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ISOLATION AND MOLECULAR IDENTIFICATION OF POTENTIALLY PATHOGENIC *Escherichia coli* AND *Campylobacter jejuni* IN FERAL PIGEONS FROM AN URBAN AREA IN THE CITY OF LIMA, PERU

Moisés CABALLERO(1), Isabel RIVERA(1), Luis M. JARA(1), Francisco M. ULLOA-STANOJLOVIC(2) & Carlos SHIVA(1)

SUMMARY

Feral pigeons (*Columbia livia*) live in close contact with humans and other animals. They can transmit potentially pathogenic and zoonotic agents. The objective of this study was to isolate and detect strains of diarrheagenic *Escherichia coli* and *Campylobacter jejuni* of urban feral pigeons from an area of Lima, Peru. Fresh dropping samples from urban parks were collected for microbiological isolation of *E. coli* strains in selective agar, and *Campylobacter* by filtration method. Molecular identification of diarrheagenic pathotypes of *E.coli* and *Campylobacter jejuni* was performed by PCR. Twenty-two parks were sampled and 16 colonies of *Campylobacter* spp. were isolated. The 100% of isolates were identified as *Campylobacter jejuni*. Furthermore, 102 colonies of *E. coli* were isolated and the 5.88% resulted as Enteropathogenic (EPEC) type and 0.98% as Shiga toxin-producing *E. coli* (STEC). The urban feral pigeons of Lima in Peru can act as a reservoir or carriers of zoonotic potentially pathogenic enteric agents.

KEYWORDS: *Escherichia coli*; *Campylobacter jejuni*; Diarrhea; Zoonoses; PCR.

INTRODUCTION

The feral pigeons (*Columbia livia*) live in urban and rural areas, in close contact with humans and other animals. In recent years, the population growth of feral pigeons has increased their interest in public health, because it could represent a reservoir of transmissible pathogens by air and through contaminated food or water[29].

Among the zoonoses that could be transmitted by pigeons, the most important are chlamydiosis, cryptococcosis, aspergillosis and enterobacteriosis, which include pathogenic *Escherichia coli* and species of *Campylobacter*, *Salmonella* and *Listeria*[1]. Five main pathotypes of diarrheagenic *E. coli* have been described, enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enteroinvasive (EIEC) and the zoonotic enterohemorrhagic (EHEC or STEC), which produce a Shiga-like toxin that leads to hemolytic uremic syndrome[33]. Some studies show that enterohemorrhagic serotype O157:H7 of E. *coli* can be present in feral pigeons[15,28]. Moreover, in Peru there have been reported outbreaks of human enterohaemorrhagic colibacilosis from unknown sources of infection[13], although prevalence of STEC in children is up to 9% while EPEC is higher[21].

Thermotolerant *Campylobacter* species have become important, especially as agents of infectious diarrhea, with even more cases per year than salmonellosis and shigellosis[11,33]. *Campylobacter jejuni* and *Campylobacter coli* are the most frequent cause of diarrhea in childhood groups, the fecal-oral route being the main route of transmission[3,6]. Even more, *Campylobacter* has been isolated from river and waste water, chickens and pets[32]. In Lima, *C. jejuni* was reported as responsible for the 13.3% of the acute human diarrhea diagnosed in local hospital centers[25].

There are no studies about the prevalence of zoonotic diarrheagenic agents in urban birds in the city of Lima, Peru. Their population has increased in recent years and the close contact with people in public places, especially with children, requires knowledge of the epidemiological status of potential pathogenic *E. coli* and *Campylobacter* in feral pigeons. Given this context, the objective of this study was to isolate and identify the presence of *Campylobacter jejuni* and diarrheagenic *E. coli* in feral pigeons from an urban area in the city of Lima, Peru, through the microbiological isolation and molecular identification by a conventional Polymerase Chain Reaction (PCR) technique.

MATERIALS AND METHODS

Sampling: droppings samples, from healthy adult feral pigeons, were collected in parks (22) of a midwest area of the city of Lima (Pueblo Libre), Peru, in the summer (December to April) of 2012. Sterile plastics with food were extended on the ground of each park, and a swab of fresh droppings from each pigeon (about 30 animals per park) was obtained. Swabs were placed in a Stuart transport medium and then were stored at 4 °C for 24 hours.

(1) *Universidad Peruana Cayetano Heredia, Facultad de Veterinaria y Zootecnia , Lima, Perú.*
(2) Universidade de São Paulo, Faculdade de Veterinária e Zootecnia, São Paulo, SP, Brasil.
Correspondence to: Luis M. Jara, Laboratorio de Biología Molecular, Facultad de Veterinaria y Zootecnia, Universidad Peruana Cayetano Heredia. Av. Honorio Delgado 430, San Martín de Porres, Lima, Perú. E-mail: luis.jara.s@upch.pe

Microbiological and molecular identification: samples for *E. coli* isolation were seeded and cultured in MacConkey agar by the streaking culture technique and incubated at 37 °C for 24 hours in aerobiosis. Samples for *Campylobacter* were suspended in 1 mL of saline solution and inoculated into a cellulose filter (0.45 µm) on blood agar and then incubated for 72 hours at 42 °C under microaerophilic conditions[22]. According to biochemical patterns and modified Gram staining with fuchsin, *E. coli* and *Campylobacter* were presumptive identified respectively[34]. The extraction of genomic DNA of each colony was performed by the kit Wizard Genomic DNA Purification for Gram-negative bacteria, according to the supplier's instructions (Promega, USA).

For the molecular identification of diarrheagenic *E. coli* pathotypes, a multiplex PCR performed with previously described primers of intimin (*eae*), Shiga toxin (*stx1*, *stx2*) and hemolysin (*hlyA*) was used[23]. As well for the *C. jejuni* identification, the previously described primers forward 5'- TGACGCTAGTGTTGTAGGAG - 3' and reverse 5'-CCATCATCGCTAAGTGCAAC-3' were used in a conventional PCR[20]. Diarrheagenic *E. coli* pathotypes were classified according to the presence of virulence factors *eae* for EPEC and *stx1*/*stx2* for STEC[21]. The prevalence was expressed as a percentage according to the pathotype found in the total of isolates.

RESULTS

From all samples seeded on blood agar, 16 colonies were isolated showing microscopic characteristics such as small size, pinpoint morphology, non-hemolytic, and Gram-negative "gull-wing" shaped bacilli at Gram staining. One hundred percent of colonies suggestive of *Campylobacter* were positive in PCR identification as *C. jejuni* (Fig. 1). Likewise, 110 colonies of *E. coli* were isolated from McConkey agar, of which only 102 were confirmed by biochemical tests. The 6.86% of the *E. coli* strains amplified had one or more virulent genes, of which 5.88% belonged to the EPEC group and 0.98% to the STEC group (Table 1, Fig. 2).

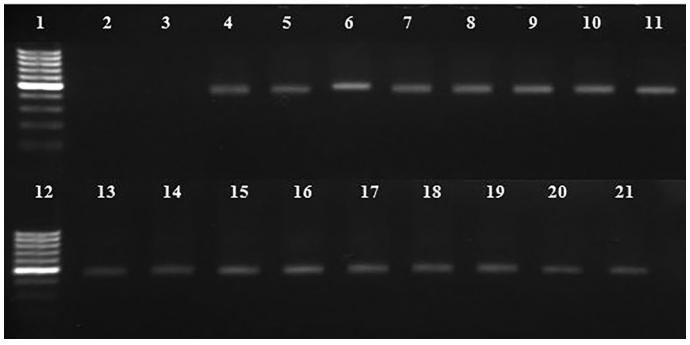

Fig. 1 - Gel electrophoresis of *C. jejuni* samples from feral pigeons. Ladder 100 bp (1); blank (2), negative control, *E. coli* ATCC 25922 (3); positive control, *C. jejuni* No. 1503-2012 (4); positive colonies (5-21). PCR product: 402 bp[16]

DISCUSSION

This study represents the first report of potentially pathogenic agents isolated from urban feral pigeons in Lima, Peru. The results indicate that these birds, settled in different parks, can serve as a reservoir of zoonotic *C. jejuni* and STEC, as well as EPEC. Although the isolation and molecular identification methodology was similar to previous studies, and there were no differences among parks tested, the prevalence obtained may differ by the number of samples collected and sampling areas, among other investigated factors.

The presence of *Campylobacter jejuni* has been documented worldwide in urban birds. In Spain this was found, with 69.1% in contrast to 1.1% of *C. coli*[32]. Also in Sweden, a study found 3% of prevalence of *C. jejuni*[19], while in Chile a 7% was found[10]. In Bolivia a 29% of frequency for *Campylobacter* spp. was obtained and 57% was confirmed as *C. jejuni*[2]. And in the Peruvian jungle, *C. jejuni* was found in 8% of the domestic chickens sampled[31].

The role of *Campylobacter* in the etiology of human enteric disease has been established in developing countries, and there is a consensus that the environment and level of sanitary care are crucial in infections[11]. In USA, the 15% of *Campylobacter* infections have been due to contact with pets[18], with a wide range of reservoirs among mammals and birds present in the surroundings. As reported, *C. jejuni* isolates from clinically

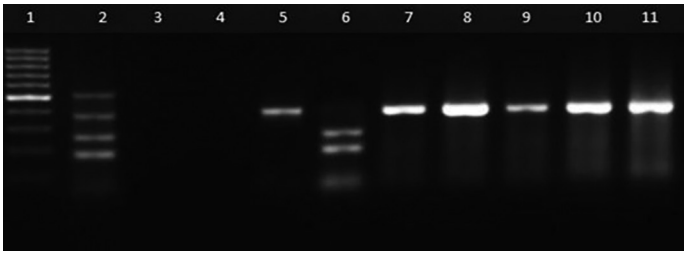

Fig. 2 - Gel electrophoresis of diarrheagenic *E. coli* samples from feral pigeons. Ladder 100 bp (1); positive control *E. coli* O157:H7 (2), negative control *E. coli* ATCC 25922 (3); blank (4), positive colonies EPEC (5,7-11); positive colony STEC (6). PCR products: 180 bp (*stx1*), 255 bp (*stx2*), 384 bp (*eae*) and 534 bp (*hylA*)[15]

Table 1
Classification of pathogenic *E. coli* isolates

Virulence factors [a]				Pathotype of *E. coli*	No. of colonies [b]
eae	*stx1*	*stx2*	*hylA*		
+	-	-	-	EPEC	6 (5.88%)
-	+	+	-	STEC	1 (0.98%)
				Total	7 (6.86%)

[a] + presence of gene; - absence of gene; [b] From a total of 102 *E. coli* isolates.

healthy homing pigeons can invade human enterocytes *in vitro*[30]. In some prevalence studies about human diarrhea in Peru, *Campylobacter* has been isolated more frequently from children. In the north of Lima, a study showed that 10% of patients with diarrhea were carriers of the bacteria, *C. coli* (5%), followed by *C. jejuni* (2.9%) and *C. lari* (2.1%) being the most frequently observed among children under the age of 5[5]. Furthermore, in clinical samples from some hospitals in Lima, 37 positive cases for *Campylobacter* spp. were obtained from children under one year-old[14]. However, a previous study indicated that exposure to the domestic poultry feces was the predominant risk factor for diarrhea in children[12].

The presence of pathogenic *E. coli* in urban feral pigeons has been reported mainly in developed countries as USA where 7.9% of the isolates corresponded to the STEC group[24]; whereas in the present study less than 1% was obtained. Other studies in Brazil showed that EPEC and ETEC were present in around 12.1% of feral pigeon droppings[29]. In Finland, the EPEC group presented a prevalence of 7%[16], similar to the results of the present study where EPEC represented 5.88% of the total isolations. Recently, in Spain they reported the EPEC group (6%) from urban pigeons, but no STEC was found, in contrast with our study where STEC was isolated[27]. According to a study in Lima, where 113 isolates of *E. coli* were collected from human diarrheic feces, 13.3% carried the *eae* gene (EPEC group); in addition, strains of ETEC, STEC, EAEC and EIEC[21] were found. Conversely, the implication of EPEC and STEC groups in neonatal diarrhea has been demonstrated in livestock, STEC being of zoonotic importance. In addition, it has been suggested that saprophytic *E. coli* strains, in exchanging genetic material from different sources, could become pathogenic if they acquire genetic sequences for specific virulence factors[17]. Thus, it is suggested that intestinal isolates from healthy birds could cause disease in susceptible hosts, depending on the combination of the virulence factors that occur[4].

It is known that direct and indirect contact with animals and food products may be of importance in the transmission of human STEC infections, especially of serotype O157:H7, which has been isolated from chickens and wild birds[7,9]. However, this study could not demonstrate the presence of *E. coli* STEC with more virulence genes than *eae* or *hylA*, which would have suggested a zoonotic enterohemorrhagic serotype. On the other hand, contamination of the environment is an important factor to consider with respect to the exposition to STEC in farm animals, which would keep the epidemiological cycle of pathogenic strains[26]. It is also known that the water that is used to irrigate many urban parks does not receive an adequate treatment, if they receive it at all, and serves as drinking water for animals, which could sustain the persistence of many enteropathogens in urban birds and its presence on human diarrhea, as has been suggested in a epidemiological study in a collective community in Israel[8].

The results of this study show, for the first time, the epidemiological status of diarrheagenic and zoonotic bacteria in urban feral pigeons of Lima, Peru. Further studies exploring previous exposure to birds or animal feces, antibiotic resistance profile in birds strains, and genotyping and phylogeny analysis between strains of human and animal source may contribute to fulfill the lack of epidemiological data in the country, and alert the national health authorities for the need of establishing preventive and control measures in order to reduce the possible causes of potential zoonoses.

RESUMO

Isolamento e detecção de *Escherichia coli* e *Campylobacter jejuni* potencialmente patogênicos em pombos selvagens de uma área urbana na cidade de Lima, Perú

Os pombos selvagens (*Columbia livia*) vivem em estreito contato com os seres humanos e outros animais. Podem transmitir agentes potencialmente patogênicos e zoonóticos. Os objetivos deste estudo foram isolar e detectar cepas de *Escherichia coli* diarreiogênica e *Campylobacter jejuni* de pombos selvagens urbanos de uma área de Lima, Peru. Amostras de fezes frescas foram coletadas em parques urbanos para o isolamento microbiológico para cepas de *E. coli* em ágar seletivo e *Campylobacter* por método de filtração. Identificação molecular de patótipos diarreiogênicos de *E. coli* e *Campylobacter jejuni* foi realizado por PCR. Vinte e dois parques foram amostrados e 16 colônias de *Campylobacter* spp. foram isolados. O 100% dos isolados foram identificados como *Campylobacter jejuni*. Além disso, 102 colônias de *E. coli* foram isoladas e 5,88% resultaram como tipo enteropatogênico (EPEC) e 0,98% como produtora de toxina Shiga (STEC). Os pombos selvagens urbanos de Lima no Peru podem atuar como reservatório ou ser portador de agentes zoonóticos entéricos potencialmente patogênicos.

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