Chlamydia Psittaci, Causative Agent of Avian Chlamydiosis and Human Psittacosis: Risk Assessment and Biosafety Recommendations for Laboratory Use

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Abstract

Human psittacosis is a zoonotic infectious disease, which is caused by the obligate intracellular bacterium Chlamydia psittaci. Transmission of the disease usually originates from close contact with infected birds, most frequently in the context of the poultry industry, and from contact with Psittaciformes (cockatoos, parrots, parakeets, and lories). The zoonotic nature of the bacterium makes it a threat to people in close contact with birds, such as veterinarians, farmers, employees of abattoirs, taxidermists, and pet (shop)keepers, but also to laboratory workers. The current article presents guidelines for appropriate laboratory containment of C. psittaci to prevent zoonotic transmission to humans in a laboratory environment.

Keywords
Biosafety, Chlamydia, Chlamydophila, psittaci, risk assessment

Introduction

Chlamydia (C.) psittaci is an obligate intracellular Gram-negative bacterium causing respiratory disease in birds. The bacterium is widespread. Its presence has been proven in about 475 bird species all over the world (Kaleta & Taday, 2003). The infection is endemic in many wild bird species, but also in commercial poultry such as chickens, turkeys, ducks, and geese (Newman et al., 1992; Verminnen et al., 2008). The zoonotic nature of the bacterium makes it a threat to people in close contact with birds, such as veterinarians, farmers, employees of abattoirs, taxidermists, and pet (shop)keepers, but also to laboratory workers examining C. psittaci. Because C. psittaci is transmitted through aerosols, short contact with a bird or its excrements can be sufficient to cause a zoonotic infection. This article presents guidelines for appropriate laboratory containment of C. psittaci to prevent zoonotic transmission to humans in a laboratory environment.

Avian Chlamydioidis

Currently there are nine different C. psittaci genotypes: seven avian (A to F and E/B) and two mammalian (WC, M56) strains, which differ from the avian strain (Everett et al., 1999; Geens et al., 2005). The avian genotypes are relatively host-specific. All genotypes should be considered to be readily transmissible to humans (Beeckman & Vanrompay, 2009; Hedema et al., 2006b).

C. psittaci is excreted both via the faeces and through nasal discharge. Shedding occurs intermittently and can be activated by stress. The excretion period is influenced by the strain virulence, infectious dose, and immune status, but shedding may continue for several months (Harkinezhad et al., 2009). Vertical transmission has been demonstrated in turkeys, chickens, ducks, parakeets, seagulls, and snow geese (Lublin et al., 1996; Wittenbrink et al., 1993). The incubation period depends on the infectious dose, virulence of the bacterium, and host species but is normally about 5 days. It can extend to several weeks, months, or even years (Gerlach, 1999; Grimes, 1994). The infection can be acute, subacute, chronic, or subclinical. Depending on the chlamydial strain and the avian host, symptoms may include ocular and nasal discharge, coughing, sneezing, dyspnoea, conjunctivitis, sinusitis, pneumonia, watery droppings, fever, anorexia, lethargy, diarrhoea, loss of body weight, and lowered egg production (Grimes & Wyrick, 1991; Longbottom & Coulter, 2003; Vanrompay et al., 1995).

Different diagnostic methods used for detection of a C. psittaci infection and their advantages and disadvantages are reviewed by Harkinezhad (2008, 2009). Briefly, diagnosis of avian chlamydiosis is made by isolation and identification of the agent. Real-time PCR and micro arrays are replacing isolation in a number of laboratories because of the improved sensitivity of recently developed tests and to increase laboratory safety. Other diagnostic methods, such as enzyme-linked immunosorbent assay for detection of the antigen, and direct visualization of the organism by the Giménez staining or by fluorescent antibody tests, are satisfactory when signs of the disease are present. Despite the controversy surrounding antibiotic treatment of animals for human consumption, antibiotic treatment is currently the only way to deal with C. psittaci infections (Van Droogenbroeck, 2010). Inappropriate, excessive, or prophylactic use of antibiotics may cause pathogens to become resistant to antibiotic treatment, resulting not only in huge losses for the animal breeders, but also in incurable human infection.
Therefore, prophylaxis becomes increasingly important. Since \textit{C. psittaci} can survive for up to 30 days in faeces and bedding materials, regular cleaning and disinfection of equipment and cages of infected birds is very important (Longbottom & Coulter, 2003; Smith et al., 2005). Infection could also be prevented with an efficient vaccine. However, no vaccines against avian chlamydioidosis are currently available. Another alternative preventive measure has been examined, which is especially suitable for mass application in poultry, namely the use of the natural antimicrobial protein ovotransferrin (OvoTF). OvoTF is an avian extracellular, iron-binding glycoprotein belonging to the family of the transferrins. The anti-\textit{C. psittaci} effect of OvoTF has been demonstrated in vitro and in vivo in specific pathogen free (SPF) turkeys (Beeckman et al., 2007; Van Droogenbroeck et al., 2008) and on a turkey broiler farm (Van Droogenbroeck, 2010).

To prevent the introduction of new strains of certain pathogens, a European Union (EU) legislation to regulate the importation of birds was established. Commission Decisions 2000/666/EC and 2005/760/EC (CEC, 2000) are primarily concerned with preventing the introduction of Newcastle disease or avian influenza. \textit{C. psittaci} is mentioned only once: “If during quarantine it is suspected or confirmed that \textit{Psittaciformes} are infected with \textit{C. psittaci}, all birds of the consignment must be treated by means of a method approved by the competent authority and the quarantine must be prolonged for at least 2 months following the last recorded case.” These requirements apply only to \textit{Psittaciformes} and not to any other avian species. A new commission regulation, EC no. 318/2007 published in the Official Journal of the EU, came into effect in 2007. An important part of this new regulation about the prevention of \textit{C. psittaci} is that it enumerates quarantine conditions. Although these regulations are essential for the reduction of \textit{C. psittaci} infections, it is unfortunate that they do not apply to many of the important birds (e.g., poultry and racing pigeons). However, intra-community trade of poultry and hatching eggs is regulated by council directive 2009/158/EG, stating that the animals need to be healthy. If there is a symptomatic \textit{C. psittaci} infection, no intra-community trade is possible. Moreover, avian chlamydiosis is on the list of the World Organisation for Animal Health (OIE). Notification of the Belgian Royal Decree (RD) of April 25, 1988, listing the animal diseases for which chapter III of the animal health law of March 24, 1987 applies. The Belgian RD of September 20, 1983 lays down the notification of all diseases that are on the list of the Belgian RD of 25/04/1988 (www.ejustice.just.fgov.be).

**Psittacosis in Humans**

Zoonotic transmission of \textit{C. psittaci} most often occurs through inhalation of aerosols from respiratory and eye secretions or dried faeces from diseased animals or asymptomatic carriers. Other sources of infection are handling the plumage and tissues of infected birds, a bite from an infected bird, and in rare cases mouth-to-beak contact (Beeckman & Vanrompay, 2009; Smith et al., 2005). Even a short exposure to birds or bird wastes, as well as seemingly unrelated activities like gardening or lawn-mowing without a grass catcher, can result in infection (Telfer et al., 2005; Williams et al., 1998). Person-to-person transmission is possible but is considered rare (Hughes et al., 1997; Ito et al., 2002; Williams et al., 1998). \textit{C. psittaci} should be handled carefully under conditions of biocontainment. The severity of the disease varies from unapparent, to a mild non-specific illness, to a systemic illness with severe pneumonia that may even result in mortality. Several severe psittacosis (pneumonia) cases were recently documented (Chorazy et al., 2006; Haas et al., 2006; Pandeli & Ernest, 2006; Strambu et al., 2006). However, “severe” psittacosis cases are probably only the tip of the iceberg. The less severe infections are often misdiagnosed due to symptoms similar to those of other respiratory pathogens (Dickx et al., 2010a; Dickx et al., 2010b; Dickx & Vanrompay, 2011; Harkinezhad et al., 2005; Vanrompay et al., 2007). A review from a clinical perspective has been published by Beeckman et al. (2009). An adequate human case definition has been given by the Centers for Disease Control and Prevention (CDC, USA) (Smith et al., 2010).

The widespread nature of \textit{C. psittaci}, combined with the transmission by aerosol, makes it a common occupational illness. People in close contact with birds, such as veterinarians, poultry farmers, abattoir workers, pet shop employees, and taxidermists are at great risk (Andrews et al., 1981; Dickx et al., 2010b; Fenga et al., 2007; Gaede et al., 2008; Harkinezhad et al., 2009; Heddena et al., 2006a; Saito et al., 2005). One category is often forgotten in this respect—the laboratory workers. Avian \textit{C. psittaci} strains are classified as risk group 3 organisms, while the non-avian \textit{C. psittaci} strains are classified as risk group 2 organisms (Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 [Official Journal of the European Communities, 17.10.2000, L 262/21-45]). \textit{C. psittaci} is a category B bioterrorism agent for the U.S. and [www.bt.cdc.gov/agent/agentlist-category.asp](http://www.bt.cdc.gov/agent/agentlist-category.asp) for the EU. All infections acquired through laboratory or laboratory-related activities are considered laboratory-acquired infections (LAIs), whether they are symptomatic or asymptomatic. Activities in product installations and animal facilities are considered laboratory-related activities. The sources of the LAIs range from tissues, faeces, nasal secretions, and blood of infected birds to blood, sputum, and tissues of infected humans as person-to-person transmission of psittacosis is possible (Hughes et al., 1997; Ito et al., 2002). For \textit{C. psittaci} the main infection route is probably inhalation of infected aerosols. While LAIs mainly affect the laboratory workers themselves, there is also the risk of transmission to family members, friends, and other citizens, and LAIs may thus become a public health concern.
LAIs in general and C. psittaci in particular are severely underreported for several reasons. An important reason for not reporting a LAI is the fear of reprisal and the stigma associated with such events. Also, when the pathogen is widespread, it may be hard to prove that the infection is indeed a LAI and did not result from avian contact outside the work environment. Since the symptoms can be very mild to absent, the infection may not be recognized as resulting from C. psittaci, so an LAI is not even considered.

From 1930 until 1978, 116 cases of C. psittaci were reported with 10 deaths (Harding & Byers, 2000). However, only one of the fatal cases occurred after 1949 (Pike, 1979), probably because of the effectiveness of tetracycline, introduced on the market in 1948 (Rawal & Rawal, 2001). The reported fatal cases occurred in France, Germany, England, Argentina, and the United States (reviewed in [Pike, 1979]). A relatively small number of laboratories worked with this agent, so the LAI rate was high (Sulkin, 1961). Despite insufficient reporting of LAIs, C. psittaci was in the top 10 of LAIs, with one of the highest case facility rates of all infectious agents. Between 1979 and 1999, C. psittaci disappeared from the top 10 reported LAIs (Harding & Byers, 2000). Most infections happened before 1955, but sporadic cases of psittacosis LAIs have been reported ever since (Sewell, 1995; Van Droogenbroeck et al., 2009).

**Appropriate Laboratory Containment of C. psittaci**

**Definition**

All manipulations with the biological agent have to be performed in containment facilities using containment equipment, implying the “contained use” of the pathogenic organism. “Contained use” is generally defined as any activity in which microorganisms are genetically modified or in which genetically modified microorganisms are cultured, stored, transported, destroyed, disposed of, or used in any other way, and for which specific containment measures are used to limit their contact with and to provide a high level of safety for the general population and the environment (Directive 2009/41/EC, Official Journal of the European Union, 2009). This refers to all activities conducted in laboratories, animal facilities, green houses, large-scale production facilities, and hospital rooms.

**Regulatory Framework**

In Belgium, the contained use of genetically modified microorganisms (GMMs) or organisms (GMOs) and/or pathogens such as C. psittaci is regulated at the regional level. The legislation is based on the implementation of European Directive 2009/41/EC which repeals Directive 90/219/EEC and its subsequent amendments Directive 94/51/EC, Directive 98/81/EC, and Decision 2001/204/EC (Official Journal of the European Communities, 2009). Interestingly, only genetically modified microorganisms are covered by the European regulatory framework. In Belgium, however, the Belgian regional legislations have been extended and also cover genetically modified organisms and pathogenic organisms for humans, animals, and plants.

Furthermore, a Federal legislation applies to people that come into contact with pathogenic organisms (genetically modified or not) due to their work. The current Belgian legislation on this topic is the Royal Decree of April 29, 1999 as an amendment of the Royal Decree of August 4, 1996 on the protection of employees against the risks of exposure to biological agents at work. This Decree implements several European Directives of which Directive 2000/54/EC was most recently implemented (Official Journal of the European Communities, 2000).

All contained-use activities require the submission of a biosafety dossier to the technical expert (in Belgium: the Division of Biosafety and Biotechnology [SBB]). Afterwards, an authorization for the notified activity is provided by the relevant competent authorities.

**Biological Risk Assessment of Contained Use Activities Using C. psittaci**

When performing a biological risk assessment of an activity that uses C. psittaci, the goal is to organize and analyze scientific information in order to estimate the probability and severity of an adverse effect. By doing so, appropriate measures can be implemented to provide maximum protection to human health and the environment.

For a C. psittaci risk assessment, first the risk group of this pathogen has to be considered. The main characteristics inherent to a hazardous agent are its capability to be infectious and cause disease in susceptible hosts, its virulence, and the availability of preventive measures and effective prophylaxis or treatments (Zaki, 2010). Based on these properties and the natural route of transmission, pathogens can be generally classified into four risk groups (Official Journal of the European Communities, 2000). These risk groups are correlated with biosafety levels, but they do not necessarily equate to them (Zaki, 2010).

According to the risk classification criteria of the World Health Organization (WHO, 2004), Australia (Standard AS/NZS 2243.3:2002), Canada (data available online at www.phac-aspc.gc.ca/publicat/lbd-lmdb1-04/index.html), the European Union (Directive 2000/54/EC), and the USA Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) (data available online at www.cdc.gov/od/ohs/biosafety/bmb14/bmb14toc.htm), avian C. psittaci strains belong to risk group 3 for humans and animals (in this specific case, birds) as they meet the standards for risk group 3: The bacterium can cause severe human disease and present a serious hazard; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available.

According to the guidelines mentioned in the former paragraph, human or animal pathogens classified as risk group 3 microorganisms are capable of causing serious
illness, and airborne transmission of the pathogens can occur. The fact that *C. psittaci* is mainly transmitted by the airborne route has influenced its classification into this risk group.

A second item that is highly important for a risk assessment is the type of activity that is being performed, taking into account the risks of exposure to potential biological hazards. These hazards apply to the possible generation of infectious aerosols, the scale of the activity, the concentrations and volumes (e.g., cultures, supernatant, etc.) being manipulated, and the type of operation (e.g., *in vitro*, laboratory animal experiments, etc.).

As *C. psittaci* is transmitted mainly by aerosols, the generation of infectious aerosols must be strongly minimized. Manipulations that may create infectious aerosols include:

- Centrifugation: Media or solutions that contain infectious *C. psittaci* bacteria may leak from the centrifuge tubes or the tubes may even break during centrifugation or manipulation, thereby releasing infectious aerosols; also opening the tubes after centrifugation may create aerosols.
- Pipetting or the use of syringes: Common pipette tips, Pasteur pipettes, and syringes may create bubbles that form aerosols when they burst.
- Vortexing or shaking (e.g., of swabs).
- Sonication.
- Production of cryostat tissue sections: When cryosections are made from frozen tissue samples, contaminated ice and tissue debris may also generate infectious aerosols.

Handling and necropsy of infected birds also present a risk for human health. Strict handling procedures and collective (proper biosafety cabinet class II) and personal protective measures (gloves, P3 full face mask that filters at least 99.95% of airborne particles) are required to reduce the risk (Deschuyffeleer et al., 2012).

Performing large-scale activities and manipulating samples with a high bacterial load or large volume increase the risk of exposure to the pathogenic bacteria.

The type of operation also has to be considered. When conducting laboratory animal experiments such as turkey vaccination trials, turkeys become experimentally infected by aerosol with the *C. psittaci* bacteria. A major risk during these experiments is the possibility of being exposed to the created aerosol and also later on, when the animals are shedding the bacteria.

Taking into account all of these aspects, a risk group can be assigned to the *C. psittaci* contained use activity and appropriate measures need to be implemented according to the recommended containment level.

**Biosafety Recommendations for Laboratory Containment of C. psittaci**

According to international legislation, the contained use of pathogens such as *C. psittaci* requires a specific containment level. In the regional decrees, different levels of containment are described, each covering specific facility design and technical requirements, safety equipment, working practices, and waste management. Based on the risk assessment mentioned above and taking into account these containment criteria, the following measures are recommended for the contained use of *C. psittaci*.

**Contained Use of C. psittaci in Laboratories**—All laboratory manipulations with avian *C. psittaci* strains require a biosafety level 3 (BSL-3). Generally, a biosafety level 3 is appropriate for agents with a known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections, and those that are indigenous or exotic in origin (U.S. Department of Health and Human Services, 2009). For *C. psittaci* bacteria, more attention is given to primary and secondary barriers to protect laboratory personnel handling the agent, the general population, and the environment from exposure to infectious *C. psittaci* aerosols.

Bacterial cultures and swabs, tissues, or other samples that might contain *C. psittaci* bacteria should be opened and manipulated only in a properly certified class II biosafety cabinet (BSC). This type of BSC not only provides personnel and environmental protection, but also protects the product itself. Only well-trained laboratory employees wearing the appropriate personal protection equipment are allowed to work with these samples. As secondary barriers, a negative pressure differential between the lab and the adjacent area and a restricted access to the facility help to maximize the protection level.

**Contained Use of C. psittaci in Animal Facilities**—A biosafety level 3 in animal facilities (ABSL-3) is recommended when performing animal experiments with birds naturally or experimentally infected by *C. psittaci* (U.S. Department of Health and Human Services, 2009). The birds need to be kept in negative pressure isolators to prevent *C. psittaci* transmission to humans. As for biosafety level 3 laboratories, access is restricted (badge-controlled) to authorized and well-trained personnel. Before entering, personal protective measures (Deschuyffeleer et al., 2012) such as putting on a protective suite, overshoes, and gloves, must be implemented. This can be done in the entryway between the external and internal entries. Afterwards, protective suits, overshoes, and gloves are placed in a medical waste bucket before leaving the entryway of the ABSL-3.

Isolators that house *C. psittaci*-infected birds need to operate under negative pressure to protect the employees handling the animals. HEPA filters need to be present to filter the incoming and outgoing air. Special gloves are attached to the isolator’s glass side and need to be sufficiently strong to avoid being torn by the pecking birds. A decontamination reservoir with chlorinated water (3 g/l chloramine-T) is connected to the isolator’s ground surface and is used to decontaminate (10 minutes) any materials (bottles filled with autoclaved drinking water for SPF birds, sampling equipment such as swabs, Eppendorf tubes, etc.) going in or going out of the isolator. Isolator types such as the IM 1500 (Montair Sevenum, the Netherlands) have been shown suitable for these kinds of experiments (Van Droogenbroeck et al., 2008; Van Loock et al., 2004).
**C. psittaci Disinfection and Waste Management**—Because *C. psittaci* has a high lipid content, it is susceptible to most disinfectants and detergents as well as heat. However, this pathogen is resistant to acid and alkali. A 1:1000 dilution of quaternary ammonium compounds, 70% isopro­ pyl alcohol, 1% lysol, 1:100 dilution of household bleach, and chlorophenols are effective (www.cfsph.iastate.edu). Many disinfectants are respiratory irritants and they should be used only in a well-ventilated area (NASPHV, 2010).

All work surfaces should be thoroughly cleaned and disinfected when manipulating infectious material and also immediately after each accidental spill. Lab coats should be autoclaved before leaving the laboratory. Contaminated materials should be autoclaved or otherwise inactivated before removal from the containment facility. Disposables used frequently, such as plastic pipettes, pipet tips, tissue culture flasks, and *Chlamydia* containers with a coverslip, need to be inactivated and afterwards discarded in solid, leak-proof, hermetically sealable containers for hazardous waste. The waste containers are incinerated by a certified company. Contaminated liquid waste produced while work­ing in the BSC can first be collected in a plastic waste bot­tle (containing a disinfectant) and should thereafter also be deposited in the containers for hazardous waste. The containers should be sufficiently labelled and marked with a biohazard sign. Needles and sharp materials should be col­lected in the appropriate containers for sharp waste. Once full, they need to be deposited in the solid, leak-proof, and hermetically sealable containers for hazardous waste.

It is not obligatory to use a class III BSC, which is connected to an autoclave. Thus, when using a class II BSC for necropsies, it is also safe to place the carcasses in two sealed plastic bags and to store them immediately in her­metically sealed (locked), solid, leak-proof containers that are going directly to the incinerator. Negative pressure iso­lators (BSL-3) for experimental infections in animals must be cleaned after the experiment using a 1:1000 dilution of quaternary ammonium compounds. However, before clean­ing (opening), isolators need to be decontaminated by for­maldehyde gas for at least 24 hours. During the sterilization process, the air inlet and outlet ventilation ports as well as the waste disposal port need to be closed by slide valves. Litter is placed in sealed plastic bags and removed using the decontamination compartment (vapor disinfection of litter bags) or the decontamination dip tank (disinfection of litter bags by liquid disinfectant), depending on the design of the isolator. Bags with litter are placed in solid, leak­proof, and hermetically sealable containers for infectious waste, which are incinerated. Besides the use of formalde­hyde, hydrogen peroxide, or peracetic acid vapour could be a suitable alternative.

**Conclusion**

The aerogenic transmission of the zoonotic bacterium *C. psittaci* contributes to the high risk of LAI infections due to working with *C. psittaci* or infected animals. Since hu­man infection may result in serious illness, prevention is key. It is therefore essential that the requirements for con­tained use are met at all levels, from the design of the facility to safety equipment and waste management and that all laboratory and animal facility working practices are fol­lowed accurately. This article presented an overview of the biosafety recommendations for the contained use of *C. psittaci* in the regulatory framework.

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**References**


Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook
(2nd Edition)

Bad Bug Book, published by the Center for Food Safety and Applied Nutrition, of the Food and Drug Administration (FDA), U.S. Department of Health and Human Services, provides current information about the major known agents that cause foodborne illness. The information provided in this handbook is abbreviated and general in nature, and is intended for practical use. It is not intended to be a comprehensive scientific or clinical reference. Each chapter in this book is about a pathogen: a bacterium, virus, or parasite or a natural toxin that can contaminate food and cause illness. The book contains scientific and technical information about the major pathogens that cause these kinds of illnesses. A separate consumer box in each chapter provides non-technical information, in everyday language. The boxes describe plainly what can make you sick and, more important, how to prevent it. Available at: www.fda.gov/food/foodsafety/foodborneillness/foodborneillness