zoonosis by physicians, the struggle in acquiring paired serum sample for proper diagnosis and the lack of feedback by health professionals regarding the patient's bird contact after the patient's successful diagnosis and treatment. Chapter 4 is going to be submitted to an Infectious Diseases scientific journal and published with the following co-authors, Dr. Marcos Vinícius da Silva, Dr. Bill Randerson Bassetti, Dr. Alessandra Pellini and Dr. Tânia Freitas Raso. In turn, chapter 5 is already published as follows: Ferreira, V.L.; Silva, M.V.; Nascimento, R.D.; Raso, T.F. Psittacosis associated with pet bird ownership: a concern for public health. *Journal of Medical Microbiology Case Reports* (DOI 10.1099/jmmcr.0.000085).

Lastly Chapter 6 brings final considerations on the studies presented in this thesis and further thoughts of research perspectives concerning human psittacosis and avian chlamydiosis in Brazil.

## 6 FINALS CONSIDERATIONS

In Brazil, in the last decade, considerable evidence indicating that *Chlamydia psittaci* is endemic among birds has been obtained (RASO, 2014). However, zoonotic transmissions as well as the impact of *C.psittaci* infections on human health needs to be better understood. The fact that the overall number of reported cases remained generally low is, to some extent, due to the absence of this pathogen in most routine diagnostic schemes. The symptoms in affected individuals are mainly non-specific and influenza-like, but severe pneumonia are not uncommon (KNITTLER et al. 2014). The present research demonstrates human psittacosis occurrence of 27% (10/37) in patients with previous contact with birds. According to Spoorenberg et al. (2016), doctors should be aware of the possibility of psittacosis in any case of community-acquired pneumonia.

In addition, efforts to control avian chlamydiosis should be enhanced by veterinarians and public health authorities. In the current research, psittacosis patients reported having contact with birds in which *C. psittaci* was laboratorial confirmed. In previous studies, Santos et al. (2014) found a 17% *C.psittaci* infection prevalence in birds from pet shops and Raso et al. (2011) 90% prevalence in cockatiels intended to the pet commerce. This provides an opportunity to anticipate disease in the human population and prevent its appearance by implementing appropriate control measures.

Unquestionably, surveillance is of little use if not shared with other groups or individuals who can act on the information to prevent or diagnose disease. In this context, veterinary-human medical partnerships can expand the horizons of medical care (RABINOWITZ; CONTI; 2010). Yet professional interaction of this type is often limited, it is possible, brings many benefits and should be encourage. As described in these thesis chapters, a multidisciplinary team composed by veterinarian and physician allowed the first epidemiological study on human psittacosis in a hospital setting in which the source of infection was also investigated.

Hopefully in the future researches, diagnosticians, laboratory scientists and practitioners will take a more holistic view of health; and change the many overlaps between human and animal medicine and health.

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