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Pet Reptiles: A Potential Source of Transmission of Multidrug-Resistant *Salmonella*

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Salmonella spp. is widely considered one of the most important zoonotic pathogens worldwide. The close contact between reptiles and their owners provides favourable conditions for the transmission of zoonotic pathogen infections, and ~6% of human salmonellosis cases are acquired after direct or indirect contact with reptiles. Moreover, antimicrobial resistance is one of the most important health threats of the twenty-first century and has been reported in *Salmonella* strains isolated from pet reptiles, which could entail therapeutic consequences for their owners and breeders. The aim of this study was to assess *Salmonella* carriage by pet reptiles in pet shops and households, and their role in the transmission of antimicrobial resistance, to inform the owners about the possible risks factors. During the period between January 2019 and December 2019, 54 reptiles from pet shops and 69 reptiles from households were sampled in the Valencian Region (Eastern Spain). Three different sample types were collected from each reptile: oral cavity, skin, and cloacal swabs. *Salmonella* identification was based on ISO 6579-1:2017 (Annex D), serotyped in accordance with Kauffman-White-Le-Minor technique, and antibiotic susceptibility was assessed according to Decision 2013/652. The results of this study showed that 48% of the pet reptiles examined from households and pet shops carry *Salmonella* spp. All the strains isolated presented resistance to at least one antibiotic, and 72% were multidrug-resistant strains, the most frequently observed resistance patterns being gentamicin-colistin and gentamicin-colistin-ampicillin. The present study demonstrates that pet reptiles could be a source of human multidrug-resistant *Salmonella* infection. In this context, the most optimal prevention of multidrug-resistant *Salmonella* infections necessarily involves strict control of the sanitary status of reptile pet shops and hygienic handling by the individual owners at home.

Keywords: reptile-associated salmonellosis, multidrug-resistant *Salmonella*, pet reptiles, One Health, zoonosis, *Salmonella*

INTRODUCTION

Salmonella is widely considered one of the most important zoonotic pathogens worldwide. This pathogen has become an important public health concern with a significant economic impact, which has been estimated at 3.6 billion dollars annually (1, 2). In Europe, salmonellosis was responsible for 94,203 human cases, of which 9.3% corresponded to Spain (3). The infection usually causes self-limited diarrhoeal illness, although severe illness and death may occur, especially in children, elderly or immunocompromised adults (4). However, the overall epidemiological pattern of human salmonellosis cases is related to *Salmonella*-contaminated food from animal origin, especially eggs and poultry meat, and ∼6% of human salmonellosis cases are acquired after direct or indirect contact with reptiles (3, 4).

In the last few years, exotic reptiles have risen in popularity as pets, with a population of over 7 million in European households (5). This increase in "living presents for children" is resulting in a trade of non-conventional species around the world, with Europe as the leading reptile importer (6, 7). The close contact between reptiles and their owners provides favourable conditions for the transmission of zoonotic pathogens infections, constituting a public health concern, as these pets have been considered as potential *Salmonella* carriers (7–11). Reptiles are natural reservoirs of *Salmonella*, which can hold a wide variety of serovars simultaneously without symptoms (12–14). However, reptile-associated salmonellosis seems to be responsible for more serious complications, with invasive disease and hospitalisation, especially in children (14, 15). From a public health standpoint, pet reptiles represent a persistent source of salmonellosis in households (16–19).

In addition, *Salmonella* multi-resistant strains emerge as a potential concern for public health safety, with implications of increased disease severity, longer hospitalisations and higher cost rates (20, 21). In this context, the World Health Organisation deemed antimicrobial resistance (**AMR**) one of the most important health threats, which could cause 10 million deaths a year by 2050, ahead of other diseases such as cancer (22, 23). In this sense, *Salmonella* has been included in the World Health Organisation priority list of twelve antibiotic-resistant bacteria (24). Interest in the role of reptiles as an antibiotic-resistant *Salmonella* reservoir has increased in recent years (7, 25, 26). Moreover, AMR had been reported in *Salmonella* isolated from captive reptiles, and their release could entail therapeutic consequences for their owners and breeders (16, 27). Moreover, the widespread use of antibiotics against *Salmonella* has been described in the international trade of pet reptiles, in order to prevent economical loses, as well as in animal welfare in crowded farms and long-distance transport (28–30). Therefore, more information on AMR in pet reptiles is needed in view of One Health (31).

In this context, the objective of the present study is to assess *Salmonella* carriage by pet reptiles in pet stores and households in Eastern Spain (Valencia Region) and gain more in-depth knowledge of their role in AMR transmission, in order to inform the owners about the possible risk factors.

MATERIALS AND METHODS

All animals were handled according to the principles of animal care published by Spanish Royal Decree 53/2013 (32).

Sample Collection

During the period between January 2019 and December 2019, a total of 349 samples from 123 different reptile species from households and pet shops reptiles were taken. Previously, the owners were contacted by advertising the project through the University community (Universidad Cardenal Herrera-CEU, and Universidad Politécnica de Valencia) and veterinary clinics of the Valencian Region (Eastern Spain).

A total of 37 species were identified from the 123 of the reptiles sampled (**Table 1**). From these species, 12 were classified as chelonians (order *Chelonia*), 16 as lizards (suborder *Sauria*) and 9 as snakes (suborder *Ofidia*) (**Table 1**). According to the individuals sampled from each group, 43.9% (54/123), 39.0% (48/123), and 17.1% (21/123) were chelonians, lizards and snakes, respectively (**Table 1**).

For each individual, whenever possible, samples from oral cavity ($n = 114$), skin ($n = 123$), and cloaca ($n = 112$) were taken using sterile cotton swabs (Cary Blair sterile transport swabs, DELTALAB®) (33). All individuals sampled were healthy and none of them presented clinical symptoms such as diarrhoea at the moment of sampling. In addition, an epidemiological questionnaire was filled in. The questionnaire contained information related to species, diet and the number of reptiles that cohabit in the same terrarium. The diet was classified as food from animal origin (including live prey, fresh meat and frozen meat), food of vegetable origin (including fruit and vegetables) and processed (including commercially manufactured reptile food). Moreover, the number of reptiles coexisting in the same terrarium was recorded as reptiles that inhabit alone, or reptiles that cohabit with two or more reptiles.

Detection of *Salmonella* spp.

The collected samples were analysed within 24 h of collection according to ISO 6579-1:2017 (Annex D) recommendations (34). Samples were pre-enriched in 1:10 vol/vol Buffered Peptone Water 2.5% (BPW; Scharlau, Barcelona, Spain), and then incubated at $37 \pm 1°C$ for 18 ± 2 h. The pre-enriched samples were transferred onto Semi-Solid Modification Rappaport Vassiliadis (MSRV; Difco, Valencia, Spain), and incubated at $41.5 \pm 1°C$ for 24–48 h. For the positive plates, the cultures obtained in MSRV were inoculated onto two specific agar plates for *Salmonella* spp. detection: Xylose Lysine Deoxycholate Agar (XLD; Liofilchem, Valencia, Spain) and a selective chromogenic agar medium specific for detection of C8-esterase activity (ASAP, bioMérieux, Marcy l'Étoile, France), then incubated at $37 \pm 1°C$ for 24 h. After incubation, one typical colony was collected and inoculated into a pre-dried nutrient agar plate (Scharlau, Barcelona, Spain), then incubated at $37 \pm 1°C$ for 24 h. Finally, an API (API-20-E; bioMérieux, Madrid, Spain) biochemical test was performed to confirm *Salmonella* spp. The *Salmonella* isolates were stored at $−80°C$ for further serotyping and antimicrobial susceptibility testing.

TABLE 1 | *Salmonella* isolated from reptiles in relation to reptile species.

Category	Reptile species	Number of reptiles examined	Number of positive reptiles (%)
Suborder *Sauria*	*Zonosaurus ornatus*	1	1 (100)
	Hemitheconyx caudicinctus	2	1 (50.0)
	Correlophus ciliatus	1	1 (100)
	Eublepharis macularius	21	11 (52.4)
	Paroedura picta	2	2 (100)
	Iguana	2	2 (100)
	Tupinambis teguixin	1	1 (100)
	Physignathus cocincinus	5	5 (100)
	Petrosaurus thalassinus	2	2 (100)
	Pogona vitticeps	4	4 (100)
	Chamaleo calyptratus	2	1 (50.0)
	Varanus glauerti	1	1 (100)
	Varanus albigularis	1	1 (100)
	Phelsuma grandis	1	0
	Pseudopus apodus	1	0
	Gecko gecko	1	0
Suborder *Ofidia*	*Python regius*	5	5 (100)
	Boa constrictor imperator	1	1 (100)
	Gongylophis colubrinus	1	1 (100)
	Acrantophis madagascariensis	1	1 (100)
	Elaphe guttata	7	5 (71.4)
	Spalerosophis diadema	2	2 (100)
	Lampropeltis getula	2	1 (50.0)
	Basiliscus plumifrons	1	0
	Heterodon nasicus	1	0
Order *Chelonia*	*Graptemys pseudographica*	5	3 (60.0)
	Testudo marginata	1	1 (100)
	Testudo hermanni	11	5 (45.5)
	Testudo horsfieldii	8	1 (12.5)
	Trachemys scripta elegans	5	0
	Pelusios	1	0
	Mauremys reevesii	3	0
	Cuora flavomarginata	1	0
	Pelomedusa subrufa	1	0
	Stigmochelis pardalis	1	0
	Testudo graeca	16	0
	Pseudemys nelsoni	1	0

Serotyping and Antimicrobial Susceptibility Testing

From each individual, the serotyping was performed from a cloacal strain, and when not present, a strain from the skin or oral cavity was analysed. Thus, all the strains were unfrozen and revived (ASAP) and the selected isolates 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 [35].

From all strains, *Salmonella* antimicrobial susceptibility was tested according to the European Committee on Antimicrobial Susceptibility Testing guidelines [36]. *Salmonella* strains were inoculated into Mueller-Hinton agar (Scharlab, S.L.) to form a bacterial lawn and were allowed to dry for 30 min at ambient (25°C) temperature; then, the antibiotic discs were applied and plates were incubated at 37°C for 24 h. The antimicrobial agents selected were those set out in Decision 2013/652 [37], including three b-lactams: ampicillin (AMP, 10 μg), cefotaxime (CTX, 30 μg) and ceftazidime (CAZ, 30 μg); two quinolones: ciprofloxacin (CIP, 5 μg) and nalidixic acid (NA, 30 μg); one phenicol: chloramphenicol (CHL, 5 μg); one potentiated sulfonamide: trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg); one polymyxin: colistin (COL, 10 μg); one macrolide: azithromycin (AZM, 15 μg); one glycylcycline: tigecycline (TGC, 15 μg); one aminoglycoside: gentamicin (GM, 10 μg); and one pyrimidine: trimethoprim (TM, 5 μg). The source for zone diameters used for interpretation of the test and plates after incubation at 37°C for 24 h was the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/clinical_breakpoints/), and where this was not possible, according to Clinical and Laboratory Standards Institute (CLSI) indications (https://clsi.org/media/2663/m100ed29_sample.pdf) [38]. The isolate strains were categorised as susceptible (S) or resistant (R), based on EUCAST imperative criteria [39]. Multidrug resistance (MDR) was defined as acquired resistance to at least one agent in two or more antimicrobial classes [40].

Statistical Analysis

A Generalised Linear Model, which assumed a binomial distribution for *Salmonella* shedding, AMR and MDR, was fitted to the data to determine whether there was an association with the categorical variables (species and order or suborder of reptile, the habitat of the reptile, sample type, diet and number of reptiles that cohabit in the same terrarium). A reptile was considered *Salmonella* positive if one or more samples collected (oral cavity, skin and/or cloacal) tested positive. A $P \leq 0.05$ was considered to indicate a statistically significant difference. Data are presented as least squares means ± standard error of the least squares means. In addition, a descriptive analysis has been done to assess the subspecies isolated in this study. Analyses were carried out using a commercially available software application (SPSS 24.0 software package; SPSS Inc., Chicago, IL, 2002).

RESULTS

From all samples collected during this study, 25.2 ± 2.3% (88/349) tested positive for *Salmonella*. The type of sample taken was significantly associated with *Salmonella* carriage ($P = 0.000$), with higher positive samples from cloaca (38.0 ± 4.6%, 43/112) than from skin (22.0 ± 3.7%, 27/123) and oral cavity (16.0 ± 3.4%, 18/114).

Salmonella spp. was detected in 48.0 ± 4.5% (59/123) of individuals sampled, with significant differences between snakes (76.0 ± 9.3%, 16/21) and lizards (69.0 ± 6.7%, 33/48), compared to chelonians (19.0 ± 5.3%, 10/54) ($P = 0.000$).

The reptiles sampled in this study inhabited households with private owners (56.1%, 69/123), as well as pet shops (43.9%, 54/123) in the Valencian Region (Eastern Spain). Significant differences for *Salmonella* isolation were found among the different reptile habitats (owners vs. pet shops) ($P = 0.000$), being higher in pet shop reptiles ($67.0 \pm 6.4\%$, 36/54) than in household pets ($33.0 \pm 5.7\%$, 23/69).

Moreover, the number of reptiles cohabiting the same terrarium were known for 111 of the 123 reptiles analysed, 49 for pet shops and 66 for households. In pet shops, significant differences were found between the number of reptiles present in the same terrarium and *Salmonella* shedding ($P = 0.008$). Thus, $89 \pm 7.4\%$ of reptiles that cohabit in terrariums with two or more reptiles were positive for *Salmonella* (16/18), while $58 \pm 8.9\%$ of reptiles that inhabit terrariums alone were positive for the bacterium (18/31). In contrast, for private owners' reptiles, no significant differences were observed between reptiles that cohabit in terrariums with two or more reptiles or alone and *Salmonella* shedding ($P = 0.064$), $21.0 \pm 7.8\%$ (16/38) and $42.0 \pm 8.0\%$ (6/28), respectively.

The diet was significantly associated with *Salmonella* carriage ($P = 0.000$), with higher frequency in reptiles that were fed with food from animal origin ($65.0 \pm 5.6\%$, 47/72), in contrast to reptiles that were fed with food from vegetable origin, and processed ($24.0 \pm 6.6\%$, 10/42, and $22.0 \pm 13.9\%$, 2/9, respectively).

From the 59 strains selected for serotyping, 51 were viable after culture and were serotyped. All *Salmonella* isolates were classified as *Salmonella enterica*. The most represented subspecies were *S. enterica* (56.9%, 29/51), *S. houtenae* (19.6%, 10/51), *S. diarizonae* (11.8%, 6/51), *S. salamae* (9.8%, 5/51) and *S. arizonae* (2.0%, 1/51). Fifteen different serovars of *S. enterica* subspecies were identified (**Table 2**). From all the strains serotyped, one *Salmonella enterica* serovar was indeterminate.

Seventy-five out of 88 *Salmonella* strains isolated were viable after culture and included in the antimicrobial susceptibility study. All strains analysed were resistant to at least one out of the twelve antibiotics tested ($n = 75/75$). The highest percentages of AMR were found to COL (97.3%, $n = 73$), followed by GM (84.0%, $n = 63$), AMP (46.7%, $n = 35$) and TGC (42.7%, $n = 32$), AZM (26.7%, $n = 20$), NAL (12.0%, $n = 9$), CHL (9.3%, $n = 7$), SXT and TM (8.0%, $n = 6$, both), and finally CAZ (6.7%, $n = 5$), CTX (4.0%, $n = 3$), and CIP (1.3%, $n = 1$) ($P = 0.000$). Antimicrobial resistance of the different *Salmonella enterica* serovars was summarised in **Table 3**.

Furthermore, a total of 72.0% (54/75) *Salmonella* isolates were resistant to two or more antimicrobials. No significant differences in MDR rates were shown between lizards (78.0%, 32/41), chelonians (73.3%, 11/15) and snakes (57.9%, 11/19) ($P = 0.206$). Although the type of sample collected was not significantly associated with MDR carriage, oral cavity (75.0%, 12/16), skin (77.3%, 17/22) and cloacal samples (67,6%, 25/37) ($P = 0.692$), significant differences were found between the type of sample and the different types of reptiles, except for lizards (**Table 4**, $P < 0.05$). Moreover, no significant differences were found between the habitat (pet shop and household), diet (food

TABLE 2 | *Salmonella* serovars isolated from private owners and pet shops.

Sample origin	Subspecies	Serovar	*n*
Private Owner	*enterica*	Albany 8,20:z4,z24	2
		Cerro 18: z4,z23	1
		Lattenkamp 45:z35:1,5	3
		Newport 6,8:e,h:1,2	1
		Paratyphi 4,12:b:1,2	1
	diarizonae	60:r:e,n,x,z15	1
		48:z53	1
		50:z52:z35	1
		47:z10:z35	1
		47:i:z53	1
	arizonae	44:z4,z23	1
	houtenae	11:z4,z23	1
	salamae	13, 22:z29:1,5	1
Pet Store	*enterica*	Cotham 28:i:1,5	2
		Fresno 9,46:z38	1
		Hadar 6,8:z10:e,n,x	3
		Hvittingfoss 16:b:e,n,x	1
		Muenster 3,15:e,h:1,5	2
		Newport 6,8:e,h:1,2	2
		Panama 9,12:l,v:1,5	1
		Pomona 28:y:1,7	3
		Sandiego 4,12:e,h:e,n,z15	1
		Vitkin 28:1,v:e,n,x	4
	houtenae	11:z4,z23	6
		16:z4,z32	2
		16:z36	1
	salamae	30:l,z28:z6	2
		21:g,s,t	1
		52:g,t	1
	diarizonae	42:k: z35	1

n: Number of strains isolated.

from animal origin, vegetable origin, and processed) and MDR *Salmonella* carriage ($P = 0.065$ and $P = 0.432$, respectively)

Overall, 25 different resistance patterns were observed. The combination of GM-COL (18.7%, 14/75) was the most frequently observed pattern, followed by GM-COL-AMP and GM-COL-TGC (10.7%, 8/75, both), COL alone (9.3%, 7/75) and GM-COL-AMP-TGC (8.0%, 6/75).

DISCUSSION

The present study demonstrates that 48% of the pet reptiles examined from households and pet shops carry *Salmonella* spp. All the strains isolated showed resistance to at least one antibiotic and 72% were multidrug-resistant strains. To our knowledge, this is the first study in the literature evaluating the prevalence and the antimicrobial resistance of this zoonotic pathogen from a considerable sample size in pet reptiles of Eastern Spain (Valencia Region).

TABLE 3 | Percentage of antimicrobial resistance of *Salmonella enterica* isolated from pet reptiles.

Salmonella enterica serovars	n	AMP	CTX	CAZ	CIP	NA	CHL	SXT	COL	AZM	TGC	GM	TM
Albany	2	100	0	0	0	100	100	100	100	0	100	100	100
Cerro	1	0	0	0	0	0	0	0	100	0	0	100	0
Cotham	2	100	0	0	0	0	0	0	100	0	100	100	0
Fresno	1	0	0	0	0	0	0	0	100	0	0	100	0
Hadar	3	66.7	0	0	0	0	0	0	100	100	66.7	100	0
Hvittingfoss	1	100	0	0	0	0	0	0	100	0	100	100	0
Lattenkamp	3	0	0	0	0	0	0	0	66.7	0	0	33.3	0
Muenster	2	100	0	0	0	0	0	0	100	50	50	100	0
Newport	3	0	0	0	0	0	0	0	100	0	33.3	66.7	0
Panama	1	0	0	0	100	0	0	0	100	0	0	100	0
Paratyphi	1	0	0	0	0	0	0	0	100	0	0	100	0
Pomona	3	100	0	33.3	0	0	0	0	100	66.7	0	100	0
Sandiego	1	100	0	0	0	0	0	0	100	100	100	100	0
Vitkin	4	50	0	25	0	0	0	0	100	0	75	75	0

n: Number of samples. The resistance was determined by disc diffusion. AMP, Ampicillin (10 μg); CTX, Cefotaxime (30 μg); CAZ, Ceftazidime (30 μg); CIP, Ciprofloxacin (5 μg); NA, Nalidixic acid (30 μg); CHL, Chloramphenicol (5 μg); SXT, Trimethoprim-sulfamethoxazole (1.25/23.75 μg); COL, Colistin (10 μg); AZM, Azithromycin (15 μg); TGC, Tigecycline (15 μg); GM, Gentamicin (10 μg); TM, Trimethoprim (5 μg).

TABLE 4 | Multidrug-resistant *Salmonella* isolated according to the type of sample collected in the different type of reptiles.

Reptile classification	Type of sample	n	MDR rate
Suborder *Sauria*	Oral cavity	9	89.0 ± 10.5
	Skin	13	79.0 ± 9.4
	Cloacal	19	69.0 ± 12.8
Suborder *Ofidia*	Oral cavity	3	0.0 ± 0.0^a
	Skin	23	100.0 ± 0.0^b
	Cloacal	13	62.0 ± 13.5^c
Order *Chelonia*	Oral cavity	4	100.0 ± 0.0^a
	Skin	6	83.0 ± 15.2^{ab}
	Cloacal	5	40.0 ± 21.9^b

Data are presented as least squares means ± standard error of the least squares means.
[a,b,c] *Different superscripts in each file means significant differences in the same reptiles' classification with a $P < 0.05$. MDR, Multidrug resistance. n, Number of samples.*

Reptiles have been known to be important carriers of *Salmonella* spp. worldwide, which may pose a health hazard as a source of human infection, particularly in children (4, 16, 41–43). However, there is a lack of consensus regarding the role of reptile shops on MDR *Salmonella* strains spreading. The results of this study showed that *Salmonella* strains isolated in reptiles from shops were twice as high as those from private owners (67 vs. 33%) (44, 45). This may be due to poor hygienic management of terrariums, especially in pet shops where they are usually occupied and ensuring a proper cleaning and disinfection procedure is not easy (45). This fact could facilitate that MDR strains remain in the shop environment among different reptile batches. In addition, the reptiles' stress related to cohabiting with individuals of different ages and origins could result in an increase in the bacterial infection, shedding in the terrarium and reptile-to-reptile transmission (45, 46). Conversely, reptiles from private owners are exposed to better hygiene practises and less stressful environments, leading to lower *Salmonella* shedding (45).

In reptiles, *Salmonella* is spread by faecal-oral route with an asymptomatic natural colonisation of the enteric tract, so in this study cloacal swabs collected were more sensitive for *Salmonella* isolation than other samples collected, such as skin or oral cavity. However, it is important to highlight that because *Salmonella* is excreted through faeces, it could contaminate the reptile's skin, oral cavity and the environment, being a source of infection for humans who handle the reptile or who are exposed to the reptile's environment (11, 46–50). Moreover, the *Salmonella* serovars most frequently detected in this study have been cited previously in reptile studies (51, 52), as well as in human outbreaks (3, 51–55). In addition, it has been reported that cold-blooded animals could be the major reservoir for the subspecies *houtenae*, *diarizonae*, *salamae*, and *arizonae* (56, 57).

The results of this study showed higher *Salmonella* prevalence among snakes and lizards compared to chelonians, in accordance with previous research (11, 31, 52, 58, 59). Particular attention has recently been given to snakes and lizards, as human interaction with these reptiles has become increasingly common in domestic environments (11, 60). In this sense, it is important to highlight that these reptiles are mainly fed with food from animal origin, which represents an important source of *Salmonella* (49, 61, 62). Previous studies carried out in the United Kingdom reported the important role of commercial feeder rodents in bacterial transmission among reptiles, and even their owners (63, 64). Thus, handling *Salmonella*-contaminated feeder rodents, as well as cross-contamination in the kitchen due to the rodents being kept in the freezer and thawed in microwaves, also in

contact with food for human consumption, have been linked to human *Salmonella* outbreaks (63, 65). In this context, to avoid *Salmonella* infection of reptiles, control of food products of animal origin has to be mandatory for the food suppliers (64).

On the other hand, special attention must be given to chelonians, due to their popularity as a pet for children. In this context, several countries, such as the US, have implemented strict bans in an attempt to curtail chelonian-associated salmonellosis; however, in Europe there are not many regulations to control its prevalence (53, 66). In the present study, *Salmonella* has been isolated from 18.5% of the chelonians tested. Seasonal effects, such as hibernation or season of sampling, have been speculated by previous studies to explain the low isolation rate of *Salmonella* in chelonians compared to other reptiles (31). Moreover, the diet may also have an important role (43, 49) because, as reported above, a large proportion of the chelonians are fed with food from vegetable origin or processed, and not from animal origin, frequently related with *Salmonella* outbreaks (11, 31, 67).

The increase in MDR *Salmonella* strains is of worldwide interest because it enhances the risk of therapeutic failure in cases of life-threatening salmonellosis in human and veterinary medicine (68, 69). In fact, it has been estimated that AMR could be the main cause of human mortality in 2050 (22). One of the most relevant outcomes in this study was the level of MDR isolated from pet reptiles, the most frequently observed resistance patterns being GM-COL and GM-COL-AMP. The high resistance against GM could be explained due to the indiscriminate use of aminoglycosides in pet reptile breeders, especially in the chelonian industry (28, 70). The use of GM as prophylactic *Salmonella* treatment in eggs to ensure sanitary conditions is a common practise in the US, the main supplier country of live reptiles for the EU (28, 71). Indeed, this practise has contributed to the finding of high-level plasmid-mediated gentamicin resistance in *Salmonella* isolated in its breeder farms (28).

Polymyxins have been widely used against Gramme-negative infections in animals, especially in animal production, the origin of several products involved in reptile feeding (72, 73). Currently, polymyxins such as COL represent the last line of defence against severe resistant infections in humans (72). Thus, it is highly restricted for animal infection treatments, and it is expected that resistance to this antibiotic will decrease in the coming years (74–76).

AMP was the third most frequent resistance shown in this study of *Salmonella* reptile strains, in line with a previous study conducted on species of gecko in Italy (10). This antibiotic is the most widely used in human medicine in Spain (76) and could thus be implicated in possible transmission of resistance from humans to reptiles, as a consequence of the direct and indirect contact between reptiles and their owners (75, 77, 78).

On the other hand, the level of resistance to NAL, CHL, SXT, TM, CAZ, CTX, and CIP in reptile *Salmonella* strains was relatively low. Resistance to CAZ contrasts with a survey conducted on geckos by Russo et al. (10), who showed a high resistance of *Salmonella* species to this antimicrobial, that is used in reptiles to treat infections or prophylactically

after a traumatic injury (79). Moreover, it is important to note that fluoroquinolones (e.g., CIP) are the drugs of choice for invasive salmonellosis infections in humans (adults) and cephalosporins (e.g., CTX and CAZ) in children (21), although both are implicated in a reduced effectiveness of *Salmonella* treatment (77).

Should be essential to inform pet reptile owners about the risks of wrongly handling these animals (60). Proper hygienic management measures should be taken, such as the use of gloves when cleaning the reptile, and even during cleaning and disinfection of the surfaces that come in contact with the pet reptile (13). Moreover, it is important to thorough hand washing after handling the reptiles, especially the wounds due to bites or scratch (13). Reptiles and its feed should keep away from the kitchen and areas where the owners prepare their own food (64). Besides, before introducing a new reptile in the household, microbiological exams should be carried out to avoid cross-infection (13). Finally, it highlights the importance of extreme caution with young children and immunocompromised patients, because they are especially susceptible to *Salmonella* spp. infections (60).

CONCLUSIONS

The present study clearly demonstrates that pet reptiles could be a source of human MDR *Salmonella* infection. The problem of MDR in reptiles could start with the shops, where *Salmonella* presence is extremely high, and seems to be linked with the origin of reptile food. In this context, the most optimal prevention of MDR *Salmonella* infections involves strict control of the sanitary status of reptile pet shops and hygienic handling of the individuals in the household. Nevertheless, it is important to highlight that the number of included samples is relatively small, which may restrict the interpretation of our results to Eastern Spain. Further studies are needed to validate our results in a larger study sample.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because All animals were handled according to the principles of animal care published by Spanish Royal Decree 53/2013 (32). Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

CM, OL, and SV: conceptualisation. OL, JV-G, CM, and SV: data curation. LL-R, CM, OL, and JV-G: methodology. CM and SV: investigation, writing–review and editing, and funding acquisition. CM and LL-R: writing–original

REFERENCES

1. USDA (United States Department of Agriculture). *USDA ERS—Cost Estimates of Foodborne Illnesses.* (2014). Available online at: https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx (accessed April 24, 2020).

2. WHO (World Health Organization). *Interventions for the Control of Non-typhoidal Salmonella spp.* 30th ed. L. Thorgeir Rome: Microbiological Risk Assessment Series (2016).

3. EFSA and ECDC. The European Union One Health 2018 zoonoses report. *EFSA J.* (2019) 17:1–276. doi: 10.2903/j.efsa.2019.5926

4. Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, et al. Reptiles, amphibians, and human *Salmonella* infection: a population-based, case-control study. *Clin Infect Dis.* (2004) 38:S253–61. doi: 10.1086/381594

5. FEDIAF (The European Pet Food Industry). *FEDIAF Annual Report 2019. RunSignup.* (2019). p. 60.

6. Bush ER, Baker SE, Macdonald DW. Global trade in exotic pets 2006-2012. *Conserv Biol.* (2014) 28:663–76. doi: 10.1111/cobi.12240

7. Bertelloni F, Chemaly M, Cerri D, Le Gall F, Ebani VV. *Salmonella* infection in healthy pet reptiles: bacteriological isolation and study of some pathogenic characters. *Acta Microbiol Immunol Hung.* (2016) 63:203–16. doi: 10.1556/030.63.2016.2.5

8. Stockman JA. Multistate outbreak of *Salmonella* infections associated with small turtle exposure, 2007–2008. *Yearb Pediatr.* (2011) 2011:288–9. doi: 10.1016/S0084-3954(10)79733-5

9. Jiménez RR, Barquero-Calvo E, Abarca JG, Porras LP. *Salmonella* isolates in the introduced asian house gecko (*Hemidactylus frenatus*) with emphasis on *Salmonella* Weltevreden, in two regions in Costa Rica. *Vector-Borne Zoonotic Dis.* (2015) 15:550–5. doi: 10.1089/vbz.2015.1785

10. Russo TP, Varriale L, Borrelli L, Pace A, Latronico M, Menna LF, et al. Salmonella serotypes isolated in geckos kept in seven collections in southern Italy. *J Small Anim Pract.* (2018) 59:294–7. doi: 10.1111/jsap.12808

11. Ramos CP, Santana JA, Morcatti Coura F, Xavier RGC, Leal CAG, Oliveira Junior CA, et al. Identification and characterization of *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens*, and *C. difficile* isolates from reptiles in brazil. *Biomed Res Int.* (2019) 2019:1–9. doi: 10.1155/2019/9530732

12. Wikström VO, Fernström LL, Melin L, Boqvist S. *Salmonella* isolated from individual reptiles and environmental samples from terraria in private households in Sweden. *Acta Vet Scand.* (2014) 56:7. doi: 10.1186/1751-0147-56-7

13. Ebani VV. Domestic reptiles as source of zoonotic bacteria: a mini review. *Asian Pac J Trop Med.* (2017) 10:723–8. doi: 10.1016/j.apjtm,.2017.07.020

14. Dudek B, Ksiazczyk M, Krzyzewska E, Rogala K, Kuczkowski M, Wozniak-Biel A, et al. Comparison of the phylogenetic analysis of PFGE profiles and the characteristic of virulence genes in clinical and reptile associated *Salmonella* strains. *BMC Vet Res.* (2019) 15:1–12. doi: 10.1186/s12917-019-2019-1

15. Walters MS, Simmons L, Anderson TC, De Ment J, Van Zile K, Matthias LP, et al. Outbreaks of salmonellosis from small turtles. *Pediatrics.* (2016) 137:1–9. doi: 10.1542/peds.2015-1735

16. Seepersadsingh N, Adesiyun AA. Prevalence and antimicrobial resistance of *Salmonella* spp. in pet mammals, reptiles, fish aquarium water, and birds in Trinidad. *J Vet Med Ser B Infect Dis Vet Public Heal.* (2003) 50:488–93. doi: 10.1046/j.0931-1793.2003.00710.x

17. Lowther SA, Medus C, Scheftel J, Leano F, Jawahir S, Smith K. Foodborne outbreak of *Salmonella* subspecies IV infections associated with contamination from bearded dragons. *Zoonoses Public Health.* (2011) 58:560–6. doi: 10.1111/j.1863-2378.2011.01403.x

18. Gay N, Le Hello S, Weill FX, de Thoisy B, Berger F. *Salmonella* serotypes in reptiles and humans, French Guiana. *Vet Microbiol.* (2014) 170:167–71. doi: 10.1016/j.vetmic.2014.01.024

19. Kuroki T, Ishihara T, Nakajima N, Furukawa I, Une Y. Prevalence of *Salmonella enterica* subspecies enterica in red-eared sliders trachemys scripta elegans retailed in pet shops in Japan. *Jpn J Infect Dis.* (2019) 72:38–43. doi: 10.7883/yoken.JJID.2018.140

20. O'Neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. In: O'Neil, editor. *The Review on Antimicrobial Resistance Chaired.* London (2014).

21. EFSA (European Food Safety Authority). The European union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. *EFSA J.* (2019) 17:1–278. doi: 10.2903/j.efsa.2019.5598

22. O'Neill J. Tackling drug-resistant infections globally. *Rev Antimicrob Resist.* (2016) 7:110. doi: 10.4103/2045-080X.186181

23. WHO. *Ten Threats to Global Health in 2019.* (2019). Available online at: https://www.who.int/vietnam/news/feature-stories/detail/ten-threats-to-global-health-in-2019 (accessed May 12, 2020).

24. Tacconelli E, Carrara E, Savoldi A, Kattula D, Burkert F. *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics.* (2017). Available online at: http://www.cdc.gov/drugresistance/threat-report-2013/ (accessed April 24, 2020).

25. Gorski L, Jay-Russell MT, Liang AS, Walker S, Bengson Y, Govoni J, et al. Diversity of pulsed-field gel electrophoresis pulsotypes, serovars, and antibiotic resistance among *Salmonella.* isolates from wild amphibians and reptiles in the California Central Coast. *Foodborne Pathog Dis.* (2013) 10:540–8. doi: 10.1089/fpd.2012.1372

26. Zhang J, Kuang D, Wang F, Meng J, Jin H, Yang X, et al. Turtles as a possible reservoir of nontyphoidal *Salmonella* in Shanghai, China. *Foodborne Pathog Dis.* (2016) 13:428–33. doi: 10.1089/fpd.2015.2107

27. Wei Z, Xu X, Yan M, Chang H, Li Y, Kan B, et al. *Salmonella* Typhimurium and *Salmonella* Enteritidis infections in sporadic diarrhea in children: source tracing and resistance to third-generation cephalosporins and ciprofloxacin. *Foodborne Pathog Dis.* (2019) 16:244–55. doi: 10.1089/fpd.2018.2557

28. Díaz MA, Cooper RK, Cloeckaert A, Siebeling RJ. Plasmid-mediated high-level gentamicin resistance among enteric bacteria isolated from pet turtles in Louisiana. *Appl Environ Microbiol.* (2006) 72:306–12. doi: 10.1128/AEM.72.1.306-312.2006

29. Giacopello C, Foti M, Fisichella V, Latella G, Aleo A, Mammina C. Antibiotic resistance in *Salmonella* isolated from tegus (*Tupinambis* spp.). *J Exot Pet Med.* (2012) 21:328–31. doi: 10.1053/j.jepm.2012.09.008

30. Goławska O, Zajac M, Maluta A, Pristas P, Hamarová L, Wasyl D. Complex bacterial flora of imported pet tortoises deceased during quarantine: another zoonotic threat? *Comp Immunol Microbiol Infect Dis.* (2019) 65:154–9. doi: 10.1016/j.cimid.2019.05.007

31. Chen CY, Chen WC, Chin SC, Lai YH, Tung KC, Chiou CS, et al. Prevalence and antimicrobial susceptibility of salmonellae isolates

from reptiles in Taiwan. *J Vet Diagnostic Investig.* (2010) 22:44–50. doi: 10.1177/104063871002200107

32. Spain. Royal Degree 53/2013, 1st of Febrary, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. *Boletín Oficial del Estado.* (2013) 34:11370–421.

33. Pees M, Rabsch W, Plenz B, Fruth A, Prager R, Simon S, et al. Evidence for the transmission of *Salmonella* from reptiles to children in Germany, July 2010 to October 2011. *Eurosurveillance.* (2013) 18:1–10. doi: 10.2807/1560-7917.ES2013.18.46.20634

34. ISO. *Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Detection of Salmonella spp. 6579-1:2017.* Genève: International Organization for Standardization (2017).

35. Grimont PAD, Weill FX. *Antigenic Formulae of the Salmonella Serovars.* 9th ed. Paris: WHO Collaborating Centre for Reference and Research on Salmonella (2007).

36. Matuschek E, Brown DFJ, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clin Microbiol Infect.* (2014) 20:O255–66. doi: 10.1111/1469-0691.12373

37. European Commission. *Commission Implementing Decision of 12 November 2013 on the Monitoring and Reporting of Antimicrobial Resistance in Zoonotic and Commensal Bacteria (Notified Under Document C(2013) 7145).* (2013). Available online at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32013D0652 (accessed May 5, 2020).

38. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty—fourth Informational Supplement.* CLSI document M100—S24. Wayne, PA: s.n (2014).

39. EUCAST. *New Definitions of S, I and R from 2019.* (2019). Available online at: https://www.eucast.org/newsiandr/ (accessed May 1, 2020).

40. EFSA and ECDC. (European Food Safety Authority and European Centre for Disease Prevention and Control). *EU Protocol for Harmonised Monitoring of Antimicrobial Resistance in Human Salmonella and Campylobacter Isolates.* Stockholm: EFSA; ECDC (2016).

41. Gambino-Shirley K, Stevenson L, Wargo K, Burnworth L, Roberts J, Garrett N, et al. Notes from the field: four multistate outbreaks of human *Salmonella* infections linked to small turtle exposure—United States, 2015. *Morb Mortal Wkly Rep.* (2016) 65:655–6. doi: 10.15585/mmwr.mm6525a3

42. Kiebler CA, Bottichio L, Simmons L, Basler C, Klos R, Gurfield N, et al. Outbreak of human infections with uncommon *Salmonella* serotypes linked to pet bearded dragons, 2012–2014. *Zoonoses Public Health.* (2020) 67:425–34. doi: 10.1111/zph.12701

43. Sodagari HR, Habib I, Shahabi MP, Dybing NA, Wang P, Bruce M. Veterinary sciences a review of the public health challenges of salmonella and turtles. *Vet Sci.* (2020) 7:1–12. doi: 10.3390/vetsci7020056

44. Nakadai A, Kuroki T, Kato Y, Suzuki R, Yamai S, Yaginuma C, et al. Prevalence of *Salmonella* spp. in pet reptiles in Japan. *J Vet Med Sci.* (2005) 67:97–101. doi: 10.1292/jvms.67.97

45. Marin C, Vega S, Marco-Jiménez F. Tiny turtles purchased at pet stores are a potential high risk for *Salmonella* human infection in the Valencian Region, Eastern Spain. *Vector-Borne Zoonotic Dis.* (2016) 16:455–60. doi: 10.1089/vbz.2016.1950

46. Lukac M, Pedersen K, Prukner-Radovcic E. Prevalence of *Salmonella* in captive reptiles from Croatia. *J Zoo Wildl Med.* (2015) 46:234–40. doi: 10.1638/2014-0098R1.1

47. Ebani VV, Cerri D, Fratini F, Meille N, Valentini P, Andreani E. *Salmonella* enterica isolates from faeces of domestic reptiles and a study of their antimicrobial *in vitro* sensitivity. *Res Vet Sci.* (2005) 78:117–21. doi: 10.1016/j.rvsc.2004.08.002

48. Marin C, Ingresa-Capaccioni S, González-Bodi S, Marco-Jiménez F, Vega S. Free-living turtles are a reservoir for *Salmonella* but not for campylobacter. *PLoS ONE.* (2013) 8:e72350. doi: 10.1371/journal.pone.0072350

49. Clancy MM, Davis M, Valitutto MT, Nelson K, Sykes JM. *Salmonella* infection and carriage in reptiles in a zoological collection. *J Am Vet Med Assoc.* (2016) 248:1050–9. doi: 10.2460/javma.248.9.1050

50. Guyomard-Rabenirina S, Weill FX, Le Hello S, Bastian S, Berger F, Ferdinand S, et al. Reptiles in Guadeloupe (French West Indies) are a reservoir of

51. Ricard C, Mellentin J, Ben Abdallah Chabchoub R, Kingbede P, Heuclin T, Ramdame A, et al. Méningite à Salmonelle chez un nourrisson due à une tortue domestique. *Arch Pediatr.* (2015) 22:605–7. doi: 10.1016/j.arcped.2013.09.019

52. Bjelland AM, Sandvik LM, Skarstein MM, Svendal L, Debenham JJ. Prevalence of *Salmonella* serovars isolated from reptiles in Norwegian zoos. *Acta Vet Scand.* (2020) 62:3. doi: 10.1186/s13028-020-0502-0

53. De Jong B, Andersson Y, Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis. *Emerg Infect Dis.* (2005) 11:398–403. doi: 10.3201/eid1103.040694

54. Jones SB, Spalloni W, Ferreccio F, Postigo J, Fernández A, Porte L, et al. *Salmonella* spp. Gastroenteritis associated to pet turtles in three infants. *Rev Chil Infectol.* (2015) 32:334–8. doi: 10.4067/S0716-10182015000400013

55. Bosch S, Tauxe RV, Behravesh CB. Turtle-associated salmonellosis, United States, 2006-2014. *Emerg Infect Dis.* (2016) 22:1149–55. doi: 10.3201/eid2207.150685

56. Briones V, Téllez S, Goyache J, Ballesteros C, Del Pilar Lanzarot M, Domínguez L, et al. *Salmonella* diversity associated with wild reptiles and amphibians in Spain. *Environ Microbiol.* (2004) 6:868–71. doi: 10.1111/j.1462-2920.2004.00631.x

57. Bauwens L, Vercammen F, Bertrand S, Collard JM, De Ceuster S. Isolation of *Salmonella* from environmental samples collected in the reptile department of Antwerp Zoo using different selective methods. *J Appl Microbiol.* (2006) 101:284–9. doi: 10.1111/j.1365-2672.2006.02977.x

58. Abalem De Sá IV, and Solari CA. *Salmonella* in Brazilian and imported pet reptiles. *Brazilian J Microbiol.* (2001) 32:293–7. doi: 10.1590/S1517-83822001000400007

59. Geue L, Löschner U. *Salmonella* enterica in reptiles of German and Austrian origin. *Vet Microbiol.* (2002) 84:79–91. doi: 10.1016/S0378-1135(01)00437-0

60. Whiley H, Gardner MG, Ross K. A review of *Salmonella* and squamates (Lizards, snakes and amphisbians): implications for public health. *Pathogens.* (2017) 6:1–15. doi: 10.3390/pathogens6010001

61. Fuller CC, Jawahir SL, Leano FT, Bidol SA, Signs K, Davis C, et al. A multi-state *Salmonella* Typhimurium outbreak associated with frozen vacuum-packed rodents used to feed snakes. *Zoonoses Public Health.* (2008) 55:481–7. doi: 10.1111/j.1863-2378.2008.01118.x

62. Vrbova L, Sivanantharajah S, Walton R, Whitfield Y, Lee C, Picard I, et al. Outbreak of *Salmonella* Typhimurium associated with feeder rodents. *Zoonoses Public Health.* (2018) 65:386–94. doi: 10.1111/zph.12442

63. Kanagarajah S, Waldram A, Dolan G, Jenkins C, Ashton PM, Carrion Martin AI, et al. Whole genome sequencing reveals an outbreak of *Salmonella* Enteritidis associated with reptile feeder mice in the United Kingdom, 2012-2015. *Food Microbiol.* (2018) 71:32–8. doi: 10.1016/j.fm.2017.04.005

64. Marin C, Martelli F, Rabie A, Davies R. Commercial frozen mice used by owners to feed reptiles are highly externally contaminated with *Salmonella* Enteritidis PT8. *Vector-Borne Zoonotic Dis.* (2018) 18:453–7. doi: 10.1089/vbz.2018.2295

65. Cartwright EJ, Nguyen T, Melluso C, Ayers T, Lane C, Hodges A, et al. A multistate investigation of antibiotic-resistant *Salmonella* enterica serotype I 4,[5],12:i:-Infections as part of an International Outbreak Associated with Frozen Feeder Rodents. *Zoonoses Public Health.* (2016) 63:62–71. doi: 10.1111/zph.12205

66. Cohen ML, Potter M, Pollard R, Feldman RA. Turtle-associated salmonellosis in the United States. Effect of Public Health Action, 1970 to 1976. *JAMA.* (1980) 243:1247–9. doi: 10.1001/jama.1980.03300380027016

67. Wojdat E, Kwiatek K, Zasadny R. Microbiological quality of petfood in Poland. *Pol J Vet Sci.* (2004) 7:207–9.

68. Threlfall EJ, Fisher IS, Berghold C, Gerner-Smidt P, Tschäpe H, Cormican M, et al. Antimicrobial drug resistance in isolates of *Salmonella* enterica from cases of salmonellosis in humans in Europe in 2000: results of international multi-centre surveillance. *Euro Surveill.* (2003) 8:41–5. doi: 10.2807/esm.08.02.00400-en

69. Münch S, Braun P, Wernery U, Kinne J, Pees M, Flieger A, et al. Prevalence, serovars, phage types, and antibiotic susceptibilities of *Salmonella* strains isolated from animals in the United Arab Emirates from 1996 to 2009. *Trop Anim Health Prod.* (2012) 44:1725–38. doi: 10.1007/s11250-012-0130-4

70. Arnafia W, Ningrum SG, Adji RS, Lukman DW, Pasaribu FH, Wayan I, et al. Aislamiento de *Salmonella* en tiendas de mascotas de reptiles y su suceptibilidad a los antibioticos en Indonesia. *Hum Vet Med Int J Bioflux Soc Res Artic.* (2016) 8:177–88.

71. Auliya M, Altherr S, Ariano-Sanchez D, Baard EH, Brown C, Brown RM, et al. Trade in live reptiles, its impact on wild populations, and the role of the European market. *Biol Conserv.* (2016) 204:103–19. doi: 10.1016/j.biocon.2016.05.017

72. Sartelli M, Weber DG, Ruppé E, Bassetti M, Wright BJ, Ansaloni L, et al. Antimicrobials: a global alliance for optimizing their rational use in intra-abdominal infections (AGORA). *World J Emerg Surg.* (2016) 11:1–32. doi: 10.1186/s13017-016-0089-y

73. Elbediwi M, Li Y, Paudyal N, Pan H, Li X, Xie S, et al. Global burden of colistin-resistant bacteria: mobilized colistin resistance genes study (1980–2018). *Microorganisms.* (2019) 7:461. doi: 10.3390/microorganisms7100461

74. Aarestrup FM. The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. *Philos Trans R Soc B Biol Sci.* (2015) 370:20140085. doi: 10.1098/rstb.2014.0085

75. ECDC/EFSA/EMA (European Centre for Disease Prevention and Control/European Food Safety Authority/ European Medicines Agency). ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals: Joint Interagency Antimicrobial Consumption and Resistan. *EFSA J.* (2017) 15:1–135. doi: 10.2903/j.efsa.2017.4872

76. MAPA (Ministry of Agriculture, Fishing and Food). Informe JIACRA España. Primer análisis integrado del consumo de antibióticos y su relación con la aparición de resistencia. AEMPS. Plan Nac. Resist. Antibióticos. (2018). p. 1–165. Available online at: http://www.resistenciaantibioticos.es/es/system/files/field/files/informe_jiacra-espana.pdf?file=1andtype=nodeandid=410andforce=0 (accessed April 24, 2020).

77. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin Microbiol Rev.* (2015) 28:901–37. doi: 10.1128/CMR.00002-15

78. EMA (European Medicines Agency). *Categorisation of Antibiotics in the European Union.* (2019). Ema/Cvmp/Chmp/682198/2017 31. Available online at: https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf (accessed May 1, 2020).

79. Cerreta AJ, Lewbart GA, Dise DR, Papich MG. Population pharmacokinetics of ceftazidime after a single intramuscular injection in wild turtles. *J Vet Pharmacol Ther.* (2018) 41:495–501. doi: 10.1111/jvp.12500