

Isolation of *Salmonella enterica* subsp. *enterica* (O:4,5:i) and *Salmonella enterica* subsp. Typhimurium from free-living domestic pigeons (*Columba livia*)

[*Deteção de Salmonella enterica subsp enterica (O:4,5:i) e Salmonella enterica subsp. Typhimurium em pombo doméstico de vida livre (Columba livia)*]

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ABSTRACT

The present study reports the isolation of *Salmonella enterica* in organs of free-living domestic pigeons. In the clinic examination, the presence of feces in the peri-cloacal and abdominal regions were observed, as well as symptoms such as cachexy, incoordination and opisthotonos. Before any therapeutic protocol was applied the bird died and a necropsy was then performed for the removal of spleen, liver, kidney and intestine for bacteriological examination and antibiotic sensitivity test. *Salmonella enterica* subsp. *enterica* (O:4,5:i-) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium were isolated from the liver and intestine and the sensitivity test demonstrated that these strains are sensitive to several antibiotics.

Keywords: isolation, necropsy, bacteriological examination, sensitivity

RESUMO

O presente trabalho relata o isolamento de *Salmonella enterica* em órgãos de um pombo doméstico de vida livre. No exame clínico foi observada a presença de fezes pericloacal na região ventral, caquexia, incoordenação motora e opistótono. Antes de iniciar um protocolo terapêutico, a ave foi a óbito, e, em seguida, foi realizada uma necropsia para remoção do baço, fígado, rim e intestino para exame bacteriológico e teste de sensibilidade a antibióticos. Foi isolado *Salmonella enterica* subsp. *enterica* (O:4,5:i-) e *Salmonella enterica* subsp. *enterica* sorovar Typhimurium do fígado e intestino, e o teste de sensibilidade demonstrou que essas cepas são sensíveis a vários antimicrobianos.

Palavras-chave: isolamento, necropsia, exame bacteriológico, sensibilidade

INTRODUCTION

The pigeons are reservoirs for several organisms that are pathogenic for animals and humans. Approximately 60 different microorganisms, including viruses, bacteria, fungi and protozoan were isolated from pigeons (Haag-Wackernagel e Moch, 2004).

Among the pathogens found in these birds is *Salmonella enterica*, a bacteria responsible for salmonellosis, which is the most importance zoonotic disease in public health, responsible for outbreaks of gastrointestinal illness in humans

(Hauri *et al.*, 2004) and was isolated from feces of domestic pigeons (González-Acuña *et al.*, 2007).

The molecular epidemiological studies also indicate that different clones of serovar Typhimurium predominate among passerines, gulls, and pigeons (Refsum *et al.*, 2002) and the serotype Typhimurium is frequently associated with disease in many different mammalian and avian host species (Rabsch *et al.*, 2002). However, *S. Typhimurium* is not adapted to any particular host animal (Grund and Stolpe, 1992).

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Due to the survival of salmonelas in a natural environment, salmonellosis continues to be an important zoonosis, and can be transmitted to humans through animal and contaminated food. The pigeon is an important source of infection (Grund and Stolpe, 1992).

Thus, the aim of the present study was to report the occurrence of *Salmonella* spp. in free-living domestic pigeons and the antimicrobial resistance profile of these micro-organisms.

CASUISTRY

An adult male free-living domestic pigeon (*Columba livia*) was brought to the Laboratory of Ornithological Studies (LABEO) of State University of Ceará (UECE), Brazil. Upon the physical exam the bird presented cachexy, incoordination, opisthotonos and the presence of feces in the pericloacal and ventral regions. Before any therapeutic protocol was applied, the bird died and a necropsy was performed aseptically for removal of spleen, liver, kidney and intestine for bacteriological examination.

The organs were macerated individually in a petri dish and transferred to tubes containing Buffered Peptone Water (DIFCO, Sparks, Maryland, USA) and incubated for 24h at 40°C. After incubation, the aliquots were transferred to tubes containing Selenite-Cystine broth (DIFCO, Sparks, Maryland, USA) and Rappaport-Vassiliadis broth (DIFCO, Sparks, Maryland, USA). Subsequently, the samples were plated onto Brilliant Green agar (DIFCO, Sparks, Maryland, USA) containing 40µg Novobiocin (SIGMA), *Salmonella-Shigella* agar and MacConkey agar (DIFCO, Sparks, Maryland, USA). Colonies suspected to contain *Salmonella* were collected from each plate for biochemical identification using TSI (Triple Sugar Iron Agar), LIA (Lisine Iron Agar), SIM (Sulfur, Indol, Motility), VP (Voges-Proskauer), MR (Methyl Red), Urea, Ornithine Descarboxylase Broth and Simmons Citrate Agar. The

temperature and period of incubation were standardized at 40°C for 24h.

Colonies with biochemical profile of *Salmonella* were submitted to slide agglutination test using a polyvalent serum against O antigen (Probac). The colonies that agglutinated during one or two minutes were considered positive for *Salmonella*, and were preserved in Nutrient Agar. Isolates were submitted to FioCruz laboratory in Rio de Janeiro, Brazil, for complete identification and serotyping.

In order to determine the antimicrobial resistance profile, the disk diffusion method on Muller-Hinton Agar was tested according to CLSI (2003). The following antimicrobial agents were tested: penicilin G (10U), tetracycline (30µg), gentamicin (10µg), chloramphenicol (30µg), ciprofloxacin (30µg), ampicillin (10µg), streptomycin (10µg), sulfonamide (300mcg), neomycin (30µg), thiamphenicol (30µg) and erythromycin + sulfonamide + trimethoprim (15mcg + 300mcg + 5mcg). Following the application of the antimicrobial disks, the inoculated plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured (millimeter) and were compared to internationally accepted measurements to determine the susceptibility or resistance of the isolate. *Salmonella* Pullorum ATCC strain was used as control.

Salmonella enterica subsp. *enterica* (O:4,5:i) (*S. enterica* O:4,5:i) was isolated from the liver sample and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S. Typhimurium*) was isolated from liver and intestine samples. The results from the antimicrobial resistance test of the *Salmonella* isolated from samples of pigeon organs are shown in Tab. 1. *S. enterica* O:4,5:i was resistant to streptomycin, penicillin, thiamphenicol and neomycin, while *S. Typhimurium* was resistant to streptomycin, penicillin and thiamphenicol.

Isolation of *Salmonella enterica*...

Table 1. Antibiotic resistance profiles of *Salmonella enterica* subsp.*enterica* (O:4,5:i-) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolated from organs of a naturally infected pigeon

Isolate	Antimicrobial agent											
	AM	CP	CHL	GM	TE	ST	SF	P	TF	NM	EST	
<i>Salmonella enterica</i> subsp. <i>enterica</i> (O:4,5:i)	S	S	S	S	S	R	S	R	R	R	S	
<i>Salmonella</i> Typhimurium	S	S	S	S	S	R	S	R	R	S	S	

AM: ampicillin (10µg); CP: ciprofloxacin (30µg); C: chloramphenicol (30µg); GM: gentamycin (10µg); TE: tetracycline (30µg); ST: streptomycin (10µg); SF: sulfonamide (300mcg); P: penicillin (10U); TF: thiamphenicol (30µg); NM: neomycin (30µg); EST: estreptomycin+sulfonamide+trimethoprim (15mcg+300mcg+5mcg); S: susceptible; R: resistant.

DISCUSSION

The pigeons have been appointed as transmitters of pathogenic microorganisms to humans (Haag-Wackernagel and Moch, 2004), birds and other animals (Jahantigh and Nili, 2010).

Several studies performed with pigeons report the presence of pathogenic agents, mainly the ones that compromise the public health and cause significant economic impact worldwide, such as *Salmonella* spp. (Trevejo *et al.*, 2003).

In this study, two distinct serovars were isolated, *S. enterica* O:4,5:i and *S. Typhimurium* from two organs: liver and intestine. Both were isolated from the liver, while only *S. Typhimurium* was isolated from the intestine.

According to Grund and Stolpe (1992), the best site to isolate the pathogen is in the incision of the bowels, mainly in the ileum, although the small and large intestines may present the bacteria as well, but in smaller number.

The intestine samples that were positive for *Salmonella* were removed from two distinct portions: cranial (small intestine) and caudal (large intestine). The pathogen was successfully isolated from both portions.

The other isolate, *S. enterica* O:4,5:i, member of the O:4 group, the same as the *S. Typhimurium*, was not, to the best of the author's knowledge, reported causing infection in pigeons or any other animal species, neither in environmental, economic nor in public health nuisance.

The antimicrobial sensitivity test performed demonstrated that both *S. Typhimurium* as well as *S. enterica* O:4,5:I presented sensitivity to almost all of the antimicrobial agents.

The resistance of *S. Typhimurium* to streptomycin and sulfonamide has been widely reported in studies performed with isolates from feces of humans with gastroenteritis, contaminated feed and animal sources (Gorman and Adley, 2005) and the high resistance rate to multiple drugs are constantly present in many serotypes that cause human salmonellosis (Lauderdale *et al.*, 2006).

The resistant microorganisms may be transferred to humans through contact with sick animals or consumption of contaminated food originated from animals. Therefore the antimicrobial resistance monitoring in animals is highly important due to the risk of resistance gene transmission between pathogenic bacteria and the transmission of these pathogens to humans through the consumption of products derived from these animals (Yang *et al.*, 2001). Therefore, the resistance profiles are the result of the use of antimicrobial agents in human medicine, as well as in the animal originated food industry (Lauderdale *et al.*, 2006).

According to the genotype and the profile of antimicrobial resistance, the strains of *Salmonella* associated with captive and free-living birds represent a potential threat to men, since they can act as reservoirs for the transmission of salmonelas to the pet owners and people that live in direct or indirect contact with such birds (Hudson *et al.*, 2000).

CONCLUSION

In conclusion, the free-living pigeons are capable of hosting *Salmonella enterica* subsp. *enterica* (O:4,5:i-) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium, which may be zoonotic and are resistant to drugs commonly used to treat human infections.

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