Journal of Animal and Veterinary Advances 13 (16): 1014-1017, 2014
ISSN: 1680-5593

Chlamydia psittaci in Parrots, Pigeons, Canaries, Peacocks and Pheasants in Albania

[1]Ymer Elezi, [2]Gezime Shehu, [3]Kastriot Korro and [4]Luljeta Qafmolla
[1]Department of Decorative Bird Diseases, Tirana, Albania
[2]Department of Agriculture, Protection of Animal Health, Tirana, Albania
[3]Faculty of Veterinary Medicine, University of Agriculture, Tirana, Albania
[4]Department of Virology, Institute of Food Safety and Veterinary, Tirana, Albania

Abstract: The study is based on the results of 557 samples taken from birds of different species in the Republic of Albania and tested for the presence of *Chlamydia psittaci*. Sampling was conducted at stores trading birds, at a breeding center in a veterinary clinic specialized for bird, public squares and in zoos. According to species, the test included 135 parrots, 210 pigeons, 60 canaries, 80 peacocks and 72 pheasants. Identify specific antigen to *C. psittaci* was made by indirect Immunofluorescence Method (IFT) using a commercial kit. Average results obtained were 20.28% and after additional testing with PCR results were 14.72%. Prevalence according to species, revised after additional testing by PCR Method was: in parrots 21.48, in pigeons 12.38, in canaries 13.33, in peacocks 16.25 and in pheasants 8.88%. But the country based sampling most of the positive birds were found in retail stores 16.21% followed by those obtained from breeders 15.66, zoos 13.94 in public parks 12.66%.

Key words: Immunofluorescence Test (IFT), PCR, seropositivity, relative sensitivity, pigeons

INTRODUCTION

Chlamydia psittaci (*C. psittaci*) is an important problem of public health because it can be transmitted from birds to humans. Clinically, the disease is manifested in birds in its acute form in apparent or subclinical but also as a chronic disease. It affects at least 30 species of birds especially parrots and pigeons but less canaries (Rohde *et al*., 2010; Rodolakis and Yousef, 2010). The main source of the infection is the wild birds such as ducks, geese, pigeons, crows, sparrows, goldfinch and other migratory birds. Some birds are asymptomatic against these bacteria while others may vary. An economic loss can be seen when the turkeys and ducks are affected whereas in parrots is observed a high mortality rate (Wheelhouse and Longbottom, 2012).

The study was conducted in the coastal territory of Albania focused primarily on birds kept in cages like birds as house parrots, canaries, peacocks and pheasants and also in pigeons for sport and leisure as well as peacocks. From these birds, samples were also taken from the breeding items from their stores trading; public parks and zoos were sent for analysis at the Institute of Food Safety and Veterinary (IFSV) and Life Pets Hospital, Tirana and Kosovo. Samples were collected in accordance with Law 10 465, dated 29/09/2011: "Veterinary service in the Republic of Albania" on the control of zoonotic diseases. This obligate intracellular bacterium represents a complex problem in diagnosis. Because of the zoonotic potential of *C. psittaci*, it is important to make a quick diagnosis, decorative birds. Detection of specific antigens like chlamydia has several advantages over techniques of isolation and serologic tests. By many researchers, immunofluorescence method is a convenient test for the diagnosis of chlamydia (Gerlach, 1997; Andersen, 1998).

Since this method, as many others by the time researchers may give non-specific results or suspected false positive, samples were additionally tested by conventional PCR (Hewinson *et al*., 1997; Prukner-Radovcic *et al*., 2005). This disease also affects humans and is called psittacosis but when birds are affected it is called *Chlamydophila psittaci*. People can get easily infected by *C. psittaci* especially when they are in contact with sick birds but can still occur even after receiving preventive protective measures or while feeding and cleaning them (Andersen, 1991, 2004).

MATERIALS AND METHODS

Monitoring was based on analysis of 557 blood samples taken from parrots, pigeons, canaries, peacocks and pheasants, which were sent to VRI, in accordance with the laws into force for the control of zoonosis in the Republic of Albania. Testing included 135 parrots, 210 pigeons, 60 canaries, 80 peacocks and 72 pheasants with

Corresponding Author: Ymer Elezi, Protection of Wildlife, Tirana, Albania

geographical distribution in several cities such as Tirana, Durres and Kavaja. Taking samples was carried out in trading shops of birds in a breeding center in a veterinary clinic specialized in birds, public park and the zoo. Storage, transportation and manipulation are performed in conformity with the rules of asepsis and technical requirements of the laboratory.

Testing was performed with the method of Immunoflurorescence Test (IFT) taking blood samples in amounts of 0.5 mL/head then the birds were marked and monitored throughout the year. This method was selected because of its high specificity which aids in identifying sub-clinical cases as well as its speed and simplicity of its implementation in the field and in the laboratory (Andersen *et al*., 1997; Borel *et al*., 2008; Wheelhouse and Longbottom, 2012). The data of the study was based in the analysis of blood samples, controlled by IFT using the kit imported from: Fuller Laboratories EC REP Mark Medi-Italy Europe Sarl. The separation of serum was made using common methods whereas its storage, dilution, incubation, testing and control of prepared material was carried out rigorously applying all the instructions on how to use the kit. In order to identify the stains in the forms of small droplets a 400x magnification was used for each tile and then the visual intensity of the elementary bodies was compared with those shown in the positive and negative control manholes. The storage of the tiles was made at a temperature 2-8°C in the dark for a period of 24 h. The positive reaction appeared to glow in fluorescent light for regular stained elementary bodies which were assessed by (1+, 2+) (Andersen and Vanrompay, 2003; Andersen, 2004).

Determination of specific chlamydial DNA by PCR: The 38 samples in total which showed a non-specific reaction method Immunofluorescence Test (IFT) were tested by PCR Method. Using DNeasy® Blood and Tissue kit (USA) in accordance with the manufacturer's instructions from all total samples suspected, DNA isolation was performed. Conventional PCR reaction by 264 base pairs of DNA sequence 5'-non-translated segment of *ompA* gene of *C. psittaci* bacteria was strong up during the use of specific primers (Borel *et al*., 2008; Hewinson *et al*., 1997; Sachse *et al*., 2009). The PCR reaction was conducted with the usage of GoTaq Flexi DNA Polymerase (Promega, USA) kit on GeneAmp PCR System 2400 device (Use of Bio Systems, USA). Further, after reinforcement, 10 μL of PCR products were seclusion by electrophoresis on 2% agarose gel stained with ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

From the analysis of 557 blood samples originating from different types of birds using Immunoflurescence Method it was showed that 113 of them were positive for chlamydia or 23.33%. The number and percentage of samples analyzed, positivity for the presence of the bacterium *C. psittaci* and their origin by location and species samples are presented in Table 1. This was the reason why 38 samples that gave positive results with higher values than the positive control tests were re-tested by PCR Method, the results of which are presented in Table 2.

The data showed (Table 1) that the samples are sorted not only on the basis of bird species but also on the basis of their place-making to show the possibility of a higher risk for an outbreak of infection. Given their priority, sampling is performed according to this chart: from poultry breeding farms 177 samples from 145 retail stores samples originating from birds brought to the clinic by their owners 47 samples, 50 samples from public park and 68 samples from zoos. According to test results, positive poultry breeding farms resulted 23.16% followed by bird's trading shops 17.24 from zoos 17.64, the birds brought to the clinic for treatment 17.20 and from public parks 16.00%. According to bird species samples with high positivity in relation to the total number of them were 31 or 22.96% total samples from parrots', from pigeons 44 or 20.95%, from canaries 11 or 18.33%, from peacocks 17 or 21.25%, from pheasants 10 or 13.88%. It is stressed that no species has resulted completely free after testing. Immunofluorescence test proved relatively easy to use and with good specificity. But although this test has demonstrated success in identifying the presence of organisms in poultry chlamydia, it generally fails to identify relevant including serotypes or their subtyping.

Table 1: Results of testing for chlamydia in birds using the IFT Method (n = 557)

Sampling location	Bird species					
	Parrots	Pigeons	Canaries	Peacocks	Pheasants	Total
Breeders	70/19 (27.14)*	90/24 (26.66)	30/6 (20.0)	35/8 (22.85)	22/3 (13.63)	177/60 (23.16)
Stores	40/8 (20.0)	60/10 (16.66)	25/4 (16.0)	10/2 (20.0)	10/1 (10.0)	145/25 (17.24)
Clinic	25/4 (16.0)	10/2 (20.0)	5/1 (20.0)	7/1 (14.28)	0	47/8 (17.02)
Public park	-	50/8 (16.0)	-	-	-	50/8 (16.0)
Zoo	-	-	-	28/6 (21.42)	40/6 (15)	68/12 (17.64)
Total	135/31 (22.96)	210/44 (20.95)	60/11 (18.33)	80/17 (21.25)	72/10 (13.88)	557/113 (20.28)

*Number of analyzed/number of positive (Percentage of positive)

Table 2: Result of PCR testing (n = 38)

Species	Doubtful positive samples Immuno-fluorescence test	PCR positive	
		Number	Percentage
Parrots	5	3	60.00
Pigeons	19	1	5.26
Canaries	4	1	25.00
Peacocks	5	1	20.00
Pheasants	5	1	20.00
Total	38	7	18.42

The positive results obtained from the IF test are considered relevant if a bird shows clinical symptoms but also it is not excluded in cases where it is in sub-clinical conditions. Because the LPS antigens of several other bacteria can react with monoclonal antibodies specific for LPS of chlamydia and can cause false-positive results for the elimination of such cases should be taken other measures prior to addition (Gerlach, 1997; Hewinson *et al*., 1997; Andersen, 1998).

The results obtained by the PCR procedure (Table 2) show that a total of 38 samples reacted with non-specific positive Immunofluorescence Test Method, only 7 (18.42%) resulted positive for the presence of chlamydia. According to the species, these results were obtained from 19 samples from pigeons and only 1 (26.50%) resulted positive. But a higher percentage was seen in samples that come from parrots, canaries, peacocks and pheasants, respectively (60, 25, 20 and 20%). This shows that the results obtained with the method of immunofluorescence test were right on samples taken from pigeons in comparison with those obtained from other species where the accuracy rate was lower (Hewinson *et al*., 1997; Verminnen *et al*., 2008; Sachse *et al*., 2009). But the results obtained by PCR are considered more reliable because of the specificity of the test. A false positive result, even accepted by some researchers is related to the ability of the kit to detect chlamydial LPS antigen (Borel *et al*., 2008; Andersen and Vanrompay, 2003; Andersen, 1998). However, the level of specific chlamydia setting may be different in different types, depending on the types of samples, location and ability to eliminate chlamydia as feces, blood or organs (Hewinson *et al*., 1997; Magnino *et al*., 2009; Vogel *et al*., 1994).

Taking into account the results of re-testing with the PCR procedure, the total number of birds positive for chlamydia will be somewhat smaller. Thus, instead of a total of 113 positive birds (20.28%) in fact seropositive were 82 heads or a total of 14.72%. According to the species, the number of positive results after re-testing with the PCR Method was in parrots 21.48% instead of 22.96 %, in pigeons 12.38% instead of 20.95%, in canaries 13.33% instead of 18.33%, in peacocks 16.25% instead of 21.25%, in pheasants 8.33% instead of 13.88%. According to location more positive samples have been taken from those shops selling birds (16.12%) followed by those taken by breeders (15.66%). The conducted monitoring shows that there has been up to 14.72%, positive birds for chlamydia (Dovc *et al*., 2004, 2007; Gerlach, 1997).

Despite the result of 20.28% which we take as valid by Immunofluorescence Test Method and further consideration of the additional 14.72% correct to PCR procedure are quite disturbing and they confirm the importance of health monitoring birds, especially in shops or in their breeding points (Borel *et al*., 2008; Gerlach, 1997; Hewinson *et al*., 1997).

By analyzing samples from different bird species, *C. psittaci* have revealed the presence in nearly all the territory of the Republic of Albania. A systematic control of chlamydia is the most acceptable solution for the prevention and awareness of bird breeders and owners of decorative birds.

CONCLUSION

As an infectious disease and zoonotic, listed alongside to other animal pathologies, it is involved in the monitoring plans, according to Law No. 10 465, dated 29.09.2011: "Veterinary service in the Republic of Albania". So, the high percentage of chlamydia in birds demonstrates the importance of reliable diagnostics for this disease as an important precondition for the protection of human health, especially to owners and breeders of decorative birds. Finally, the choice of method of immunofluorescence test as many methods of other status, combined with additional tests including PCR procedure remains an effective way to control chlamydia in decorative birds.

ACKNOWLEDGEMENTS

A study was conducted in collaboration with the Institute of Veterinary and Food Safety and the support of the World Association for the Protection of Wildlife in Tirana in the framework of the program: monitoring of the prevalence of chlamydia in decorative birds.

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