Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Oct - Dec 2016 / v.18 / n.4 / 701-704
http://dx.doi.org/10.1590/1806-9061-2016-0296

Screening of Feral Pigeons (Columba livia) for Pathogens of Veterinary and Medical Importance

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■Keywords

Chlamydia psittaci, Mycoplasma sp., Newcastle disease virus, *Salmonella* sp.

Submitted: April/2016
Approved: August/2016

ABSTRACT

Pathogens of veterinary and medical importance were investigated in 240 feral pigeons (*Columba livia*) captured in warehouses in São Paulo State, Brazil for one year. Rapid serum agglutination test (RST) was performed for the detection of antibodies against *Mycoplasma synoviae*, *Mycoplasma gallisepticum* and *Salmonella* Pullorum/Gallinarum. Positive samples were submitted to hemagglutination inhibition (HI) and tube seroagglutination tests, respectively. Molecular techniques (RT-PCR and PCR) were performed for Newcastle Diseases Virus (NDV) and *Chlamydia psittaci* diagnosis. Additionally, HI test was applied to detect antibodies against NDV. Serological results by RST were 3.3% positive for *M. synoviae*, 2.5% for *M. gallisepticum*, and 0.4% for *S.* Pullorum/Gallinarum, all negative on the confirmatory tests performed. NDV RNA or antibodies were not detected. *C. psittaci* DNA was detected in 13% of the samples. Further research on pigeon health status should be conducted because this species is highly adaptable and their numbers are rapidly rising around the world, posing risks for animals and human beings.

INTRODUCTION

The rock pigeon (*Columba livia*) is native to Asia and Europe and is the original genetic stock for domesticated pigeons. Today, it can be considered cosmopolitan, because, with the exception of the northern and southern polar regions, they are present in all continents and in all the countries of the world (Sick, 1993; Vogel *et al*., 1994).

Currently, the overpopulation of pigeons in different regions of the world causes several economic losses and health problems: droppings damage buildings, pigeons searching for food destroy vegetation, and the role of this species in the transmission of diseases to humans and to domestic animals has been documented (Haag-Wackernagel & Moch, 2004; Bucher & Ranvaud, 2006; Alexander, 2011). Some of the pathogens that can be transmitted by pigeons are particularly important for animal health (Alexander, 2011). For instance, *Salmonella* Pullorum, *Salmonella* Gallinarum, *Mycoplasma synoviae*, *Mycoplasma gallisepticum* and Newcastle Disease Virus (NDV), are listed in the Brazilians National Poultry Health Program (PNSA) due to the economic losses caused in commercial flocks (Brasil, 2009).

Pigeons may also pose a risk to humans. *Chlamydia psittaci* is the most widespread bacterium with zoonotic potential in feral pigeon populations. The disease caused by *C. psittaci* infection in humans is called psittacosis. An extensive literature search identified 47 case reports of psittacosis, which route of transmission was traced to a contact with feral pigeons (Haag-Wackernagel & Moch, 2004; NASPH, 2010). Given the scope of the problems mentioned above, the aim

of this study was to investigate microorganisms of veterinary and medical importance in feral pigeons where their overpopulation is a matter of concern for public and animal health authorities.

MATERIALS AND METHODS

Study area and pigeon sample collection

The pigeons surveyed in this study were trapped around warehouses used for the storage and market of agricultural products in São Paulo State, Brazil. During one year, 20 pigeons were monthly trapped in cages: 10 pigeons per month in the city of Tatuí, and 10 pigeons in the city of São Paulo, both in the state of São Paulo, totaling 240 birds.

Blood samples were collected from the brachial vein of each bird using sterile material, disposed in proper collections tubes, and stored at 4ºC. In the laboratory, blood samples were centrifuged at 1000 x g at room temperature for 10 min to obtain the serum samples required for testing. Cloacal swabs samples were also collected from each bird, stored in PBS (Phosphate Buffer Saline) and immediately frozen (-80ºC) until diagnostic testing.

Serological examination

Serum samples obtained were submitted to rapid serum agglutination test (RST) using commercial antigens in order to evaluate the presence of antibodies against *S.*Pullorum/Gallinarum (SP/SG), *M. synoviae* (MS), and *M. gallisepticum* (MG). The commercial antigens Pulor test, the Synovitest, and Myco-Galli test (Biovet®, São Paulo, Brazil), respectively, were used. Positives serum samples were submitted to a Federal Reference Laboratory for diagnostic confirmation using tube agglutination test (TA) and haemagglutination inhibition test (HI). All tests were performed according to the guidelines of the OIE (2008; 2012a) and PNSA (Brasil, 2009). For indirect NDV diagnosis, serum samples were also submitted to the Federal Reference Laboratory to be tested by HI, according to standard methodologies (OIE, 2012b; Brasil, 2009).

Molecular examination

For the molecular analysis of NDV, RNA was extracted from cloacal swabs samples using a commercial kit (Nucleospin Viral RNA Isolation®, Macherey-Nagel, GmbH & Co. KG, Germany), according to the manufacturer's protocol. Ulster vaccine strain (Fort Dodge®, São Paulo, Brazil) and ultrapure water were used as positive and negative controls, respectively. Reverse transcription polymerase chain reaction (RT-PCR) was based on primers targeting a conserved region of the NDV genome as described elsewhere (Oberdörfer & Werner, 1998), and as used in other studies (Jestin & Jestin, 1991). Primer sequences were: P1F (5'TTGATGGCAGGCCTCTTGC3') and P2R (5'GGAGGATGTTGGCAGCATT3'), cDNA synthesis and polymerase chain reaction (PCR) were carried out according to Jestin & Jestin (1991).

For the *C. psittaci* diagnosis, DNA was extracted from cloacal swabs samples using a commercial kit (Nucleid Acid and Protein Purification Kit®, Macherey-Nagel, GmbH & Co. KG, Germany), according to the manufacturer's protocol. *C. psittaci* genomic DNA from monk parakeets (*Myiopsitta monachus*) (Cpsi/Mm/BR01, GenBank number JQ926183.1) and ultrapure water were used as positive and negative controls, respectively. PCR was based on primers targeting a conserved region of pmp gene sequences, as designed by Laroucau *et al.* (2001). Primers sequences were: CpsiA (5'ATGAAACATCCAGTCTACTGG3') and CpsiB (5'TTGTGTAGTAATATTATCAAA3'). Samples were analyzed by electrophoresis on 1.5% agarose gel (Uniscience®, Brazil), stained with Gel RedTM (Uniscience®, Brazil) at 0.5µg/10 mL and run at 80 volts/60 min. Samples showing 362 base pairs (bp) and a 300 bp DNA fragment under UV light were considered to be positive for NDV and *C. psittaci*, respectively.

RESULTS

Out of 240 pigeon sera evaluated by RST, antibodies against *M. synoviae* were detected in 3.3% (8/240), antibodies against *M. gallisepticum* in 2.5% (6/240), and antibodies against *S.* Pullorum/Gallinarum in 0.4% (1/240) of the samples. All serum samples positive for *Mycoplasma* and *Salmonella* in the RST screening were submitted to HI and TA, respectively, but none was positive in these confirmatory tests. Neither NDV occurrence was detected in the samples evaluated by RT-PCR (0/240), nor antibodies by HI (0/187). *C. psittaci* DNA was detected in 13% (31/240) of the samples analyzed.

DISCUSSION

Currently, large populations of feral pigeons are present in urban areas all over the world, and the extensive food supply is one of the main factors that provide the ecological basis for this situation (Haag-Wackernagel, 1995). In Brazil, as in others countries,

the number of pigeons in urban and rural areas is an old-standing problem, which is difficult to solve. The overpopulation of feral species in warehouses used for the storage or marketing of agricultural products is considered a hazard for human and animal health.

Avian mycoplasmosis is caused by several pathogenic mycoplasmas, of which *M. gallisepticum* and *M. synoviae* are the most important (OIE, 2008). In the present survey, pigeons were seropositive for *M. synoviae* and *M. gallisepticum* by RST. Nonetheless, RST is a screening test and may yield false positive results (Kleven, 2008). For that reason, positive RST samples were submitted to confirmatory test (HI), which results were negative. It should be noted, however, that pigeons may play a role in the dissemination of *Mycoplasma* species pathogenic for poultry. Gharaibeh & Hailat (2011) conducted an experiment in which a pathogenic field strain of *M. gallisepticum* isolated from broilers was inoculated in pigeons. Although the pigeons did not show any clinical signs or seroconversion, *M. gallisepticum* was reisolated up to 7 days post-inoculation, demonstrating that this species may act as temporary biological carriers.

The bacteria *S.* Pullorum and *S*. Gallinarum may also cause avian diseases and economic losses in poultry production. In this study, antibodies against *S*. Pullorum/Gallinarum were detected in 0.4% of the pigeon serum samples evaluated by RST, but were negative in the confirmatory TA test. Espinosa-Argüelles *et al.* (2010). The also employed RST to determine the seroprevalence of S. Pullorum/Gallinarum in wild doves (*Zenaida asiatica* and *Zenaida macroura*) in Mexico. Out of the 201 doves evaluated, 26.3% were serum positive. It should be considered that wild birds are a potential source of contamination, and should be further investigated, particularly because of their mobility and close association with animal production facilities.

Still on the subject of pathogen dissemination, feral pigeons have been implicated as NDV carriers of in different regions in the world (Sousa *et al*., 2010; Alexander, 2011; Schuler *et al*., 2012). In the present study, however, the occurrence of NDV was not detected, neither by RT-PCR nor by the HI test performed. Nonetheless, the potential of transmission of an infectious agent that is airborne or shed via excreta is considerably greater in birds living in high-density populations, as Columbiformes (Vickers & Hanson, 1980; Sick, 1993). Thus, despite the negative results, long-term surveillance programs of NDV strains in these birds in Brazil should be encouraged.

Concerning public health issues, it is known that feral pigeons are commonly infected with the zoonotic bacterium *C. psittaci* (Magnino *et al*., 2009). In Brazil, the main studies related to this pathogen to date were conducted in free-living psittacine birds (Raso *et al*., 2006). In the present research, *C. psittaci* DNA was detected in 13% of the pigeon cloacal swabs samples analyzed. Molecular techniques using different genomic targets have been employed by several authors, with positive results ranging from 3.4 to 50% (Magnino *et al.,* 2009). On the other hand, Dickx *et al*. (2010) found a low infection rate of 1.6% (1/61) in feral pigeons evaluated in the city of Ghent, Belgium. The authors attribute their results to the low breeding activity of the pigeons, probably due to the fact that latent infection can be reactivated after stressful episodes as breeding (Flammer, 1997). In our study, however, the infection rate was higher and breeding activity was observed all year round. On the other hand, Geigenfeind *et al.* (2012) detected *C. psittaci* DNA only in 8.4% (17/202) of feral pigeons of all ages living in a pigeon loft in the city of Basel, Switzerland. It should be mentioned that this city has an ongoing project since the 1980s with the aim of establishing a small, but healthy population of feral pigeons. This purpose was achieved specially by reducing the food supply provided by humans (Haag-Wackernagel, 1995). Relative to C. p*ittaci* positive results, caution needs to be taken, as psittacosis cases associated with pigeons contact have been already been reported (Haag-Wackernagel & Moch, 2004). Therefore, continuous diseases surveillance programs of feral pigeon populations may contribute for the development of prophylactic measures to prevent pathogen dissemination.

STATEMENT OF ANIMAL RIGHTS

All procedures performed in this study followed the guidelines of the Bioethics Commission of the School of Veterinary Medicine of São Paulo University (Number 1605/2009) and were authorized by ICMbio/SISBIO (Brazilian Chico Mendes Institute for Biodiversity Conservation/Biodiversity Authorization and Information System, Number 18919-1).

FINANCIAL SUPPORT

This study was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico/ Ministério da Agricultura, Pecuária e Abastecimento (CNPq/ MAPA) under grant Proc. 578709/2008-2; and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) under grant 2010/01288-0.

ACKNOWLEDGEMENT

We thank Biovet®, São Paulo, Brazil, for providing the commercial antigens used in the seroassays and the Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP) for the assistance and support with the fieldwork.

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