Comparative Immunology, Microbiology and Infectious Diseases 43 (2015) 22–27

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Epidemiological survey of zoonotic pathogens in feral pigeons (*Columba livia* var. *domestica*) and sympatric zoo species in Southern Spain

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ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form
23 September 2015
Accepted 17 October 2015

Keywords:
Avian influenza viruses
Captive zoo animals
Feral pigeons
Flaviviruses
Salmonella spp.
Toxoplasma gondii

ABSTRACT

A cross-sectional study was carried out to determine the prevalence of pathogenic zoonotic agents (flaviviruses, avian influenza viruses (AIVs), *Salmonella* spp. and *Toxoplasma gondii*) in feral pigeons and sympatric zoo animals from Córdoba (Southern Spain) between 2013 and 2014. Antibodies against flaviviruses were detected in 7.8% out of 142 ($CI_{95\%}$: 3.7–11.8) pigeons, and 8.2% of 49 ($CI_{95\%}$: 0.9–15.4) of zoo animals tested. Antibodies with specificity against West Nile virus (WNV) and Usutu virus (USUV) were confirmed both in pigeons and in zoo birds. Even though seropositivity to AIVs was not detected in any of the analyzed pigeons, 17.9% of 28 ($CI_{95\%}$: 3.7–32.0) zoo birds tested showed positive results. *Salmonella* spp. was not isolated in any of 152 fecal samples collected from pigeons, while 6.8% of 44 zoo animals were positive. Antibodies against *T. gondii* were found in 9.2% of 142 ($CI_{95\%}$: 4.8–13.6) feral pigeons and 26.9% of 108 ($CI_{95\%}$: 19.6–34.1) zoo animals. This is the first study on flaviviruses and *T. gondii* in feral pigeons and captive zoo species in Spain. Antibodies against WNV and USUV detected in non-migratory pigeons and captive zoo animals indicate local circulation of these emerging pathogens in the study area. *T. gondii* was widespread in species analyzed. This finding could be of importance for Public Health and Conservation of endangered species present in zoo parks. Pigeons and zoo animals may be included as sentinel species for monitoring zoonotic pathogens in urban areas.

1. Introduction

Feral pigeon (*Columba livia* var. *domestica; Columbidae* family) is one of the bird species most frequently found in urban and peri-urban areas. During the last decades, their populations have increased exponentially in different countries, reaching densities higher than 2000 birds/km^2 in many European cities [1,2]. Favorable environmental conditions and abundant food, which imply loosing the breeding season, and the absence of predators are the main factors implicated in the high increase of their populations [3]. Feral pigeon is considered a pest species in many urban areas due to the destruction architectonic and urban heritage, damage to agriculture and risk of disease transmission to other sympatric species, including humans. In this sense, control measures including surgical or chemical sterilization, removal of eggs, physical repellents, use of birds of prey or capturing and controlled elimination are frequently implemented in urban areas [2,4].

Zoological parks are a very favorable habitat for pigeons, where they are found in higher densities than in urban areas. High populations imply significant economic losses associated to the consumption of food intended for captive zoo animals. Furthermore, the risk of disease transmission from pigeons to zoo sympatric species can be of concern for Animal Health and Conservation. In this sense, the importance of zoos for surveillance

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http://dx.doi.org/10.1016/j.cimid.2015.10.003
0147-9571/

of zoonotic and emerging diseases has been previously suggested [5].

Even though feral pigeons are considered reservoirs of zoonotic diseases including Newcastle disease, histoplasmosis, ornithosis, salmonellosis, or cryptococcosis [6,7], information about zoonotic diseases in this species in Spain is yet very limited. High prevalence of *Chlamydophila psittaci* infection was found in central (Madrid: 52.6%) and southwest (Murcia: 35.9%) areas [8,9]. Prevalence of *Campylobacter* spp. infection ranged between 1.1% and 69.1% in Barcelona and Madrid, respectively [9,10]. Also, 1.5% of *Salmonella* spp. and 0.25% of *Yersinia intermedia* infections was detected in pigeons from Barcelona city [10]. In addition, 70.3% of pigeons analyzed in Alicante (southwest Spain) showed *Cryptococcus neoformans* infection [11].

The aim of this study was to determine the prevalence of selected zoonotic pathogens (flaviviruses, avian influenza viruses (AIVs), *Salmonella* spp. and *Toxoplasma gondii*) in feral pigeons, and to assess the risk of transmission to sympatric zoological species in the Córdoba Municipal Zoo Park (CMZP, Southern Spain).

2. Materials and methods

2.1. Study design and sampling

A total of 152 feral pigeons were captured in the CMZP (Southern Spain) between November 2013 and May 2014. Captures were performed within the course of a pest control program undertaken by the local authorities following European guidelines (UNE 171210: 2008). Animals were handled and sampled according to regulations of the Regional Government of Andalusia.

Blood samples were taken from the brachial vein, after those animals were humanely euthanized by overdose sodium pentobarbital (Dolethal®, Vetoquinol Laboratories, Lure, France) (0.5 ml/kg) as recommended for birds [12]. Samples were placed into sterile tubes without anticoagulant and centrifuged at 3000 rpm for 10 min. Sera were stored at −20 °C until analysis. All individuals were necropsied and inspected for the presence of macroscopic lesions. Pigeons were classified as juveniles (<8 months) and adult (>8 months) according to previously established criteria [13]. Sera from 108 zoo animals belonging to 34 species (Table 1), which share habitat with the sampled pigeons, were also collected. In addition, digestive content (2–3 g) from all pigeons and 44 fresh fecal samples from 35 different zoo species were collected for *Salmonella* spp. isolation.

2.2. Laboratory analysis

Sera from 142 pigeons and 49 captive zoo animals were tested for antibodies against one epitope of the preMembrane-Envelope (prM-E) protein common to flaviviruses of the Japanese Encephalitis antigenic complex (JEV) using a commercial blocking ELISA (bELISA 10.WNV.K3 INGEZIM West Nile COMPAC®, Ingenasa, Madrid, Spain). ELISA-positive sera were confirmed by virus neutralization test (VNT) for the detection of specific neutralizing antibodies against West Nile virus (WNV; Is98 strain), Usutu virus (USUV; It12 strain) and Meaban virus (MBV; Brest ART707 strain). VNTs were performed as previously described [14,15]. Samples that showed neutralization and absence of cytopathic effect at dilutions ≥10 for WNV and USUV and ≥20 for MBV, were considered positive. Interpretation of results was based on comparison of VNT titers obtained in parallel against the 3 flaviviruses. The neutralizing immune response observed was considered specific when VNT titers for a given virus was ≥4-fold higher than titers obtained for the other viruses. Samples showing VNT titers differences ≤2-fold between the viruses examined were considered positive for flavivirus but not conclusive for any specific virus.

The presence of antibodies against the nucleoprotein of AIVs type A was determined using a commercial (bELISA) (1.0.FLU.K3 INGEZIM INFLUENZA A®, Ingenasa, Madrid, Spain) according to the manufacturer's recommendations. A total of 148 pigeons and 28 zoo birds were analyzed.

Fecal droppings and fecal samples were processed as described in Annex D of the Spanish standard UNE-EN ISO 6579:2002 for *Salmonella* isolation. *Salmonella*-presumptive colonies were tested with the Mucap (Biolife, Milano, Italy) and indole tests. *Salmonella* serotyping was carried out according to the Kauffman-White scheme [16] by the Agri-food laboratory of Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural (Generalitat de Catalunya, Spain).

Sera from 142 feral pigeons and 108 zoo animals, including samples from 28 carnivores, 43 ungulates, 35 birds and 2 monkeys were examined to detect antibodies against *T. gondii* using the modified agglutination test (MAT) as previously described [17]. Serum was tested at 1:25, 1:50, 1:100 and 1:500 dilutions and both positive and negative controls were included in all tests. Sera with titers ≥1:25 were considered positive.

2.3. Statistical analysis

The prevalence of the different pathogens was estimated from the ratio of positive to the total number of samples tested, with the exact binomial confidence intervals of 95% ($CI_{95\%}$). Correlation between estimated prevalences and independent variables (species, sex and age) were analyzed by means of a Pearson's chi-square test or, when there were less than six observations per category, by the Fisher's exact test. The differences between variables were analyzed by Tukey tests. Values with $P < 0.05$ were considered as statistically significant. Statistical analyses were performed using SPSS 22.0 (Statistical Package for Social Sciences (SPSS) Inc., Chicago, IL, USA).

3. Results

Antibodies against flavivirus of the JEV serocomplex were found in 11 of 142 pigeons (7.8%; $CI_{95\%}$: 3.7–11.8) tested by bELISA. No statistically significant differences were observed among sex or age. Six of the 11 positive samples were confirmed by VNT (Table 2). Specific antibodies against WNV were detected in three pigeons (3/142: 2.1%; $CI_{95\%}$: 0.0–4.3), while USUV infection was also confirmed in three individuals (3/142: 2.1%; $CI_{95\%}$: 0.0–4.3). No MBV-specific antibodies were detected in the pigeon sera tested.

Seroprevalence of flaviviruses in zoo animals was 8.2% (4/49; $CI_{95\%}$: 0.9–15.4). Presence of antibodies was found in ostrich (*Struthio camelos*), white stork (*Ciconia ciconia*), emperor goose (*Chen canagica*) and black wildebeest (*Connochaetes gnou*) (Table 2). Specific antibodies against WNV (1:40) and USUV (1:20) were confirmed in white stork and ostrich, respectively. Moreover, the seropositive emperor goose found positive in bELISA presented neutralizing antibodies against WNV (1:80), USUV (1:40) and MBV (1:20), which are indicative of at least two independent flavivirus infections (by a flavivirus belonging to the JEV serocomplex like WNV or USUV and tick-borne flavivirus like MBV).

Antibodies against AIV were not found in any of the 148 pigeons tested (0.0%; $CI_{95\%}$: 0.0–1.9). Seropositivity was observed in five out of 28 (17.9%; $CI_{95\%}$: 3.7–32.0) sera from captive zoo birds, including mallard (*Anas platyrhynchos*), emperor goose, African sacred ibis (*Threskiornis aethiopicus*), rosy-billed pochard (*Netta peposaca*) and black-bellied whistling duck (*Dendrocygna autummalis*) (Table 1).

Salmonella spp. was not isolated from any of the 152 (0.0%; 95% CI: 0.0–1.9) pigeons analyzed. In contrast, in three fecal samples from the 44 captive zoo animals tested (6.8%) positive isolation results were obtained (Table 1). In particular, *Salmonella* Panama