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# *CHLAMYDIA PSITTACI* IN THE PARROTS, PIGEONS AND CANARIES IN THE CITY OF TIRANA

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***Abstract***

*The growth of decorative birds in Albania in the recent years has brought an increased frequency of chlamydial infection not only in the flocks of birds but, also to humans. According to the data from the Public Health Institute (PHI) in Tirana, is it has been observed an increase in the vulnerability of people, mainly in to those who are in the young age who have been in contact with decorative birds. This study, the first of its kind conducted in Albania, was based on a serological control of 573 blood samples which were taken from pigeons, parrots and canaries in Tirana, Albania. Sampling was carried out in all four seasons of the year 2011 and their control was carried out by the Institute of Veterinary Food Safety (FSV) Tirana. In order to check them it was used an indirect immunofluorescence test (IFT), combined with chlamydia's isolation in chicken egg embryo cells, as a comparative method, in which there were identified at least 51 cases with Ch. psittaci. Relative specificity of fluorescent antibody's test in the serum was approximately 95.3% and the relative sensitivity was about 60.3%. Results of this study, which as mentioned above were conducted for the first time in Albania, showed that immunofluorescence tests performed using diagnostic kits of Medical servicce-2000, combined with primary isolation of chlamydia in embryo of chicken eggs,were very specific and very useful for the identification of useful option for veterinary service.*

***Keywords:*** *fluorescent antibody, immunodepression, immunofluorescence overhead, relative sensitivity seropositivity.*

## INTRODUCTION

Cage birds parrots, canaries, and pigeons showed different sensitivity to *C.p*sittaci, causing their injuries, but also a risk for the spread of this disease with zoonotic nature. (Barnes, RC: 1989 and Pospisil, L., et al: 1996). Even in Albania from year to year, this category of birds has been increasing, serving as a potential risk in to the spread of the chlamydial infection not only in flocks of birds, but also in humans. (Pospisil, L., et al: 1996). Evidence shows that during the period 2001-2011 there have been reported about 635 cases of individuals infected with chlamydia (PHI: 2011) who mostly were pople in their young age or individuals involved decorative bird breeding. Expressed clinical signs were depending on chlamydia's pathogenicity, type, race, and physiological condition, age of the bird, route and time frame of exposure, stressful factors, immunodepresive and situation of the birds (Lublin, A., et al: 1993) and the presence of other infections in an interaction. Psittacosis' soft "explosion's" can often go unnoticed because there are no clear symptoms. The most obvious are those of airways and diarrhea (Avian Disease Manual, 1983). In adult wild birds,C. psittaci is often seen without clinical signs and they can serve as asymptomatic carriers**.** The infection can develop in the acute form, sub-acute, or it can be chronic. In the acute form the disease can cause severe damages, which can be fatal, especially for some species, while at younger birds, it appears to be highly sensitive. The most typical sings are seen on young birds, which often appear weak, showing anorexia, they faint, lie in a special position, from their eyes and nose there is a purulent flow, they usually are contracted and stay with disheveled feathers (Gerlach 1986).

Diagnostic methods used for detection of chlamydic infection in the birds are numerous. For specificity, sensitivity, speed and relativity

and its simplicity, the identification of chlamydial antibodies, especially in sub-clinical cases, is made by using the indirect immunofluorescence test which is considered to be the most effective test, whereas the primary isolation of chlamydia of chicken embryos **(**Andersen, A.A.1991) can also be used as comperative method (Kennedy G, et *al: 1985*). In different species such as, pigeons, parrots and canaries, it has been seen that their immunological responses towards their organism can be different (Geens, T. et al. 2005). The study showed that organs taken from dead birds which were infected with *C. psittaci* were important in identifying the infection by using diagnostic kits. The aim of the study was to develop and evaluate a rapid antemortem diagnostic technique for detection of *C. psittaci* in the serum of birds. Also as a quick and accurate method it can allow a veterinary doctor to immediately begin treating the sickening birds thus, preventing the infection to spread from domestic birds to humans, Greub,G.2010**)** which is the basic objective of the veterinary medicine.

## MATERIALS AND METHODS

For conducting the study, parrots and canaries, were selected inclusive, and for the control of pigeons were included and those races where as indicated had cases of people affected by this infection.The perennial scope had as an objective the study of the dynamics of the disease, as well as climatic factors correlating with other environmental issues. The process of taking the blood samples from decorative birds was carried out in batches, at 0.5 ml / head, which were marked and have been monitored throughout the year, 573 samples which prevailed according to their species were: 118 heads of parrots, 348 pigeons and 107 canaries, of which 30% were selected from young birds, varying from 2-4 months old. As a laboratory method it was selected the *indirect immunofluorescence test* (IFT), which because of its high specificity helps in all sub-clinical cases, when a seropositive birds lack a complete clinical framework. It is fast and simple to implement in the field and laboratory procedures, and also combined with primary isolation method of chlamydia in the chicken embryo, it can obtain rapid and accurated data (Andersen, AA, 2008). The IFT control procedure was based on the principle of reaction of 573 blood samples, using IgG Antibody Kit, imported by Medical Servicce-2000. The separation of serum was made using the usual metthod, by following rigorously all steps to preserve the kit, dilution, incubation, testing and control of the material prepared. At the end the reading of the small droplet shaped spots was made using a 400x magnification for each tile, and then they were compared with the visual intensity of basic troops, shown in the positive control well and negative.The tiles were stored in a dark room in a temp 2-8 degree celsius for a period of 24 hours. In the positive responses appeared a fluorescent glow, sharp, regular elementary troops and stained, which was rated at (1 +, 2 +). The isolation of chlamydia cells in chicken embryos was carried out according to standard procedures, injected into the viteline sac, up 0.5 ml.i emulsion prepared with positive material from positive birds and suspected, from the lungs, liver, spleen, trachea and air sacs injured birds and sacrificed, in chicken embryos aged 6-7 days. Then they were placed for incubation at 39 degree celsius. After that it was carefully observed the replication of chlamydia, and evaluation of results was done from the fetus state which, in case it results positive, usually dies within 5-12 days after inoculation. After the histopathological control of infected embryos with the typical chlamydie infection, the material was collected and homogenised with a 20% suspension sacus vitelinus. The Identification of the agent was carried out by preparation of an infected sakus vitelinus antigen.

## RESULTS AND DISCUSSIONS

Study shows that in areas chlamydic infection monitoring circulating in the following levels: in parrots: 12.7%, pigeons: 8.4%, and canaries: 7.47%. Chlamydia focus in birds' bodies but, more sensitive and higher concentrations they are found in the lungs. IFT method, combined with the isolation of chlamydia′s in embryos of chicken eggs, as well as histopathological control of the heads of damage, proved to be effective for disease control, the practical implementation of field and laboratory procedures. Chlamydia chicken embryo grew

and multiplied in the first pass, where the largest concentration of them is in the core of an egg. Death of embryos, under the action of chlamydia is high in percentage; the most pathogenic were chlamydia which was separate from the parrots and less pigeons and canaries. Identification of *C.psittaci* is done by the clinically healthy birds, and those coupled other pathologies, where the intensity of touching the bodies, in the first case has been much lower than in cases with pathology combined with other pathology, aspergilosis, parasites etc. The morbidity rate of birds only by chlamydia are observed to be as 10, (7 parrots and 3 pigeons), but always accompanied with other causes, which have enabled C.psittaci where after histopathology 'Basic ' are found localized in the brains of damaged birds. The clinical disease has been evident and obvious mainly in young birds, which make possible a veterinary service, whereas adults show a sub-clinical form, serving as an asymptomatic carrier, helping to permanent recycling of infection. The survey data show that chlamydia are ordinarily resident in the bodies of birds, which are activated when lowered their sustainability as a result of stress and other resources that accompany infection, findings that are compatible with those of many foreign authors. Recognition of favorable factors and the development cycle chlamydia′s can serve as options for preventive measures by the veterinary service to control the disease in poultry in general and particularly decorative, and as a zoonosis, prevention and protection of human health.

Table 1. Information about the positivity of chlamydiosis in parrots by the breeds (in%)

| Race | Spring | | | Summer | | | Autumn | | | Winter | | | Annual | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection |
| Total | **28** | **4** | **14.2** | **26** | **6** | **23** | **34** | **3** | **8.8** | **30** | **2** | **6.6** | **118** | **15** | **12.7** |
| Amazon | **8** | 2 | 25 | **7** | 3 | 42.8 | **10** | 1 | 10 | **10** | 1 | 10 | **35** | 7 | 20 |
| Ondule | **8** | 1 | 12.5 | **7** | 2 | 28.5 | **10** | 1 | 10 | **10** | 1 | 10 | **35** | 5 | 14.3 |
| Calopsitte | **6** | 1 | 16.6 | **6** | 1 | 16.6 | **7** | 1 | 14.3 | **5** | 0 | 0 | **24** | 3 | 12.5 |
| Cacatoe | **6** | 0 | 0 | **6** | 0 | 0 | **7** | 0 | 0 | **5** | 0 | 0 | **24** | 0 | 0 |

Table 2. Information about the positivity of chlamidiosis in pigeons by breeds (in %)

| **Race** | Spring | | | Summer | | | Autumn | | | Winter | | | Annual | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Birds Tested | Positive + | % infec-tion | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection |
| **Total** | **84** | **8** | **8.5** | **89** | **11** | **12.3** | **95** | **6** | **6.3** | **80** | **3** | **3.3** | **348** | **28** | **8.04** |
| **Rancing** | **44** | 5 | 11.3 | **44** | 7 | 15.9 | **41** | 3 | 7.3 | **40** | 2 | 5 | **169** | 17 | 10.05 |
| **Other** | **40** | 3 | 7.5 | **45** | 4 | 8.8 | **54** | 3 | 5.5 | **40** | 1 | 2.5 | **179** | 11 | 6.14 |

Table 3. Information about the positivity of chlamydiosis in canaries by breeds (in%)

| **Race** | Spring | | | Summer | | | Autumn | | | Winter | | | Annual | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection | Birds Tested | Positive + | % infection |
| **Total** | **29** | **3** | **10.3** | **26** | **3** | **11.5** | **29** | **2** | **6.9** | **23** | **0** | **0** | **107** | **8** | **7.5** |
| **Serina** | **19** | 2 | 10.5 | **16** | 2 | 12.5 | **19** | 1 | 5.3 | **13** | 0 | 0 | **67** | 5 | 7.5 |
| **Belge** | **10** | 1 | 10 | **10** | 1 | 10 | **10** | 1 | 10 | **10** | 0 | 0 | **40** | 3 | 7.5 |

The presentation of data, in tables No. 1, 2, 3, in overall it has been identified a total of 51 heads of seropositive birds, of which 15 heads of parrots, 28 heads of pigeons, 8 heads of canaries, and by using the primary isolation method of chlamydia in chickens embryos, there were prepared 15 samples, of which 5 were parrots, 7 were pigeons and 3 canaries. Selecting the method of indirect immunofluorescence in determining the positive samples resulted to be very useful especially in sub-clinical cases, when complete clinical signs were missing, mostly in adult birds, and combined with the method of isolation chlamydia in chicken embryos, because the death of embryos occurred when they were at the age of 12-18 days, making

completely compatible with IFT score. (Andersen, A.A.2008). From the histologic control of typical chlamydia infection in chicken embryos, in general for all species it was observed vascular congestion of membranes sacus -vitelinus, (Andersen, AA 2008). An important element was observed and the dynamics of infection, which according to the species stands as follows : parrot′s, average annual level of infection has been 12.7%, in spring 14.2%, in summer 23%, in fall 8.8%,and in winter 6.6%. (Table.1). Pigeon′s, average annual level of infection was about 8.04%, 8.5% in spring, in summer 12.3%, in fall 6.3%, while in winter, 3.3%, where is almost is hidden completely. (Table.2) Canarie′s average annual level of infection was 7.47%, in spring 10.3%, in summer 11.5%, in autumn 6.89%, while in the winter it was 0, which means that is completely extinct (Table.3). Although in different species such as parrots, pigeons, canaries the frequency was different, it was strictly related to seasonal conditions of the weather. In spring the infection has shown a tendency to increase, in summer where the weather has been so hot it has shown it maximum value whereas in autumn, with the weather cooling the value has tended to decrease, while in the winter when the weather has been cold the infection is reduced, hidden or wiped out, facts which coincide with that of the foreign authors (Lublin, A., et al, 1995), etc. It is important to be considered as support for increasing the frequency of infection during the months with warm and hot weather there were also many other factors, individually or in the correlations between them have contributed to the situation. Those can be from the: increase of contact between birds with warm weather, activation of arthropods and hematophag insects which help spread the movement vectors of infection in decorative birds, which have also been observed on a case by case basis on the infected birds. While monitoring the incidence of pigeons (not decorative ones) and seropositive races, which make up about 30% of the samples, as a source of infection we should also evaluate the contact with water weeds, which have been polluted with eksements of porter birds. On the other hand unfamiliar areas affected by infection, and the transit during the races(Salinas, J., et al.1993), but also the use of uncontrolled food products with unsafe origin, low hygiene standards of breeding, stresses and strains circulation with high virulence, etc.. Should also be taken more into consideration (Geens, T., et al., 2005). During monitoring, it was noted that infection to young people is organized in acute form causing damage to former company because of their high sensitivity. Young birds have shown signs of weakness typical, anorexia, purulent leak from eyes and nose. Inactivity, stay in position to collect, disheveled feathers (Gerlach, 1986). In cases with mild to developments birds lacked clear symptoms, the most obvious would be those of the respiratory tract and diarrhea (Avian Disease Manual, 2007) making them serve as asymptomatic carriers, recycling permanently the infection. The isolation of chlamydia from chicken egg embryos aged 6-7 days, were made from 15 samples, of which 5 parrots, 7 pigeons and 3 canaries, which resulted seropositive IFT method. To avoid horizontal transmission of infection through eggs, before infecting, 6 embryos were checked aged 6-7 days, two for each species, with IFT method, which resulted in negative from chlamydia. Control embryos was carried out for 14-15 days in a row and the death rate of embryos infected with suspension by the parrots was 100% Infected embryos suspension pigeons was 4, or 57.7%, whereas embryos infected with suspension canary bodies 1, or 33%. Mortality dynamics was observed during the period 3-14 days after infection, and mortality to parrots, 70-80% of them occurred 3-8 days after infection, 10-12 pigeons after infection, while the canary in the day of 13 after infection. Histopathological control of dead embryos dominated by the presence of hemorrhage in the body, in the head region of the feet, thickening of the lining of an egg and the slowdown in their growth and development. Surviving embryos, especially those with suspension by pigeons and canaries, microscopic researches have been identified the 'Basic corpus'. By monitoring the people affected with C. psittaci, in the past three years, there have been a total of 132 cases, and after controlling almost 45 of them, it was found that the disease has been correlated directly from their contact with seropositive birds, parrots,

canaries and doves, and its frequency was variable, with age, the level of exposure and patogenicity were the determining factors for the occurrence and form infection clinic. (POSPISIL, L., et al., 1996)

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The circulating levels of decorative birds with chlamydia infections are considered to be relatively low.*C.psittaci* are mainly focused on birds' bodies but, more sensitive and higher concentrations are to be found in the lungs. The IFT method, combined with the isolation of chlamydia in embryos of chicken eggs, as well as histopathological control of the damaged heads, proved to be effective for controlling the disease, the practical implementation of field and laboratory procedures. Identification of 'elementary bodies' can be done with materials which are taken from bodies stained with May-method Grynvald-Giemsa and by controlling them under a microscope, shows that they are located mainly within the cytoplasm of the cell in the form of meal pomegranate and being a little pink in color. It was observed that the death of the embryos was higher in chlamydia isolated from parrots and less from those in doves and canaries. Identification was done by the clinically healthy birds, where the intensity was much lower however, in cases with combined pathology with mycotic causes, aspergilosis, parasites etc intensity was higher. Morbidity cases of birds just by chlamydia alone are 10 in total, (7 parrots and pigeons 3) but, cases coupled with other causes have dominated. The clinical disease has been evident and obvious mainly in young birds which make possible orientation in veterinary service, whereas adults show a sub-clinical form, serving as asymptomatic carriers, helping to permanent recycling of infection. The study shows that chlamydia are ordinarily resident in the birds' bodies which, are activated when their sustainability is lowered as a result of stress and other resources that accompany infections. The findings are compatible with those of many foreign authors. Recognition of favorable factors and the development cycle in chlamydia can serve as options for preventive measures by the veterinary service to control the disease in poultry in general and particularly decorative birds, and as a zoonosis, in prevention and protection of human health.

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