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Salmonella enterica subsp. *enterica* serotypes isolated for the first time in feral cats: The impact on public health

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ABSTRACT

Stray cat populations can represent a significant threat of the transmission of zoonotic diseases such as salmonellosis. The objective of this study was to assess *Salmonella* carriage by free-living cats in Gran Canaria island and the *Salmonella* serovars involved, in order to inform to those responsible for the colonies about the possible risk factors. One hundred rectal swabs of feral cats were taken. *Salmonella* strains were serotyped in accordance with Kauffman-White-Le-Minor technique. Of a total of 100 animals under study, 19% were found to be positive to *Salmonella* spp. This is the first report that described the zoonotic serovars *S.* Nima, *S.* Bredeney, *S.* Grancanaria and *S.* Kottbus in cats. The present study demonstrates that feral cats may represent a source of risk for the spread of different *Salmonella* zoonotic serovars. It has been reported that there is a certain correlation between *Salmonella* isolates from pets and wild animals. Further studies are needed from other animal species and environmental sources to make this correlation.

1. Introduction

Salmonella is considered one of the most important zoonotic pathogen agents causing an estimated number of 93.8 million cases of gastroenteritis worldwide annually [1,2]. Despite the fact that salmonellosis is mostly noticed as a food-borne disease, it has been estimated that about 9% of the cases are due to direct contact with animals [1]. Pets may play a significant role in transmission of the pathogen in the environment and household. They could excrete *Salmonella*, which can infect other animals and humans through the environment [3].

In 2020, the number of households with pets in Europe was 88 million, being cats the most commonly pet, with a population of over 110 million [4]. Moreover, free-living cats, that live outdoors in public or private urban areas, usually live in colonies, using resources of the human activity to feed or predating small animals such as birds, rodents and reptiles [5–7].

Populations of free-living cats could represent an important threat to public health, being an important factor in the transmission of zoonotic diseases due to the close contact with humans and other pets [8]. Previous studies, carried out in different countries, have reported cats as a source of rabies, toxoplasmosis, tularemia, murine typhus [9,10] and salmonellosis, among others [5,11–13]. The close contact between these animals and humans provides favourable conditions for the transmission of zoonotic pathogens infections, constituting a public health concern, as cats have been considered as potential *Salmonella* carriers [3]. Free-living cats lack the preventive care necessary to control these diseases and therefore pose a potential risk to other domestic animals and to human health. The control of the health status of these animals under a One Health viewpoint is necessary due to their direct impact on public health [5].

In Canary Islands (Spain), trap-neuter-return (TNR) campaigns, has been carried out in order to control the overpopulation of these free-living cats colonies, which consist of trapping all or most of the cats in a colony, sterilising them and returning them to their territory, marked with a cut on the ear or a tattoo to identify them as sterilised. The use of this campaigns to assess the status of *Salmonella* in free-living cats

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colonies could elucidate the risk of *Salmonella* in this populations. In this context, the objective of this study was to assess *Salmonella* carriage by free-living cats colonies in Canary Islands and the *Salmonella* serovars involved, in order to inform to those responsible for the colonies about the possible risk factors.

2. Materials and methods

2.1. Ethic statement

In this study was not applicable because the rectal samples obtained were taken after sedation of the animals from the campaigns carried out on the island of Gran Canaria by official institutions for control purposes (Trap-Neuter-Return).

2.2. Samples collection

The present study was conducted between November 2018 and January 2019. One hundred rectal swabs were taken (VWR, Transport Swabs, Italy) from apparently healthy feral cats under sedation prior to sterilisation.

The samples analysed came from the following municipalities on the island of Gran Canaria: Ingenio (n = 35), Santa Brígida (n = 30) and Las Palmas de Gran Canaria/Arucas (n = 35). For the sampling, collaboration was requested from the different veterinary centres involved in the mass sterilisation campaigns with the implementation of the TNR programme on the island.

2.3. Detection and serotyping of Salmonella

Rectal swabs were inoculated in 9 mL of buffered peptone water (Merck, Germany) used as a non-selective pre-enrichment medium. Subsequently, it was incubated at 37 °C for 18 ± 2 h. Then, 0.1 mL of the samples from the pre-enrichment media were inoculated into 9 mL of Rappaport–Vassiliadis (RV) broth (Sigma-Aldrich, USA) and incubated at 41.5 ± 1 °C for 24 ± 3 h. Using Kolle handles, a loop-full (1 μL) was inoculated onto Xylose Lysine Deoxycholate Agar (XLD; Sharlau, Spain) and Hektoen Enteric Agar (HK; Oxoid, UK). The plates were incubated at 37 ± 1 °C for 24 ± 3 h.

Suspected *Salmonella* colonies were inoculated onto MacConkey agar to obtained sufficient material for its identification. These were then confirmed by biochemical tests: oxidase, Christensen's Urea Agar, Kligler Iron Agar and IMVIC (Indole, Methyl Red, Voges–Proskauer and Citrate) tests.

One strain of *S.* Enteritidis from the private strain collection of the University Institute of Animal Health was used as positive control.

Finally, *Salmonella* strains isolated were serotyped at the National Reference Laboratory for Animal Health (Algete, Madrid, Spain). The method used for serotyping was antigenic agglutination with specific antisera according to the White-Kauffmann-Le Minor scheme [14].

3. Results

Through identification by biochemical tests, of 100 cats sampled, we obtained 22% of possible isolates of *Salmonella* spp.

No strain fermented the lactose and they were oxidase and urease-negative. The IMViC test gave negative results in the Indole and Voges–Proskauer tests, and positive in the Methyl Red and the Citrate tests.

By biochemical tests, of the 35 cats sampled from the municipality of Ingenio, 20 were positive for *Salmonella* spp., 57.14% of positivity. Of the 30 cats sampled from the municipality of Santa Brígida, two tested positives for *Salmonella* spp., representing an isolation rate of 6.66%. None of the stray cats sampled from Las Palmas de Gran Canaria/Arucas was positive for *Salmonella* spp.

These 22 isolates were sent to the Central Veterinary Laboratory of Algete (Madrid, Spain). Of the 22 isolates, only 19 were found to be *Salmonella* spp. and three were discarded (two from Sta Brígida and one from Ingenio), obtaining a 19% isolation rate of salmonellosis in feral cats in our geographical area. All isolates belonged to *S. enterica* subsp. *enterica.* Five different serovars were isolated: *Salmonella* Nima (57.89%), *Salmonella* Bredeney (15.78%), *Salmonella* Grancanaria (10.52%) *Salmonella* Florida (10.52%) and *Salmonella* Kottbus (5.26%) (Table 1).

4. Discussion

Salmonellosis in cats is a zoonosis of variable prevalence according to studies carried out by different authors. Spain et al. [5] did not detect differences in the prevalence of *Salmonella* spp. between refuge cats [0.7% (1/149)] and domestic cats [0.9% (1/114)]. Also, Hill et al. [11] found a similar prevalence of *Salmonella* spp. between shelter and domestic cats (1.3% and 0.8%, respectively).

In our study, we obtained an isolation rate of 19%. These results show that stray cats in our area can act as asymptomatic carriers of various *Salmonella* serotypes. The isolation rate obtained in our study make us consider the public health risk that these cats could pose.

Our results differ from those obtained in the study by Hariharan et al. [15] in Grenada, where the isolation of *Salmonella* spp. was not achieved in any of the 47 rectal swabs of the feral cats studied. Nevertheless, Mushgil et al. [16] found an isolation rate of 10.16% in a study conducted in apparently healthy feral cats, data close to those obtained in our study.

Some authors cite that the main sources of *Salmonella* infection in cats are ingestion of raw meat, commercial diets without standard industrial cooking processing, some processed foods and some contaminated commercial dry foods, as well as pig ear treats, hunting of rodents and birds, exposure to reptiles and environmental contamination [7,12,16–21]. Reptiles are considered natural reservoirs of *Salmonella* spp. and can infect cats by ingestion or by simple contact with their faeces [22,23]. Therefore, we consider that contact with the fauna (rodents and lizards) typical of the area, as well as possible feeding with raw meat, could be the cause of the high percentage of these isolates.

In this sense, with the 19% isolation rate obtained in our study in feral cats, we could think that our animals could have been infected through the consumption of wild animals typical of our area, such as rodents, lizards and birds, which are found in their environment and could be a source of *Salmonella* infection [22].

Different studies confirm that cats are carriers of different species of *Salmonella* zoonotic serovars and that in most cases *Salmonella* infection in cats is asymptomatic [5,11,24,25]. In a study carried out in Belgium

Table 1
Relation of the sample to its serovar.

N = 100 Positive samples code = 19	Serovar Identified	Isolation %
G-26	*S.* Kottbus 6,8:e,h:1,5	**5.26%**
G-2	*S.* Florida 6,14,25:d:1,7	**10.52%**
G-23	*S.* Florida 6,14,25:d:1,7	
G-3	*S.* Grancanaria 16:z39:-	**10.52%**
G-8	*S.* Grancanaria 16:z39:-	
G-18	*S.* Bredeney 4,12:1,v:1,7	**15.78%**
G-20	*S.* Bredeney 4,12:1,v:1,7	
G-21	*S.* Bredeney 4,12:1,v:1,7	
G-10	*S.* Nima 28:y:1,5	**57.89%**
G-13	*S.* Nima 28:y:1,5	
G-14	*S.* Nima 28:y:1,5	
G-15	*S.* Nima 28:y:1,5	
G-19	*S.* Nima 28:y:1,5	
G-24	*S.* Nima 28:y:1,5	
G-25	*S.* Nima 28:y:1,5	
G-29	*S.* Nima 28:y:1,5	
G-30	*S.* Nima 28:y:1,5	
G-65	*S.* Nima 28:y:1,5	
G-12	*S.* Nima 28:y:1,5	

by Van Immerseel et al. [26], *Salmonella* spp. were recovered of one of the 278 rectal swabs of apparently healthy domestic cats, corresponding to 0.35%.

All our isolates were obtained from asymptomatic cats, although it has also been described that feline salmonellosis can appear with different clinical conditions [27].

It should be noted that from our biochemical results (22 *Salmonella* spp. isolates), only 19 were serotyped as *Salmonella* spp. (discarding two strains from Sta Brígida and one from Ingenio), therefore, all our serotypes come from Ingenio.

The hypothesis that all our isolates were from this municipality could be related to the environmental temperature. For Reimschuessel et al. [28], temperature was a determining factor in *Salmonella* isolation. They obtained a higher percentage of isolations when the ambient temperature was 26.7 ºC. Ingenio was the only municipality in our study located in the dry zone with high temperatures. The average ambient temperature recorded in Ingenio in November 2018 was 20 ºC, with a maximum of 25 ºC and a minimum of 15 ºC; similar to those described by these authors, although we are aware that cats can be intermittent excretors and that the size of the sample with which we have worked is too small to make this statement. The rest of the municipalities studied were in humid areas. The average temperature recorded in November 2018 in Santa Brígida was 15 ºC, with a minimum of 12 ºC and a maximum of 17 ºC. In Las Palmas de Gran Canaria/Arucas in January 2019, the average temperature was 18 ºC, with a maximum of 19 ºC and a minimum of 17 ºC. We think that the differences in *Salmonella* isolation rates between humid areas (Las Palmas de Gran Canaria/Arucas and Santa Brígida) and warm areas (Ingenio) could be due to temperature. This could be an additional factor to explain the higher percentage of positives in Ingenio compared to the other municipalities.

Different studies carried out on domestic cats confirm that cats have been associated with a wide variety of serotypes, most often with *S.* Typhimurium. Other serotypes isolated with lower frequency were Heidelberg, Dublin, Enteritidis, Choleraesuis, Agona, Arechavelata, Copenhagen, Hessarek, Paratyphi B var. Java and Newport [7]. Furthermore, the *S.* Derby and *S.* Indiana serotypes have been identified by Lingling et al. [29], and Reimschuessel et al. [28] have also isolated *S.* Javiana, *S.* I 4,5,12:i:- and *S.* Infantis.

Regarding the isolation of *Salmonella* serovars obtained from rectal swabs of feral cats, Mushgil et al. [16] cite *S.* Anatum (3.38%), *S.* Montevideo (3.38%), *S.* Typhimurim (1.69%) and *S.* Brenrderup (1.69%).

Our work shows for the first time the existence of serotypes of *S. enterica* subsp. *enterica* that have not been described previously in cats (neither domestics nor ferals), such as *S.* Kottbus (5.26%); *S.* Florida (10.52%); *S.* Grancanaria (10.52%); *S.* Bredeney (15.78%) and *S.* Nima (57.89%).

Regarding the *S.* Kottbus serotype, we must mention that it represented 5.26% of the total *Salmonella* isolates obtained in our study.

A high zoonotic potential is attributed to this serovar, which was one of the 20 most widely identified serovars in humans in Europe until 2016, when it was replaced by the Braenderup serovar [30]. We would point out that this document does not mention the other serovars identified in our cats, although they have been identified in humans; it has been cited by Mohle et al. [31] in relation to a case of food poisoning in humans due to the consumption of alfalfa sprouts. In Gran Canaria, an outbreak of *Salmonella* Kottbus was described in humans whose source was bottled water; researchers suggest that the outbreak was due to contamination by pigeon excreta [32].

This *Salmonella* is of low incidence in Spain. In this regard, Palmera-Suárez et al. [32] mentioned that no outbreak of this serovar had been diagnosed in this country in the period between 1996 and 2006.

We have not found reports of it in cats, but it has been isolated in Hermann's tortoise [33], as well as in ducks, horses, pigs, poultry and lambs [34].

Regarding *S.* Florida, this represented 10.52% of the total *Salmonella* isolates obtained in our study. We have not found previous references to this in cats or humans. In animals, we have found it reported only in iguana [35] and guinea pigs [36].

Salmonella Grancanaria showed an isolation rate of 10.52% in our study. It was first identified in *Gallotia sthelini* by Monzón et al. [22]. Studies carried out by this author on Gran Canaria on human samples obtained in hospitals showed that, as in the rest of the national territory, the Enteritidis serotype predominated followed by Typhimurium. The author of the study highlights that some isolated serotypes on the island of Gran Canaria are rare, or not at all frequent, in the Spanish continental area, being referred to as exotic serotypes. This could have their origin in the cosmopolitan character of our Autonomous Community, where there is a movement of individuals from other latitudes. It has been reported that the Grancanaria serotype has produced gastroenteritis in humans in Belgium, which highlights its zoonotic nature. This author has already mentioned the need to elucidate the role that the Canary lizard could play as a vector of salmonellosis on the island, and in our case as a vector of salmonellosis for stray cats.

In our study, Bredeney serotype showed an isolation rate of 15.78%. It has been isolated from water carcasses, fish, lettuce, cattle and poultry faeces [37] and guinea pigs [36]. This serotype is associated with gastroenteritis in humans and has been reported in certain regions such as Romania, Alabama, England and Wales. In 1998, it was the most commonly isolated serotype in humans in Ireland [38].

Finally, the most isolated serotype in our work was *S.* Nima, representing 57.89% of the total *Salmonella* isolates.

This serotype has been isolated from animals such as cattle [37] and humans [39]. It has also been isolated in poultry farms, causing mortality in chickens [40]. Reptiles and amphibians have been reported as sources of infection for humans [41]. Pedersen et al. [42], isolated it from a chameleon. *S.* Nima has been isolated from the snakes *Thamnophis radix* and *Pituophis melanoleucus* [43]. *S.* Nima has also been isolated from the perianal swab of Columbia Rainbow Boa (*Epicrates cenchria maurus*) and Anaconda (*Eunectes murinus*) [44].

The source of *Salmonella* Nima in the environment of our feral cats could be through our animals' access to raw meat, either chicken or reptiles, as with other *Salmonella* serotypes. *S.* Typhimurium infection in cats can arise from different sources, such as consumption of raw meat and hunting of rodents and birds. The most likely source of *S.* Heidelberg and *S.* Enteritidis is the consumption of raw chicken. Cats may consume raw pork or sheep meat from human-generated waste and may become infected with *S.* Heidelberg. *S.* Dublin is rare in cats, although it is possible for cats to become infected by consuming raw meat, being exposed to livestock faeces or even hunting rodents [7].

It has been reported that there is a certain correlation between *Salmonella* isolates from companion animals and those from wild fauna (birds, reptiles and mammals) [45]. In our study it was impossible to make this correlation, since we lacked the necessary information, except for *S.* Grancanaria, which has been previously isolated from native lizards [22].

According to the results provided by our study, cats could be a source of risk for the spread of different *Salmonella* serotypes, some studies support that cats and reptiles could be potential risk factors for *Salmonella* infection of humans [46].

5. Conclusions

The present study clearly demonstrates that feral cats could be a source of risk for the spread of different *Salmonella* zoonotic serovars: *S.* Nima, *S.* Bredeney, *S.* Grancanaria and *S.* Kottbus. *S.* Nima is the serotype most isolated in feral cats, being one of the isolates with the highest incidence in humans. Nevertheless, further studies are needed with a larger number of samples, not only from cats, but also from other animal species and environmental sources to make this correlation.

Declaration of Competing Interest

None of the authors have any conflicts of interest to declare.

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