

Relatório Final de Estágio

Mestrado Integrado em Medicina Veterinária

***Chlamydophila psittaci* in a captive flock of budgerigars
(*Melopsittacus undulatus*) at an inner-city zoo in France – Case report
and review of recent literature concerning common diseases of small
and medium sized psittacine birds in zoo collections.**

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Orientador:

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Co-Orientadores:

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Abstract

An epizootic of avian Chlamydophilosis occurred at an inner-city zoo in France, affecting a flock of budgerigars (*Melopsittacus undulatus*) resulting in 51 deaths in nine months reducing the adult population down to 44% of the initial flock size. Budgerigars from every age and both sexes exhibited clinical signs of depression, cachexia, dyspnoea, sinusitis and diarrhoea. In most of the cases death was sudden with no previous manifestation of disease. Gross pathologic findings were splenomegaly, hepatomegaly and yellow “plaques” in the abdominal and thoracic air-sacs. *Chlamydophila psittaci* infection was diagnosed by real time PCR analysis and immunochromatography assay. Free-ranging domestic pigeons (*Columba livia domestica*) were presumed to be the main source of the infection. Low temperature, high humidity, temporary overcrowding and concomitant parasitic infections were thought to be the stressors compromising the flock’s immunity. It is the author’s intention to provide a brief review on recent literature regarding common diseases affecting small and medium sized psittacines in zoological collections focusing on practical aspects such as diagnosis, treatment protocols and preventive measures in captive populations. A higher emphasis will be given to the pathogenesis of *Chlamydophila psittaci* since it was the pathogenic agent mainly highlighted in the group studied by the author.

Key words.

Outbreak; *Chlamydophila psittaci*; Chlamydophilosis; Budgerigars; Psittacines; Avian Diseases;

Resumo

Em França, num zoo no centro de uma cidade, ocorreu um surto de Clamidiofilose Aviária que afectou um bando de periquitos australianos (*Melopsittacus undulatus*) resultando na morte de 51 indivíduos em nove meses reduzindo a população adulta para 44% do número inicial. Periquitos de todas as idades e de ambos os sexos exibiram sinais clínicos de depressão, caquexia, dispneia, sinusite e diarreia. Em muitos dos casos a morte foi repentina sem manifestações prévias da doença. Os principais achados patológicos foram esplenomegalia, hepatomegalia e “placas” amarelas nos sacos aéreos abdominais e torácicos. A infecção por *Chlamydophila psittaci* foi diagnosticada através de PCR em tempo real e teste de imunocromatografia. A suspeita do principal foco de infecção recaiu sobre os pombos selvagens (*Columba livia domestica*). As temperaturas baixas, a elevada humidade e a sobrepopulação temporária com concomitante infecção parasitária terão sido os factores predisponentes que comprometeram a imunidade do bando. É intenção do autor fazer uma breve revisão da literatura recente sobre doenças que mais comumente afectam psitacídeos de pequeno e médio porte em colecções zoológicas. Focando aspectos práticos, tais como diagnóstico, protocolos de tratamento e medidas de prevenção em populações em cativeiro. Maior ênfase será dada à patogénese da *Chlamydophila psittaci* uma vez que foi o agente patogénico que mais destaque teve no grupo estudado pelo autor.

Palavras chave.

Surto; *Chlamydophila psittaci*; Clamidiofilose; Periquitos-Australianos; Psitacídeos; Patologias de Aves;

Résumé

Une épizootie de Chlamydophilose aviaire a touché un parc urbain en France, affectant un groupe de perruches ondulées (*Melopsittacus undulatus*) aboutissant à la mort de 51 animaux sur neuf mois, réduisant la population adulte de 44%. Les perruches ondulées de tous âges et de tout sexe ont présenté des signes de dépression, de cachexie, de dyspnée, des sinusites et de la diarrhée. Dans la plupart des cas la mort fût soudaine, sans signes avant coureurs laissant suspecter la maladie. L'examen macroscopique lors des autopsies a montré des splénomégalias, des hépatomégalias ainsi que des « plaques » jaunes au niveau des sacs aériens thoraciques et abdominaux. L'infection à *Chlamydophila psittaci* a été diagnostiquée par analyse PCR en temps réel et immunochromatographie. Les pigeons sauvages (*Columba livia domestica*) sont probablement la principale source de contamination. Les températures basses, l'humidité importante, la surpopulation dans l'enclos ainsi que les infections parasitaires concomitantes sont autant de facteurs de stress qui compromettent l'immunité du groupe. L'intention de l'auteur est de fournir un bref résumé des récentes publications à propos des maladies communes touchant les petits et moyens psittacidae en parc zoologique. Ceci en s'attardant sur les aspects pratiques de tels diagnostics, les protocoles de traitements ainsi que les mesures de préventions pouvant être mises en place pour les populations captives. Une attention particulière sera portée à la pathogénie de *Chlamydophila psittaci* puisque c'est le pathogène principalement mis en évidence dans le group eétudié par l'auteur.

Mots clefs

Chlamydophila psittaci; Chlamydophilose; Perruche ondulée; *Psittacidae*; Maladie aviaire;

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***I dedicate this adventure to my mother who, with only 1.6m of sweetness,
is the strongest, courageous and most intelligent woman I know.***

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Abbreviation List

ARKS - Animal Record Keeping System	
AZT - Azithromycin	
CITES - Convention on International Trade in Endangered Species	
CNS - Central Nervous System	
CTC - Chlorotetracycline	
DGAV - Direcção Geral de Alimentação e Veterinária	
DIE - World Organisation for Animal Health	
DNA - Deoxyribonucleotide Acid	
DXC - Doxycycline	
e.g. - <i>Exempli gratia</i>	
EAZA - European Association of Zoos and Aquaria	
EEC - European Economic Community	
EEP - European Endangered Species Programme	
ELISA - Enzyme Linked Immunosorbent Assay	
ENR - Enrofloxacin	
FMUP - <i>Faculdade de Medicina da Universidade do Porto</i>	
HA - Hemagglutination	
HI - Hemagglutination inhibition	
HRM - High Resolution Melt	
I.U. - International Units	
IChr - Immunochromatography	
ID - Immunodiffusion	
IF - Immunofluorescence	
IgG - Immunoglobulin G	
IgM - Immunoglobulin M	
IUCN - International Union for Conservation of Nature	
LCL - Levinthal-Cole-Lillie	
LPS - Lipopolysaccharides	
MJP - <i>Ménagerie du Jardin des Plantes</i>	
m - Meter	
m² - Square meter	
m³ - Cubic meter	
mg - Miligram	
mg/Kg - Miligram per Kilogram	PO - <i>Per oral</i>
OCT - Oxytetracycline	SID - Once per day
OIE - World Organisation for Animal Health	µg/ml - Microgram per mililiter
PBFD - Psittacine Beak and Feather Disease	°C - degree Celsius
PCR - Polymerase Chain Reaction	% - Percentage
PFDV - Beak and Feather Disease Virus	
rMOMP - Recombinant Major Outer Membrane Protein	
rt-PCR - Real Time Polymerase Chain Reaction	

1. Introduction

The first part of the author's final practices was at the *Ménagerie du Jardin des Plantes* (MJP), Paris, France from mid-February until mid-May of the present year. The MJP presently directed by Monsieur Michel Saint-Jalme is a small sized urban zoo classified as an historical monument since 1993. It was founded in 1793 after the French Revolution being the second oldest zoological park in the world. The MJP is located by the Seine in the centre of Paris and it belongs to the French National Museum of Natural History, before the Royal Medicinal Plant Garden created by King Louis XIII, therefore having a privileged location being surrounded by all sources of scientific knowledge and history. Nowadays the MJP does not have large animals on its collection like elephants, giraffes, lions, gorillas, etc. because it is impossible to properly maintain these animals. Efforts are being made to house smaller and medium sized species of mammals, birds, reptiles and amphibians in spacious and environmentally improved enclosures (landscaped and vegetated the closest possible to their natural habitats). The MJP adheres to the European Association of Zoos and Aquaria (EAZA) and to their breeding programs. In this institution the author had the opportunity to follow the work of three veterinarians (Norin Chai, Muriel Kohl and Aude Bourgeois) participating actively in most of the procedures. In the Table I - a, - b, - c, Appendix I are listed all the procedures assisted by the author during this period. Not listed in this tables is the laboratory component of the work, which was also an important part of the author's study time. The author was able to perform coprocological and cytological analysis and collaborating with conservationists and scientific projects while creating the zoo's cryobank. The author also participated in the 2013 Conference of the *Association Francophone des Vétérinaires de Parcs Zoologiques* and in workshops organized by the non-profit organization "Yaboumba", which emphasises continued medical training and communications on clinical and research work done on wild and exotic species. Whilst working with avian experts at the MJP the author gained experience in artificial breeding of numerous avian species. Avian endoscopy was performed regularly at the institution and the author was able to gain technical experience in this field. At the MJP, animal trainers perform medical training on Bornean Orang-Outan (*Pongo pygmaeus pygmaeus*) and by following their daily routine the author was able to understand the fundamentals of enrichment and training.

The second part of the author's final practices, with the duration of three weeks, took place at Oporto, Portugal where the author could follow the work of the veterinarian Dr.^a Maria Luísa Guardão in two different institutions, the animal facility of the *Faculdade de Medicina da Universidade do Porto* (FMUP) and at the veterinary clinic UPVet in the author's university.

The new animal facility of the FMUP, where animal welfare is the leitmotiv, is directed by Dr.^a Maria Luísa Guardão and was founded in January 2012 housing different animal species such as mice, rats, guinea pigs, rabbits and pigs. In 2012 approximately 5000 animals passed through the doors of this facility. This facility is very well organized being divided in a conventional area and a specific pathogenic free zone where nude, knockout and transgenic mice are housed. During this period the author learned and practiced how to perform complete physical examinations, various animal restraint and drug administration techniques on different species along with the understanding of the veterinary responsibilities in these facilities. The veterinary clinic UPVet was founded in 1998 and presently is directed by the veterinarian Professor Dr. Augusto Matos. This is a small an exotic companion animal's veterinary practice that was created to become a plus asset for the training of veterinary students. The veterinarian in charge of the exotic animal's care is Dr^a. Maria Luísa Guardão. During this period the author was able to perform physical examinations, minor procedures along with the establishment of diagnosis and therapeutic plans in small exotic mammals.

Training in two different settings with exotic pets and captive wildlife is a major asset to the author's career in veterinary medicine, as these thematics are seldom addressed in the basic training schedule.

The main goal of this dissertation is to review and update on current literature regarding common infectious and non-infectious diseases affecting Psittaciformes (Parrots and cockatiel) in zoological collections. Along with the description and discussion of a case report that has been a concern at the MJP during the author's practice period.

1.1. Birds in Zoological Collection

Parrots and Cockatiels are abundant in aviaries (for hobbies or commercial purposes) and zoos, also besides being a reference for larger species of the group, the family of Psittaciformes harbours some of the most commonly represented species in captivity, such as the budgerigar (*Melopsittacus undulatus*), the cockatiel (*Nymphicus hollandicus*) and the African Grey Parrot (*Psittacus erithacus*). The information presented in this dissertation may be of scientific and clinical value to students, clinicians and aviculturists. Clinical medicine of birds in zoos balances between companion bird's and aviary's medicine. There are so many similarities as there are differences involving these three fields of veterinary medicine. Zoo keepers are in daily contact with their birds, creating a close bond and understanding their individual behaviour and personality. They figure as the “alarm” for abnormal behaviours and sick birds as an attentive

owner would do. All birds are kept in enclosures which are environmentally enriched and try to approach the species natural habitat. The main goal is to preserve instinctive behaviours and to be attractive not only to the bird but also to the general public that visits the Zoo. Normally birds, and especially parrots, are housed in flocks in large enclosures making some procedures (e.g. deworming) and diagnostic measures (e.g. considering some diseases as group pathologies) similar to the clinical approach in commercial aviary medicine. In Zoos, the avian patient can be taken to the veterinary clinic for many reasons, such as an emergency (e.g. Trauma, or when it has signs of disease, etc), follow ups, scheduled procedures (e.g. surgical sexing, castration, etc), or *post mortem* examination. In order to have access to the clinical history of the zoo patient, veterinarians use the Animal Record Keeping System (ARKS) where all the detailed information (e.g. date and place of birth, parents' information, movements, clinical procedures, etc) is kept. It is undeniable that zoos have an important role in the conservation of some species and education of the general public. Zoo veterinarians have the responsibility to share their knowledge acquired with the animals kept in captivity (*ex-situ*) with other actors of the 'One World – One Health' dynamics in order to contribute to the conservation of the World's biodiversity. Most of the zoos in Europe work along with EAZA, with the International Union for Conservation of Nature (IUCN) and with the European Endangered Species Programme (EEP) which is an integrative part of the EAZA. Species under EEP are managed by a coordinator assisted by a committee of experts who will give recommendations on reproductive management, group husbandry and needs in research. The overall goal of this management is the maintenance of a healthy genetic pool in captivity with foresight to *in-situ* conservation and reintroduction.

1.2. *Psittaciformes* - Parrots and Cockatoos

Parrot is the alternative common name given to species belonging to the *Psittacidae* Family, *Psittaciformes* Order and *Aves* Class⁽²⁵⁾. Parrots are one of the three families within the order *Psittaciformes*, the other two being the *Cacatuidae* (Cockatoos) and the *Strigopidae*⁽²⁵⁾. The *Psittacidae* Family subdivided in two subfamilies, the *Psittacinae* and the *Lorinae*, comprehends 78 genera and 332 species, 86 of these species are threatened and almost certainly 11 of those species and eight subspecies are extinct since 1600⁽²⁵⁾. Parrots are long-lived birds, particularly the larger species, and are usually characterized as stocky, large-headed birds with powerful, short, curved, articulated bill, highly developed tongue and jaw musculature, and short legs^(25,51). The plumage is exuberantly colourful in many species and their length size can range from eight up to 100 cm⁽²⁵⁾. Cockatoos have many common anatomical aspects with the parrots

like zygodactyl feet, massive hooked bills and medium to longish tail but they differ in the way that many species of cockatoos have a well-developed crest, their average length size ranges from 30 to 65cm and their natural distribution is within Australasia while parrots are dispersed throughout the southern hemisphere⁽²⁵⁾. The *Cacatuidae* Family is also subdivided in three subfamilies such as *Calyptorhynchinae*, *Nymphicinae* and *Cacatuinae*, and has 21 species placed in six genera and seven of these species are considered threatened⁽²⁵⁾. Presently, the *Strigopidae* Family has only four species within the two genera *Nestoridae* and *Strigopidae*, although one of these species is critically endangered (*Strigops habroptila*), two are endangered (*Nestor meridionalis meridionalis* and *Nestor meridionalis septentrionalis*) and one is considered vulnerable (*Nestor notabilis*)⁽⁴⁰⁾. At the MJP there are two Keas (*Nestor notabilis*), parrots with a natural distribution in New Zealand with a very particular management (e.g. they are alpine and omnivorous)⁽⁴⁰⁾. Keas have a long, thin and curved beak, their colour is olive-green and the ventral part of the wings are orange, their average length size is 48 cm⁽⁴⁰⁾. The popularity of parrots and cockatoos as pets is growing, mostly due to their capacity to imitate various sounds and human speech and their high level of intelligence which makes them very attractive to humans as companion animals. In addition to their highly social nature these species are extremely affectionate, bonding closely with people especially with their owners or keepers at a zoo. They are as well valued for their beauty and variety of the plumage colours, their natural ability to perform acrobatic moves, the possibility of choosing a bird in a wide range of sizes and the fact that these birds are easily trained, bred and kept in captivity. The author defines small and medium sized psittacine birds as all animals belonging to the order Psittaciformes, whose average whole body length does not exceed 50 cm. In this essay, emphasis will be given to the budgerigar, a parrot native to Australia^(25, 49). Nowadays there are a vast variety of colour phenotypes. But there are characteristics that are horizontal in all budgerigars, such as dark strips on the back of the wings and back of the head, a long tail, a bluish spot extending backward from near the corner of the mouth, and dark spots surrounding the front part of the neck. There is only little sexual dimorphism in budgerigars, except that the cere is merely blue in males and brownish or pinkish in females, but as colour variations are frequent, definitive sexing should be done using known scientific methods⁽⁴⁹⁾.

1.3. Capture and Restraint

Capturing a bird is often a critical procedure and should be performed with caution since during this process birds can easily injure themselves. The increased muscular activity may lead

to dyspnea, hyperthermia and collapse. Subsequently efforts should be made to carry out a rapid, non-traumatic capture. In the enclosure, birds can be caught using a net and after taken to the clinic in a transportation box or in a small cage, depending on the distance, size and condition of the bird. It is important to be sure that all entrances and exits of the examination room are closed⁽⁴⁹⁾ The primary aim during capture should be to immobilise the head and the beak, and at the same time prevent the wings from flapping. In medium sized birds a finger and thumb are placed around the bird's neck in order to immobilize the mandible as the other hand restrains the legs. A second operator is needed to perform a full clinical examination. One can also use a light towel (care should be taken not to squash small-sized birds with the weight of the towel), this way there's no need for extra help. In small sized birds, the head can be held between the middle and index finger resting the body in the palm of the hand and restraining the hind legs using the thumb of the same hand. The other hand is thus free for performing the clinical examination. Medium sized parrots can be handled the same way as their smaller conspecifics, using a thick leather glove for protection against bite wounds^(49, 51).

1.4. Clinical examination, Sedation and Anaesthesia

Before restraining the animal, its posture, attitude and locomotion should be evaluated. Psittacines are preys in the wild and therefore tend to mask any signs of disease, appearing normal on an initial evaluation. In order for the bird to manifest signs of disease it is left in its environment and the veterinarian can observe from a distance. Respiratory rate and mental status, should be monitored constantly during handling. The physical exam can be performed with or without general anaesthesia or sedation. It is very unlikely that a bird is calm enough enabling the examination without these drugs or without being stressed, unless it is already very depressed or had been submitted to medical training since most of the times the birds in zoo collections never leave their enclosures turning very frightened in strange environments. Several drugs are currently available to safely sedate, immobilize and/or anesthetize birds, but care should be taken to accurately weigh the risks and benefits of sedation or anaesthesia in debilitated animals. It is out of the scope of this work to elongate about the particularities of avian anaesthesia. Dehydration, shock, cardiovascular and respiratory diseases, extreme emaciation or obesity, space occupying lesions of the abdomen reducing air sac capacity, and full crop predisposing to regurgitation and obstruction of the glottis, increase the anaesthetic risk. Impaired hepatic and renal function will decrease or unable the excretion of some anaesthetic drugs. Birds should be accurately weighed prior to administration of any medication and dosages

should be calculated carefully. In very small animals some drugs may need to be diluted before administration and care should be taken to choose compatible diluents, in order to avoid adverse reactions. All parrots must be carefully assessed and a thorough clinical examination (Table II, Appendix II) performed before induction of anaesthesia or sedation. The exam room for birds should have subdued lighting and noise-minimizing equipment to avoid extra stress to the animal. Environmental factors most often factor into the pathogenesis of avian disease. Flock dynamics, husbandry and management should be taken into consideration when assessing an individual bird. Easy diagnostic measures, such as faecal analysis for parasite infestation, digestive flora, food utilization and stress hormone levels can help orientate further, more invasive diagnostics. Final diagnosis of disease in flock medicine is often only achieved after thorough examination of a representative number of individual animals in the group. All results obtained from these examinations are compiled and put into relation with all extrinsic factors influencing the population, in order to achieve accurate diagnosis and treatment plans. Verification of treatment efficacy and formulation of preventive measures are essential for future changes.

2. *Chlamydophila psittaci* in a captive flock of budgerigars (*Melopsittacus undulatus*) at an inner-city Zoo in France

2.1. Case description

Between September 2012 and early-May 2013, 51 captive budgerigars died or were euthanatized due to severe illness or severe lesions caused by cannibalistic behaviours involving other birds of the same flock or free-ranging birds and rats that may have had access to the budgerigar's enclosure. In September 2012 a population of 91 budgerigars (Table III, Appendix III) were sharing an aviary with two Nanday Parakeet (*Nandayus nanday*), two Cockatiels (*Nymphicus hollandicus*), and two Ring-Necked Parakeet (*Psittacula kramuri*), consisting in an outside aviary of about 150 m³ with unlimited access to an inside enclosure of 5 m³. The enclosure of these parrots was located right next to other individual aviaries housing larger sized Psittaciformes. Before this outbreak, the inside enclosure was cleaned daily using a commercial detergent and a chlorine solution for disinfection. All food and water bowls were changed on a daily basis. Nests were cleaned and disinfected with 1% Virkon®, Qaliam, France, once per year. All enclosure entrances and exits were equipped with disinfectant doormats. Symptoms of disease were most severe during winter time, when prolonged periods of temperatures below 0°C

combined with high ambient humidity occurred. The birds were routinely dewormed three to four times per year.

2.2. Clinical findings

Budgerigars presented mostly signs of respiratory (dyspnoea, sinusitis, nasal discharge, partial or total obstruction of the nares, tail bobbing and open mouthed respiration) and gastrointestinal (watery diarrhoea, and greenish or yellowish faeces) disease. Some also presented signs of systemic disease such as emaciation, depression, inability to fly, hypothermia and ruffled feathers. Hyperkeratinization and abnormal coloration of the beak and obstruction of the cloaca with a mix of faeces, urates, food debris and feathers were also found in some of the birds (Figure 1, Appendix III). Mortality was observed in parrots of every age and sex, with many parrots found dead without premonitory symptoms. Apart from the budgerigars and two monk parakeets (*Myopsitta monachus*) found dead at the time of the outbreak with unrelated clinical signs, no other birds were clinically ill.

2.3. Post mortem examination, sample's collection and complementary exams

Complete necropsies were performed only in 23 budgerigars (budgerigars' necropsy findings are in the Table IV, Appendix III and featured in Figure 2, Appendix III) and in two monk parakeets. The main gross lesions in the two monk parakeets, and similar to the ones found in the budgerigars were congested lungs (2/2), green diarrhoea (2/2), cloacal obstruction with faeces, urates, food debris and feathers (1/2), splenomegaly (1/2), congestion of the spleen (1/2) and the kidneys (1/2). Two budgerigars' carcasses were sent to the laboratory (*Laboratoire D'Anatomie Pathologic Veterinaire*, France) for histopathologic examination. The principal histopathologic lesions found in the two carcasses sent for examination, were airsacculitis and hepatitis with inflammatory infiltration mainly by mononuclear cells (lymphoplasmocytes and macrophages). The renal interstitium showed a similar inflammatory infiltration pattern. Splenic histiocytosis was also seen, but there were no signs of viral body inclusions in any organs. Impression smears of spleen and liver of five budgerigars stained with Diff-Quick coloration were performed at the MJP in-house laboratory and confirmed the mononuclear infiltrate. The liver parenchyma showed also heterophilic infiltration and intracytoplasmatic basophilic inclusions. Liver, spleen and kidney samples from one budgerigar along with 22 cloacal swabs (six from budgerigars, two from the Hyacinth Macaw (*Anodorhynchus hyacinthinus*), two from Great Green Macaws (*Ara ambiguous*), two from Military Macaws (*Ara militaris*), eight from

Blue-and-Yellow Macaws (*Ara ararauna*) and two from Keas (*Nestor notabilis*) were sent to the laboratory (Scanelis – Laboratoire d'Analyses Vétérinaires, France) for real time PCR analysis of *Cp. psittaci* infection (Figure 1, Appendix III). It was performed 19 immunochromatography (IChr) assays (Speed® Chlam, Virbac France, France) in budgerigars and monk parakeets off faecal samples, organs (spleen, lungs, liver) samples as well as cloacal swabs. Direct coprology and faecal flotation with Sheather's solution were performed in eight budgerigars and two monk parakeets. Two budgerigars were analyzed for infection with *Mycobacterium avium* (kit Quick-TB®, Réctifs RAL, Martillac, France). Two adult budgerigars were positive for coccidia and the remaining psittacines were all negative for endo-parasitic infection. No animal was found positive to *Mycobacterium avium* infection. There was only one doubtful result, in a budgerigar when performing the IChr assay in a cloacal swab. Cloacal swabs belonging to two budgerigars were tested positive for *Cp. psittacine* infection by rt-PCR. One of them was the same budgerigar that tested positive for *Cp. psittacine* with IChr. All large psittacines were tested negative for this pathogenic agent.

2.4. Treatment and control measures

All budgerigars presented at the clinic showing signs of ongoing disease (depression, dyspnoea and/or watery diarrhoea), were isolated and medicated with a multi vitamin and mineral complex in the drinking water (Vitavia®, Virbac France, France and Bicalphos® 4%, Virbac France, France), a macrolid antibiotic (150000 I.U. erythromycin and 150000 I.U. tetracycline per litre drinking water SID for five days; Erytavicol®, Laboratoire Vétoquinol S.A., France), an inhalation mixture administered three times per day, consisting in a herbal respiratory decongestant (Balsoléene®, Laboratoire Cooper, France), an aminoglycoside (80 mg gentamicin per litre of inhalant; G4®, Virbac France, France) and a mucolytic agent (0,15 mg acetylcystine per 250 ml of inhalant; Mucomyst®, Bristol Myers Squibb, France). Specific treatment against *Aspergillus spp.* with enilconazole (Imaveral®, Janssen Santé Animale, France) and itraconazole (5 mg/Kg SID for a minimum period of 14 days – up to six months; Itrafungol®, Janssen Santé Animale, France) was initiated⁽⁵⁾. A coccidiostatic (17 mg/Kg toltrazuril PO, SID for three days; Baycox 2,5%®, Bayer Santé Animale, France) was used in all juveniles presenting with clinical eimeriosis. All animals were dewormed twice at an interval of 15 days (0,2 mg/Kg ivermectin pour-on; Virbamec® pour-on, Virbac France, France). Despite all efforts made to save the clinically ill animals, most died within two days of treatment. From February 2013 until early-May 2013, six surviving adults were placed in a critical care

incubator with controlled temperature (21°C), the antibiotic was changed to sulphonamides in order to treat a now persistent coccidiosis (320 mg trimethopim/sulfadoxine per litre of drinking water SID; Borgal 24%®, Virbac France, France) and were also given a multi vitamin complex in the drinking water (Vitavia®, Virbac France, France). One budgerigar testing positive for *Cp. psittaci* was isolated and treated using Doxycycline (25-35 mg/Kg SID PO for 21 days; Ronaxan P.S 5%®, Laboratoire Coophavet, France) and dewormed using Ivermectin (0,2 mg/Kg pour-on; Virbamec® pour-on, Virbac France, France)⁽³²⁾. Hygienic measures for protection against zoonotic transmission were taken and all personnel in contact with the budgerigar flock were asked to wear protective clothing. The use of disinfectant doormats was continued. The medical counsellor at the institution was notified and all personnel were informed about the risks of zoonotic transmission of *Cp. psittaci*.

2.5. Discussion

Zoo records revealed that from September 2012 to May 2013 there was a dramatic decline in the budgerigar's adult population number down to 44% of the initial flock's size. These results exceeded by far a seasonal decline of the population by natural means and an epizootic outbreak of Chlamydophilosis was suspected. Temporary overcrowding and concomitant parasitism were thought to be the stressors compromising the flock's immunity. Mortalities were higher between December 2012 and March 2013, pointing that along with a compromised immunity these birds may also have suffered stress due to the low environmental temperatures of a prolonged winter. Attacks and cannibalistic behaviours performed by other parrots, free-ranging birds or rodents, especially when birds were depressed, led either to these birds' death or to severe lesions which were incompatible with life, warranting euthanasia (Figure 1, Appendix III). There was a discrepancy between the mortalities among the budgerigar's flock and the number of performed necropsies and sexing of the carcasses. This could be explained by the advanced decomposition of some of the carcasses and severe predatory lesions. The first clinical case (December 2012) was a verified coccidiosis in a subadult. Aspergillosis was in a first approach, because of suggestive post-mortem findings. The antifungal treatment failed and ended despite all efforts in the death of nearly every budgerigar isolated for that treatment pointing out that sensitivity testing is a crucial part of treatment choices. It is suspected that in these case the interior lesions were either too advanced to respond to the treatment or the *Aspergillus* isolate was resistant to both molecules. A viral component to the disease outbreak was excluded as histopathologic findings did not support the theory. Even

after finding intracytoplasmatic inclusions in spleen and liver impression smears in the in-house laboratory, the clinical suspicion tended increasingly towards a *Cp. psittaci* infection. Chlamydophilosis is characterized as being a clinically unapparent or a latent infection and because clinical signs and pathological findings are not pathognomonic, the definitive diagnosis was only reached after microbiological tests. The antibiotic therapy improved the clinical status of six budgerigars that were isolated in a critical care incubator. Antibiotic therapy, stable environmental temperatures, impeccable hygiene and supplemented feed act as a symptomatic treatment as they improve the immune status. According to Tully (2001) Chlamydophilosis could be confirmed after the identification of *Cp. psittaci* antigen by IChr and rt-PCR from cloacal swabs of budgerigars presenting clinical signs compatible to those of Chlamydophilosis. The large amount of negative results to *Cp. psittaci* infection by IChr analysis may suggest that the outbreak was acute and the IgG antibodies did not have time to rise. This also contributed to the delay of the definitive diagnosis. There was only one budgerigar isolated for treatment, positive to *Cp. psittaci* infection. This animal suffered concurrent deformation of the beak due to an earlier ecto-parasitic infection, suggesting some level of individual susceptibility to Chlamydophilosis due to prolonged immunosuppression (Figure 1, Appendix III). This budgerigar was isolated and received treatment for Chlamydophilosis. The five budgerigars tested negative for *Cp. psittaci* infection were released in their enclosure although care has to be taken in the future, because *Cp. psittaci* is excreted intermittently. So paired serology or repeat rt-PCR analysis should be performed in order to confirm that the budgerigars are not excreting the pathogenic agent anymore. The two monk parakeets that died suddenly without premonitory signs presented similar post mortem findings as the budgerigars. Although this two birds were negative to *Cp. psittaci* infection by IChr analysis, random samples could have been taken from other monk parakeets in the population for PCR analysis and bacterial isolation to exclude with certainty a *Cp. psittaci* infection. Pigeons were presumed to be the main source of infection and our suspicions could be verified as Chai (2003) communicated that there is a 50% serologic prevalence of *Cp. psittaci* in domestic pigeons (*Columba livia domestica*) in Paris. Other free-ranging birds could also have been the source of infection^(15, 33). Nests should be cleaned and disinfected frequently between clutches. This way it is possible to diminish the presence of a *Cp. psittaci* reservoir in the enclosure. Outside aviaries should also have been cleaned thoroughly on a regular basis, but all preventive measures need to involve pest control. As long as enclosures are contaminated with feces from feral animals, a diminution of pathogen prevalence is not to be expected and individual treatment of any birds housed in immediate contact to free-roaming

animals will be in vein. Individual treatment for *Cp. psittaci* involves administration of oral antibiotics over a long period of time and daily handling of every animal. The immunosuppression caused by these measures is contra productive in debilitated animals, even more so when these animals are in free contact with the pathogen in their environment. Group treatment through drinking water or medicated feed is a possibility but serum levels are difficult to control and antibiotic resistance may develop. Isolation of the entire flock and individual treatment of all animals is the gold standard in therapy of such outbreaks, but unfortunately not practical when considering flock size and housing requirements. A complete screening of the whole flock was discussed, but financial barriers prohibited this. In this case, this was very unfortunate, as it would have helped in the understanding of the epidemiology of the disease outbreak.

3. Common diseases of small and medium sized psittacines in zoo collections

3.1. Chlamyophilosis

Everett *et al.* 1999, revised the taxonomy of the order *Chlamydiales*, creating four different families, one of these being the *Chlamydiaceae* family which is currently divided into two genera, *Chlamydia* (*C. trachomatis*, *C. muridarum*, and *C. suis*) and *Chlamydophila* (*Cp. pneumonia*, *Cp. pecorum*, *Cp. abortus*, *Cp. caviae*, *Cp. felis*, and *Cp. psittaci*)^(7, 27, 56). The bacteria belonging to the *Chlamydiaceae* family are small obligate intracellular living bacteria containing RNA and DNA with a unique life cycle being able to persist latently in the cells of the host animal, creating long-life asymptomatic carriers^(7, 56). These bacteria can affect several animals, including man^(37, 56). *Cp. psittaci* has seven serovars (A-F and E/B) relatively host-specific and all of them are able to be transmitted to humans and causing disease^(7, 61). In Psittaciformes serovars A and B were isolated, serovar A being considered highly pathogenic⁽⁶²⁾. Relating to this recent scientific knowledge it only makes sense to designate Chlamyophilosis to describe infections caused by any species belonging to the *Chlamydiaceae* family, putting aside the ambiguous designations of chlamydiosis, psittacosis, ornithosis, parrot fever, parrot disease and pneumonia pseudotypcosa. Chlamyophilosis is a zoonotic disease, also considered an occupational hazard that has the ability to cause severe clinical disease in infected humans of every age but especially people belonging to risk groups. Consequently it is a mandatory reportable disease to the official veterinary services in many countries, including Portugal where it is reported to the *Direccao-Geral de Alimentação e Veterinaria* (DGAV), and it is also a reportable disease to the World Organisation for Animal Health (OIE)^(37, 46, 59). In France avian

Chlamyphilosis is not a reportable disease in non-parrots birds, the veterinarians do not need to notify the French official veterinary authorities⁽¹⁷⁾. In Germany and in the Netherlands there is a duty of notification for Chlamyphilosis in psittacines and of information for Chlamyphilosis in non-psittacines or man⁽⁵⁸⁾. The author did not find anything regarding to this distinction between psittacines and non-psittacines birds in the Portuguese law. Zoos under the “BALAI” directive have to notify *Cp. psittaci* infections when have intention to trade the infected animal with other zoos under the same directives. Humans can be infected by *Cp. pneumonia*, *C. trachomatis* (these two bacteria infect only humans) and *Cp. psittaci*⁽³⁷⁾. *Cp. psittaci* strains from Psitaciformes, ducks and turkeys cause the most severe disease in humans, in contrast to pigeon strains that rarely cause clinical disease^(33, 51, 54). Psittacine birds seem to be the major source of human infection, although outbreaks did occur in which the infection sources were poultry⁽⁶²⁾. In humans, Chlamyphilosis presents as anything from a mild flu-like illness to a severe generalized infection that may lead to death^(51, 54). After an incubation period from one to three weeks there can be fever, unspecific pneumonias, muscle aches, neurological signs, cardiovascular disorders and occasionally diarrhoea^(51, 58). If untreated the disease may progress to severe mental confusion, renal failure and death⁽⁵¹⁾. The incidence of psittacosis in men seems to be increasing in industrialized countries and is related to the importation of exotic birds⁽⁶¹⁾. Nowadays there are no endemic or epidemic situations in Europe for *Cp. Psittaci* however isolated cases of zoonotic transmission are regularly happening but most of the times are under diagnosed and/or poorly documented^(26, 58).

3.1.1. Pathogenicity and Epidemiology

It is important to have in mind that there are two known variables that determine the course of an infection, the relative susceptibility of the host and the natural reservoirs. The disease's transmission is mostly aerogenous by inhalation of feather dust and fecal aerosol. Other secretions and excretions like ocular and nasal secretions, pharyngeal secretions or crop milk, can also contain the pathogen^(54, 58). Oral transmission is possible but not relevant⁽⁵⁸⁾. Vertical transmission occurs very occasionally and has been documented in budgerigars and other birds, however this is controversial and there are authors that indicate the inexistence of evidence that vertical transmission occurs via the ovaries^(26, 33, 51, 54). Transmission by hematophageous insects or mites is also possible⁽⁵⁴⁾. There are authors that state that direct horizontal transmission like the vertical one is possible but not relevant. In a crowded environment the transmission of the disease is much more enhanced due to the intimate contact of infected birds (with or without

clinical signs) and susceptible individuals. Also, the highly social behaviour of psittacines, like pruning (transferring infectious particles from the pharynx onto the plumage) or when they pass food mouth-to-mouth (along with pharyngeal secretions) enables transmission. *Cp. psittaci* can remain infective in the dried faeces for several months and usually it can be detected in the faeces ten days prior to the onset of clinical signs, but sometimes positive-individuals can be misdiagnosed because *Cp. psittaci* can be intermittently excreted by apparently healthy parrots with a latent infection^(26, 51, 54). Cockatiels are frequent carriers of *Cp. psittaci* and can shed the agent in the faeces for more than one year following an active infection⁽⁵⁴⁾. The susceptibility of a bird to Chlamydophilosis varies with type of species (generally psittacines from the New World, like macaws and Amazon parrots, are more susceptible than psittaciformes from South Asia, Australia and related islands, and the African parrots are even less susceptible than the Asian ones) and with various endogenous and exogenous stressors such as age (young birds, and birds during the onset of their sexual maturity are generally more predisposed to infections than adults), immune status (laying birds, or birds with concomitant diseases), matting and rearing of offspring and conditions of the surrounding environment (low temperature, high humidity, poor-sanitized, crowded and stressful enclosures)^(33, 49, 54). It is important to say that anthroponozoonotic transmission is very rare⁽⁵⁸⁾. The incubation period can last from three to seven days up to three months (or carrier state), but the illness may appear years after exposure (e.g. for budgerigars seven years were suggested)^(54, 58, 59).

3.1.2. Clinical Signs, Necropsy Findings and Histopathology

The diagnosis of Chlamydophilosis is a challenge to the veterinarian because clinical presentation is non-specific and no different from those of other systemic avian diseases, creating a vast list of possible diagnosis. It can cause a completely asymptomatic infection, or an acute and often fatal illness (in the acute phase death normally ensues within 8 to 14 days)^(49, 54). In birds with latent infection clinical disease is often precipitated when subjected to stress (artificial environments and invasive husbandry and climate changes), mainly by human-induced conditions and procedures (e.g. captures) but also when the bird is affected with endogenous and exogenous stressors^(26, 33, 51, 54). In the acute phase of the disease dyspnoea, ocular-nasal discharge, sinusitis (budgerigars), conjunctivitis (uni or bilateral) and systemic signs of disease (hypothermia, apathy, tremors, dehydration, anorexia, emaciation, and a ruffled plumage) are often present^(33, 54). Signs of liver disease, gastro-intestinal signs (mainly various types of watery diarrhoea) and CNS signs can also be seen depending on the patient's condition and the

virulence of the infecting serovar⁽⁵⁸⁾. Spontaneous recovery is rare and survivors, who will be fully susceptible to new infection, have poorly formed feathers, lose body condition rapidly and characteristically they will have intermittent diarrhoea^(51, 54). Sub-acute to chronic phase of the disease is characterized by anaemia and in psittaciformes it is often characterized by CNS signs of the clonic-tonic type, including tremors, opisthotonus, torticollis, convulsive movements, etc, and if the birds are left untreated they will usually die within a few weeks^(54, 33). Once the animals present CNS signs it is most certainly a terminal phase of the disease. Although this neurological signs can occur in many Psittaciforme birds, classic examples are the African grey parrot as well as several *Cacatua spp.* and *Amazona spp.*⁽³³⁾. Cockatiel may also show flaccid paresis and paralysis^(54, 33). Australian parakeets, particularly in the genus *Neophema*, may have a circumscribed ocular infection under the form of a recurrent keratoconjunctivitis with no other, or only subtle systemic signs and frequently cannot be treated successfully^(54, 33). In zoo collections and group aviaries subtle symptoms are easy to miss and the first evidence of a latent Chlamydophilosis is a sudden high mortality rate, especially in newborns. At necropsy the gross lesions can vary as widely as the clinical disease. Gross lesions often form a characteristic pattern involving several organs such as the spleen (splenomegaly, friable, grey foci in the parenchyma suggesting focal necrosis), liver (hepatomegaly, friable, yellowish discoloration, rounded edges, focal necrosis, and surface haemorrhages giving the surface a mottled appearance), air sacs (opacification, exudates and adherent yellowish plaques), heart (purulent, serous, or fibrinous pericarditis and myocarditis) and intestines (enteritis, congestion, and strands of fibrin adhering to the surfaces of the abdominal viscera)^(33, 49, 51, 54). Splenomegaly is frequently a common finding in Chlamydophilosis, but is somewhat overemphasized because it may be either absent owing to the stage of the disease at the time of death or it can be seen in a variety of other avian diseases. In psittaciformes and pigeons the occurrence of a mildly fibrinous airsacculitis is more indicative of Chlamydophilosis^(33, 54). In sub-acute and chronic cases there may be consolidation of the lungs, with or without necrotic foci in the liver and spleen, diffuse airsacculitis with caseous deposits adhering to the air sacs, proliferation of connective tissue (up to cirrhosis) in the liver and kidney, pancreatic necrosis (budgerigars and pigeons), and a marked emaciation^(49, 51, 54). In sexually active males *Cp. psittaci* induces orchitis and epididymitis resulting in permanent infertility^(54, 33). CNS lesions can be recognized only by histology and consist of nonpurulent meningitis, which can spread to the cerebrum causing diffuse encephalitis^(33, 54). Histologically, it is possible to find foci of hyperplasia in lymphoreticular spleen tissues, increase of monocytes and of kupffer-cells in the liver in the

presence of necrosis, pneumonia with proliferation of epithelial cells, in conjunctivas there is infiltration with different cell types and macrophage-like cells with intracytoplasmic inclusions⁽³⁰⁰⁾. These intracytoplasmic inclusions are pathognomonic, and can be seen in various organs but are especially common in serosal membranes^(33, 54).

3.1.3. Diagnosis

It is very difficult to access immediately a positive diagnose of *Cp. psittaci* infection once clinical signs and necropsy findings are not pathognomonic of Chlamydophilosis. Also, secondary bacterial, fungal or viral infections can alter the lesions obscuring the diagnosis. However, Chlamydophilosis should always be considered as the possible cause of non-specific disease, particularly if there is high mortality involved⁽⁵¹⁾. The pathogenic agents that comprehend the differential diagnosis list of this disease include, *Aspergillus spp.*, Herpesvirus, Paramyxovirus, Influenza A virus, Newcastle virus, Reovirus, *Mycoplasma spp.*, *Pseudomonas spp.*, and bacteria belonging to the *Enterobacteriaceae* family^(33, 51, 54). Nowadays, the gold standard for chlamydial diagnosis seems to be a triad of diagnostic methods, including bacteriological culture, PCR and serology. Serology seems to be the diagnostic method of choice' especially when "screening" large bird populations for epidemiological studies and have often worked best when the bird was exhibiting clinical signs^(22, 62). However, serology alone is not particularly useful in diagnosing a current *Cp. psittaci* infection in psittacines because of the high prevalence of this infection in these birds and the long-term persistence of anti-chlamydia antibodies. So, in order to determine whether a parrot is infected, serology should always be used in conjunction with culture isolation, and/or nucleic acid amplification, or ultimately by performing paired serology (two-three weeks intervals)⁽⁶²⁾. Serial faecal samples or combined choana/cloaca cultures should be collected for three to five consecutive days and sent to a lab in a specific transport media in order to increase the probability for *Cp. psittaci* culture and isolation⁽⁵⁹⁾. PCR sometimes may only reflect a carrier status, as this technique does not differentiate viable from non-viable nucleic acid, therefore, serology could be helpful to confirm the clinical diagnosis' and to determine the efficacy of the subsequent antibiotic treatment. On the other side, PCR and ELISA techniques are particularly useful if circumstances suggesting that the chlamydophila might have lost their ability to multiply in susceptible cells or faeces⁽³⁷⁾.

In the table V, Appendix IV some of the existent laboratory diagnostic techniques of Chlamydophilosis are summarized along with their advantages, disadvantages, and specific considerations. *Cp. psittaci* is more frequently isolated from pharyngeal swabs than from cloacal

or faecal swabs^(32, 37). Outside the natural hosts, chlamydia are sensitive to inactivation and are difficult to isolate, which may result in false-negative results⁽³⁷⁾. Vanrompay *et al.* (2000), discussed the possibility of using a recombinant antibody ELISA using recombinant major outer membrane protein (rMOMP) as an antigen. Former antibody ELISAs use whole organisms or chlamydial LPS as antigen, but LPS should be avoided because it is serologically related to the LPS of *Enterobacteriaceae* producing false-positive results⁽⁶²⁾. Anti-MOMP antibody titers appear at an early stage during the infection (within seven days for a primary infection), and they can remain for several months⁽⁶²⁾. The recombinant ELISA does not differentiate between IgG and IgM, however, this is possible by using an immunoglobulin serotype-specific monoclonal antibody against psittacine IgG and IgM⁽⁶²⁾. High resolution melt (HRM) curve analysis following PCR is a relatively new technique used to detect nucleotide sequence variations, and because it is a one-step procedure the risk of contamination is far less than a multi-step procedure, such as nested PCR or ELISA, since the entire process is completed in a single closed tube⁽⁵⁶⁾. Robertson *et al.* (2009) proved that PCR-HRM curve analysis using the oligonucleotide set 16S produces a rapid, cost effective, reliable diagnostic technique to detect and differentiate at least six species of the *Chlamydiaceae* family. In addition, the technique may be useful for the study of Chlamydiae in species where the occurrence of the disease has not been fully examined, by the detection of unknown *Chlamydiaceae* species and identification of them to the closest species used as reference⁽⁵⁶⁾.

3.1.4. Treatment

Doxycycline is nowadays the drug of choice for the treatment of *Cp. psittaci* infections in birds and it has been replacing the use CTC in the past years⁽³²⁾. In Table VI, Appendix IV, the antibiotics usable for treatment of Chlamydiae are described. Tetracyclines are only effective against actively metabolizing microorganisms and thus incapable to treat latently or persistently infected birds in which the pathogenic agent is located inertly in macrophages^(49, 54). Birds suffering from active disease and treated with these drugs improve rapidly leading to immediate cessation of shedding and cessation of clinical signs in days^(51, 54). Elimination of *Cp. psittaci* is possible, but not likely under practical conditions, however, this treatment can reduce the infectious pressure in the environment minimizing the infection risk of humans and other animals⁽⁵⁴⁾. To eliminate latent infection it is recommended to prolong the treatment for periods of 30-45 days and the serum level of tetracyclines must be over 1µg/ml continuously during the treatment period^(51, 32). Resistant strains are still rather rare, but those with reduced sensitivity are

emerging in increasing numbers⁽³³⁾. Strains can develop resistance to tetracyclines if exposed to sub-therapeutic levels for prolonged periods of time⁽⁵⁴⁾. Normally, tetracyclines are administered PO, through medicated food, but with non-compliant patients, daily gavage or parenteral administration might be considered. Treatment of parrots with these drugs through the drinking water is unsatisfactory as many psittacine species do not drink sufficiently often to maintain a therapeutic blood level continuously for the required period, and because these birds are very exigent and if the taste of the medicated water is different from the one they are used to, they will not drink it^(32, 33, 51). Apart from the specific treatment with tetracyclines, symptomatic therapy in acutely sick birds is frequently necessary and the birds should be kept isolated, heat and fluid supplemented, hepatoprotective therapy and immunity enhancers should be administered in relation to the clinical signs⁽⁵⁴⁾. The success of the treatment has to be checked by sampling for antibody or antigen detection⁽⁵⁸⁾. Doxycycline can be given orally or parentally (intramuscular and/or intravenous), for the critically ill patient and birds that cannot be treated orally^(59, 32). The great disadvantage of injection, besides the quantity of the drug to be injected requiring more than one injection sites, is the necessity of catching the birds, increasing the stress on a fragile bird. A doxycycline-medicated food was found to provide > 1 µg/ml plasma concentration in a group of psittacines, it was not necessary to catch the birds and they could eat their normal diet without needing special care, except for some multivitamin supplementation, avoiding losing weight by refusing non-palatable food⁽³³⁾. The disadvantages are gastrointestinal disorders and direct calcium chelation of the antibiotic with food components (extremely problematic in young fast-growing birds)^(22, 33). Comparing with other tetracyclines, doxycycline has a higher oral bioavailability and a longer elimination half-life, which results in lower dosing regimens (improving compliance) and less-frequent dosing (improved palatability)⁽³³⁾. Guzman *et al.* (2010) showed that in cockatiels despite a reduction of the treatment duration from 45-21days, with Doxycycline at a dosage of 25-35 mg/Kg, per orally, was still able to clear *Cp. psittaci* infection by maintaining serum levels of the antibiotic above 1 µg/ml. The trial did not show any advantage in the use of azithromycine as compared to doxycycline⁽³²⁾.

3.1.5. Prevention and Control

In zoological collections the main goals are to prevent infection in susceptible bird populations and to eliminate any possibility of zoonotic transmission to members of the personnel or general public. The veterinarian should always consider treating clinically sick and seropositive parrots. Since the disease may be transmitted to man, it is probably wise to treat

suspected cases as positive, even though the diagnosis is only tentative⁽⁴⁹⁾. To prevent infection and re-infection a strict hygiene is required when dealing with birds. Cleaning and disinfecting protocols help minimize the environmental contamination and thus reduce the transmission rate. A thorough cleaning protocol using water at high temperatures (30°C – 40°C) reducing dust, followed by disinfection based on ethanol or quaternary ammonium compounds is strongly recommended⁽⁵⁸⁾. Phenol and formalin are poor choices for inactivating these bacteria⁽⁵⁸⁾. Efficient UV-lights kill *Chlamydiales* within 3 minutes but are unpractical choices for large enclosures⁽⁵⁸⁾. For personnel security, infected birds should only be handled by appropriately protected handlers and all manipulations around infected birds are to be kept to a strict minimum. Protective clothing and masks are required to wear by all personnel in the contaminated area. There are no efficient vaccines available at the moment but there are experimental studies with a *Cp. psittaci*-MOMP-vaccine produced by genetic engineering^(37, 58). Zoos should trade only with psittacines who are tested negative for *Cp. psittaci* infections⁽⁵⁸⁾. The article seven of the council directive 92/65/EEC, 13 July 1992, defines that member states shall ensure that birds to be traded do not come from a holding nor have been in contact with animals from a holding in which Chlamydia infection (*Cp. psittaci*) has been diagnosed. It also defines that the period of prohibition since the last recorded case and the period of treatment under veterinarian supervision must be of at least two months⁽¹⁹⁾. So it is imperative to ask the institution of origin for recent health certificates stating that their bird populations are negative for all official diseases, Chlamydia infection included. When trading animals from non-approved sources quarantine is required, diagnostic examination by means of triple swabs of the conjunctiva, pharynx and cloaca along with blood samples tested for notifiable diseases and for other highly pathogenic agents⁽⁵⁸⁾. Once a psittacine is tested positive for *Cp. psittaci* a follow-up protocol needs to be initiated. The bird must be re-tested with triple swabs (choana/cloaca/conjunctiva) for bacteria isolation and PCR, combined with serology to discard false-positive results. If the individual is really positive to *Cp. psittaci*, as it was mentioned before, the veterinarian must apply national laws, the bird or birds are to be isolated and the zoo's medical counsellor should be informed. In cases of infection in large psittacines collections, it is important to screen the other individuals for *Cp. psittaci*, and start treatment in all positive individuals. Sometimes euthanasia can be an option, but it should be definitely be the last resource in dealing with Chlamydia infection. Some authors recommended that in consideration of the human health hazard and the difficulty of treatment, euthanasia of the affected bird and contact birds should be seriously considered⁽⁵¹⁾. This is true in domestic poultry

destined for human consumption, in severely ill individuals, and in cases where owner compliance is inexistent. It is almost impossible to establish a seronegative population for *Cp. psittaci* infections because this pathogenic agent is distributed worldwide. Free-ranging birds especially free-living urban pigeons are reservoirs for these bacteria. Fifty percent of the population of free-ranging birds and domestic pigeons in Paris are serologically positive for *Cp. psittaci*, a prevalence suggesting that a high number of latent carriers could be shedding the bacteria⁽¹⁵⁾. Periodical pathogen screenings in zoo collections are one way to keep track of a populations' health status and detect potentially zoonotic pathogens, but their practical value can be contested as the financial situation might not allow for such extensive analysis. Random sampling of a representative number of individuals might be a choice, but there are good chances that due to intermittent shedding of pathogens the results biased. Necropsies are an excellent way to screen a population and diagnose latent disease amongst a population.

4. Aspergillosis

Avian aspergillosis is the most common mycotic disease caused by fungi of the genus *Aspergillus sp.*⁽³¹⁾. *A. fumigatus* and *A. flavus* seem to be the most frequently isolated aetiological agents of psittacine aspergillosis but infections in these birds were also related to *A. nigris*, *A. oryzae*, *A. ochraceus*^(16, 25, 29). It is an infectious, non-contagious and non-zoonotic disease, even though prolonged contact with contaminated feather dust can lead to infection in susceptible human beings. Fifteen different mycotoxins have been associated with clinical avian aspergillosis^(2, 31). The fungus is distributed worldwide infecting captive, free-living and companion birds^(31, 33, 49, 54). Its occurrence is higher in captive than in free-ranging birds with major mortality in poultry, psittacines, raptors and aquatic birds^(12, 31, 50). High prevalence values have been reported in African Grey Parrots and Blue-Fronted Amazon Parrots (*Amazona aestiva*)⁽⁵⁴⁾. Birds are highly susceptible to the fungus and apparently fail to carry out an effective immune response⁽²²⁾. Transmission occurs through inhalation (mostly) or ingestion of conidia present in the environment^(22, 31). Fungi of the genus *Aspergillus sp.* are ubiquitous and are found in the air, water, soil, food, and decomposing vegetables^(14, 22). As parrots are continuously exposed to environmental spores predisposing factors such as, stressful environments, overcrowding, unhygienic and/or poor ventilated enclosures, thermal stress, malnutrition, trauma, immunosuppression, concomitant disease, prolonged antibiotic or corticosteroid therapies, respiratory irritants, age and poor condition are necessary to lead to clinical infection^(14, 22, 34, 65). Vertical transmission and reactivation of latent infections have been reported^(31, 34).

The course of the disease can be acute (generally with a fatal outcome) or chronic (more common), local or systemic^(33, 34, 54). Acute forms in young psittacines are often associated with immunosuppressive viruses and chronic forms occur more frequently in adult psittacines⁽¹⁴⁾. *Aspergillus sp.* can affect many organs having greater tropism for the upper-respiratory tract (trachea, syrinx and bronchi), although the course of disease is more severe when the lower-respiratory tract (air-sacs and alveoli) is affected⁽²²⁾. The main target organs are lungs and air sacs, but in the systemic form, liver, kidney, encephalon, bones, skin and eyes can also be affected^(22, 31). The nasal cavity is an important entrance and multiplication site in psittacine birds, lesions usually are granulomatous and accompanied by substantial destruction of the adjacent tissue⁽⁵⁴⁾. Clinical signs of respiratory and systemic disease, and sudden death are the majority of the findings⁽⁴²⁾. Aspergillosis typically causes severe airsacculitis^(22, 31). There are two characteristic forms of lesions, a nodular or granulomatous form with internally septate hyphae, typical of organs with non-aerated parenchyma and a non-encapsulated superficial diffuse form containing sporangia with a distinctive morphology, typically located especially in the air-sacs but also in the lungs⁽¹⁴⁾. Histopathologic findings include granulomatous pneumonia, fungal airsacculitis, signs of concurrent infections, bronchopneumonia, tracheobronchial mycetomas, acute necrosis with thrombosis⁽⁵⁴⁾. Main differential diagnosis include Nocardiosis, Avian Tuberculosis, Pseudotuberculosis, Poxvirus, Trichomoniasis, *Escherichia coli*, Pasteurellosis, Chlamydophilosis, Neoplasia^(34, 47). In psittacine birds, the ante mortem diagnosis of aspergillosis is challenging and usually based on clinical signs combined with the results of diagnostic tests such as radiography, endoscopy, routine hematologic and biochemical analysis, and biopsy⁽²⁰⁾. Detection of *Aspergillus sp.* is achieved by cytology, histology and culture⁽⁴²⁾. Serological testing to detect antibodies against *Aspergillus sp.* using electrophoresis are very sensitive, but direct or indirect ELISA tests as well as the recent galactomannan assay are also useful for detecting circulating *Aspergillus sp.* antibodies^(14, 20, 31, 42). The search for antigens can be made by electrophoresis or by searching *Aspergillus sp.* galactomannan antigen in the serum with a commercially available immunoenzymatic assay (Platelia® *Aspergillus*)^(14, 21, 31, 35, 42). Given the low sensitivity of the Platelia® *Aspergillus*, the usefulness of this test for diagnosing aspergillosis in birds, when used alone, appears to be limited⁽⁴²⁾. The treatment of aspergillosis is difficult and often not efficient because of the advanced stage of the disease when birds eventually present with clinical symptoms⁽⁴²⁾. Symptomatic treatment and husbandry management are part of the integrative treatment of clinical aspergillosis^(33, 54). Present fungicidal therapeutic options include 5-fluorocystine, nystatine, amphotericin B, miconazole, itraconazole,

ketoconazole, voriconazole, clotrimazole, fluconazole and terbinafine^(9, 31, 54). Beernaert *et al.* (2010) summarized the administration routes and doses of antifungal agents used in the treatment of avian aspergillosis⁽⁹⁾. Flushing areas with amphotericin B or chlorhexidine may be helpful in superficial forms especially if combined with systemic therapy, intratracheal administration of amphotericin B has been used to treat tracheal and pulmonary aspergillosis. Surgical removal of the mycotic lesions, flushing of the affected abdominal cavity with an amphotericin B solution, and the use of immunostimulants have been suggested^(33, 54). Rherbrecht *et al.* (2002) proved that in humans voriconazole compared with amphotericin B has more efficacy fewer secondary effects⁽³⁴⁾. Beernaert *et al.* (2009) showed acquired resistance in-vitro of *A. fumigatus* to itraconazole and voriconazole⁽⁸⁾. Prognosis of avian aspergillosis is often poor to grave due to delayed diagnostic and treatment failure⁽⁸⁾. Prevention is centred on limiting fungal growth in the environment and reducing stress⁽³⁴⁾. Enclosures must be well ventilated, nests and incubators frequently cleaned and only fresh food should be available to birds (to avoid food in decomposition to be eaten)⁽³⁴⁾.

5. YERSINIOSIS

Yersiniosis is a worldwide zoonotic disease, occurring mostly in moderate climates⁽³⁰⁾. In birds this disease is mainly caused by the *Enterobacteriaceae* bacteria *Yersinia pseudotuberculosis*^(12, 33, 54, 68). *Y. pseudotuberculosis* has six different serotypes being serotype I and II the most frequently found in birds^(12, 18, 33, 54). This bacteria is considered native to Northern and Central Europe and its occurrence is very rare in Portugal⁽⁴⁹⁾. The prevalence of yersiniosis increases in winter in part because the bacteria is psychrophile^(33, 51, 68). Wild and domestic mammals and birds, especially rodents and pigeons, are reservoirs of these bacteria transmitting the disease to birds per contamination of food and water sources^(12, 22, 49, 67). Psittacines are not known to have greater susceptibility than other birds but the disease has been described in budgerigars^(49, 51). Predisposing factors such as cold weather and concurrent immunosuppression are associated with outbreaks of clinical yersiniosis in birds⁽¹⁸⁾. Susceptibility is likely to be dependent on the immune state and/or the general health of individuals⁽¹⁸⁾. In acute cases, birds may be presented with severe systemic signs such as weakness, depression, ruffled feathers, emaciation, fever, dehydration and also watery or hemorrhagic diarrhea⁽¹²⁾. Chronic infections may be associated with neurological signs and granuloma formation in organs and skeletal musculature⁽³³⁾. Cork *et al* (1999) described that in pigeons and Psittaciformes the clinical course of the disease was more chronic in nature and

involved the liver and spleen. Pathologically, the disease is characterized by enteritis, miliary necrosis in the liver, lungs, spleen and kidneys, hepatomegaly, splenomegaly and congestion of various internal organs^(12, 49, 51, 54). Histology reveals coagulation necrosis of organ tissue, possibly surrounded by heterophils and in chronic cases it is possible to see granulomas^(33, 54). Differential diagnosis includes avian tuberculosis, salmonellosis, coligranulomatosis and pasteurellosis⁽³³⁾. Yersiniosis can only be confirmed by necropsy and bacteriological examination (MacConkey and blood agar)^(33, 51). Coloration of tissue smears within the macroscopic lesions using a Ziehl-Neelsen technique helps differentiate the disease from tuberculosis⁽⁵¹⁾. The organism can be readily identified on impression smears of liver lesions as gram-negative bacilli, which have a characteristic bipolar appearance with Romanowsky stains⁽²²⁾. Serum can be used to isolate bacteria (cultivation with cold enrichment, Congo red agar, PCR, Puls-field gel electrophoresis), search for specific antibodies (rapid nested PCR, random amplified polymorphic DNA-PCR) or to detect *Y. enterocolitica* enzyme A with a disc test based on the bacteria's resistance to ticarcillin⁽¹²⁾. For serotyping it can be used tube agglutination⁽¹²⁾. Birds with peracute and acute forms of yersiniosis usually die before therapy can be initiated⁽⁵⁴⁾. Treatment is based on antibiotics, fluoroquinolones (Ofloxacin) being the drug of choice but third generation cephalosporins can also be used^(12, 28). *Yersiniae* exhibit varying antibiotic resistances through broad spectrum enzyme A production depending on the serogroupes involved⁽¹²⁾. Many *Y. enterocolitica* strains are resistant to β -lactams due to chromome-mediated β -lactamase-production, others are partially resistant to cephalosporins or oxacillin due to cephalosporinase (enzyme B)⁽¹²⁾. Treating chronic cases is difficult if granulomas are present because it prevent antibiotics from reaching the *Yersinia* organisms nestled in the center of necrotic debris⁽⁵⁴⁾. It is as well known that the massive rodent's infestation in Paris, like in other European cities, allied with the possibility of infectious disease transmission to other animals, man included, increases the concern about these pathogens. Therefore yersiniosis is a constant concern in zoos where healthy rodents and pigeons shedding bacteria are difficult to totally eliminate⁽⁵¹⁾. The implementation of pest control programs in zoological institutions is imperative, not only due to the food competition and the predatory risk these animals represent to the zoo collection, but mostly for their roles as disease reservoirs. Pest proof food storage and strict work hygiene are required in order to avoid the spread of potentially zoonotic diseases through zoo collections. Vaccination can be carried out in exposed areas but as most vaccines are designed for pet animals, neither their innocuousness nor their efficacy is scientifically proven in wild animal species^(12, 22, 51, 66). When humans are affected by the disease, presenting fever,

nausea, headache, abdominal pain diagnosis is difficult as symptoms mimic other abdominal disease⁽⁶⁷⁾. Gastrointestinal infections are usually self-limiting and do not need antimicrobial therapy⁽²⁸⁾. Humans (and non-human primates) are infected either by contaminated water and food, or by direct contact with the hosts and their parasites^(12, 67).

6. Salmonellosis

Avian Salmonellosis is a zoonotic infectious disease caused by bacteria of the *Enterobacteriaceae* family, genus *Salmonella spp.*, with the sub-species of *Salmonella enterica* causing three distinct diseases in birds: dysentery or pullorum disease (*Salmonella enterica sp. pullorum*), aviary typhoid (*Salmonella enterica sp. gallinarum*) and paratyphoid infection (caused by different serovars of *Salmonella enterica*, also related to foodborne illness)^(4, 11, 41, 43). Salmonellosis is one of the most important foodborne illnesses and in Portugal is a mandatory reportable disease to DGAV^(4, 10, 44). There are more than 1800 different *Salmonella spp.* spread worldwide with birds, reptiles, small mammals and arthropods being the reservoirs^(4, 10, 11). Domestic pigeons are important in the spread of salmonellae in cities, due to their abundance⁽⁶⁷⁾. Various wildlife species are susceptible to infections by *Salmonella spp.* regardless of whether they live in captivity or freely in the wild, although the frequency of *Salmonella spp.* infections in captive birds is much lower when compared with captive mammals and reptiles⁽⁴³⁾. Despite that *Salmonella spp.* can cause serious disease in healthy captive psittacines being *S. typhimurium* and *S. pullorum* the most common isolated serotypes^(3, 4, 10, 30). Recently, Allagayer *et al.* (2009) identified for the first time in a cloacal swab of a young healthy Hyacinth macaw, *Salmonella braenderup*. Marietto-Gonçalves *et al.* (2010) verified the presence of *Salmonella enterica* serovar Enteritidis in three captive specimens of Blue-Fronted Amazon parrots (*Amazona aestiva*) and Tunca *et al.* (2012) isolated *Salmonella gallinarum* from budgerigars presenting signs of clinical disease. In psittacines, the course of the disease is variable, from peracute, acute, chronic to asymptomatic^(38, 60). Young budgerigars usually suffer the peracute and acute form of the disease whereas adult psittacines usually are affected by the acute and chronic forms⁽⁶⁰⁾. Signs of acute disease usually consist of septicemia, diarrhea, fever, depression, and hypothermia^(10, 38). In the chronic form the bird shows arthritis, panophthalmitis, osteomyelitis, anorexia (food and water), loss in body condition, crop stasis, with or without diarrhea^(10, 38, 68). Gross pathology is characterized by hepatomegaly, splenomegaly, congestion of kidneys and lungs, peritonitis and pericarditis^(38, 60). Peritonitis and pericarditis are the most common findings in young budgerigars at necropsy, while in adult budgerigars scattered grey–

white necrotic foci can be found in the liver⁽⁶⁰⁾. Histopathologically, lesions in young budgerigars are characterized by fibrinonecrotic peritonitis and/or pericarditis and necrotic hepatitis⁽⁶⁰⁾. In adult budgerigars with acute infection, hepatic necrosis with focal heterophile infiltration was present, whilst lesions in the chronic cases were granulomatous in nature with infiltration of macrophages, lymphocytes and histiocytes⁽⁶⁰⁾. The diagnosis can be made by bacterial isolation (cloacal swabs are more efficient than feces samples) or serology methods (avidin-biotin peroxidase complex, immunofluorescence, serum agglutination and enzyme-immunoassays methods)^(3, 4, 11, 24, 30, 43, 60). Intermittent shedding is common in salmonellosis so care should be taken when evaluating results of bacterial isolation⁽¹³⁾. The commercial antigen for pullorum disease was shown to be a useful tool for rapid detection in the screening of *Salmonella spp.* in Psittaciformes⁽⁴³⁾. Treatment should be supportive (fluidotherapy, strict hygiene and stress-reducing environment as well as antiparasitic treatment), antibiograms should be used before choosing an appropriate antimicrobial as *Salmonella spp.* are showing increasing resistance profiles to several of the high-end antibiotics in human medicine⁽¹¹⁾. The results of antibiotic sensitivity tests revealed that fluoroquinolones show moderate to high sensitivity against almost all the bacterial isolates⁽³⁾. The difficulty of eliminating or controlling *Salmonella* in the environment is a critical point in disease management. Wild reservoirs, such as free-ranging songbirds and rodents have been repeatedly documented as *Salmonella spp.* carriers and implicated in the transmission of these pathogens to mammals (man included) and to birds (psittacines included)⁽¹⁰⁾. Serologic testing of all incoming and outgoing birds in a collection could be helpful in detecting silent carriers and possibly help prevent the spread of infections⁽³⁰⁾.

7. Psittacine Beak and Feather Disease

Psittacine beak and feather disease (PBFD) is the most common viral infection affecting wild and captive psittacine birds^(1, 39). The causative agent is a circovirus (BFDV) currently present worldwide^(39, 50, 64). This virus is recognized as a threat for endangered psittacine birds in Australia, New Zealand and South Africa^(39, 48). Shearer *et al.* (2008) confirmed that all psittacines are susceptible to the disease, even if Amazon parrots seem to be less susceptible to PBFDV infection in comparison to other New World parrots^(50, 1). Currently, BFDV has 14 strains, some of them host-specific^(53, 45). Khalesi *et al.* (2005) showed that isolates derived from lorikeets may be able to infect other psittacine bird species. Transmission is mainly horizontal (oral, intracloacal or intranasal routes) but vertical transmission has been suggested⁽⁵⁴⁾. Varsani *et al.* (2011) suggested that with animal trades all over the world the distribution as well as

strains host specificity becomes blurred. The same authors suggested that captive birds housed in close proximity with free-ranging birds enables for multiple infections with BFDV and to the consequent emergence of recombinant strains. Surveys in both wild and captive psittacine populations have shown virus prevalence rates vary between 10% and 94%, depending on the method of detection⁽⁹¹⁵⁾. The main clinical features in the acute and chronic course of the disease are progressive and irreversible feather dystrophy and loss, spread symmetrically over the body, and a severe immunosuppression that may result in lethal secondary infections^(53, 55). Clinical signs of the disease may also include weight loss, lethargy, hepatic disease and beak deformities^(48, 50, 53, 55). The first sign of PBFD is a replacement of normal down and contour feathers with dystrophic feathers that stop growing shortly after emerging from the follicle⁽⁵³⁾. The disease can affect psittacines from both sexes and all ages, although the acute form of infection is mostly found in juveniles aged between 0-3 years old and their susceptibility to disease is thought to be due the rapid feather growth rather than the virus strain^(6, 52, 55). Frequently, in young psittacines the BFDV infection is diagnosed during their first moult⁽⁶³⁾. Schoemaker *et al.* (2000) described an atypical course of PBFD in African grey parrots, characterized by severe immunosuppression and anaemia in the absence of feather disorders. Raue *et al.* (2004) demonstrated not only that in captive psittacines that there might be a high prevalence of asymptomatic carriers. At necropsy, internal lesions are variable and differ with age and the type of secondary infection⁽⁵⁴⁾. The cloacal bursa may be small with poorly developed folds and the thymus may reveal small lobes with pale necrotic tissue⁽⁵⁴⁾. In mature birds the spleen is frequently small and depleted of lymphocytes, and occasionally necrosis of the reticular cells can be observed⁽⁵⁴⁾. As avian polyomavirus has similar clinical manifestations in psittacines, it constitutes the principal differential diagnosis for PBFD⁽⁵⁴⁾. Non-infectious causes of similarly appearing feather lesions include nutritional deficiencies, endocrine abnormalities and drug reactions⁽⁵⁴⁾. The disease should be suspected when a parrot has progressive feather loss involving malformed feathers. Feather pulp or follicular epithelium from psittacines with clinical signs of dystrophic, non-viable feathers should be examined (biopsy) for basophilic intracytoplasmatic or intranuclear inclusion bodies⁽⁵⁴⁾. The major capsid protein of BFDV (C1) can induce antibody response in psittacines infected with this virus producing titres sufficient for reliable serological tests⁽³⁶⁾. Specific detection of antibodies against BFDV by ELISA and immunoblotting assays is an option⁽³⁶⁾. The quantitative tests HI assays are recommended by some authors as the gold standard for detecting anti-BFDV antibody in the serum and are being currently used for seroepidemiological studies in wild and captive

psittacines^(36, 39). Although, PCR testing has been considered the method of choice for diagnosis of BFDV and it is more sensitive and specific than HA for the detection of BFDV in feathers^(39, 53). Nested PCR assays can increase sensitivity of an assay but its interpretation have a high level of complexity and can produce, by many reasons pointed by Khalesi B., *et al.* (2005), false-positive and false-negative results. The advantages on having a two-stage method for BFDV testing in psittacines have been pointed. Non-quantitative PCR assay is reliable for the diagnosis of BFDV infection in blood and feathers of psittacines with PBFD lesions, being more sensitive with blood samples^(39, 53). There is no specific treatment for this disease. The animal should be kept in a controlled environment and a symptomatic treatment should be performed⁽⁵⁴⁾. In cases that psittacines don't respond to the treatment, and because beak lesions are very painful, euthanasia is suggested⁽⁵⁴⁾. Psittacines in zoological collections should be tested for PBFDV latency and carriers as well as shedding animals isolated or eliminated from the collection. An outbreak of BFDV in an aviary is difficult to contain since this virus is environmentally stable, very infectious and resistant to many control measures^(55, 63). In order to stem the spread of BFDV inside captive bird populations, it is important to combine monitoring (carriers or birds in the viral incubation phase) and quarantine⁽⁵³⁾. Young psittacines, which seem to be most susceptible to the BFDV should not be exposed to areas where other psittacines have been identified as positive to PBFD virus infection. As no natural reservoir of BFDV is known in Europe, successful elimination should be possible in this region, provided that sensitive and reliable diagnostic tests are available⁽³⁶⁾. Development of new diagnosis techniques along with effective vaccines is very difficult because of the virus inability to propagate in tissue or cell cultures, therefore recombinant antigens are needed⁽³⁶⁾. Massano & Ortiz-Catedral (2012) suggested, for New Zealand populations but with applicability worldwide, that pro-active screening and monitoring of BFDV infection rates in aviaries as well as in wild populations are necessary to limit the risk of transmission among threatened and endangered parrot populations.

8. Conclusion

Health issues in captive bird flocks are common and sometimes the clinical approach can be challenging, as most diseases spread rapidly amongst the population and environmental factors play a key role in managing the disease. It is important to establish gold standard diagnostics and treatment plans to effectively manage captive populations. Testing the innocuousness of existing vaccines to certain pathogens in exotic species is a relevant research issue as the efficiency of these vaccines is unknown for most of the species kept in zoological

collections. Regular screening of flocks for certain pathogens can help establishing a population's health status at a given time, but as most clinical diseases are multifactorial, this method needs to be addressed with caution. Efficient pest control though is absolutely imperative and reduces pathogen pressure in the environment, but it is practically impossible to completely eliminate pests from zoological collections. Thus another approach to the pest species problem is a policy for systematic necropsy on all rodents and free-ranging animals found dead at the premises of a zoo collection. Pathogen prevalence values in pest species populations help addressing disease outbreaks in captive populations and enable for the establishment of efficient preventive management tools.

9. References

- 1 Shearer P, Bonne N, Clark P, Sharp M, Raidal S (2008) "Beak and feather disease virus infection in cockatiels (*Nymphicus hollandicus*)" **Avian Pathology** 37(1), 75-81
- 2 Abad A, Molina J, Margareto J, Sendino J (2010) "What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis" **Revista IberoAmericana de Micologia** 27(4), 155-182
- 3 Akhter J, Hossain M (2010) "Isolation and Identification of Microflora from Apparently Healthy Caged Parrots of Dhaka Zoo of Bangladesh" **Bangladesh Journal of Veterinary Medicine** 8(1), 5-10
- 4 Allgayer M, Oliveira S, Mottin V, Loiko M, Weimer T (2009) "Isolamento de Salmonella Braenderup em arara-azul (*Anodorhynchus hyacinthinus*)" **Ciência Rural, Santa Maria** 39(8), 2542-2545
- 5 André JP (2005) "**Formulaire in Guide Pratique des Maladies des oiseaux de cages et de volières**", Editions Med'Com, Paris, France, 239-248
- 6 Bassami M (2001) "Genetic Diversity of Beak and Feather Disease Virus Detected in Psittacine Species in Australia" **Virology** 279, 392-400
- 7 Beeckman DAS, Vanrompay DCG (2009) "Zoonotic *Chlamydophila psittaci* infections from a clinical perspective" **Clinical Microbiology and Infection** 15(1), 11-17
- 8 Beernaert LA, Pasmans F, Van Waeyenberghe L, Dorrestein GM (2009) "Avian *Aspergillus fumigatus* Strains Resistant to both Itraconazole and Voriconazole" **Antimicrobial Agents and Chemotherapy** 53(5), 2199-2201
- 9 Beernaert LA, Pasmans F, Van Waeyenberghe L, Haesebrouck F, Martel A (2010) "Aspergillus infections in birds: a review" **Avian Pathology** 39(5), 325-331
- 10 Boseret G, Losson B, Mainil J, Thiry E, Saegerman C (2013) "Zoonosis in pet birds: Review and perspectives" **Veterinary Research** 44(36)
- 11 Brack M (2003) "Salmonellosis" in **Transmissible Diseases Handbook**, Infectious Diseases Working Group EAZWV
- 12 Brack M (2004) "Yersiniosis" in **Transmissible Diseases Handbook**, Infectious Diseases Working Group EAZWV, fact sheet no 31
- 13 Butron O, Brightsmith D (2010) "Testing for *Salmonella* spp. In released parrots, wild parrots, and domestic fowl in lowland Peru" **Journal of Wildlife Diseases** 46(3), 718-723
- 14 Cacciuttolo E, Rossi G, Nardoni S, Legrotttaglie R, Mani P (2009) "Anatomopathological aspects of avian aspergillosis" **Veterinary Research Communications** 33, 521-527

- 15 Chai N (2003) "Prevalence of *Chlamydophila psittaci* in domestic pigeons (*Columba livia domestica*) in Paris" **Personal communication.**
- 16 Churria C, Reynaldi F, Origlia J, Marcantoni H, Piscopo M, Loyola M, Reinoso E, Petriccelli M (2012) "Pulmonary Aspergillosis due to *Aspergillus flavus* Infection in a Captive Eclectus Parrot (*Eclectus roratus*)" **BJVP** 5(1), 4-6
- 17 Code rural (2012) "Liste des maladies réputées contagieuses" in Article D223-21.
- 18 Cork S *et al.* (1999) "Visceral lesions caused by *Yersinia pseudotuberculosis*, serotype II, in different species of bird" **Avian Pathology** 28, 393-399
- 19 Council Directive 92/65/EEC (1992) "Article 7" **Official Journal of the European Communities**
- 20 Cray C *et al.* (2009) "Galactomannan Assay and Plasma Protein electrophoresis Findings in Psittacine Birds with Aspergillosis" **Journal of Avian Medicine and Surgery** 23(2), 125-135
- 21 Cray C *et al.* (2009a) "Application of Galactomannan Analysis and Protein Electrophoresis in the Diagnosis of Aspergillosis in Avian Species" **Journal of Zoo and Wildlife Medicine** 40(1), 64-70
- 22 Davies R (2000) "Avian Liver Disease: Etiology and Pathogenesis" **Seminars in Avian and Exotic Pet Medicine** 9(3) 115-125
- 23 Dean D, Kandel RP, Adhikari HK, Hessel T (2008) "Multiple *Chlamydiaceae* Species in Trachoma: Implications for Disease Pathogenesis and Control" **PLoS Med** 5(1), 57-59
- 24 Deem S, Noss A, Cuéllar R, Karesh W (2005) "Health evaluation of free-ranging and captive Blue-Fronted Amazon Parrots (*Amazona aestiva*) in the Gran Chaco, Bolivia" **Journal of Zoo and Wildlife Medicine** 36(4), 598-605
- 25 del Hoyo J, Elliot A, Sargatal, J. eds (1997) "**Handbook of the Birds of the World**" in vol.4 Sandgrouse to cuckoos, Lynx Editions, Barcelona, 274, 278-279, 362-363, 380, 384, 398-399, 412-421, 434-445
- 26 Dovč A, Slavec B, Knific R, Rojs O, Račnik J (2007) "Study of *Chlamydophila psittaci* outbreak in budgerigars" **Bulletin of the Veterinary Institute in Pulawy** 51, 343-346
- 27 Everett KD, Bush RM, Andersen AA (1999) "Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. Nov. and *Simkaniaceae* fam. Nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms" **International Journal of Systematic Bacteriology** 49, 415-440
- 28 Fabrega A, Vila J (2012) "*Yersinia enterocolitica*: Pathogenesis, virulence and anti-microbial resistance" **Enfermedades Infecciosas y Microbiología Clínica** 30(1), 24-32
- 29 Fraga M, Medeiros M, Neves D (2011) "Estudo de *Aspergilli* durante o período de quarentena de Psitacideos do Centro de Triagem de Animais Silvestres (CETAS) IBAMA, Seropédica, RJ" **Revista Brasileira de Medicina Veterinária** 33(2), 68-72
- 30 Grimes J, Arizmendi F (1995) "*Salmonella typhimurium* agglutinins in exotic bird sera in the USA" **Journal of Veterinary Diagnosis and Investigation** 7(270), 269-270
- 31 Guillot J (2013) "A propos des aspergilloses aviaires" **Personel communication.**
- 32 Guzman DSM, Figueroa OD, Tully T, Ciembor P, Morgan T, Walden M, Poston R, Flammer K, Mitchell M, Ritchie B (2010) "Evaluating 21-day Doxycycline and Azithromycin Treatments for Experimental *Chlamydophila psittaci* Infection in Cockatiels (*Nymphicus hollandicus*)" **Journal of Avian Medicine and Surgery** 24(1), 35-45
- 33 Harrison GJ, Harrison LR (1986) "Zoonotic Diseases" in **Clinical Avian Medicine and Surgery** W.B Saunders Company, Philadelphia
- 34 Herbrecht R, Denning D, Patterson T *et al.* (2002) "Voriconazole versus Amphotericin B for primary therapy of invasive aspergillosis" **The New England Journal of Medicine** 346(6), 408-415
- 35 Ivey E (2000) "Serological and Plasma Protein Electrophoretic Findings in 7 Psittacine Birds with Aspergillosis" **Journal of Avian Medicine and Surgery** 14(2), 103-106

- 36 John R, Raue R, Grund C, Kaleta E, Müller H (2004) "Recombinant expression of a truncated capsid protein of beak and feather disease virus and its application in serological tests" **Avian Pathology** 33(3), 328-336
- 37 Kaleta EF, Eva MA (2003) "Avian host range of *Chlamydophila* spp. Based on isolation, antigen detection and serology" **Avian Pathology** 32(5), 435-462
- 38 Kanashiru A, Castro A, Cardoso A, Tessari E, Tavechio A (2002) "Persistência de *Salmonella* sp. Após antibioticoterapia em psitacídeos pertencentes a um criadouro comercial" **Arquivo do Instituto de Biologia de São Paulo** 69(2), 99-101
- 39 Khalesi B, Bonne N, Stewart M, Sharp M, Raidal S (2005) "A comparison of haemagglutination, haemagglutination inhibition and PCR for the detection of psittacine beak and feather disease virus infection and a comparison of isolates obtained from lorids" **Journal of General Virology** 86, 3039-3046
- Lindsay T, Morris R (2000) "**Field Guide to New Zealand Wildlife**", HarperCollins Publisher, Auckland.
- 41 Liu G, Rahn A (2002) "The envolving Genome of *Salmonella enterica* Serovar Pullorum" **Journal of Bacteriology** 184(10) 2626-2633
- 42 Loch G (2005) "Evaluation of the Serological test PLATELIA® Aspergillus for the Diagnosis of Aspergillosis" in **Proceedings, 8th European AAV Conference, 8th Scientific ECAMS Meeting**
- 43 Marietto-Gonçalves G, Almeida S, Lima E, Okamoto A, Pinczowskip P, Andreatti Filho R (2010) "Isolation of *Salmonella enterica* Serovar Enteritidis in Blue-Fronted Amazon Parrot (*Amazona aestiva*)" **Avian Diseases** 54(1), 151-155
- 44 Ministério da Agricultura, Mar, Ambiente e Ordenamento do Território (2012) "**Lista das doenças de declaração obrigatória a nível nacional**".
- 45 Ogawa H *et al.* (2010) " A novel genotype of beak and feather disease virus in budgerigars (*Melopsittacus undulatus*)" **Virus Genes** 41(2), 231-235
- 46 OIE (2012) "Criteria for the inclusion of diseases, infections and infestations on the OIE list" in 1.2 **Terrestrial Animal Health Code**.
- 47 OrosZ S (2000) "Overview of Aspergillosis: Pathogenesis and treatment options" **Seminars in Avian and Exotic Pet Medicine** 9(2), 59-65
- 48 Ortiz-Catedral L *et al.* (2010) "A new isolate of beak and feather disease virus from endemic wild red-fronted parakeets (*Cyanoramphus novaezelandiae*) in New Zealand" **Archives of Virology** 155, 613-620
- 49 Petrak ML (1982) "**Diseases of Cage and Aviary Birds**", 2º Ed, Lea & Febiger, Philadelphia
- 50 Piasecki T, Wieliczko A (2010) "Detection of Beak and Feather Disease virus and avian Polyomavirus DNA in Psittacine birds in Poland" **Bulletin of Veterinary Institute Pulawy** 54, 141-146
- 51 Price CJ (1988) "**Manual of Parrots, Budgerigars, and other Psittacine Birds**" in British Small Animal Veterinary Association, Cheltenham
- 52 Rahaus M *et al.* (2003) "Psittacine Beak and Feather Disease: a First Survey of the Distribution of Beak and Feather Disease Virus Inside the Population of Captive Psittacine Birds in Germany" **Journal of Veterinary Medicine** B50, 368-371
- 53 Raue R *et al.* (2004) "Nucleotide sequence analysis of a C1 gene fragment of psittacine beak and feather disease virus amplified by real time polymerase chain reaction indicates a possible existence of genotypes" **Avian Pathology** 33(1), 41-50
- 54 Ritchie B, Harrison G, Harrison L (1994) "**Avian Medecine : Principles and Application**" LakeWorth, Fla
- 55 Ritchie P *et al.* (2003) "Evidence for specificity of psittacine beak and feather disease viruses among avian hosts" **Virology** 306, 109-115
- 56 Robertson T, Bibby S, O'Rourke D, Belfiore T, Lambie H, Noormohammadi AH (2009) "Characterization of *Chlamydiaceae* species using PCR and high resolution melt curve analysis of the 16S rRNA gene" **Journal of Applied Microbiology** 107, 2017-2028

- 57 Saracel B, Ak S (2006) "A Comparative Studie of Detecting *Chlamydophila psittaci* in Pet Birds Using Isolation in Embryonated Egg and PCR" **Avian Diseases Digest** 1(1), 489-493
- 58 Tschirch W (2002) "Chlamydiosis (Psittacosis, ornithosis)" in **Transmissible Diseases Handbook**, Infectious Diseases Working Group EAZWV, 601-604
- 59 Tully T (2001) "Update on *Chlamydophila psittaci*" **Seminars in Avian and Exotic Ped Medicine** 10, 20-24
- 60 Tunca R, Toplu N, Kirkan S, Avci H, Aydogan A, Epikmen E, Tekbiyik S (2012) "Pathomrphological, immunohistochemical and bacteriological findings in budgerigars (*Melopsittacus undulatus*) naturally infected with *S. Gallinarum*" **Avian Pathology** 41(2)
- 61 Vanrompay D, Harkinezhad T, Walle M, Beeckman D, Droogenbroeck C, Verminnen K, Leten R, Cauwerts K (2007) "*Chlamydophila psittaci* Transmission from Pet Birds to Humans" **Emerging Infectious Diseases** 13(7), 1108-1110
- 62 Vanrompay D, Lublin A, Vanlooock M, Mulenga S (2000) "Serology of *Chlamydiaceae psittaci* Infections in Psittaciformes by Use of a Recombinant Enzyme-Linked Immunosorbent Assay: Recombinant DNA Technology at Work" **Seminars in Avian and Exotic Pet Medicine** 9(1), 43-49
- 63 Varsani A *et al.* (2010) " A unique isolate of beak and feather disease virus isolated from budgerigars (*Melopsittacus undulatus*) in South Africa" **Archives of Virology** 155, 435-439
- 64 Varsani A, Regnard G, Bragg R, Hitzeroth I, Rybicki P (2011) "Global genetic diversity and geographical and host-species distribution of beak and feather disease virus isolates" **Journal of General Virology** 92, 752-767
- 65 Verstappen F (2005) "Aspergillosis in american parrots after corticosteroid therapy for smoke-inhalation injury" **Journal of Avian Medicine and Surgery** 19(2), 138-141
- 66 Vincent P, Leclerq A (2008) "Sudden onset of Pseudotuberculosis in Humans, France, 2004-05" **Emerging Infectious Diseases** 14(7), 1119-1122
- 67 Weber W (1979) "Pigeon Associated People Diseases" **Bird Control Seminars Proceedings**, Paper 21
- 68 Zhang S *et al.* (2008) "*Yersinia pseudotuberculosis* strains can be divided into 21 different O-serotypes or O-genotypes on the basis of the difference in the lipopolysaccharide (LPS) O side chain (O-antigen) or in the O-antigen cluster" **Journal of Veterinary Diagnostic Investigation** 20, 356-359

Appendix I

Table I - a: Mammals' casuistic of the author's externship at the *Ménagerie du Jardin des Plantes*, Paris, France (2013).

Common Name	Scientific Name	N° of Animals*	Procedure(s) in one or more animals
Anoa	<i>Bubalus depressicornis</i>	1	Capture; Testicular trauma; Follow-ups and wound management;
Arkal	<i>Ovis aries arkal</i>	1	Capture; Emergency care; Drugs administration;
Bennett's Wallaby	<i>Macropus rufogriseus</i>	4	Capture; Anaesthesia; Orchiectomy; Follow-ups and drugs administration; Podal Surgery ; Radiography; Euthanasia; Necropsy;
Bharal	<i>Pseudois nayaur</i>	1	Capture; Podal pathology; Follow-up;
Binturong	<i>Arctictis binturong</i>	5	Capture; Obstetric and gynecological examination; Sexing; Vaccination; Exploratory laparotomy; Reproductive pathology; Follow-ups and drugs administration;
Brown Capuchin	<i>Cebus apella</i>	1	Capture; Physical exam; Blood sampling;
Capybara	<i>Hydrochoerus hydrochaeris</i>	4	Deworming;
Collared Mangabey	<i>Cercocebus torquatus</i>	1	Capture; Anaesthesia; Echocardiography; Radiography;
Dromedary	<i>Arabian camel</i>	3	Capture; Anaesthesia; Dental treatment; Reproductive management; Emergency care - trauma management - surgery; Follow-ups drug's administration;
Gaur	<i>Bos gaurus</i>	1	Podal pathology; Follow-ups and drugs administration;
Guinea Pig	<i>Cavia porcellus</i>	37	Capture; Deworming; Pododermatitis' treatment; Dermatologic pathology - abscess treatment; Trauma - Neurological disease; Emergency treatment - minor surgery; Follow-ups and drugs administration; Echography; Radiography; Necropsy;
Long-nosed Potoroo	<i>Potorous tridactylus</i>	1	Capture; Anaesthesia; Ophtalmologic Surgery; Pododermatitis; Radiography; Follow-ups, wound management and drug's administration;
Markhor	<i>Capra falconeri</i>	1	Capture; Emergency care; Podal pathology; Follow-ups and drugs administration; Anaesthesia; Radiography; Euthanasia; Necropsy
Pallas's Cat	<i>Otocolobus manul</i>	1	Capture; Follow-ups and drugs administration; Trauma management;
Patagonian Mara	<i>Dolichotis patagonum</i>	13	Capture; Inventory; Placing of identification chip; Deworming; Emergency care; Anaesthesia; Podal Pathology; Dystocia - obstetric and gynecologic exam; Euthanasia; Necropsy;
Poitou Donkey	<i>Equus asinus</i>	1	Capture; Vaccination;
Przewalski's Horse	<i>Equus ferus przewalskii</i>	2	Capture; Anaesthesia; Hoof trimming; Orchiectomy; Horse's identification with liquid nitrogen; Dental treatment;
Pudú	<i>Pudu mephistophiles</i>	1	Capture; Anaesthesia; Podal pathology - Hoof trimming; Follow-up;
Raccoon	<i>Procyon lotor</i>	3	Capture; Anaesthesia; Orchiectomy;
Rock Squirrel	<i>Otospermophilus variegatus</i>	3	Capture; Anaesthesia; Emergency care; Orchiectomy; Penile Trauma; Oral Surgery Follow-ups and drugs administration;
Rocky Mountain Goat	<i>Oreamnos americanus</i>	1	Capture; Emergency care; Horn trauma; Follow-up;
Snow Leopard	<i>Uncia uncia</i>	3	Follow-ups and drug's administration; Ophthalmologic exam and treatment; Necropsy;
Vicuña	<i>Vicugna vicugna</i>	1	Capture; Sedation and Anaesthesia; Dental treatment; Deworming;
Visayan Warty Pig	<i>Sus cebifrons</i>	2	Anaesthesia; Chemical castration;
West Caucasian tur	<i>Capra caucasica</i>	1	Capture; Dystocia - obstetric and gynecological examination; Anaesthesia; Follow-ups and drugs administration;
Yack	<i>Bos grunniens</i>	3	Deworming;

*Total of mammals: 96

Appendix I

Table I - b: Avian' casuistic of the author's externship at the *Ménagerie du Jardin des Plantes*, Paris, France (2013).

Common Name	Scientific Name	N° of Animals*	Procedure(s) in one or more animals
African Sacred Ibis	<i>Threskiomis aethiopicus</i>	32	Capture; Anaesthesia; Endoscopy (sexing); Placing of identification chip; Deworming;
Blue Crane	<i>Anthropoides paradiseus</i>	1	Capture; Anaesthesia; Beak Surgery; Deworming;
Blue-and-Yellow Macaw	<i>Ara ararauna</i>	8	Capture; Cloacal swab;
Budgerigar	<i>Melopsittacus undulatus</i>	27	Emergency care; Radiography; Follow-ups and drugs administration; Cloacal Swab; Euthanasia; Necropsy;
Channel-billed Toucan	<i>Ramphastos vitellinus</i>	1	Capture; Emergency care; Follow-ups and drugs administration; Necropsy;
Chicken	<i>Gallus gallus domesticus</i>	1	Capture; Deworming;
Common Moorhen	<i>Gallinula chloropus</i>	2	Capture; Anaesthesia; Endoscopy (sexing);
Crested Pigeon	<i>Ocyphaps lophotes</i>	25	Capture; Deworming;
Fulvous Whistling Duck	<i>Dendrcygn a bicolor</i>	2	Capture; Deworming;
Great Green Macaw	<i>Ara ambiguus</i>	2	Capture; Cloacal swab;
Grey-winged Trumpeter	<i>Psophia crepitans</i>	1	Capture; Emergency care; Radiography; Orthopedic surgery; Podal pathology; Anaesthesia; Wound management; Follow-ups and drug's administration; Euthanasia; Necropsy; Pediatric emergency care;
Guira Cuckoo	<i>Guira guira</i>	1	Necropsy;
Hawaiian Goose	<i>Branta sandvicensis</i>	2	Capture; Deworming;
Hyacinth Macaw	<i>Anodorhynchus hyacinthinus</i>	1	Capture; Deworming; Cloacal swab;
Kea	<i>Nestor notabilis</i>	2	Capture; Cloacal swab;
Laughing Kookaburra	<i>Dacelo novaeguineae</i>	2	Capture; Beak trimming;
Military Macaw	<i>Ara militaris</i>	2	Capture; Cloacal swab;
Monk Parakeet	<i>Myiopsitta monachus</i>	2	Necropsy;
Nanday Parakeet	<i>Nandayus nenday</i>	1	Emergency care; Radiography; Euthanasia;
Northern Lapwing	<i>Vanellus vanellus</i>	1	Capture; Ophthalmic pathology - Ophthalmic exam; Follow-ups and drugs administration;
Red-billed Blue Magpie	<i>Urocissa erythrorhyncha</i>	1	Capture; Deworming;
Red-headed Vulture	<i>Sarcogyps calvus</i>	1	Capture; Emergency care; Radiography; Drugs administration; Follow-up;
Scarlet Ibis	<i>Eudocimus ruber</i>	2	Capture; Trimming of nails;
Southern Screamer	<i>Chauna torquata</i>	1	Capture; Cloacal swab; Podal Pathology; Anaesthesia; Orthopedic surgery; Follow-ups; Wound-management;
Stone Curlew	<i>Burhinus oedicnemus</i>	2	Capture; Deworming;
Superb Starling	<i>Lamprotomis superbus</i>	2	Capture; Anaesthesia; Endoscopy (sexing); Placing of identification chip;
Wood Duck	<i>Aix sponsa</i>	1	Capture; Ophthalmologic pathology - Ophthalmologic exam; Follow-ups and drugs administration;

*Total of birds: 126

Appendix I

Table I - c: Reptiles' casuistic of the author's externship at the <i>Ménagerie du Jardin des Plantes</i> , Paris, France (2013).			
Common Name	Scientific Name	N° Animals*	Procedure(s) in one or more animals
Boa Constrictor	<i>Boa constrictor</i>	1	Capture; Emergency care; Follow-ups and drugs administration; Necropsy;
European Green Lizard	<i>Lacerta viridis</i>	5	Capture; Deworming; Necropsy;
Gaboon Viper	<i>Bitis gabonica</i>	1	Necropsy;
Galápagos Tortoise	<i>Chelonoidis nigra</i>	2	Capture; Shell repair;
Green Tree Python	<i>Morelia viridis</i>	1	Capture; Emergency care; Neurologic pathology; Follow-ups and drugs administration;
Indian Python	<i>Python molurus</i>	3	Capture; Biometric mesures; Deworming;
Knight Anole	<i>Anolis equestris</i>	1	Capture; Ophthalmologic exam - Ophthalmologic pathology; Anaesthesia; Follow-ups and drugs administration;
Malagasy Tree Boa	<i>Boa mandrita</i>	1	Capture; Emergency care; Trauma - wound management; Follow ups;
Radiated Tortoise	<i>Astrochelys radiata</i>	7	Dermathologic treatment; Follow-up;
Reticulated Python	<i>Python reticulatus</i>	2	Capture; Biometric mesures; Deworming;
Rock Monitor	<i>Varanus albigularis</i>	4	Capture; Ophthalmologic exam; Anaesthesia; Ophthalmologic surgery; Deworming; Follow-ups and drugs administration; Emergency care; Oral swab; Geriatric management; Euthanasia; Necropsy;
Spur-thighed Tortoise	<i>Testudo graeca</i>	6	Capture; Biometric mesures; Deworming;
*Total of reptiles: 34			

Appendix II

Table II: Physical examination of psittacines in a zoological collection - *What not to miss?*

Steps of the exam	Checking for	Possible causes and Observations	Ref.*
Behaviour	Ruffled feathers and wings far from the body(1); Eyes closed, head tucked under the wings, isolated and uninterested in the surrounding environment(2); Tail bobbing up and down with the breathing and breathing with opened mouth(3); Signs of regurgitation and abnormal droppings(4); Muscle tremors(5); Lameness;	(1)Difficulties in body temperature regulation; (2)Depression; (3)Respiratory distress; (4)The examiner can confirm the existence of regurgitation if sticky clusters of regurgitated seeds or a sticky caramel-like substance are on the bottom of the cage or surrounding the bird's mouth; this may indicate behavioural problems, stress or gastro-intestinal tract disorders; (5)Hypocalcaemia, poisoning or pruritis;	49,51,54
Head	Large atypical feathers(6); Abrasions; Fractures; Abnormal contour of the skull; Abnormal positioning of the head(7);	(6)Integumentary disorders; (7)Orthopedic or neurological disorders;	
Ears	Exudates(8); Stenosis; Foreign bodies; Wounds;	(8)Cause feathers' discoloration;	
Eyes	Swollen; Blepharospasm; Blepharitis; Hyphema(9); Corneal ulcers; Masses (abscesses, tumors, hematoma); Protrusion of the nictitating membrane(10); Epiphora(11); Chemosis; Abnormal pupillary reflex(12); Cataracts(13); Blindness;	(9)Trauma; (10)Can be as a result of infection; (11)Often a sign of terminal disease in birds the size of an African grey parrot or larger; (12)Pupillary light reflex is difficult to access in the normal bird as it is under voluntary control. So if the response is rapid this indicates CNS damage; (13)Found in Cockatiels, budgerigars, cockatiels, aged parrots;	49,51
Cere & Nostrils	Swellings; Proliferation of exuberant tissue(14); Occlusions; Exudates; Asymmetry;	(14)Infestations by <i>Knemidokoptes spp.</i> ;	49
Beak	Fractures; Abnormalities of growth or shape; Wry beak; Atrophy; Malocclusion; Fragility; Flakiness; Abnormal colouration; Hyperkeratinization(15); Tumors(16);	(15)Infestations by <i>Knemidokoptes spp.</i> ; (16)Such as carcinoma, sarcoma and osteosarcoma often cause distortion of the beak and must be differentiated from bacterial, viral and fungal granulomas;	49,51
Mouth & Tongue	Stomatitis; Foreign bodies; Burnings; Tears; Tumors; Abscesses; Abnormal odors;	The most obvious lesions are caseous abscesses involving the tongue or choana but tumors are occasionally seen;	49,51,54
Crop	Foreign bodies; Impaction; Dilatation(18); Masses(17);	(17)Neoplastic masses inside the wall are usually accompanied by a fetid odor;	49,54
Pectoral area	Abnormal configuration; Body condition; Masses(18); Hemorrhage; Bradycardia; Subcutaneous accumulation of air(19);	(18)This area is a common site for superficial tumors particularly lipoma in budgerigars; The normal heart rate is 300-500 beats per minute so it has limited value in small psittacines; Normal respiratory rate is 85 breaths per minute. Chirping sound may suggest infection of the air sacs; (19)Rupture of an air sac;	49, 51
Abdomen	Abnormal consistencies of the liver or abdominal organs; Egg retention; Herniation; Abnormal distension(20);	(20)Internal tumour, cyst or peritonitis;	
Tail & Vent	Tumors in the uropygial or preen gland(21); Impaction of the preen gland; Broken tail feathers; Soiled vent feathers(22); Prolapse, impaction or tumour in the cloaca;	Some parrots may not have preen gland. (21)Squamous cell carcinoma, adenoma and abscesses; (22)Gastro-intestinal disease;	49, 54
Wings	Abnormal positioning(23); Wounds; Masses; Swollen joints; Fractures;	(23)Neoplasia, metabolic bone disease or fracture;	
Pelvic Limbs	Fractures; Dislocations; Temperature; Swelling of the joints(24); Abnormal shape; Overgrowth of the nails; Toes deformities;	(24)Urates deposits, bumble-foot, staphylococcal arthritis;	
Skin and feathers	Malformed, broken or loss of feathers; Feather picking generalized or restricted to a specific area; Excessive moult; Dried mucus in the feathers(25); Stress lines(26); Abnormal coloration; Feather cysts(27); Wounds; Ectoparasites;	(25)Dried mucus in the feathers is an indication of recent regurgitation particularly in the budgerigar; (26)Stress lines occur where the barboles fail to hook together due to malformation and lead to structural weakness; (27)Not common in psittacines;	51
*Ref.: References			

Appendix III

Table III: Demographic evolution of the budgerigar's population in the *Ménagerie du Jardin des Plantes*, Paris, France. Data of September 2012 until May 2013. Is also referred the number of necropsies performed every month.

	Timeline of Chlamydiosis Outbreak									Total
	2012				2013					
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	
Nº A.I.	91	89	89	84	73	62	54	44	41	40
Nº D. (M.F.U)	2 (0.0.2)	0 (0.0.0)	5 (3.2.0)	11 (3.0.8)	11 (3.4.4)	8 (4.3.1)	10 (5.4.1)	3 (2.1.0)	1 (1.0.0)	51
Nº P.N.	0	0	4	3	1	2	10	2	1	23

N° A.I.: N° of alive individuals; **N° D. (M.F.I):** Number of deaths (Male.Female.Undefined Sex); **N° P.N.:** Number of performed necropsies;

Table IV: Necropsy findings in the necropsies performed to the budgerigars by the author during the externship at the MJP (2013).

Total number of budgerigars submitted to necropsy from mid-February until mid-May = 14	
.External exam	
Slimness to cachexia (9/14)	Nasal secretion with discoloration of the feathers surround the beak (5/14)
Beak hiperkeratinization (5/14)	Cloaca befouled with greenish feces and urates, suggesting diarrhea (5/14)
Abnormal coloration of the beak (6/14)	Cloaca with an obstruction formed by feces, urates, food and feathers (4/14)
Partial to total nares' obstruction (6/14)	
.Internal exam	
Gastrointestinal Tract	
Distended crop full with dry seeds (3/14)	Intestines full of liquid green feces (5/14)
Distended crop with seeds involved in a whitish secretion (2/14)	Intestines full of black feces (5/14)
Enlargement of the proventriculus (3/14)	Typhlitis (2/14)
Enteritis (8/14)	White miliar punctuations in the intestine (1/14)
Rectum calcification (1/14)	
Pancreas	
Enlarged pancreas with white coloration (3/14)	
Liver	
Hepatomegaly (7/14)	Yellow areas (1mm) scattered diffusely throughout the parenchyma (5/14)
Congestion (5/14)	White areas (1mm) scattered diffusely throughout the parenchyma (1/14)
Friability (7/14)	Cirrhosis (1/14)
Heterogeneous coloration (4/14)	Liver with yellow coloration suggesting hepatic lipidosis (1/14)
Spleen	
Splenomegaly (10/14)	Pallid (2/14)
Congestion (5/14)	
Heart	
Auricular congestion (2/14)	
Lungs & Air Sacs	
Congestion of the lungs (6/14)	Opacification of the thoracic air sacs (3/14)
Fibrous plaques with yellow coloration in the thoracic air-sacs (8/14)	Fibrous plaques with yellow coloration in the thoracic air-sacs (6/14)
Urogenital Tract	
Congestion (6/14)	Testicles enlargement suggesting orchitis (4/14)
Ureters full of urates (5/14)	

Appendix III

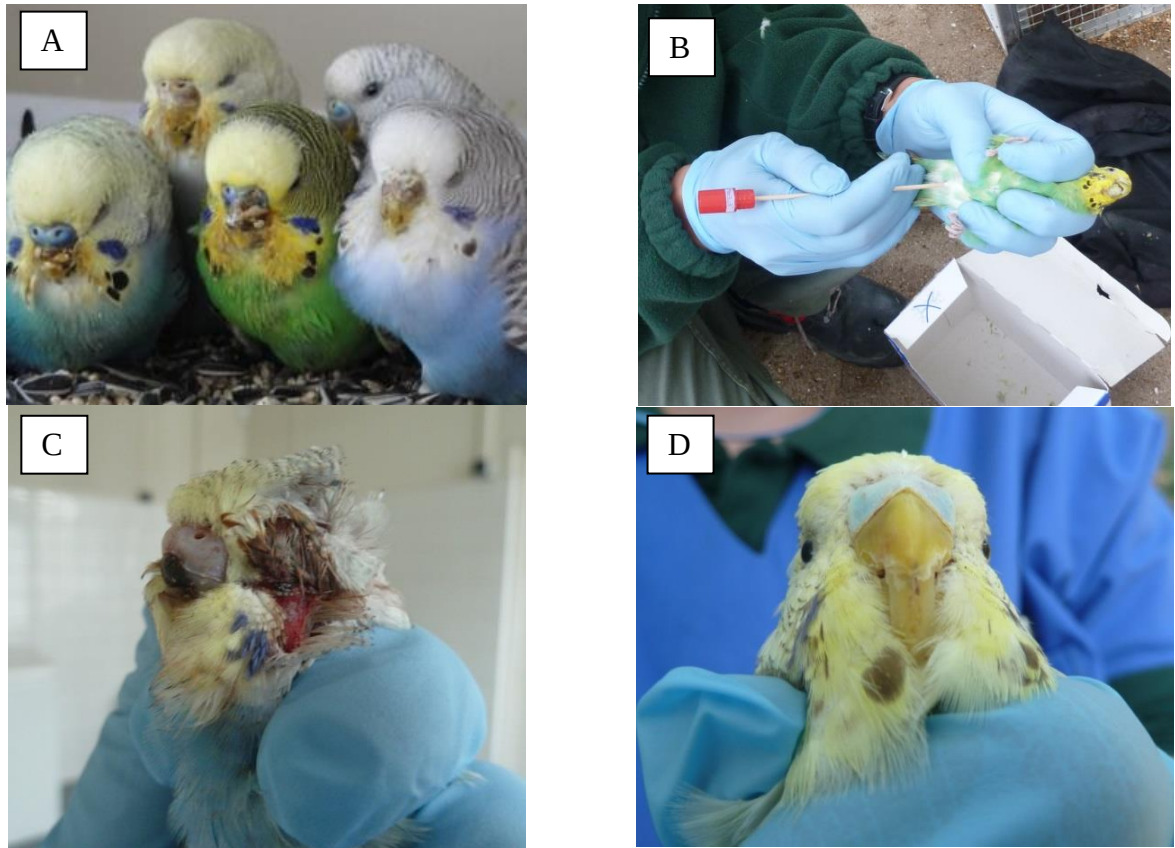


Figure 1: **A)** Budgerigars showing signs of disease (depression, ruffled feathers, nasal discharge, signs of regurgitation) ; **B)** Cloacal swab in a budgerigar showing how to handle a small psittacine; **C)** Budgerigar attacked by other parrots (absence of the beak and ocular heamatoma); **D)** Budgerigar with beak's deformation (sign of ectoparasitism).

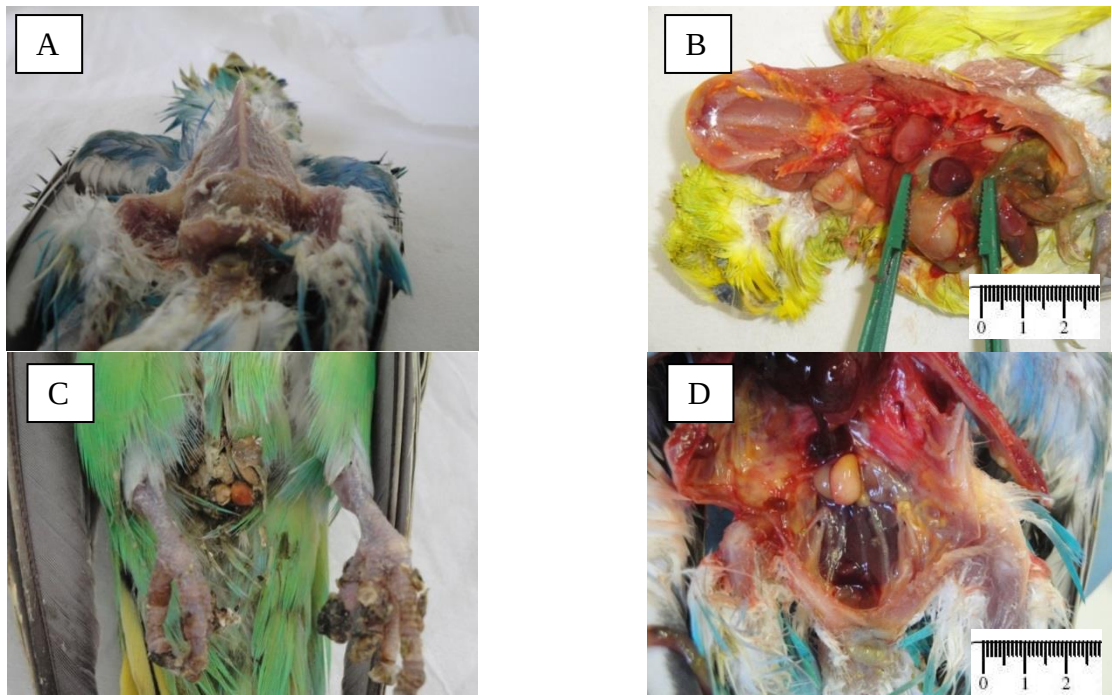


Figure 1: **A)** Budgerigar showing severe emaciation; **B)** Bugarigar presenting with splenomegaly, watery diarrhoea and yellow plaques in the abdominal and thoracic airsacs; **C)** Budgerigar with cloacal obstruction; **D)** Budgerigar's necropsy showing enlarged testicles, congested lungs, yellow "plaques" in the abdominal and thoracic air-sacs and ureters full of urates.

Appendix IV

Table V: Laboratory methods currently used for diagnostic of Chlamydophilosis.

Technique	Sample collection	Observations	Ref.
Citology	<i>Smear</i> Feces-, choanal-, cloaca- and conjunctiva swabs. Liver, spleen air-sac membranes or exsudates from the pericardium.	<i>Advantage:</i> -Quick test; <i>Disadvantage:</i> -Intracitoplasmatic bodies are difficult to detect; -A positive test is confirmatory while a negative one does not rule out chlamydophilosis.	49, 51, 54, 33
	<i>Smear plus IF</i>	More sensitive than simple staining.	54, 33
Bacterial Culture	Liver, spleen, lungs, kidneys, cloaca swabs and feces.	<i>Advantage:</i> -Direct demonstration of the bacteria; -Sensitive; <i>Disadvantage:</i> -False-negative results occurs when the bacteria are no longer viable; -May be difficult to culture due to it obligate intracellular nature; -Results are poorest with desiccated fecal samples, subjected to bacterial deterioration and contamination; -Administration of several antibiotics (chloranphenicol, tylosin, erythromycin and tetracyclines) can also interfere with the results;	49, 51, 54, 59, 33
Antigen Detection Systems	<i>ELISA</i> Cloacal- (preferable), choanal-, conjunctival-swabs and feces.	<i>Advantage:</i> -Antigen test kit available; -Sensitive and quick to perform; <i>Disadvantage:</i> -False-positive when there is insufficient numbers of bacteria in the sample;	54
	<i>PCR</i> Conjunctival-, choanal- and cloacal- swabs. Feces, tissue biopsy, whole-blood samples, organs post-mortem, cell cultures and embryonated eggs.	<i>Advantage:</i> -It is the most sensitive and specific test available and can provide rapid results; -Requires minimal sampling; -Rapid results; -High sensitivity and specificity; <i>Disadvantage:</i> -Do not differentiate between viable and nonviable organisms infecting the bird, environmental contamination, or segments of the organism passing through the G.I tract or being immunologically neutralized by the immune system.	57, 58, 59
	<i>Nested PCR</i>	More sensitive than simple PCR; Can distinguish clearly Chlamydiaceae species or strains;	59
	<i>TapMan*</i> Whole blood.	Detect rRNA and/or ompA gene for the family Chlamydiaceae, being able to determine if it is or not a viable organism infecting the bird.	59
	<i>Multiplex tests*</i> Whole blood.		
Antibody Detection Systems	<i>ELISA</i>	A fourfold rise in titer with paired samples taken ten days apart is considered conclusive for a positive result. <i>Advantage:</i> -Sensitivity and the possibility of automatization; <i>Disadvantage:</i> -Under treatment with tetracyclines the production of humoral antibodies can be severely inhibited, and no increase in titer may occur; -Serum containing low levels of antibody makes difficult to find and define correct cut-off levels;	51, 37, 33
	<i>BELISA</i> Blood serum.	<i>Advantage:</i> -Can detect infected birds that excrete or not the agent; -Test kit available; <i>Disadvantage:</i> -False-negative results may occur in: a) Fresh infections (no antibody production as yet); b) Following treatment (inhibition of antibody production and no shedding of the agent); c) Cross reactions with other bacteria;	54
	<i>HI</i>	This test use mouse erythrocytes.	33
	<i>ID</i>	It is quick and simple only requiring agar gel.	
	<i>Immuno-chromatography</i> Cloacal- (preferable), choanal-, conjunctival-swabs and feces.	It is a quick and simple test.	33

*: Experimental techniques; **IF**: Immunofluorescence; **ELISA**: Enzyme Linked Immunosorbent Assay; **PCR**: Polymerase Chain Reaction; **BELISA**: Blocking Enzyme-Linked Immunosorbent Assay; **HI**: Hemagglutination inhibition; **ID**: Immunodiffusion; **Ref.**:References

Appendix IV

Table VI: Antibiotics used for the treatment of <i>Cp. Psittaci</i> infections in Parrots.				
Antibiotics	Admn route	Dose	Observations	Ref.
Tetracyclines				
	CTC	PO	500 ppm in feed, SID for 30 days - SSP 2400 ppm in feed, SID for 45 days - MSP	Therapeutic levels in 10 days. Not allowed other food components besides the medicated food (especially if it contains calcium). Fresh water available all the times. Excreted mainly by the kidneys.
	OCT	IM	75 - 100 mg/Kg PV	Therapeutic levels in hours. Stop shedding <i>Cp. psittaci</i> in 24h post-injection. Birds can remain on their normal diet.
			25 - 35mg/Kg PV, in feed, SID for 21 days	
		PO	400 - 600 mg/Kg PV, per litre of drinking water	Toxicity can occur with this protocol (depression, inactivity, anorexia, biliverdin-stained urine, and altered enzymes).
	DXC	IM	75 - 100 mg/Kg PV, SID, every 5 days for 45 days	Induce blood levels of 1µg/mL that last for 7 days. Shedding of <i>Cp. psittaci</i> ceases within 24 hours. Excreted extra-renally (feces, bile, etc) and metabolites are microbiologically almost inert.
		IV	25 - 50 mg/Kg PV, SID-BID	Dose for the initial critical presentation, after which the bird should be switched to the PO or IM formulation.
Macrolid				
	AZT	PO	40 mg/Kg PV, SID for 30 days	
Fluoroquinolone				
	ENR	PO	250 ppm, in feed for 21 days in SSP 500 ppm, in feed for 21 days in LSP	Necessary minimum blood level of 0,5 mg/L for at least 14 days.
Legend: Admn route: administration route; Ref.: References; CTC: Chlortetracycline; OCT: Oxytetracycline; DXC: Doxycycline; AZT: Azithromycin; ENR: Enrofloxacin;				