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ORIGINAL ARTICLE

Zoonotic Agents in Feral Pigeons (*Columba livia*) from Costa Rica: Possible Improvements to Diminish Contagion Risks

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Abstract

Most studies on zoonotic agents in pigeons have been conducted in the Palearctic region, but the scarcity of data is notorious in the Neotropical region, where these birds can breed all year around and are in close contact with humans. In this study, we used a combination of culture-dependent and culture-independent methods to identify infectious agents in 141 fecal samples from pigeons collected at four urban parks from Costa Rica. Of these we identified 34 positive samples for *Salmonella enterica* subsp. enterica serovar Braenderup (24.1%), 13 for *Chlamydophila psittaci* (9.2%), 9 for enteropathogenic *Escherichia coli* (6.4% *eae*A, 0% *stx*-1 and 0% *stx*-2), and 2 for *Campylobacter jejuni* (1.4%). These populations of pigeons pose low risk for healthy adult humans, however, they may pose a health risk to immunocompromised patients or children. This study provides scientific data, which can be incorporated into educational programs aiming to reverse the public attitude toward pigeon feeding and to rationally justify population control efforts.

Keywords: *Campylobacter jejuni*, *Chlamydophila psittaci*, ecosystem health, enteropathogenic *Escherichia coli*, infectious agent, *Salmonella enterica*, zoonoses

Introduction

The high annual reproductive success, tameness, and selection against aggressiveness in males that characterize domesticated pigeons contribute to their thriving success in cities (Magnino et al. 2009), where they find abundant food and places for roosting and nesting (Ali et al. 2013).

Massive populations of feral pigeons on urban infrastructure creates opportunities for their frequent contact with humans (Haag-Wackernagel and Moch 2004, Bradley and Altizer 2007). Pigeon droppings can create a potential risk of transmission of zoonotic microbes and human pathogens through contamination of drinking water sources and agricultural crops (Lillehaug et al. 2005), while occupational exposure and casual interactions (Graczyk et al. 2007) have also been shown to be sources of transmission. Indeed, up to 110 human pathogens, including *Salmonella enterica*, *Campylobacter jejuni*, *Campylobacter coli*, *Chlamydophila psittaci, Aspergillus* spp., *Candida parapsilosis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Toxoplasma gondii*, have been found in feral pigeons from Europe, North America, South America, and Africa (Lillehaug et al. 2005, Magnino et al. 2009, Vázquez et al. 2010, Hoelzer et al. 2011, Osman et al. 2013).

This public health issue has not been addressed in Central America, where feral pigeon populations can breed year-round with shorter clutch intervals (Hetmański and Wołk 2005) and the dynamics of pathogen transmission are likely influenced by the unique socioeconomic, geographical, and climate aspects of the region (Hoelzer et al. 2011). In this study, we used a combination of culture-dependent and culture-independent

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methods to determine the presence of *C. psittaci* and *Salmonella* spp., in addition to virulence factors of zoonotic strains of *Escherichia coli and C. jejuni* in pigeons from four urban parks of the Central Valley of Costa Rica.

Materials and Methods

Sampling

We investigated the health status of 141 adult feral pigeons and their possible role on maintaining various zoonotic agents (Table 1). The birds were captured in four urban parks using a net and corn or peanuts as bait. The parks were visited twice between April 2013 and May 2014. The samples were taken during the rainy season of 2013 and the dry season of 2014 as follows: Guadalupe (April 2013 and March 2014), Tres Rios (June 2013), Alajuela (July and December 2013), and San Jose (October 2013 and May 2014). It was not possible to capture birds in Tres Rios in the second visit.

All of the sampled parks are popular public places located in the heart of their respective downtowns, but three of them are characterized by a large numbers of visitors, street vendors, and a tradition of feeding the resident pigeons (Plaza de la Cultura, Guadalupe and Alajuela). On the other hand, Tres Ríos is a more rural city surrounded by agricultural and livestock activities and less of a tradition of feeding pigeons.

Birds were first examined for signs of illness such as poor body condition, traumas, ectoparasites, and secretions. Next, a cloacal swabbing and a cloacal wash with phosphate-buffered saline were taken. The cloacal washes were cryopreserved at −80°C before use. All procedures were performed with authorization from the National Service for Animal Health (SENASA) and the Animal Welfare Committee of the Veterinary Medicine School of the National University of Costa Rica. In all cases veterinarians handled pigeons according to national animal welfare laws.

Isolation, identification, typing, and antimicrobial susceptibility testing of Salmonella

Cloacal swabs were transported into buffered peptone water (1:10) and analyzed within 24 h. Once in the laboratory, the swabs were enriched at 42°C for 18 h in ONE-Broth Salmonella Broth (Thermo Fisher Scientific) and aliquots from the latter were then plated onto plates of *Brilliance*™ Salmonella Agar (Thermo Fisher Scientific). After 24 h of incubation at 37°C under aerobiosis, bright purple colonies were subcultivated on blood agar plates and preliminarily identified as *Salmonella* sp. using a latex test targeting flagellar antigens (Oxoid). This procedure has been validated and approved by AFNOR according to the ISO 16140 standard for detection of *Salmonella* in food, animal feed, and

Table 1. Pigeons Communities Tested and Number of Animals Taken from Each One

Location	*No. of pigeons*
Guadalupe, San José	55
Plaza de la Cultura, San José	41
Tres Ríos, Cartago	16
Alajuela, Alajuela	29
Total	141

Table 2. Methods Used for Determining Infectious Agents

Agent/gene	*Method*	*Primer/probe*	*Annealing temperature (°C)*	*References*
Campylobacter jejuni	Real-time PCR (TaqMan probe)	5′-CTGGTGGTTTTGAAGCAAAGATT-3′ 5′-CAATACCAGTGTCTAAAGTGCGTTTAT-3′ 5′-(6FAM)AATTCCAACATCGCTAATG-MGB-3′	60	Best et al. (2003)
*eae*A	PCR	5′-TCAATGCAGTTCCGTTATCAGTT-3′ 5′-GTAAAGTCCGTTACCCCAACCTG-3′	55	Vidal et al. (2004)
stx-1	Real-time PCR (Sybr Green)	5′-CATTACAGACTATTTCATCAGGAGGTA-3′ 5′-TCGTTCAACAATAAGCCGTAGATTA-3′	55	Chui et al. (2010)
stx-2	PCR	5′-CTTCGGTATCCTATTCCCGG-3′ 5′-CTGCTGTGACAGTGACAAAAACGC-3′	55	Vidal et al. (2004)
Chlamydophila psittaci (detection)	Real-time PCR (TaqMan probe)	5′-GCCATCATGCTTGTTTCGTTT-3′ 5′-CGGCGTGCCACTTGAGA-3′ 5′-(6FAM)TCATTGTCATTATGGTGATTCAGGA-MGB-3′	60	Ménard et al. (2006)
C. psittaci (genotyping)[a]	Real-time PCR (HRM-Eva Green)	5′-TGTGCAACTTTAGGAGCTGAGTTC-3′ 5′-GCTCTTGACCAGTTTACGCCAATA-3′	60	Mitchell et al. (2009)

[a]Only applied in positive samples for *C. psittaci* detection.

environmental samples. Preliminary identifications were confirmed phenotypically using the API20 system (bioMérieux, Marcy l'Etoile, France) and genotypically with the Seeplex Diarrhea-B1 ACE Detection kit (Seegene). Isolates with confirmed identifications were serotyped according to the Kauffman-White scheme and then subtyped by pulsefield gel electrophoresis (PFGE) using the Pulsnet International Protocol for *Salmonella*.

Antibiotic susceptibility and categorization of these isolates was determined with a VITEK2 system using AST-N279 cards for the analysis of aerobic Gram-negative bacilli (bioMérieux). These cards include the following antibiotics: ampicillin (AMP), ampicillin/sulfabactam (SAN), piperacillin/tazobactam (PTZ), cephalothin (CEP), cefoxitin (FOX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), imipenem (IMP), meropenem (MER), amikacin (AMI), gentamicin (GEN), nalidixic acid (NAL), ciprofloxacin (CIP), and nitrofurantoin (NIT).

Molecular detection of pathogens and virulence factors

Total DNA extraction was performed by a pressure filtration method with a QuickGene® Mini 80 nucleic acid isolation instrument and a QuickGene DNA tissue kit S (Fujifilm Life Science, Tokyo, Japan) following manufacturer's instructions. Thereafter, we used different PCR methods to detect *C. psittaci*, genes associated to zoonotic strains of *E. coli* (*stx*-1, *stx*-2, and *eae*A genes) and *C. jejuni*, following the protocols described in Table 2. All samples positive for *C. psittaci* were genotyped by high resolution melting analysis (Table 2).

Statistical analyses

A nonparametric test (Mann–Whitney *U*) was applied (SPSS vs. 14.0) to establish differences among sampling areas for the presence of each agent. Sampling dates were classified into dry (December–April) and wet (May–November) seasons, and the same nonparametric test was performed.

Results

None of the 141 pigeons trapped and examined showed clinical signs related to illness. In the laboratory, the following zoonotic pathogens were detected: 34 samples from a single park (Guadalupe) and collected on the same day were positive for *S. enterica* subsp. enterica serovar Braenderup (24.1%), 13 samples were positive for *C. psittaci* (9.2%) (Guadalupe, Plaza de la Cultura and Tres Ríos), 9 for enteropathogenic *E. coli* (EPEC) (6.4% *eae*A, 0% *stx*-1 and 0% *stx*-2) (Plaza de la Cultura, Tres Ríos and Alajuela), and 2 for *C. jejuni* (1.4%) (Guadalupe and Plaza de la Cultura) (Table 3).

With regards to *Salmonella* sp., the PFGE pattern detected using *Xba*I as restriction enzyme (CRINJBPX01.0002) exhibited >80% similarity to a PFGE pattern recovered from diseased humans in San José, Costa Rica (CRINBBPX01.001). Four different antibiotic susceptibility patterns were seen among the 34 isolates: three of these patterns are linked to low resistance levels and a single isolate was categorized as resistant to several beta-lactams, a fluoroquinolone, and the tested nitrofuran (Table 4).

Six of the 13 *C. psittaci* were typed as genotype B ($n = 4$) or E ($n = 2$).

No difference in these results was found between sampling areas and between seasons.

Discussion

Due to its enhanced capacity to survive in the environment (Bleasdale et al. 2009), salmonellosis continues being an important zoonotic disease. There are over 2500 serotypes of zoonotic Salmonella and pigeons have been shown to harbor *S. enterica* O:4,5:i and *Salmonella* Typhimurium (Botti et al. 2013, Rocha-e-Silva et al. 2014). In this study, we detected a clonal infection by *S. enterica* Braenderup in pigeons. Along with the serovars Typhimurium and Lomita, *S. enterica* Braenderup has been previously detected in pigeons from Egypt (Osman et al. 2013), though it is rarely found in animal reservoirs.

According to the CDC (2017), the serovar Braenderup has been recently found in live poultry (2016), nut butter (2014), and mangoes (2012). Moreover, there are reports of human cases of food poisoning and enteric fever by this serovar (Osman et al. 2013) and the PFGE profile CRINJBPX01.0002 has been seen in Latin America in human *Salmonella* strains from Paraguay (Dr. Natalie Weiler Gustafson, Central Public Health Laboratory, Paraguay, pers. comm.). Though the risk of human infection with our isolates exists, their potential infections are likely to be easily controlled with antibiotics due to their widespread susceptibility to common drugs.

In a previous study, only 4% of the pigeons sampled at the park in Guadalupe harbored bla_{TE}M (Blanco-Peña et al. 2017). We observed four susceptibility profiles among a rather limited number of individuals from the same strain collected on the same day and location, hence we hypothesize that the resistance genes linked to this phenotypic trait are susceptible to lateral transfer. If this turns out to be true, the role of pigeons in the dissemination of resistance genes

Table 3. Results of Zoonotic Agents and *Escherichia coli* Associated with Virulence Factors in Pigeons from Costa Rica

Location	n	*eaeA*, n *(%)*	stx-*1*[a], n *(%)*	stx-*2*[a], n *(%)*	C. jejuni, n *(%)*	C. psittaci, n *(%)*	S. enterica, n *(%)*
Guadalupe, San José	55	0 (0)	n.d.	n.d.	1 (1.8)	5 (9.1)	34 (61.8)
Plaza de la Cultura, San José	41	4 (9.8)	0 (0)	0 (0)	1 (2.4)	7 (17.1)	0 (0)
Tres Ríos, Cartago	16	3 (18.7)	0 (0)	0 (0)	0 (0)	1 (6.2)	0 (0)
Alajuela, Alajuela	29	2 (6.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	141	9 (6.4)	0 (0)	0 (0)	2 (1.4)	13 (9.2)	34 (24.1)

[a]*stx*-1 and *stx*-2 were analyzed only in *eae*A-positive samples.
n.d., not determinate.

TABLE 4. ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL ISOLATES IDENTIFIED AS *SALMONELLA ENTERICA*

	MICs(μg/mL)														
	Beta-lactams								*Aminoglycosides*		*Quinolones*		*Nitrofurans*		
Isolates	*AMP*	*SAN*	*PTZ*	*CEP*	*FOX*	*CTX*	*CAZ*	*FEP*	*IMP*	*MER*	*AMI*	*GEN*	*NAL*	*CIP*	*NIT*
G1-R2, G1-R9, G1-R10, G1-R13, G1-R14, G1-R15, G1-R18, G1-R21, G1-R30, G1-R34, G1-R36	2	2	4	**2**	1	1	1	0.3	0.3	**2**	**1**	2	0.3	**64**	20
G1-R3, G1-R11, G1-R16, G1-R20, G1-R22, G1-R26, G1-R37	2	2	4	**2**	1	1	1	0.3	0.3	**2**	**1**	2	0.3	32	20
G1-R4, G1-R5, G1-R6, G1-R7,G1-R8, G1-R12, G1-R17, G1-R19, G1-R23, G1-R24, G1-R27, G1-R28, G1-R29, G1-R31, G1-R32, G1-R33, G1-R35	2	2	4	**2**	1	1	1	1	0.3	**2**	**1**	2	0.3	32	20
G1H4	**32**	**32**	4	**64**	8	4	1	0.5	2	**2**	**1**	8	0.3	**128**	**80**

MICs above the breakpoint defined for enteric bacteria appear in *bold*.
AMI, amikacin; AMP, ampicillin; CAZ, ceftazidime; CEP, cephalothin; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; GEN, gentamicin; IMP, imipenem; MER, meropenem; MIC, minimum inhibitory concentration; NAL, nalidixic acid; NIT, nitrofurantoin; PTZ, piperacillin/tazobactam; SAN, ampicillin/sulfabactam.

should be considered in future discussions related to the environment and human safety.

There are sporadic reports of isolation of *Chlamydophila abortus*, *Chlamydophila pecorum*, and *Chlamydia trachomatis* from pigeon droppings or cloacal swabs (Sachse et al. 2012). By contrast, numerous studies have defined urban pigeons as common carriers of *C. psittaci* (Heddema et al. 2006a, Dickx et al. 2010, Geigenfeind and Haag-Wackernagel 2010). However, in this study only *C. psittaci* was analyzed. The set of primers and the probe were tested in silico (BLAST search) and showed highly specificity to *C. psittaci* (data not shown). Our results confirm that the frequency of finding of *C. psittaci* DNA matches other PCR-based reports, where the positivity rate may reach up to 50% (Magnino et al. 2009). The obtained prevalence (9.2%) is comparable to those found in pigeons and doves from other Neotropical areas, such Galapagos Archipelago (0–6%) (Padilla et al. 2004), Venezuela (16.7%) (Arraiz et al. 2012), and Brazil (16.8%) (de Lima et al. 2011). All genotyped samples were classified as genotype B and E, the most frequently found in pigeons (Andersen 1991, Vanrompay et al. 1993, Čechová et al. 2016). Genotype B, which has been previously found in *Columba livia* from Costa Rica (Dolz et al. 2013), is regarded as an underestimated cause of psittacosis in humans (Heddema et al. 2006b). On the other hand, an increased exposure for humans to genotype B *C. psittaci* has been observed in people inhabiting buildings with high pigeon densities (Arraiz et al. 2012).

The lack of differences of *C. psittaci* prevalence between the seasons is particularly noteworthy. Previous studies in Palearctic scenarios showed that the prevalence of *C. psittaci* increases during late autumn, when their breeding period ends (Tanaka et al. 2005, Vázquez et al. 2010). In Central America, which belongs to the Neotropical region, the constant availability of resources allows feral pigeons to breed all year around with shorter clutch intervals (Hetmański and Wołk 2005). Further studies should be performed to confirm the absence of seasonal and temporal trends of this pathogen in pigeons.

Although *E. coli* is one of the most predominant facultative anaerobic bacteria in the human gastrointestinal tract, several pathogenic strains have emerged and may cause disease in humans. In Central America, EPEC is one of the main causes of childhood diarrhea (Becker-Dreps et al. 2014). Our results indicate that EPEC, but not Shiga toxin producing *Escherichia coli* (STEC), are part of the transient gut microflora of some pigeons from the study area. The prevalence of EPEC found in this study agrees with previous reports from Finland (7%) and Spain (6% in urban and 4% in rural pigeons respectively) (Kobayashi et al. 2002, Sacristán et al. 2014). By contrast, the low prevalence of STEC in the study area could indicate that pigeons may have a minor epidemiologic role in comparison with other regions with higher percentages (Grossmann et al. 2005, Farooq et al. 2009).

C. jejuni, the most common food-borne zoonotic pathogen in the United States and Europe, causes human gastroenteritis and high prevalences have been detected in pigeons in Spain (69%), Italy (48%), and Iran (17%) (Vázquez et al. 2010, Gargiulo et al. 2014, Abdollahpour et al. 2015). Previous studies state that *C. jejuni* is not an important cause for human gastrointestinal diseases in Central America (Hotez et al. 2014), which is in agreement with the low prevalence found in this study (1.4%).

Viable forms of the detected microbes in the environment are very likely in Costa Rica (Rose et al. 2001, Bleasdale

et al. 2009). Our results support the notion that the populations of feral pigeons studied pose low risks for healthy adults, in sharp contrast to immunocompromised patients or children, whose risk may be nearly 1000-fold greater (Haag-Wackernagel and Moch 2004).

Urban parks are very important in Costarican culture. They are a central gathering place where people spend time on a regular basis sitting or walking while they meet with friends and family. Parks preserve the biggest trees in the urban areas and are decorated with flowers, fountains, lamps, trails, benches, and sometimes tables. In crowded cities, parks bring together people of different ages and conditions. It is also common that human visitors bring their pet dogs to the park, creating a space where domestic and wild animals may have the opportunity to interact. It is also important to note that elderly populations and children are among the most frequent users of parks. As such, pigeons can come in very close contact with them, their food, or clothing, especially in parks where feeding pigeons is a common practice.

Taking into account the close contact between humans, domestic animals, and pigeons in shared public parks, the findings from this study suggest that a more critical appraisal of these zoonotic pathogens in pigeons and humans is warranted.

Conclusions

Given the rapid growth of human populations, research for conservation and restoration of urban ecosystems is becoming more urgent (Vitousek et al. 1997, Niemelä 1999, Marzluff and Ewing 2001, Miller and Hobbs 2002). Unfortunately, potential zoonotic pathogen surveillance in wild animals is not widely included in national monitoring plans and is only occasionally performed for research purposes (Botti et al. 2013), limiting the usefulness of results such as those generated here in disease risk analysis and control plans.

We urge governmental institutions to reverse the public attitude toward pigeon feeding and convince the public that feeding is counterproductive and ultimately harms the feral pigeons, since it leads to overpopulation and poor-quality living conditions (Senar et al. 2017), which can favor the transmission of pathogens between pigeons and/or from pigeons to humans. In addition, it is necessary to consider other management measures, as the use of architectural devices to limit the access of the pigeons in private and public structures (Ferri et al. 2011) and the reduction of the availability of food and of nesting and roosting sites (Haag-Wackernagel 2002).

Pigeon feces can also facilitate the spread of pathogens in areas utilized by people and these birds (*e.g.*, boulevards, seats, and tables). Therefore, it is important to develop hygiene measures in the country to diminish the risk of contagion with pathogen agents that could be spreading this way.

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Author Disclosure Statement

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