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Prevalence of *Salmonella* strains in wild animals from a highly populated area of north-eastern Italy

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Abstract

Introduction. *Salmonella* is a ubiquitous pathogen that can infect host species, like wild birds, rodents, and/or arthropods, which may transmit infection to domestic animals and human population.

Aim. In order to assess the related risk, a cross-sectional study was performed on 1114 carcasses of wild animals from a north-eastern area of the Emilia-Romagna Region, Italy.

Materials and methods. During *post mortem* examination, intestine samples were cultured. A statistical analysis demonstrated that there is no correlation between the presence of sub-clinically infected animals and greater human population density. In contrast, a significant correlation between the number of carcasses positive for *Salmonella* spp. and greater spatial density of pig, poultry, and cattle farms was observed ($p < 0.01$).

Results. The results of the present study show that wild animals with omnivorous feeding habits are particularly exposed to *Salmonella* colonization and, consequently, to spreading the organism. Regarding drug resistance, this study confirms the resistance to antimicrobials is increasing in commensal and environmental isolates.

Key words
- *Salmonella*
- infection
- wild animals
- humans
- risk

INTRODUCTION

Salmonella is a ubiquitous pathogen that can infect a wide range of host species and cause various diseases. More than 2500 serovars of *Salmonella* genus have been identified [1]. Salmonellosis is an infectious disease of global concern that is transmitted between species, sometimes by a vector, from animals other than humans to humans or from humans to other animals. Indeed, *Salmonella* species (spp.) are able to infect a wide range of domestic and wild animal species and have been isolated from the intestinal content of birds and mammals including wildlife. Infectious pathogens of wildlife origin have gained interest and are considered to be of increasing global importance, mainly because of their role in livestock health and productivity, as well as their zoonotic potential [2]. In particular, the capacity of *Salmonella* spp. to persist in the environment may facilitate the infection of wild birds, rodents, and/or arthropods, which may in turn transmit these pathogens to domestic animals [3]. Cross-infection from wild birds is possible, frequently if they are feeding in farms or stables. This study focuses on wild animals living in the north-east of the Emilia-Romagna Region, Italy, and was aimed to investigate the presence of *Salmonella,* including the antibiotic-resistant strains in wild animals. In particular, this study highlights the problem of whether foxes (*Vulpes vulpes*) and other wild animals could have any role in the spread of *Salmonella* in domestic animals or vice versa.

MATERIALS AND METHODS

A cross-sectional study was performed in the Province of Ferrara analysing carcasses of wild animals provided as a part of a Regional Program named "Wildlife Health Surveillance System for prevention of human and animal infections" instituted by the Emilia-Romagna Region since 2007. To detect the presence of *Salmonella* spp. only adult subjects were considered. The hunted or dead animals found in the territory considered were given to the local laboratory of the Istituto Zooprofilattico

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Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER). The carcasses were refrigerated overnight on the same day they were collected and cultured on the following day.

Salmonella was searched according to standard culture methods (ISO 6579:2002 protocol) [4] in about 25 grams of intestine taken from each carcass. The identification of *Salmonella* spp. was performed using biochemical tests (API 20 E System Biomerieux™ and BBL Enterotube Becton Dickinson™) and characterized phenotypically using the serum agglutination test according to the Kauffmann–White scheme [5]. The antimicrobial susceptibility test on the isolated strains was performed using the Kirby-Baüer method on Müeller-Hinton agar with 12 antimicrobial agents (amoxicillin + clavulanic acid, ciprofloxacin, cefotaxime, trimethoprim + sulfamethoxazole, chloramphenicol, ampicillin, streptomycin, tetracycline, gentamicin, nalidixic acid, colistin and cephalothin). The isolates were classified as a susceptible, intermediate or resistant strain according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [6]. Isolated strains resistant to four or more antimicrobials were considered as a "multidrug resistant" (MDR) strain. The serological characterization of the *Salmonella* isolates was performed using two methods: somatic antigens were determined by slide agglutination testing, whereas flagellar antigens were identified by the tube agglutination method, according to the Spicer technique modified by Edwards and Morris. The individual antigenic profile was then used for serological characterization of strains according to the scheme by Kauffmann-White-Le Minor [7, 8].

Statistical analysis: for each municipality of the Province of Ferrara, the number of positive carcasses of wild animals and the number of pig, poultry, and cattle farms were assessed. Descriptive univariate and bivariate (chi-squared) statistical analysis was performed, and odds ratio with the 95% confidence interval were calculated.

RESULTS

A total of 55 pig, poultry, and cattle farms were considered in the present study. From January 2010 to September 2013 period, a total of 1 114 samples were collected: 49.10% magpies (*Pica pica*), 23.43%, foxes (*Vulpes vulpes*): 23.43%, hooded crows (*Corvus corone cornix*) 2.06% jays (*Garrulus glandarius*), 5 brown hares (*Lepus europaeus*), 5 hedgehogs (*Erinaceus europaeus*), 4 pigeons (*Columbia livia*), 2 starlings (*Sturnus vulgaris*), and 1 of the followings: swan (*Cygnus cygnus*), pheasant (*Phasianus colchicus*), wild duck (*Anas platyrhynchos*), porcupine (*Hystrix cristata*), green woodpecker (*Picus viridis*) and Eurasian collared dove (*Streptopelia dedecaocto*).

Overall 32 isolates of *Salmonella* were obtained from the 1 114 samples tested (2.87%). Twenty-two *Salmonella* spp. 22 were found in foxes (68.75%), 7 in magpies (21.88%), 2 in hooded crows (6.25%), and 1 in a hedgehog (3.12%). *S. Enteritidis* was the serovar isolated more frequently *(Table 1)*.

All 32 isolates were susceptible to ciprofloxacin, cefotaxime and CAF. Out of all the isolates tested, 7 (21.87%) were susceptible to all the antimicrobials tested. The remaining 25 isolates (78.12%) were found to be resistant to at least one antibiotic and 11 (34.38%) were found to be resistant to more than one antimicrobial. Among the isolates resistant to four or more antimicrobials (multi-drug resistant, MDR), the most common resistance phenotype observed was cephalothin (53.13%), followed by ampicillin (34.38%), streptomycin (21.88%), tetracycline (15.63%), amoxicillin and clavulanic acid (12.5%). MDR was found among the following serovars: Hadar, Newport, Typhimurium monophasic variant, and Bredeney. The possible correlation between the location of the carcasses positive for *Salmonella* spp. and the territorial distribution of the pig, poultry, and cattle farms was investigated. The presence of positive carcasses in the municipalities of the Province of Ferrara was associated with the presence of farms in the same municipality (OR 11.20; 95% CI: 3.90-32.20).

DISCUSSION

This study showed *Salmonella* spp. in wild animals in the Province of Ferrara. The proportion of positive samples was much lower than what observed in previous studies [2, 9-11]. Compared to a recent study carried out in North-western Italy [10], we obtained about half of the proportion of *Salmonella* positive samples, and still lower than the proportion of positivity among mammals and birds reported by Millán *et al.* [11]. However, it should be noted that many of these studies were carried out on live animals and/or faeces samples and not on the carcasses. This difference could partly justify the different positivity rates observed between our study and the literature [12, 13].

In contrast, according to Millán *et al.*, our study demonstrated that the behaviour and feeding habits of animals influences the likelihood of their being infected with *Salmonella*, as demonstrated by several studies [14-16]. In fact, we isolated the *Salmonella* from foxes and *Corvidae* that probably acquired *Salmonella* scavenging on contaminated carcasses. The presence of farming animals (pig, poultry, and cattle) was related with *Salmonella* spp. detection in samples from wild animals. This finding is probably related to the fact that the species mostly positive (fox, magpie, hooded crow, hedgehog) normally living in the vicinity of the farms due to predation or to take advantage of animal feed.

Concerning the susceptibility to antimicrobials of the isolates, our results show that, in the Ferrara Province, the multiresistant serovars isolated are Newport, Hadar, and, as well as reported in other studies, the monophasic variant of Typhimurium [17]. All the animals sampled in this study can be considered healthy carriers of *Salmonella* because of the absence of pathological lesions attributable to salmonellosis (haemorrhagic enteritis, glaucomatous hepatitis, etc.). So, we suppose that the phenomenon is not the result of a local epidemic of salmonellosis, but is caused by a subclinical infection originating from environmental bacteria. The results of the present study show that, although it is well known that farm animals are a major reservoir of *Salmonella*, wild animals with omnivorous feeding habits can be exposed to *Salmonella* colonization and,

Table 1
Antimicrobial susceptibility of the *Salmonella* serovars detected in the different wild animal species (S = susceptible; I = intermediate; R = resistant)

Serovar	Species common name (scientific name)	Amoxicillin and Clavulanic acid (30 µg)	Ciprofloxacin (5 µg)	Cefotaxime (30 µg)	Trimethoprim and Sulfamethoxazole (25 µg)	Chloramphenicol (30 µg)	Ampicillin (10 µg)	Streptomycin (10 µg)	Tetracyclin (30 µg)	Gentamicin (10 µg)	Nalidixic acid (30 µg)	Colistin sulphate (10 µg)	Cephalotic (30 µg)
S. Bredeney	Magpie *(Pica pica)*	I	I	S	S	S	R	R	R	S	R	S	I
S. Braenderup	Magpie *(Pica pica)*	S	S	S	S	S	I	S	S	S	S	S	S
S. Braenderup	Red fox *(Vulpes vulpes)*	S	S	S	S	S	S	S	I	S	I	I	I
S. enterica O11-F	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	I	S	I	I	I	I
S. enterica Subsp. *houtenae* group O:43 (U)	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	R	S	I	I	I	R
S. Enteritidis	Hedgehog *(Erinaceus europaeus)*	I	S	S	S	S	I	I	S	S	S	S	S
S. Enteritidis	Hooded crow *(Corvus corone cornix)*	I	S	S	S	S	R	I	S	S	S	S	I
S. Enteritidis	Red fox *(Vulpes vulpes)*	I	S	I	S	S	R	R	I	S	S	S	I
S. Enteritidis	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	I	S	S	I	I	I
S. Enteritidis	Red fox *(Vulpes vulpes)*	S	S	I	S	S	I	I	S	I	I	I	R
S. Enteritidis	Red fox *(Vulpes vulpes)*	S	S	S	S	S	S	I	S	S	S	I	R
S. Enteritidis	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	I	S	S	S	I	R
S. Enteritidis	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	I	S	S	I	I	R
S. Hadar	Red fox *(Vulpes vulpes)*	R	S	S	S	S	R	R	R	S	R	S	R
S. Hessarek	Red fox *(Vulpes vulpes)*	S	S	I	S	S	I	S	S	S	S	I	R
S. Hessarek	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	S	S	I	S	I	R
S. Hessarek	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	I	S	R	S	I	R
S. Livingstone	Red fox *(Vulpes vulpes)*	I	S	I	S	S	I	I	I	S	S	S	S
S. Mbandaka	Magpie (*Pica pica)*	I	S	S	S	S	I	I	S	S	S	S	S
S. Newport	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	R	S	S	S	S	S
S. Newport	Red fox *(Vulpes vulpes)*	R	S	S	R	S	R	S	R	I	I	S	R
S. Newport	Red fox *(Vulpes vulpes)*	R	S	S	R	S	R	S	R	S	I	S	R
S. Typhimurium	Red fox *(Vulpes vulpes)*	S	S	S	S	S	R	S	I	S	I	I	R
S. Typhimurium	Magpie *(Pica pica)*	I	S	S	S	S	R	I	S	S	S	R	I
S. Typhimurium	Hooded crow *(Corvus corone cornix)*	S	S	S	S	S	I	I	S	S	S	R	S
S. Typhimurium	Magpie *(Pica pica)*	S	S	S	S	S	R	I	S	S	S	S	I
S. Typhimurium	Magpie *(Pica pica)*	S	S	S	S	S	I	I	S	S	S	S	R
S. Typhimurium	Magpie *(Pica pica)*	S	S	S	S	S	R	R	S	S	S	S	S
S. Typhimurium var. 5-	Red fox *(Vulpes vulpes)*	S	S	S	S	S	I	I	S	S	I	I	R
S. Typhimurium monophasic variant	Red fox *(Vulpes vulpes)*	S	S	S	S	S	S	I	S	I	S	S	R
S. Typhimurium monophasic variant	Red fox *(Vulpes vulpes)*	R	S	S	S	S	R	R	R	S	I	R	R
S. Zaiman	Red fox *(Vulpes vulpes)*	S	S	I	S	S	I	I	S	S	I	I	R

consequently, potentially involved in the spread of the organism. The role of wild animals as carriers and faecal spreaders of *Salmonella* spp. in the environment should not be neglected as they can act as good sentinel species in predicting the presence of *Salmonella* serovars implicated in foodstuff contamination, animal and human infections.

Furthermore, Regional Monitoring Plan on Wildlife should be updated based on new scientific knowledge, the results of the previous years, and any emerging issues which should be included in it.

Among the weaknesses of this study, there is the study design, that is based on the estimate of the *Salmonella* spp. from the sampling of carcasses of dead animals, without any sampling in live animals, that should have provided different figures.

In conclusion, we have seen a relationship between isolates of *Salmonella* spp. in carcasses of wild animals detected in proximity to farms. Secondly, we detected resistant and multiresistant *Salmonella* serovars commonly found in farming animals. This let us hypothesize that the widespread abuse of antibiotics in animals could influence the spread of pathogenic resistant *Salmonella* serovars also in wildlife.

Authors' contribution statement

Silva Rubini: study design, data collection, data analysis.

Cinzia Ravaioli: data collection, data analysis.

Sara Previato: data collection, data analysis.

Mario D'Incau: processing and analysis of samples.

Massimo Tassinari: collection of samples.

Enrica Guidi: statistical processing and data analysis.

Silvia Lupi: statistical processing and data analysis.

Giuseppe Merialdi: study design, territorial coordination of sample collection

Mauro Bergamini: study design, data analysis, drafting of the final manuscript.

All co-authors reviewed and assisted in the editing of the final version of the manuscript.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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