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Prevalence, Serotype Diversity, Antimicrobial Resistance, and Risk Factors Associated with *Salmonella* Detection in Chelonians in Algeria

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Abstract

Background/objectives: Chelonians are recognized as silent carriers of different *Salmonella* serovars. Despite the public health relevance of these animals, data on the prevalence, serovars, antimicrobial resistance, and influencing epidemiological factors are lacking in Algeria. This study aims to investigate, for the first time, the prevalence and the circulating *Salmonella* serotypes; to determine the antimicrobial resistance profiles; and to study epidemiological risk factors associated with *Salmonella* occurrence in Algerian chelonians. **Methods:** Ninety-three (93) fecal swabs of terrestrial and freshwater turtles in different regions of eastern Algeria were analyzed for *Salmonella* and tested for phenotypic antimicrobial susceptibility. **Results:** An overall prevalence of 55.9% (52/93) was detected, with differences depending on the species. Of 52 positive isolates, 51 were serotyped, and all underwent antimicrobial susceptibility testing. The majority of the isolates belonged to *Salmonella* Langford (15/51, 29.4%), to 4,5,12:b:e,n,x (8/51, 15.7%), and to *Salmonella enterica* subsp. *salamae* II (9,12:z29:1,5) (6/51, 11.8%), while serovars Kenya (3/51) and Reubeuss (2/51) were identified for the first time in chelonians. Serovars associated with human infection were also identified in this study. All isolates were sensitive to all antimicrobials tested, except for one (1) isolate (*S.* Langford) resistant to colistin. In the univariable analysis, animal species, study regions, and sex were significantly ($p < 0.05$) associated with detection of *Salmonella*, while region was the only associated factor in the final multivariable model. **Conclusions:** This study highlights the high prevalence of *Salmonella* in these animals, indicating a potential health risk for humans. Although only one colistin-resistant *Salmonella* strain was identified, chelonians may still act as potential reservoirs and disseminators of other antimicrobial-resistant bacteria. This risk can be further exacerbated by the limited recognition of these animals as a source of salmonellosis by the authorities in Algeria.

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Academic Editor: Carlos M. Franco

Received: 15 September 2026
Revised: 5 November 2026
Accepted: 6 November 2026
Published: 9 November 2026

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Keywords: Algerian chelonians; antimicrobial resistance (AMR); prevalence; *Salmonella* serovars; risk factors

1. Introduction

Chelonians, which include turtles and tortoises, are unique reptiles that are frequently kept in zoological collections and are becoming increasingly popular as pets [1]. Several species of chelonians are also kept and traded for their cultural and traditional value and consumed for their supposed medicinal benefits [2]. In Algeria, a study conducted in the province of El Tarf on tortoise ownership revealed that most tortoises were collected from forest habitats, followed by agricultural areas and local markets [3].

Non-typhoidal (NT) *Salmonella* is considered a major zoonotic pathogen. Even though human salmonellosis is typically associated with the consumption of contaminated food of animal origin, contact with infected animals, particularly in the case of inadequate hygiene practices, can be a significant source of *Salmonella* infection [4,5]. This Gram-negative bacterium, typically mobile and facultatively anaerobic, belongs to the Enterobacteriaceae family and is widely distributed. It can infect various hosts, primarily through the fecal-oral route [6] and is well adapted to reptiles [7]. Reptiles are natural *Salmonella* reservoirs; they carry a wide variety of *Salmonella* serovars in their digestive systems without showing any symptoms, and they excrete the pathogen through their feces, though it is not fully understood how frequently and at what rate turtles shed *Salmonella* or what conditions might favor shedding [8,9]. However, the potential mechanisms determining invasiveness and immune evasion, along with processes by which *Salmonella* persists in reptile hosts without generating disease, are among the many characteristics of *Salmonella* in reptiles that are still poorly understood [10]. On the other hand, controlling antimicrobial resistance (AMR) is one of the most significant public health issues and has become a priority in most nations [11]. High levels of AMR have been found in *Salmonella* strains isolated from reptiles, and the function of turtles as disseminators has come under closer examination in recent years. Resistance to several commonly used antimicrobials, including erythromycin, sulfisoxazole, gentamicin, amoxicillin, and ampicillin, has previously been reported among isolates recovered from a wide range of reptile species [12]. According to recorded data, approximately 72% and 100% of the *Salmonella* strains isolated in pet chelonians in Spain were multidrug-resistant (MDR) and resistant to at least one antibiotic, respectively [13]. Over the past ten years, reptile-associated bacteria may be resistant to clinically significant antimicrobials and, on occasion, to additional last-resort antimicrobials, including carbapenems and polymyxins. However, due to variations in reptile species, geographic origin, ecological environment, husbandry procedures, sampling strategies, bacterial targets, and laboratory techniques, reported prevalence differs significantly between studies [14]. Moreover, antimicrobial-resistant *Salmonella* isolates have been reported in both domesticated and wild reptiles [15].

Epidemiological investigations conducted following outbreaks involving reptile-associated serovars are the main source of knowledge on turtle-associated salmonellosis [16]. However, there are still numerous epidemiological gaps in knowledge of *Salmonella* carriage in turtles, and new serovars, such as *Salmonella* Agbeni, continue to be reported and identified [17]. Paratyphi B var. Java, Litchfield, and Urbana are among the *Salmonella* serovars that have been most frequently linked to turtles [12]. Although several investigations have examined *Salmonella enterica* carriage in free-living chelonians in the past, prevalence rates vary significantly according to species, location, and sampling technique [18].

Data on the occurrence of *Salmonella* in chelonians remains scarce in Algeria. To the best of our knowledge, this study is the first assessment of *Salmonella* carriage in Algerian chelonians and aims to determine its prevalence. The objective of the present study was also to identify the circulating *Salmonella* serovars, characterize the antimicrobial resistance profiles of the detected isolates, and investigate potential epidemiological risk factors associated with *Salmonella* in these animals.

2. Results

2.1. Prevalence

Out of a total of 93 sampled Greek tortoises (*Testudo graeca*), African spurred tortoises (*Centrochelys sulcata*), and European pond turtles (*Emys orbicularis*), 52 *Salmonella* strains were isolated, corresponding to an overall prevalence of 55.9%. In detail, 51 (51/75, 68%) strains were cultured from *T. graeca* and 1 (1/17, 5.9%) from *E. orbicularis*. No strain was isolated from *C. sulcata*.

2.2. Serotyping and Antimicrobial Resistance Results

Serotyping was performed on 51 *Salmonella* strains (one isolate could not be recovered for serotyping after freezing). Due to the unavailability of all antisera, complete serotyping could not be performed in some isolates, and certain antigenic variants could not be differentiated; therefore, only the antigenic formula is reported.

A total of 11 different serotypes and antigenic formulae were identified, including *Salmonella enterica* subsp. *enterica* serovar Langford (15 isolates), *S. enterica* subsp. *enterica* serovar Bardo (5 isolates), *S. enterica* subsp. *enterica* serovar Potsdam (4 isolates), *S. enterica* subsp. *enterica* serovar Kenya (3 isolates), *S. enterica* subsp. *enterica* serovar Reubeuss (2 isolates), *S. enterica* subsp. *salamae* II (serovar 9,12:z29:1,5) (6 isolates), *S. enterica* subsp. *salamae* II (serovar 13,22:z29:1,5) (3 isolates). In addition, isolates exhibiting the antigenic formulae were also identified: 4,5,12:b:e,n,x (8 isolates), 4,12:b:e,n,x (3 isolates), one (01) isolate was identified as poly D:b:e,n,z15, and one (01) as poly E:r:- (Figure 1, Table 1).

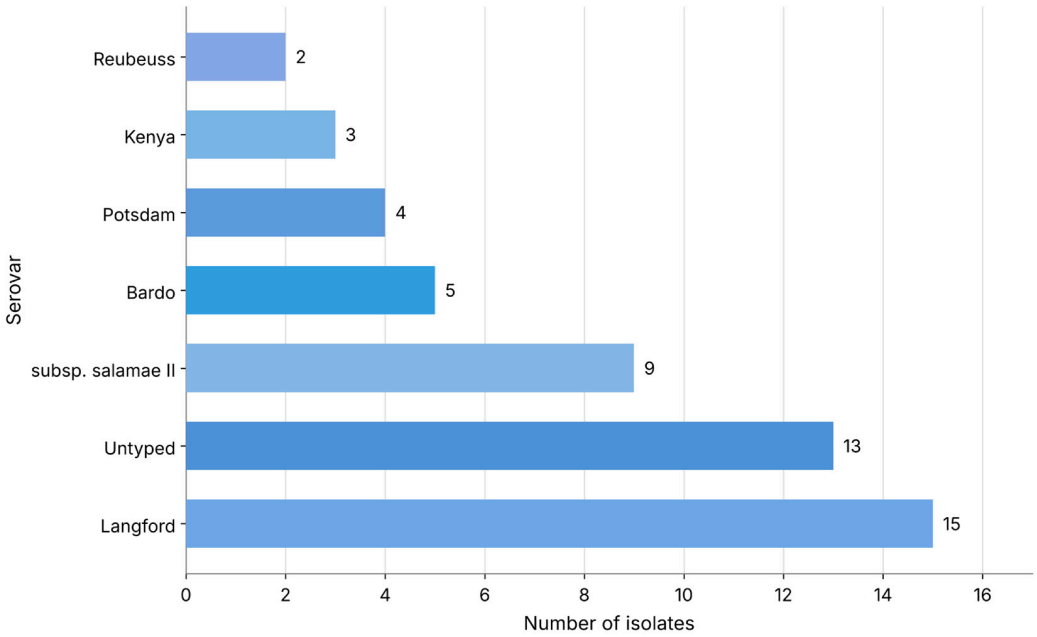


Figure 1. Serovars and untyped *Salmonella* isolates identified in the present study.

Table 1. Distribution of *Salmonella* serotyping results according to life stage, species, sex, and sampling site.

Animal Species	Antigenic Formulae	Serovar/ Untyped	Total of Isolates	Male	Female	Juvenile	Adult	Batna [1]	El Tarf [2]	El Kala [3]
Testudo graeca	4,12:b:e:n,x	Untyped	3	0	3	2	1	2	1	0
	6,7:l,v:e,n,z15	Potsdam	4	4	0	3	1	4	0	0
	Poly D b:e,n,z15	Untyped	1	1	0	0	1	1	0	0
	8:e,h:1,2	Bardo	5	3	2	1	4	5	0	0
	8:g,m,t:-	Reubeuss	2	2	0	2	0	2	0	0
	28:b:e,n,z15	Langford	15	7	8	6	9	13	1	1
	13,22:z29:1,5	subsp. salamae II	3	1	2	0	3	3	0	0
	6,7:l,z13:e,n,x	Kenya	3	3	0	1	2	3	0	0
	9,12:z29:1,5	subsp. salamae II	6	1	5	3	3	5	0	1
	Poly E r:-	Untyped	1	1	0	1	0	1	0	0
	4,5,12:b:e,n,x	Untyped	8	6	2	3	5	8	0	0
Emys orbicularis	Not determined (isolate not recovered)	Untyped	1	1	0	0	1	0	0	1
Total			52	30	22	22	30	47	2	3

Legend. [1]: Center + Timgad + N'Gaous; [2]: Center; [3]: Zoo.

Results obtained from Fisher's exact test showed a significant relationship between the distribution of *Salmonella* serovars and sex ($p = 0.009$). Several serovars were detected in one sex, with Kenya, Potsdam, and Reubeuss isolated only from males, whereas 4,12:b:e,n,x was found only in females. *Salmonella enterica* subsp. *salamae* II (9,12:z29:1,5) was more frequently isolated from females (22.7%) than males (3.3%). In contrast, there were no significant relationships between the distribution of the serotypes and species ($p = 0.058$), age ($p = 0.472$), and study region ($p = 0.836$). However, the small sample size of some serotypes warrants caution when interpreting these results.

Cefoxitin disk-diffusion screening identified 13 isolates as resistant, which were subsequently subjected to broth microdilution testing using the EUVSEC2 panel. The subsequent MIC results showed that all tested *Salmonella* isolates were susceptible to all tested antimicrobials, except for one isolate (*S. enterica* subsp. *enterica* serovar Langford), which was resistant to colistin (MIC = 4 µg/mL). This isolate was classified as non-wild type (NWT) based on the phenotypic result (Table 2).

Table 2. Antimicrobial susceptibility of *Salmonella* isolates ($n = 51$) determined by broth microdilution (Sensititre EUVSEC3/EUVSEC2 panels).

Antimicrobial Class	Antimicrobial	Panel	No. Tested	ECOFF (µg/mL)	MIC Range (µg/mL)	No. (%) > ECOFF
Penicillins	Ampicillin	EUVSEC3	51	>8	≤1–2	0 (0.0)
3rd-gen. cephalosporins	Cefotaxime	EUVSEC3	51	>0.5	≤0.25	0 (0.0)
	Ceftazidime	EUVSEC3	51	>2	≤0.25–0.5	0 (0.0)
Carbapenems	Meropenem	EUVSEC3	51	>0.125	≤0.03–0.06	0 (0.0)
Quinolones	Nalidixic acid	EUVSEC3	51	>16	≤4–8	0 (0.0)
Fluoro-quinolones	Ciprofloxacin	EUVSEC3	51	>0.064	≤0.015–0.03	0 (0.0)
Aminoglyco-sides	Gentamicin	EUVSEC3	51	>2	≤0.5–1	0 (0.0)
	Amikacin	EUVSEC3	51	>4	≤4	0 (0.0)
Tetracyclines	Tetracycline	EUVSEC3	51	>8	≤2–4	0 (0.0)
Glycylcyclines	Tigecycline	EUVSEC3	51	>1	≤0.25–0.5	0 (0.0)
Macrolides	Azithromycin	EUVSEC3	51	>16	4–8	0 (0.0)
Phenicols	Chloramphenicol	EUVSEC3	51	>16	≤8–16	0 (0.0)
Polymyxins	Colistin	EUVSEC3	51	>2	≤1–4	1 (2.0) ‡
Sulfonamides	Sulfamethoxazole	EUVSEC3	51	>256	16–64	0 (0.0)
Diaminopyrimidines	Trimethoprim	EUVSEC3	51	>2	≤0.25–0.5	0 (0.0)
3rd-gen. cephalosporins	Cefotaxime	EUVSEC2	13	>0.5	≤0.25	0 (0.0)
	Ceftazidime	EUVSEC2	13	>2	≤0.25–0.5	0 (0.0)
4th-gen. cephalosporins	Cefepime	EUVSEC2	13	>0.125	≤0.06–0.12	0 (0.0)
Cephamycins	Cefoxitin	EUVSEC2	13	>8	2–8	0 (0.0)
Carbapenems	Ertapenem	EUVSEC2	13	>0.03	≤0.015	0 (0.0)
	Imipenem	EUVSEC2	13	>1	0.25–0.5	0 (0.0)
	Meropenem	EUVSEC2	13	>0.125	≤0.03–0.06	0 (0.0)
Penicillins	Temocillin	EUVSEC2	13	>16	4–8	0 (0.0)
	Cefotaxime + clavulanate	EUVSEC2	13	—	≤0.06–0.25	— †
	Ceftazidime + clavulanate	EUVSEC2	13	—	≤0.12–0.5	— †

MIC, minimum inhibitory concentration; ECOFF, epidemiological cut-off value. A value preceded by "≤" indicates growth was inhibited at the lowest concentration tested. Isolates with an MIC above the ECOFF are considered non-wild type (microbiologically resistant). ECOFFs follow EUCAST and Commission Implementing Decision (EU) 2020/1729 for *Salmonella* spp. † Cefotaxime + clavulanate and ceftazidime + clavulanate are ESBL confirmatory tests. ‡ The single isolate above its ECOFF was an *S*. Langford isolate (colistin MIC 4 µg/mL; MIC 4 > 2 µg/mL).

2.3. Epidemiological Risk Factors

2.3.1. Univariable Firth Logistic Regression Analysis

Salmonella carriage was significantly associated with the species ($p < 0.001$), sex ($p = 0.048$), and the sampling area ($p < 0.001$). The highest positivity proportion was observed in *T. graeca* (52/75, 68%), followed by *E. orbicularis* (1/17, 5.9%), whereas no *Salmonella* isolates were detected in the *C. sulcata* analyzed (0/1). This translated into significantly lower odds of *Salmonella* carriage in *E. orbicularis* (OR = 0.043, 95% CI: 0.005–0.19, $p < 0.001$), whereas no significant association was found between *C. sulcata* and *Salmonella* carriage. Females had significantly higher odds of being positive for *Salmonella* than males (OR = 2.45, 95% CI: 1.01–6.27). Animals from Batna (N'Gaous) (OR = 22.03, 95% CI: 6.50–96.58) and El Tarf (center) (OR = 29.29, 95% CI: 1.88–4403.52) presented the highest odds compared with animals from El Kala (Zoo), whereas age was not significantly associated with *Salmonella* carriage (Table 3).

Table 3. Univariable Firth penalized logistic regression analysis of epidemiological factors associated with *Salmonella* carriage in chelonians.

Variable	Category	Tested	Positive (%)	OR	95% CI	*p*-Value
Species	*Testudo graeca*	75	51/75 (68%)	Reference		<0.001
	Emys orbicularis	17	1/17 (5.9%)	0.043	0.005–0.19	
Sex	Male	61	30/61 (49.2%)	Reference		0.048
	Female	31	22/31 (71%)	2.45	1.01–6.27	
Age	Adult	60	30/60 (50%)	Reference		0.410
	Juvenile > 10 cm	25	15/25 (60%)	1.48	0.59–3.82	
	Juvenile (<10 cm)	5	5/5 (100%)	11.00	1.16–1467.68	
	Juvenile (=10 cm)	2	2/2 (100%)	5.00	0.39–700.91	
Region	El Kala (Zoo)	23	3/23 (13%)	Reference		<0.001
	Batna (Timgad)	4	1/4 (25%)	2.51	0.20–22.39	
	Batna (Center)	14	7/14 (50%)	5.86	1.36–30.09	
	Batna (N'Gaous)	49	39/49 (79.6%)	22.03	6.50–96.58	
	El Tarf (Center)	2	2/2 (100%)	29.29	1.88–4403.52	

2.3.2. Multivariable Firth Penalized Logistic Regression Analysis

A multivariable Firth-penalized logistic regression analysis was fitted. In the full model including species, sex, age, and sampling region, none of the investigated variables was significantly associated with *Salmonella* carriage, with the lowest *p*-value being 0.087. Using the backward elimination method, the final multivariable Firth-penalized logistic regression model identified the region as the only associated factor for *Salmonella* carriage. Chelonians from Batna (N'Gaous) were about 21-fold higher in odds ratio (AOR = 21.10; 95% CI: 6.32–91.24; $p < 0.001$), and those from Batna (Center) were significantly more likely to be positive for *Salmonella* carriage compared to the reference group (chelonians from El Kala Zoo) (AOR = 7.09, 95% CI = 1.61–37.35, $p = 0.009$), while it was almost 31 times higher among chelonians in El Tarf (Center) (AOR = 30.71; 95% CI: 1.98–4615.77; $p = 0.013$). In contrast, there was no significant difference between chelonians in Batna (Timgad) and chelonians in El Kala (Zoo) (AOR = 2.63; 95% CI: 0.21–23.4; $p = 0.411$). The final model was overall significant (likelihood ratio test: $\chi^2 = 31.78$; df = 4; $p < 0.001$) (Figure 2).

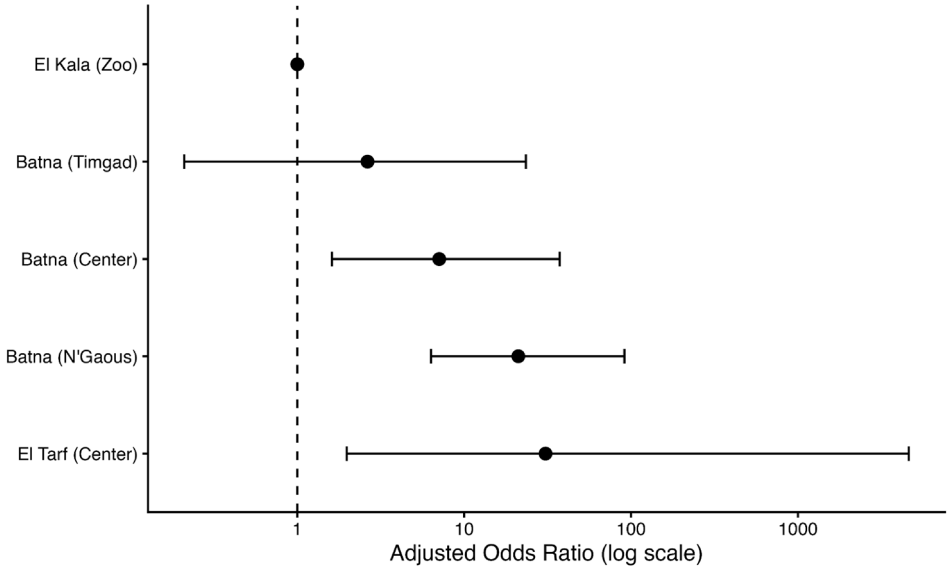


Figure 2. Forest plot showing adjusted odds ratios and 95% confidence intervals for epidemiological factors associated with *Salmonella* carriage in chelonians based on the multivariable Firth logistic regression model including sample region as the only associated factor (reference category: El Kala (zoo)). The vertical dashed line indicates the reference value (AOR = 1).

3. Discussion

Turtles are the reptiles most frequently associated with human salmonellosis, despite generally exhibiting lower *Salmonella* prevalence rates than snakes and lizards [10]. The public's misconception that turtles are safe pets, especially for young children, increases the risk of human salmonellosis associated with these reptiles [19].

Herein, we detected *Salmonella* in clinically healthy turtles and tortoises. The present study found a high overall proportion of *Salmonella* carriage in chelonians in Algeria (52/93, 55.9%). This research shows a low prevalence of *Salmonella* in *E. orbicularis* (5.8%), which is consistent with the study of Namroodi and Staji (2026) [20] in Iran, who reported a prevalence of 16.6%. In Spain, Marin et al. (2013) [21] also reported a low prevalence of *Salmonella* in *E. orbicularis* turtles (7/83, 8.0%), whereas in another study by Marin et al. (2022) [11], no positive isolates were found (0%) in this species. On the other hand, the present study found a high prevalence of *Salmonella* in *T. graeca* tortoises (68%), which is consistent with the finding of Giacopello et al. (2012) [22] in Italy, where *Salmonella* spp. Wwere detected in 50.7% of examined animals. Hidalgo-Villa et al. (2008) [23] reported a prevalence of 100% and 84.5% in *T. graeca* in R'mila and Jiblet, Morocco, respectively. In addition, Dipineto et al. (2012) [24] reported a prevalence of 42.9% in *T. graeca*, and Percipalle et al. (2011) [25] reported a prevalence of 36.8% in the same species in Italy. However, Marin et al. (2022) [11] found a prevalence of 25% in this species in Spain.

This study presents results consistent with those of Hidalgo-Vila et al. (2007) [26], who reported that the terrestrial species (*T. graeca*) had the highest prevalence of salmonellosis at 100%; in contrast, the aquatic species (*E. orbicularis*) had a low proportion of infected animals (15.4%). However, our results were higher than those reported by Costinar et al. (2022) [27] in Romania, where populations of *E. orbicularis* and *T. graeca* were analyzed for *Salmonella*, and an overall prevalence of 7.31% was detected.

Only one fecal sample from the African spurred tortoise (*C. sulcata*), collected in the El Kala National Zoological Park, was analyzed for *Salmonella* with negative results. Therefore, it is not possible to draw conclusions on the suitability of this host for *Salmonella* carriage, since only one tortoise of this species was found living in captivity. This result does not align

with those of Doutre and Boché (1976) [28] in Senegal, where they reported a prevalence of 20% in this species.

In a recent study in Algeria on the Mediterranean Pond Turtles (*Mauremys leprosa*), *Salmonella* was the most frequently isolated genus, 21.66% (13/60) among aerobic cloacal/fecal bacteria [29].

The prevalence of *Salmonella* in turtles may be influenced by a number of factors. Because *Salmonella* spends less time on the skin and in the cloaca in the aquatic environment [26], aquatic turtles appear to host the germ less frequently than terrestrial species [30]. However, contaminated water can be an important source and means of infection [31]. In water, *Salmonella* bacteria display good resistance [32].

Terrestrial turtles typically engage in geophagia, which involves consuming the excrement of other animals or turtles [33]. This behavior could also explain why *Salmonella* is more commonly detected in terrestrial turtles [34]. In addition, *Salmonella* can persist in terrariums long after the infected animals have been removed from there due to its high environmental survival rate [35].

Given that the majority of studies identified *Salmonella* through cloacal swabs, the difference in *Salmonella* detection between tortoises and freshwater turtles may be explained by the fact that in aquatic animals, *Salmonella* stays in the cloaca for shorter periods of time before being flushed out, whereas in terrestrial habitats, *Salmonella* remains for longer periods of time and is directly transmitted between individuals [11,26]. The finding that crocodiles, which are aquatic or semiaquatic, have the lowest prevalence of *Salmonella*, along with freshwater turtles, supports this hypothesis [8].

The absence of detection of *Salmonella* in a sample should always be considered a snapshot with little value for permanent assessment. It has been repeatedly demonstrated that reptiles with *Salmonella* in their gastrointestinal tracts shed these bacteria irregularly. Shedding is influenced by a range of factors, including husbandry conditions such as climate, hygiene, and diet. In addition, several studies have shown that reptiles may exhibit increased bacterial shedding under stressful conditions [10].

Stressful situations can disrupt immunomodulation and the immune system, which may increase the level of *Salmonella* shedding in the environment [36] and susceptibility to infections [37]. According to a study carried out on *T. graeca* specimens that were illegally brought into Italy from Tunisia, the high incidence of the infection was linked to high densities during transportation, confined spaces, and poor sanitary conditions [22].

A total of eleven different serotypes were identified in the present study. To the authors' best knowledge, this is the first report of *S. enterica* subsp. *enterica* serovar Reubeuss in chelonians and, more broadly, in reptiles. Furthermore, this study represents the first report of *S. enterica* subsp. *enterica* serovar Kenya in chelonians in the published literature, while *S. enterica* subsp. *enterica* serovar Bardo was identified only once before in a study of pet reptiles in Japan [38].

Our results found a larger proportion of isolates belonging to Langford serovar than those reported by Marenzoni et al. (2015) [39] in Italy, where only one isolate of *Salmonella* was identified as Langford. Other serovars found here were previously found in chelonians, including serovar Potsdam, previously identified in eight tortoises (*T. graeca*) [22] in Italy, in three isolates [11] in Spain, and in one isolate [40] in Korea. In addition, the relatively low proportion of isolates of *S. enterica* subsp. *salamae* II contrasts with those of Nowakiewicz et al. (2012) [41], who identified 26 strains, and Giacopello et al. (2012) [22], who reported 18 isolates belonging to the same subspecies. Similarly, Piasecki et al. (2014) [42] identified *S. enterica* subsp. *salamae* II at a proportion of 18%, while only one strain was identified as *S. enterica* subsp. *salamae* II (serovar 9,12:z29:1,5) in Spain [11]. The identification of these uncommon serovars suggests that chelonians are colonized with *S. enterica* serotypes

that are concurrently present in the environment, possibly due to anthropogenic influences. A recent systematic review by Muslin et al. (2025) [8] highlighted the high diversity of serovars found in reptiles and the influence of host and environmental characteristics.

Some of the *Salmonella* serovars found in this study have been cited previously in reptile studies as well as human outbreaks; for instance, *S. enterica* subsp. *enterica* serovar Potsdam was related to previous case reports and outbreaks. In Australia, Unicomb et al. (2003) [43] reported an outbreak of *Salmonella* Potsdam associated with salad dressing in a restaurant. In addition, Cheng et al. (2018) [44] reported a case of lumbar vertebral osteomyelitis caused by *Salmonella* Potsdam in an adult woman, indicating that this serovar can be related to other invasive infections beyond gastroenteritis. Similarly, there is strong evidence of *S. enterica* subsp. *enterica* serovar Bardo zoonotic and foodborne transmission; Latin America and the Caribbean were initially the only regions to report *Salmonella* Bardo among human isolates [45]. However, the National Institute for Communicable Diseases in South Africa reported at least three separate outbreaks related to *Salmonella* Bardo between July and September 2018, which were related to the consumption of goat meat [46]. Moreover, a recent study by Jeong et al. (2025) [47] identified one isolate as *Salmonella* Bardo from patients with diarrhea in South Korea. *S. enterica* subsp. *salamae* II (13,22:z29:1,5) was also isolated from human stools in 2 children and 1 adult in 2002 in Poland [48]. Although these had a zoonotic impact, it should be noted that all *Salmonella* serovars have the potential to infect humans and cause salmonellosis [49].

Interestingly, we did not find isolates belonging to the most relevant serovars in public health or serovars causing health problems in positive chelonians, in contrast with previous studies such as Candela et al. (2005) [50], who reported severe necrosis and nodular lesions associated with *Salmonella* Typhimurium infection in a case study of a *T. graeca* tortoise. Specific serovars may be more pathogenic to some reptiles than others; however, this has not been thoroughly studied, making it difficult to distinguish the effects attributable to bacterial strain from those related to host species, immunological status, and environment [10].

The MIC results indicated that isolates were susceptible to all tested antimicrobials, except for one isolate, *Salmonella* Langford, which was resistant to colistin. These results describe the isolate as non-wild-type (NWT) rather than clinically resistant based on ECOFF classification.

Although 13 isolates were detected as resistant to cefoxitin in the initial disk diffusion susceptibility test, the MIC results showed total susceptibility to cefoxitin, highlighting a discrepancy between the two methods.

Similarly, only two *Salmonella* isolates ($n = 2$, 0.4%) were resistant to colistin among the population of captive reptiles [35]. Bertelloni et al. (2022) [51] reported that 11.22% (11/98) of *Salmonella* isolates from reptiles showed resistance to colistin. However, Marin et al. (2022) [11] reported that of the 12 antibiotics studied, no resistance was found against colistin in chelonians.

The number of studies on phenotypic colistin resistance in *Salmonella* is limited. Past works were usually based on the disk diffusion test, a method not suitable for colistin resistance detection [52]. Recent surveys registered percentages of colistin-resistant *Salmonella* strains ranging between 1% and 12% [51], and few data are available on colistin resistance in *Salmonella* belonging to uncommon serovars or subspecies [53]. However, in a study conducted by Marin et al. (2021) [13], the highest percentages of antimicrobial resistance among *Salmonella* isolates were observed for colistin (97.3%, $n = 73$). Colistin is a member of the polymyxin class of antimicrobials, which has been used in both human and veterinary medicine for over 50 years. It is mostly administered orally to entire groups of pigs and poultry and, in some cases, calves, for treatment and prophylaxis of diarrhea due to Gram-negative infections [54]. The use of colistin in food-producing animals may

contribute to the selection of colistin-resistant bacteria, which can be disseminated into the environment through animal excrement and manure. These resistant bacteria and associated resistance genes may spread to soil and aquatic ecosystems, potentially exposing wildlife, including reptiles, to colistin-resistant bacteria [55]. In humans, it is currently the last line of defense against highly resistant infections [56]. As a result, its use in treating animal infections is severely restricted, and resistance to this antibiotic is expected to decrease in the upcoming years [57,58]. Similar to our study, McWhorter et al. [59] reported that only one isolate, serovar Wandsbek, was isolated from a wild reptile and was resistant to ampicillin. Conversely, previous studies reported multiple resistances to various classes of antibiotics. Giacopello et al. [22] showed that *Salmonella* strains isolated from *T. graeca* exhibited tetracycline resistance (57.1%), followed by streptomycin (13.2%). Colon et al. [60] demonstrated phenotypic resistance to streptomycin (28.6%) and azithromycin (23.8%). In addition, Percipalle et al. (2011) [25] reported an intermediate tetracycline resistance level (37.6%) and a percentage of multidrug-resistant strains of 5.3% in *Salmonella* isolates cultured from tortoises (*T. graeca* and *Testudo hermanni*). Our results are also inconsistent with the study of Marin et al. (2022) [11], where, in Hermann's tortoise and Greek tortoise, 50% of the isolated strains were resistant to antimicrobials, with the highest resistance rates observed for gentamicin (62%, $n = 18$) and ceftazidime (45%, $n = 13$). In contrast, Barbour et al. (2007) [61] showed that *Salmonella* strains isolated from *T. graeca* and *T. horsfieldii* tortoises exhibited 17% resistance to penicillin. Furthermore, isolates from Russian land tortoises were resistant to amoxicillin-clavulanic acid [41]. A recent study in Algeria on zoo reptiles reported that *Salmonella* spp. generally showed high resistance to β-lactam antibiotics, particularly AMP and AMC, with variable resistance to cephalosporins and marked susceptibility to aminoglycosides and, in most cases, fluoroquinolones [62]. In Spain, pet reptiles were also found to have a high level of antimicrobial resistance, with 72% of *Salmonella* isolates being multidrug resistant and 100% ($n = 75$) of isolates being resistant to at least one of the 12 antimicrobials tested [13].

The low levels of resistance found in the present study may be partly related to the predominantly wild origin of the sampled chelonians and their potentially limited exposure to anthropogenic reservoirs of antimicrobial resistance bacteria. This hypothesis is supported by studies of *Enterobacteriaceae* of wildlife inhabiting relatively pristine environments, where antimicrobial resistance bacteria or resistance determinants were detected at lower levels than in humans and domestic animals exposed to more anthropogenically influenced environments [63,64]. Abrahão et al. (2020) [65] reported low antimicrobial resistance among *Salmonella* isolates obtained from wild tegus (*Salvator merianae*) in Fernando de Noronha Island and suggested that this population's geographical isolation may limit contact with antimicrobial-resistant *Salmonella* strains originating from livestock on the mainland.

In general, compared to animals that produce food, the antibiotic resistance of *Salmonella* identified in reptiles remained low [35].

No PCR investigation for *mcr* genes or other molecular characterization of resistance mechanisms was performed in the present study; therefore, the finding is interpreted as a phenotypic result consistent with a non-wild-type isolate, and the presence and mechanism of colistin resistance cannot be confirmed. Therefore, this result should be discussed with caution.

Our results highlight a statistically significant association between the study area and *Salmonella* detection. The region of Batna (N'Gaous) appeared as a highly influential region and showed a high positivity level and a significant association with *Salmonella* detection ($p < 0.0001$). Land use may play a significant role in the ecology of *Salmonella*. Batna (N'Gaous), where most of the wild tortoises were sampled, is known for its rich agricultural landscape and diverse livestock, including sheep, goats, cows, and, in par-

ticular, poultry [66]. The proximity of cattle or poultry farms, the application of animal dung to pastureland, and other zoomorphic factors may also contribute to environmental contamination of the turtles' habitat [67]. Poultry are well known as reservoirs of *Salmonella* and can contribute to environmental contamination and to the potential transmission to animals, including tortoises. However, as these environmental factors were not systematically investigated in the present study, their potential contribution to *Salmonella* carriage remains hypothetical and warrants further investigation.

The present study showed that the prevalence of *Salmonella* also varies according to the animal species. In *T. graeca*, the probability of detection was significantly higher ($p < 0.0001$) compared to *E. orbicularis* and *C. sulcata*, with *Salmonella* positivity level being 68% in *T. graeca*, while only 5.9% of *E. orbicularis* turtles were positive, and the only *C. sulcata* sampled was negative. Similarly, Hernandez et al. (2021) [18] showed that there were significant differences in *Salmonella* prevalence by turtle species; painted turtles and sliders had significantly lower prevalence rates compared to snapping turtles. *Salmonella* prevalence is significantly higher in tortoises than in freshwater turtles. This could indicate that fecal-oral transmission of *Salmonella* is less effective in freshwater turtles or that tortoises are more susceptible to intestinal *Salmonella* carriage. Nonetheless, *Salmonella* spp. can survive in aquatic environments and are frequently isolated from water sources, which are capable of efficiently mediating their transmission in aquatic animals [68–70].

Furthermore, the present findings demonstrate that sex is also significantly associated with *Salmonella* detection ($p = 0.04$), with a higher positivity proportion in females (71%, 22/31) than in males (48.3%, 30/62). Our results align with the study of Colon et al. (2022) [60], which showed a significant relation between *Salmonella* detection and sex of animals ($p < 0.001$). In addition, Hernandez et al. (2021) [18] reported that the prevalence of *Salmonella* in aquatic turtles was significantly higher in females (33%) than in males (18%) ($p = 0.045$). Conversely, Casalino et al. (2021) [7] found that *Salmonella* distribution was not influenced by sex ($p = 0.1$). Turtles usually experience stress during the mating season due to the constant fighting between males and the frequent pursuit and biting of females by males [71], which may increase the shedding of *Salmonella* in the environment. Moreover, the higher detection rate among females might be due to physiological or immunological responses, among other factors. A study conducted by Terry et al. (2023) [72] on wild Red-eared slider turtles (*Trachemys scripta elegans*) showed that high reproductive investment in females during the early nesting season was associated with high progesterone levels and reduced baseline innate immunity. However, such changes in the immune system have not been studied well enough in relation to *Salmonella* carriage or shedding in chelonians.

Our results showed no significant association between age and *Salmonella* detection ($p > 0.05$). These results are consistent with those of Casalino et al. (2021) [7], where no significant difference was observed between young and older individuals ($p = 0.140$). In contrast, Hernandez et al. (2021) [18] show that the prevalence of *Salmonella* in aquatic turtles was significantly higher in juveniles (36%) than in adults (20%) ($p = 0.0153$). Similarly, Mitchell and Shane (2001) [9] reported that the prevalence of *Salmonella* infection was statistically significantly higher in juveniles than in adults. In the present study, the absence of a significant association between age and *Salmonella* detection can be explained by the fact that *Salmonella* are considered a part of the normal reptile gut microbiota, and some of them are colonized with *Salmonella* very early in life, possibly through the eggshell as well as during pregnancy and birth, indicating that *Salmonella* spp. can contribute to the physiological microbiome early in life [10]; consequently, carriage may occur across different age groups and may not be determined by age alone. Despite the present findings, small turtles (carapace length < 10 cm) pose a higher risk of disease transmission. Consequently, the U.S. Food and Drug Administration (FDA) implemented regulations in

1975 prohibiting the sale and distribution of small turtles and turtle eggs as pets [19]. In a study conducted by Bosch et al. (2016) [73] of fifteen turtle-related human salmonellosis outbreaks affecting several states in the United States, the investigation revealed that most of the reported turtles were small, and 88% of sick individuals reported that the turtles had a shell length of less than 10 cm.

Results obtained from Fisher's exact test showed a significant relationship between the distribution of *Salmonella* serotypes and sex ($p = 0.009$). Several serotypes were detected in one sex, with Kenya, Potsdam, and Reubeuss isolated only from males, whereas 4,12:b:e,n,x was found only in females, and *S. enterica* subsp. *salamae* II (9,12:z29:1,5) was more frequently isolated from females (22.7%) than males (3.3%). Direct evidence about the sex-specific distribution of *Salmonella* serovars in chelonians is still scarce; Conversely, Clancy et al. (2016) [74] found that serovar distribution in captive reptiles differed according to reptile phylogenetic group, sample type, origin, and health status, with some *Salmonella* subspecies showing strong association with certain reptile groups, rather than sex.

Before drawing definitive conclusions and conducting risk evaluations, further studies and investigations are needed. *Salmonella*'s exact mechanisms of shedding, interaction, and invasiveness are still poorly known. Moreover, the specific virulence of chelonian-associated serovars in humans, the role of *Salmonella* in the normal gut microbiome of chelonians, and the role of strains in *Salmonella* epidemiology and pathogenicity for humans and chelonians are also not well understood.

This study has some limitations: due to the conservation status of animals and their restricted seasonal periods of activity, sampling was constrained by animal availability, and individuals were sampled irrespective of their health status. The small size of sampled animals, like *Cenrochelys sulcata*, in some regions like El Tarf (center), and age categories, for which the estimates were associated with wide confidence intervals. In addition, the unequal representation of species and sampling regions may have influenced the prevalence estimates, reducing their precision. Therefore, the findings should be interpreted with caution.

Larger sample sizes and the inclusion of animals from different geographical areas could provide greater serotype diversity and reveal a broader range of antimicrobial resistance profiles.

4. Materials and Methods

4.1. Study Areas

The study was conducted in northeastern Algeria, specifically in Batna, in the extreme northeastern region of El Tarf, and in the National Zoological Park of El Kala. Batna is located between 6°7′ and 6°13′ east longitude and 35°34′ and 35°31′ north latitude, 435 km southeast of the capital, Algiers, with a semi-arid climate [75]. El Tarf is situated in the extreme northeastern region of Algeria, at 36°46′ N latitude and 8°19′ E longitude, with an average altitude of 287 m above sea level and a semi-humid climate [76]. El Kala National Zoological Park is among the earliest established parks in Algeria. located in the extreme northeast and covers an area of 80,510 hectares. It is bounded by latitudes 36°56′ N to 36°34′ N and longitudes 8°12′ E to 8°41′ E [77]. The area is characterized by diverse terrain, including lakes, hilly landscapes with relatively dense vegetation, and marshes, within a Mediterranean climate [78].

4.2. Animals and Sample Collection

Stool samples were collected from chelonians during two activity seasons (autumn and spring) (in the months of April, May, September, and October) of each year between 2023 and 2025. The study was conducted on wild, captive (National Zoological Park, El Kala),

traded (pet shop), and companion (household) chelonians and was focused on terrestrial tortoises, represented by the Greek tortoise (*T. graeca*) and the African spurred tortoise (*C. sulcata*), as well as freshwater turtles, including the European pond turtle (*E. orbicularis*).

Sampling was constrained by the availability of animals, and individuals were sampled regardless of their health status. All animals appeared healthy and showed no clinical signs at sampling. A total of 93 fresh stool and cloacal swab samples were collected in sterile containers and swabs from chelonians (75 *T. graeca*, 17 *E. orbicularis*, 1 *C. sulcata*) in two states of eastern Algeria: in Batna (67 samples: N'gaous: $n = 49$, Batna center: $n = 14$, Timgad: $n = 4$) and in El Tarf (26 samples: El Kala (zoo): $n = 24$, El Tarf center: $n = 2$) (Figure 3). The carapace length of the turtles and tortoises was recorded at sampling (<10 cm, =10 cm, or >10 cm). Carapace length was categorized using a 10 cm cutoff, corresponding approximately to the CDC's 4-inch public health threshold for small turtles [79].

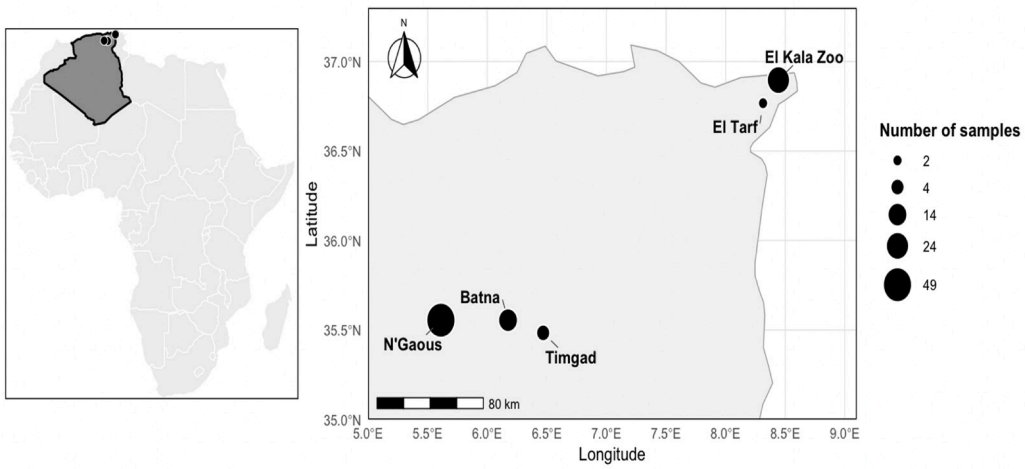


Figure 3. Map of northeastern Algeria representing study areas (Batna, N'Gaous, Timgad, El Tarf, and El Kala Zoo) and the number of fecal samples.

Each turtle was captured, sampled, marked, and released to avoid repeated sampling (chelonians were marked by making incisions in the shell—specifically on the peripheral scutes of the carapace—using a saw, following a precise coding system). Carapace length was used as a morphological indicator to estimate developmental stage and classify turtles into approximate age groups, as chronological age was not determined. No animals were sacrificed or anesthetized.

After collection, all samples were coded and transported in a cooler to the microbiology laboratory, where bacterial isolation was immediately initiated. Data, including species, collection region, sex, and age group, were also recorded.

4.3. Bacterial Isolation

Stool samples were pre-enriched in buffered peptone water (BPW) (Merck, Germany) (25 g of fecal material were pre-enriched in 225 mL of BPW, and cloacal swabs were pre-enriched in 9 mL of BPW) and incubated at 37 °C for 18 h. After incubation, 100 µL of pre-enriched broth was enriched in 10 mL of Rappaport-Vasiliadis (RV) broth (Condalab, Torrejón de Ardoz, Madrid, Spain) and incubated at 41.5 °C for 24 h. Following incubation, RV cultures were streaked onto Xylose-Lysine Deoxycholate (XLD) agar plates (HiMedia, Thane, Maharashtra, India) and incubated at 37 °C for 24 h. Presumptive colonies were identified using standard biochemical tests, including triple sugar iron (TSI), urease, indole, and catalase assays. Final confirmation was performed using the API 20E identification

system (BioMérieux, Marcy-l'Étoile, France). One presumptive *Salmonella* colony for each isolate was selected for serotyping.

4.4. Serotyping

Serological typing of *Salmonella* was performed according to ISO/TR 6579-3 [80] using the rapid slide agglutination technique according to the White-Kauffmann-Le Minor scheme, targeting specific somatic (O) and flagellar (H) antigens, respectively.

4.5. Antimicrobial Susceptibility Testing

Minimal inhibitory concentrations (MIC) were determined using the two-fold broth microdilution reference method, according to ISO 20776-1:2019 [81], using EUVSEC3 and EUVSEC2 Sensititre plates (Trek Diagnostic Systems; Thermo Scientific, Waltham, MA, USA). Panel EUVSEC2 was used only for isolates that had previously shown resistance to cefoxitin during antimicrobial susceptibility testing with disk diffusion methods in accordance with Commission Implementing Decision 2020/1729/EU.

Disk-diffusion testing was initially performed on Mueller–Hinton agar to assess susceptibility to several antibiotics, including cephalosporin, as a preliminary screen for antimicrobial resistance. Bacterial suspensions were adjusted to a 0.5 McFarland standard and uniformly inoculated onto the agar surface using a sterile swab. Cefoxitin disks (30 µg) were applied, and the plates were incubated at 37 °C. Inhibition zone diameters were measured and interpreted according to the EUCAST [82] criteria, with zone diameters of <15 mm classified as resistant.

The panel EUVSEC3 included amikacin, ampicillin, azithromycin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, nalidixic acid, sulfamethoxazole, tetracycline, tigecycline, and trimethoprim. Besides, panel EUVSEC2 included cefepime, cefotaxime, cefotaxime/clavulanic acid, cefoxitin, ceftazidime, ceftazidime/clavulanic acid, ertapenem, imipenem, meropenem, and temocillin. Interpretation of quantitative data was performed as described by Commission Implementing Decision 2020/1729/EU, as well as the European Committee on Antimicrobial Susceptibility Testing EUCAST using epidemiological cutoff values (ECOFF). The EUVSEC3 panel was used to test all 51 *Salmonella* isolates; the EUVSEC2 panel (extended β-lactam panel) was used to test the 13 isolates, initially classified as resistant to cefoxitin by the disk diffusion method. Isolates with MICs above the relevant ECOFF were classified as non-wild type (NWT), whereas those with MICs at or below the ECOFF were classified as wild type (WT). *Escherichia coli* ATCC 25922 was used as the quality-control strain.

4.6. Statistical Analysis

Initially, Excel 2016 (Microsoft Office 365) was used for data collection and organization of 93 sampled turtles. Firth penalized logistic regression was used to assess the association between certain epidemiological factors (study area, animal species, genus, and age group) and the isolation of *Salmonella* from a given animal in both univariable and multivariable analyses. Odds ratios (OR), adjusted odds ratios (AOR), and their corresponding 95% confidence intervals (CI) were calculated. Fisher's exact test was used to assess the association between *Salmonella* serotypes and investigated variables. A p-value < 0.05 was considered statistically significant. All analyses were performed using RStudio Desktop software (version 2026).

5. Conclusions

The present findings confirm that chelonians are important carriers of *Salmonella* and often harbor and excrete the bacterium without exhibiting clinical symptoms, with partic-

ularly high prevalence observed in *T. graeca* tortoises. The study determines a variety of *Salmonella* serovars, including uncommon ones such as *S*. Kenya and *S*. Reubeuss, which, to the best of the authors' knowledge, have not previously been reported in reptiles. Furthermore, serovars that were previously associated with human infections were also identified. However, zoonotic transmission was not investigated in the present study. Although only one colistin-resistant *Salmonella* strain was identified in this study, chelonians may act as sentinels for environmental antimicrobial resistance. Therefore, continued surveillance of antimicrobial resistance in chelonians is essential to better understand their potential role, along with that of other reptiles, in the emergence and dissemination of antimicrobial-resistant bacteria. Several epidemiological factors appeared to influence the detection of *Salmonella*, although these associations should be interpreted with caution in some cases. In Algeria, the risk posed by these animals as sources of *Salmonella* is underestimated. Their potential role as sources of human salmonellosis highlights the need for surveillance and improved biosecurity measures. Pet owners, vendors, zoo personnel, and wildlife enthusiasts should be cautious, take control measures, and limit direct contact to prevent the potential zoonotic risk associated with these animals.

Author Contributions: Conceptualization, R.B.; methodology, J.A., M.U.-R. and A.A.; data curation, R.B.; investigation, R.B.; formal analysis, M.U.-R., V.V.E. and F.B.; writing—original draft preparation, R.B.; writing—review and editing, R.B., J.A., M.U.-R., V.V.E. and F.B.; funding acquisition, V.V.E.; software, R.B. and S.B.; supervision, H.S., F.M. and L.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Pisa, Fondi di Ateneo 2022.

Institutional Review Board Statement: Not applicable. This study did not involve sacrifice, invasive procedures, or experimental interventions on animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is reported in the manuscript.

Acknowledgments: The authors wish to thank the technician Nisrin Maasoumi Nouha and Estefanía Rivero Muñoz for their assistance in completing all serotyping and MIC analysis at the Foodborne Zoonoses and Antimicrobial Resistance Unit at the VISAVET Health Surveillance Center (UCM). We also acknowledge the staff of the National Zoological Park of El Kala for their assistance throughout this study in facilitating access to animals and providing information.

Conflicts of Interest: The authors declare no competing interests.

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