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MICROBIOLOGY

Prevalence and antimicrobial resistance of *Salmonella* spp. isolated from free-ranging wild boars in the State of São Paulo, Brazil

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ABSTRACT: *European wild boars (Sus scrofa) are considered exotic invasive species worldwide. Invasions of wild boars are a growing public health concern, as wild boars may represent an important reservoir of zoonotic pathogens, including bacteria of the genus Salmonella. The aim of this study was to determine the prevalence and serovars of Salmonella spp. in free-ranging wild boars legally hunted in the state of São Paulo, Brazil, and the susceptibility of those Salmonella spp. to antimicrobials. Fecal samples and mesenteric lymph nodes were acquired from 63 wild boars. The prevalence of Salmonella spp. in free-ranging wild boars was 9.5 % (6/63; confidence interval: 4.4 % – 19.2 %). Six serovars were isolated: S. enterica subsp. enterica ser. 4,5,12:-:1,2, S. enterica ser. Cerro, S. enterica ser. Madelia, S. enterica ser. Typhimurium, S. enterica ser. I (4,5,12:i:-) and S. enterica ser. Muenster. Analysis of antimicrobial resistance of Salmonella spp. showed that the majority of serovars were fully susceptible to the tested antimicrobials. Only S. enterica ser. Typhimurium and S. enterica ser. Muenster showed a resistance pattern to at least one antimicrobial analyzed. To our knowledge, this study is the first report the prevalence and serovars of Salmonella spp. in free-ranging wild boars in the State of São Paulo, Brazil. Results indicate a low prevalence with variability of Salmonella serovars, with some pattern of antimicrobial resistance. This study highlights the potential role of wild boars as carriers of Salmonella and could pose a risk to wild and domestic animals as well as humans.*

Key words: *salmonellosis, public health, Sus scrofa, wildlife, reservoir.*

Prevalência e resistência antimicrobiana de *Salmonella* spp. isoladas em javalis de vida livre no Estado de São Paulo

RESUMO: *Os javalis europeus (Sus scrofa) são considerados uma espécie exótica invasora em todo o mundo. As invasões de javalis são uma preocupação crescente de saúde pública, pois os javalis podem representar um importante reservatório de patógenos zoonóticos, incluindo bactérias do gênero Salmonella. O objetivo deste estudo foi determinar a prevalência e os sorovares de Salmonella spp. em javalis de vida livre caçados legalmente no Estado de São Paulo, Brasil, e a suscetibilidade dessa Salmonella spp. aos antimicrobianos. Amostras fecais e linfonodos mesentéricos foram adquiridos de 63 javalis. A prevalência de Salmonella spp. em javalis selvagens foi de 9,5% (6/63; intervalo de confiança: 4,4% - 19,2%). Seis sorovares foram isolados: S. enterica subsp. enterica ser. 4,5,12:-:1,2, S. enterica ser. Cerro, S. enterica ser. Madelia, S. enterica ser. Typhimurium, S. enterica ser. I (4,5,12:i:-) e S. enterica ser. Muenster. As análises de resistência antimicrobiana de Salmonella spp. evidenciaram que a maioria dos sorovares era pansensível aos antimicrobianos testados. Apenas S. enterica ser. Typhimurium e S. enterica ser. Muenster mostraram um padrão de resistência a pelo menos um antimicrobiano analisado. A saber, este estudo é o primeiro relato da prevalência e de sorovares de Salmonella spp. em javalis de vida livre no Estado de São Paulo, Brasil. Os resultados indicaram baixa prevalência com variabilidade de sorovares de Salmonella, com algum padrão de resistência antimicrobiana. Este estudo destaca o papel potencial dos javalis como portadores de Salmonella spp. e pode representar um risco para os animais domésticos e selvagens, bem como para os humanos.*

Palavras-chave: *salmonelose, saúde pública, Sus scrofa, selvagem, reservatório.*

INTRODUCTION

European wild boars (*Sus scrofa*) are considered exotic invasive species in several countries, including Brazil (BARRIOS-GARCIA & BALLARI, 2012; PEDROSA et al., 2015). An increase in wild boar populations has been observed worldwide (FREDRIKSSON-AHOMAA, 2019). In Brazil, studies on distribution of wild boars in 2015, has shown their occupation of more than 472 Brazilian municipalities. São Paulo was the most affected state, with *Sus scrofa* present in 24.18 % (156/645) of its

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municipalities, followed by the states of Rio Grande do Sul (11.08 %; 55/496) and Minas Gerais (10.66 %; 91/853) (PEDROSA et al., 2015).

Because of the growth in the wild boar populations and their negative impacts on native flora and fauna, agriculture and public health, the hunting of wild boars has been allowed since 2013 by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) as a method of population control in Brazil (BRASIL, 2013).

In the last decade, concern over the public health implications of wild boars has grown, as they may represent an important reservoir of zoonotic pathogens, including bacteria of the genus *Salmonella* (WACHECK et al., 2010). Moreover, antimicrobial resistance occurs frequently in the environment, directly affecting populations of domestic and wild animals (LITERAK et al., 2010), and, consequently, can reduce therapeutic options for bacterial infections in humans (EIBACH et al., 2016). This problem can be aggravated by occasional migrations of wild boars close to domestic herds and human habitats because this greatly increases the possibility of pathogens being naturally transmitted between the wild boars and either domestic animals or humans (MEIER & RYSER-DEGIORGIS, 2018).

A disease that is prevalent in wild boar populations would be extremely difficult to control and eradicate and could cause health damages to livestock and economic embargos on the exportation of animal products (PIRES et al., 2013). Hence, the epidemiological surveillance of *Salmonella* spp. in wild boars contributes to the epidemiological understanding of the pathogen and the occurrence of its resistance to antimicrobials (FISCHER et al., 2014; QUESADA et al., 2015).

Considering inadequate data on the prevalance and resistance pattern of *Salmonella* spp. in wild boar populations throughout Brazil, further studies are needed to provide more data. The aim of this study was to determine the prevalence and serovars of *Salmonella* spp., as well as their susceptibility profiles to antimicrobials in free-ranging wild boars hunted in the State of São Paulo, Brazil.

MATERIALS AND METHODS

Sample collection

Convenience samples were collected between January 2018 and February 2019. Fecal samples and mesenteric lymph nodes were collected from wild boar legally hunted on farms located in the State of São Paulo. The study area was composed of the following municipalities: Barretos, Guaraci, Olímpia, Colina, Jaboticabal and Torrinha (Figure 1). The study region was characterized by the cultivation

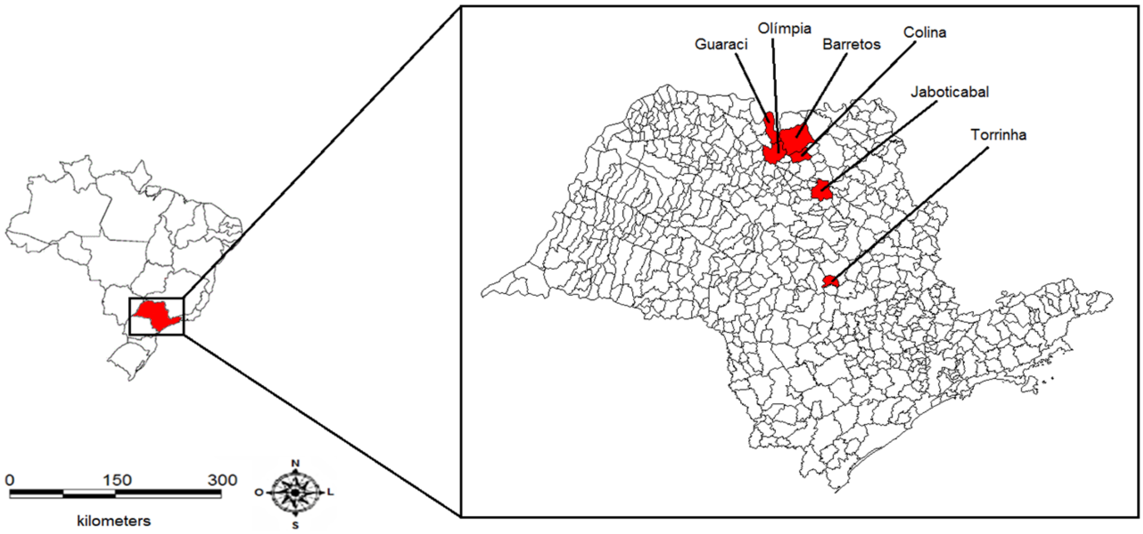

Figure 1 - Municipalities of origin of free-ranging wild boar samples collected between January 2018 and February 2019, in the State of São Paulo, Brazil.

of sugarcane and orange. Additional features of the study area included running watercourses, pastures, native forests with swamp formations, and the presence of domestic animals, mainly cattle and dogs.

The feces were collected using rectal swabs and placed in falcon tubes containing 10 ml of 1 % peptone water. After each hunt, each wild boar carcass was subject to standard necropsy, and the mesenteric lymph nodes were collected through incision by which approximately 5 g of tissue was obtained and placed in a sterile vial. Both samples were refrigerated until laboratory tests were performed, at a temperature between 2 ºC and 8 ºC, for up to 24 h.

Isolation and identification of Salmonella spp.

Isolation and identification of the *Salmonella* genus (microbiological and molecular assays) were performed at the Ornithopathology Laboratory of the Faculty of Agricultural and Veterinary Sciences of FCAV/Unesp. Presumed *Salmonella* colonies were subjected to polymerase chain reaction (PCR) targeting the *invA* gene to confirm the genus, as described by FRATAMICO & STROBAUGH (1998). Positive samples in both microbiological and molecular assays were submitted to the Enterobacteria Section of Adolfo Lutz Institute, São Paulo, SP, Brazil, for serovar identification (BRASIL, 1995).

Assessment of susceptibility to antimicrobials

The antimicrobial sensitivity of *Salmonella* strains was evaluated by Kirby-Bauer disk diffusion and minimal inhibitory concentration (MIC) tests. *Escherichia coli* strain ATCC® 25922™ was used for quality control. Twenty antimicrobials from nine different classes were used: nalidixic acid 30 µg/mL, enrofloxacin 5 µg/mL, ciprofloxacin 5 µg/mL, chloramphenicol 30 µg/mL, aztreonam 30 µg/mL, streptomycin 10 µg/mL, gentamicin 10 µg/mL, sulfonamide 300 µg/mL, trimethoprim 1.25 µg/mL, sulfamethoxazole 23.75 µg/mL, imipenem 10 µg/mL, cefoxitin 30 µg/mL, cefotaxime 30 µg/mL, cefepime 30 µg/mL, ceftiofur 30 µg/mL, ampicillin 10 µg/mL, amoxicillin 10 µg/mL, amoxicillin and clavulanic Acid 10 and 20 µg/mL, respectively; tetracycline 30 µg/mL, phosphomycin 200 µg/mL and polymyxin E 0.125-32 µg/mL.

The MIC test was performed only for polymyxin E, and strain resistance was assumed when antimicrobial concentration was higher than 2 µg/mL. The results were compared with the standards of the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017). Intermediate profiles were assumed to be resistant to the disk diffusion test (FIROOZEH et al., 2011). Serovars resistant to three or more antimicrobials from different classes were considered multiresistant (SCHWARZ et al., 2010).

Statistical analysis

Descriptive statistics of the variables studied were performed with frequency distribution and calculation of the 95 % confidence interval (CI) of prevalence rates.

RESULTS

Fecal samples (n = 63) and mesenteric lymph nodes samples (n = 11) were collected from 63 wild boars legally hunted in the State of São Paulo. From the wild boars, 46 were from Barretos, eight from Guaraci, four from Olímpia, three from Colina and one each from Torrinha and Jaboticabal.

In the present study, the prevalence of *Salmonella* spp. in free-ranging wild boars in the State of São Paulo was 9.5 % (6/63; CI: 4.4 % – 19.2 %), with six different serovars isolated (Table 1). Four serovars were recovered in feces (6.35 %; 4/63; CI: 2.49 % - 15.22 %), and three serovars from lymph nodes (27.27 %; 3/11; CI: 9.74 % - 56.56 %). Two serovars were isolated from the wild boar 4: *S. enterica* ser. Typhimurium and *S. enterica* ser. Cerro. In Barretos, five samples were positive (83.3 %; 5/6; CI: 44 % - 97 %), and only one was positive for Torrinha, (6.6 %; 1/6; CI: 2.99 % – 56.3 %). All suggestive *Salmonella* isolates were positive by PCR targeting *invA* gene.

Analysis of antimicrobial resistance of *Salmonella spp.* isolated from wild boars showed that the majority of serovars were full susceptible to the tested antimicrobials (66.6 %; 4/6; CI: 30.0 % – 90.3 %). Only two strains (33.3%; 2/6; CI: 47.8% - 108.2%), belonging to serovars *S. enterica* ser. Typhimurium and *S. enterica* ser. Muenster, showed a resistance pattern to at least one antimicrobial analyzed. The serovar *S. enterica* ser. Typhimurium showed resistance to enrofloxacin, tetracycline and ampicillin, and was classified as a multiresistant strain (resistant to three or more antimicrobials of different classes). While *S. enterica* ser. Muenster was resistant to ciprofloxacin and nalidixic acid. The isolates tested in the MIC analysis for polymyxin E did not show resistance.

DISCUSSION

The results obtained in this study confirmed the presence of *Salmonella* spp. in wild boars (9.5 % - 6/63) in some regions of the State of São Paulo,

Table 1 - *Salmonella* prevalence and serovars identified in wild boars (*Sus scrofa*) in the North, Northeast and Midwest regions of the State of São Paulo.

Municipality	Prevalence (n/N)[1] CI95[2]	Animal ID[3]	Isolated serotypes	Origin of samples
Torrinha	6.6 % (1/6) (2.9 % - 56.3 %)	Wild boar 1	*S. enterica* subsp. *enterica* ser. 4,5,12:-:1,2	Feces
Barretos	83.3 % (5/6) (44 % - 97 %)	Wild boar 2	*S. enterica* ser. Cerro	Feces
		Wild boar 3	*S. enterica* ser. Madelia	Feces
		Wild boar 4	*S. enterica* ser. Typhimurium	Feces
			S. enterica ser. Cerro	Lymph node
		Wild boar 5	*S. enterica* ser. I (4,5,12:i:-)	Lymph node
		Wild boar 6	*S. enterica* ser. Muenster	Lymph node

1- positive wild boars/total of wild boars tested; 2- 95 % confidence interval; 3- wild boar identification.

Brazil. Even though a low prevalence of *Salmonella* spp. was observed among the wild boars sampled in this study, results indicate that free-ranging wild boars can still be a source of *Salmonella* spp. infections for livestock and humans, and highlight the potential role wild boars can play as carriers of this zoonotic agent (CARLSON et al., 2011; HILBERT et al., 2012).

Wild boar carcass contaminated with *Salmonella* spp. could transmit the pathogen to humans through consumption of raw or undercooked meat (SANNÖ et al., 2018). The risk of *Salmonella* being transmitted to humans from wild boar carcasses is corroborated by inadequate hygiene practices in the handling and preparation of wild boar carcasses, such as evisceration in the soil and washing of the carcass after evisceration, which contribute to the higher frequency of carcass contamination (MIRCETA et al., 2017).

Among the isolates, our results confirm the presence of *Salmonella enterica* serovars that are of importance to public health, such as *S. enterica* subsp. *enterica* ser. 4,5,12:-:1,2 and *S. enterica* ser. I (4,5,12:i:-). Both are atypical and emergent monophasic serovars, that share almost all antigen with *S. enterica* ser. Typhimurium (ECHEITA et al., 2001), a serovar also isolated in this study.

Salmonella enterica ser. Typhimurium and monophasic *S. enterica* ser. Typhimurium (1,4,[5],12:i:-) are the two most commonly reported *Salmonella* serovars in human outbreaks (EFSA, 2017). In Brazil, between the years of 2000 and 2018, *Salmonella* spp. was the pathogen most frequently associated with outbreaks (FINGER et al., 2019).

The prevalence of *Salmonella* spp. in wild boars observed in this study matches the prevalence observed in other regions. THAKUR et al. (2011) reported 5 % (8/161) prevalence in wild boars in the USA, while MOLINA-LÓPEZ (2015) and MOLINO et al. (2019) reported a prevalence of 4.2 % (11/263) and 7.7 % (81/1041), respectively, in Spain. Currently, the only other study in Brazil is for the State of Goias which found 1 % (1/100) prevalence of *Salmonella* spp. in samples of wild boar feces collected from commercial farms (FARIA, 2016). However, studies outside of Brazil have measured higher prevalence of *Salmonella* in wild boars. For instance, VIEIRA-PINTO (2011) measured a prevalence of 22.1 % (17/77) in Portugal, and CHIARI (2013) measured a prevalence of 24.8 % (326/1313) in Italy.

The presence of cattle on pasture possibly contributed to the occurrence of *Salmonella* spp. in this study, because cattle shared the same habitat as wild boars. In Spain, NAVARRO-GONZALEZ et al. (2012) concluded that wild boars from cattle-free areas had a lower *Salmonella* spp. prevalence than of those that co-habited with cattle.

Salmonella spp. in wildlife has often been associated with serovar *S. enterica* ser. Typhimurium, but a greater diversity of serovars have been isolated in recent years (PAULSEN et al., 2012; MOLINO et al., 2019). The isolation of other serovars in this study, such as *S. enterica* ser. Muenster, *S. enterica* ser. Cerro and *S. enterica* ser. Madelia, reflects the high variability of *Salmonella* serovars found in wildlife. The *S. enterica* ser. Madelia is usually found in the fish production chain, for instance, in schrimp (BESHIRU et al., 2019). While *S. enterica* ser. Cerro and *S. enterica* ser. Muenster have been described in cattle and poultry (KOVAC et al., 2017; NOUICHI et al., 2018; LÚCIO et al., 2019; JIBRIL et al., 2021).

Variability in the presence of different *Salmonella* serovars and the prevalence of *Salmonella* is attributed to different sources of exposure such as: waste disposal (BALL et al., 2020), livestock, and other wild species such as birds and reptiles (PAULSEN et al., 2012). Variability is further the result of through direct contact, the sharing of resources used by food animals (specially pastured livestock), water cross-contamination and omnivore feeding habits such as the consumption of small animals carrying *Salmonella* spp. or contaminated carcasses (AVAGNINA et al., 2012; NAVARRO-GONZALEZ et al., 2012).

In wild fauna, investigations of antimicrobial resistance are highly variable in their results and depend mainly on the host species, etiologic agent and geographic location. Humans may be the main source of antimicrobial resistance found in wild life, owing to the indiscriminate use of antimicrobials (NAVARRO-GONZALEZ et al., 2012; EIBACH et al., 2016).

Similar to the present study, resistance to ciprofloxacin and nalidixic acid was observed in *S. enterica* ser. Enteritidis isolated from the feces of wild boar present in northeastern Spain (NAVARRO-GONZALEZ et al., 2012). FARIA (2016) reported *Salmonella* spp. resistant to tetracycline and ampicillin, as well as to the chloramphenicol, neomycin, amoxicillin and sulfonamide in wild boar fecal samples from a commercial breeding system in Brazil. Animals submitted to industrialized production systems carry bacteria with greater levels of virulence and resistance factors, because of the greater pressure of natural selection, as was corroborated by SCHIERACK et al. (2013).

Furthermore, the resistance pattern of *Salmonella* spp. to antimicrobials in different samples of farm animals has also been reported, as several studies have shown that *Salmonella* spp. have antimicrobial resistance, especially to the nalidixic acid, ciprofloxacin, tetracycline and ampicillin, as was observed from milk samples in Ghana (KUNADU et al., 2018), chicken carcasses in China **(**HU et al., 2017**)**, and pig carcasses in Colombia (RONDÓN-BARRAGÁN et al., 2015).

Although *Salmonella* spp. are resistant to antimicrobials occurring in different animal species around the world, especially with regard to ciprofloxacin and nalidixic acid in free-living animals, it is possible that the number of virulence and resistance genes in *Salmonella* spp. are limited owing to lower exposure of wildlife to antimicrobial drugs. However, anthropic action, owing to the need for greater productivity, can release wastewater into water bodies, as well as discard antimicrobial residues, thus promoting environmental contamination and, consequently, exposing animals to microorganisms with a greater possibility of resistance to these drugs.

CONCLUSION

This study is the first to report the prevalence and serovars of *Salmonella* spp. in free-ranging wild boars in the State of São Paulo, Brazil. Results showed variability of serovars and, despite low *Salmonella* spp. prevalence, highlights the potential role of wild boars as carriers of *Salmonella* spp. transmission to wild and domestic animals and humans. Considering the widespread occurrence of free-ranging wild boars in the State of São Paulo and their potential to act as healthy carriers of a wide range of *Salmonella* spp. serotypes, the epidemiological role of this species in relation to salmonellosis should be further investigated.

The pattern of multiresistance to antimicrobials expressed by *Salmonella* spp. in this study highlights the risk that the indiscriminate use of antimicrobial drugs. Environmental contamination with these drugs can result in multiresistant strains of pathogens, with which wildlife, like wild boars, come into contact.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL DECLARATION

The study was approved by the Animal Use Ethics Commission (CEUA), from the Faculdade de Ciências Agrárias e Veterinárias (FCAV) Jaboticabal, São Paulo State University, protocol no. 014836/18, and the Chico Mendes Institute for Biodiversity Conservation (ICMBio), protocol no. 62641-2.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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