Investigation of *Salmonella* types and its antibiotic resistance profile and determination of parasite carrier in free-living hedgehogs in Istanbul, Türkiye

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Abstract

Hedgehogs, which are kept as pets worldwide, are prohibited from being sold or kept as pets in Türkiye. The aim of the study was to investigate *Salmonella* and endo-parasite carriage in hedgehogs and to determine the antibacterial resistance profiles of isolated *Salmonella* agents. Free-range hedgehogs living in parks and gardens of Istanbul that were brought to veterinary clinics by animal lovers for check-up and treatment were used for sampling. Thirty faecal samples were taken from the animals. For bacteriological examinations, samples were seeded on selective media, and then the isolates were identified by conventional methods as *Salmonella* spp. Identification and antibiotic susceptibilities of the isolates were determined by the BD Phoenix Automated Microbiology System. All faecal samples were also investigated by PCR for *Salmonella* spp. specific gene regions. The aminoglycoside and quinolone resistance profiles of the strains were analysed by PCR. On parasitological examination, samples were analysed for parasites' eggs, oocysts, and larvae. Two *Salmonella* isolates were determined as *Salmonella enterica* serovar Typhimurium *(S.* Typhimurium*),* whose antigenic formula was 4,5,12:i:1,2. The isolates were phenotypically resistant to ciprofloxacin but did not harbour any of the genes examined. Out of 30 faecal samples, 19 (63.3%) were positive for one or more genera of parasites. The hedgehogs were infected with the species of *Capillaria* spp. (60%), *Crenosoma* spp. (13.3%), *Eimeria* spp. (10%) and *Acanthocephala* spp. (6.6%). This study is the first report of *Salmonella* carrier and internal parasite fauna of hedgehogs in Istanbul, Türkiye.

Erinaceus concolor, faeces, antibiotic resistance, PCR, intestinal parasites

Today, exotic animals like hedgehogs are increasingly being kept as pets at home. They are small insectivorous mammals that feed on earthworms, beetles, caterpillars, slugs, grasshoppers, and other insects but also take different food types, including birds, bird eggs, and lizards (Youssefi et al. 2013; Orkun et al. 2019; Kazemi-Darabadi et al. 2018). *Erinaceus concolor* (Southern, White-Breasted hedgehog) is a species that has a high distribution throughout Türkiye (Özen 2010). They are listed in "The Wild Animals Protected by the Republic of Türkiye Ministry of Agriculture and Forestry" in our country. It is prohibited to remove the hedgehog from its natural environment, to sell or keep it as a pet in home (Resmi Gazete 2015). However, wild hedgehogs, whose natural habitat has deteriorated due to the environmental degradation caused by intensive development and construction, have started to live in areas populated by humans for feeding. The hedgehogs that come to eat cat food left in the streets, parks, and gardens have come into close contact with both stray animals and humans. They may be the host or potential reservoir for many kinds of bacterial, viral, and parasitic agents (Sykes and Durrant 2005). The infectious agents can be transmitted through an arthropod vector, contaminated food, water, faeces and the environment (Polley 2005). Diagnosis of diseases due to infectious agents in hedgehogs is essential for animal and human health as some of them are zoonotic.

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Hedgehog species have been found to carry and transmit numerous zoonotic pathogens, parasitic infections and bacterial diseases that affect wildlife, livestock, pets and humans worldwide (Riley and Chomel 2005; Hutterer 2005). It has been reported that hedgehogs subclinically infected with *Salmonella* agents can shed the organism intermittently, and serious infections occur in humans exposed to these bacteria (Kottwitz et al. 2011).

Between 2011 and 2013, the first outbreak caused by *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) was reported in humans in the United States and researchers indicated that 80% of patients had contact with a pet hedgehog (Anderson et al. 2017). The second outbreak in the USA was between 2018 and 2019. Hoff et al. (2022) reported that the strains were the same as the first outbreak and emphasised that these *Salmonella* strains persisted in hedgehogs and would continue to be a health problem for hedgehog owners if measures were not taken to prevent contamination. The CDC investigated the third outbreak associated with domestic hedgehogs in 2020 and reported that isolates from Canada and the United States were genetically closely related to *S.* Typhimurium isolated from both outbreaks (CDC 2019). Likewise, outbreaks of salmonellosis after direct contact with hedgehogs have been reported in United Kingdom (Monecke et al. 2013), Norway (Hasseltvedt et al. 2000; Handeland et al. 2002), and Japan (Ichimi et al. 2018). *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) and *S.* Typhimurium, the most common causes of *Salmonella* infection in humans, are also the most frequently reported *Salmonella* serovars in hedgehogs. Hedgehogs may host several endoparasite species of varying clinical importance (Mullineaux and Keeble 2016; Bexton and Couper 2019). Common endoparasitic worms are lungworms (*Crenosoma striatum*), lung threadworms (*Capillaria aerophila*), and intestinal threadworm species (*Capillaria erinacei* and *C. ovoreticulata*) (Gaglio et al. 2010; Rautio et al. 2016). Researchers have reported the protozoon oocyst of *Cryptosporidium parvum*, *Eimeria* spp. and *Isospora* spp. (*Isospora rastegaiev*) in hedgehogs (Epe et al. 2004; Beck 2007; Çırak et al. 2010). *Toxoplasma gondii* (Apicomplexa: *Sarcocystidae*) and *Trichinella* (Nematoda: *Trichinellidae*) are parasites with low host specificity able to infect various animals, including humans (Polley 2005). Tissue cysts of *Toxoplasma gondii* and the larvae of *Trichinella spiralis* have been reported in hedgehog muscles (Hofmannova et al. 2016; Hofmannova and Jurankova 2019). Hedgehogs may play an important role as a wildlife reservoir for the source of infections for domestic animals or humans in urban and rural areas (Polley 2005).

Free-living helminths are ubiquitous, abundant in soil and water, and can be associated with food products. Simultaneous presence of bacteria and helminths in the environment has been reported to provide an important environmental shelter for bacteria. Helminths not only provide a shelter for further survival of bacteria but can also act as vectors of foodborne pathogens and facilitate transmission to vertebrate hosts (Lacharme-Lora et al. 2009; Foka et al. 2019).

During the literature review on the planning phase of this study, no study was found to investigate *Salmonella* and parasite prevalence in hedgehogs in Istanbul, Türkiye. To our knowledge on parasitological examination, only three studies were performed in other cities of Türkiye, one of them on the helminths and the other two were on ectoparasites of hedgehogs (Çırak et al. 2010; Girişgin et al. 2015; Goz et al. 2016).

In this study, it was aimed to investigate the carriage of *Salmonella* and endoparasite in the faeces of hedgehogs and to determine the antibacterial resistance profiles of *Salmonella* isolates.

Materials and Methods

The study was approved by the Animal Use and Care Committee at the Faculty of Veterinary Medicine, İstanbul University-Cerrahpaşa (Eth No: 72796624-604.01.02).

Thirty faecal samples were collected from hedgehogs brought to veterinary clinics with complaints of trauma, weakness and diarrhoea by animal lovers in different districts of Istanbul (Ataşehir, Avcılar, Bahçeşehir, Bakırköy,

Beylikdüzü, Kadıköy, Küçükçekmece, Üsküdar) between 2018 and 2019. As soon as the samples reached the laboratory, they were inoculated into the media for bacteriological culture, and part of the samples was used for parasitological examination.

Bacteriological examination

For pre-enrichment, 1 g of faeces specimens were transferred into tubes containing 10 ml buffered peptone water (BPW) and then incubated at 41 °C for 18 h. For selective enrichment, 1 ml from each culture was transferred to 9 ml Tetrathionate Brilliant Green broth (TTB, Oxoid, Basingstoke, UK) and incubated at 37 °C for 24 h. At the end of incubation, 1.5 ml of the culture was taken into microcentrifuge tubes and stored at −80 °C for PCR. A loop full of suspensions was spread on Brilliant Green agar (BG, Oxoid CM 329, Basingstoke, UK), Xylose Lysine Desoxycholate agar (XLD, Oxoid CM 469, Basingstoke, UK) and MacConkey agar (Oxoid, Basingstoke, UK) plates, which were incubated at 37 °C for 24 h. At the end of the incubation period, black colonies and black-centred (H_2S positive) colonies with peripheries changed from pink to red in XLD agar; white and red colonies surrounded by red zones in BG agar and colourless, semi-transparent yellowish colonies in MacConkey agar were evaluated as presumptive *Salmonella* colonies (Quinn et al. 1999). Agents isolated were identified with BD Phoenix Automated Microbiology System (BD Diagnostics, New Jersey, USA) and then serotyped by Etlik Central Veterinary Control and Research Institute (Ankara) according to the Kauffmann-White scheme.

Polymerase chain reaction (PCR)

One ml of specimens stored in TTB at −80 °C was transferred into a microcentrifuge tube. DNA extraction was performed according to the protocol by Eyigor et al. (2007). *Salmonella* genus-specific primers based on the invA gene (5'-GTG AAA TTA TCG CCA CGT TCG GGC AA-3' and 5'-TCA TCG CAC CGT CAA AGG AAC C-3') were used. The 25 µl PCR mixture (Thermo Scientific, Dreieich, Germany), which contained 0.3 µl of Taq DNA polymerase (5 U/µl), 2.5 µl of 10X PCR buffer (3.5 mM $MgCl_2$), 2.5 µl of deoxynucleoside triphosphate (dNTP) mixture (2 mM), 1 µl of each primer (5 pmol/µl), 5 µl of template DNA and 12.2 µl of deionised water, was taken into small microcentrifuge PCR tubes (200 µl). Amplification was performed in a total of 30 cycles as 1 min of predenaturation at 94 °C, 30 s of primer binding at 56 °C, 2 min of synthesis cycle at 72 °C and 10 min of the last synthesis stage at 72 °C. Amplified PCR products were electrophoresed in 1.5% agarose gel. The band of 284 bp was evaluated to be positive for *Salmonella* spp. (Kahya et al. 2013).

Antimicrobial susceptibility testing

The antibiotic susceptibility of the isolates was determined phenotypically by the BD Phoenix automated microbiology system. Antibiotics were used according to a Gram-negative result with NMIC/ID-435 Panel (BD Diagnostics, USA). Antibiotics commonly used in veterinary and human medicine were used.

The strains were also analysed by PCR for the presence of resistance genes originating from plasmid-derived enzymes (*aac(3)-Ia, aac(3)-IIa, aac(3)-III, aac(3)-IV, aac(6)-Ib, strA, strB, aadA, aphA1, aphA2, ant(2")-Ia*) and plasmid-mediated 16S rRNA methylase enzymes (*armA ve rmtB*) that inactivate aminoglycoside. The presence of plasmid-mediated quinolone resistance genes (*qnrA, qnrB, qnrC, qnrD, qnrS, qepA, oqxAB* and *aac(6')1b-cr*) was examined with multiplex PCR. Targets, primer bases and band lengths are given in Table 1.

Parasitological examination

Direct smear and saturated salt (specific density of 1.20 g/ml) flotation techniques were applied to faeces and examined under the microscope. The eggs, oocysts, and larvae were identified according to shape, size, and morphological features (Taylor et al. 2007). Because of the difficulty of identifying the species of eggs and oocysts according to the phenotypic features, parasites were referred to at the level of genus.

Results

From 30 faecal samples, 2 strains of *Salmonella* spp. were isolated by conventional methods. The isolates were determined as *S.* Typhimurium by BD Phoenix and then serotyped in Etlik Veterinary Control Central Research Institute *Salmonella* Reference Laboratory as *S.* Typhimurium 4,5,12:i:1,2. *Salmonella*-specific DNA was detected in two samples which were also culture-positive (Fig. 1).

According to the antibiotic susceptibility testing, two strains were found to be resistant only to ciprofloxacin (Table 2). The antibiotic-resistance genes belonging to aminoglycoside and quinolone were not detected.

According to the parasitological examination, 19 of 30 (63.3%) hedgehog faeces were positive for eggs of *Capillaria* spp., *Acanthocephala* spp., oocyst of *Eimeria* spp. and larvae of *Crenosoma* spp. The total frequency of infection with *Capillaria* spp., *Crenosoma* spp., *Eimeria* spp., and *Acanthocephala* spp., were 60%, 13.3%, 10% and 6.6%, respectively.

Table 1. Targets, primer bases, and band lengths to be used to detect gene regions of enzymes that inactivate aminoglycosides and PMQR gene regions.

Antibiotic	Targets	Dizi (5'-3')	Band lengths	References
Gentamycin	*aac(3)-Ia*			
	(aacC1)	ACCTACTCCCAACATCAGCC		
		ATATAGATCTCACTACGCGC	169 bp	Costa et al. 2008
	aac(3)-IIa			
	(aacC2)	ACTGTGATGGGATACGCGTC		
		CTCCGTCAGCGTTTCAGCTA	237 bp	
	aac(3)-III			
	(aacC3)	CACAAGAACGTGGTCCGCTA		
		AACAGGTAAGCATCCGCATC	185 bp	Saenz et al. 2004
	aac(3)-IV			
	(aacC4)	CTTCAGGATGGCAAGTTGGT		
		TCATCTCGTTCTCCGCTCAT	286 bp	Costa et al. 2008
Amikacin	*aac(6)-Ib*			
	(aacA4)	ATGACCTTGCGATGCTCTATGA		
		CGAATGCCTGGCGTGTTT	486 bp	Zhang et al. 2014
	armA	ATT CTG CCT ATC CTA ATT GG		
		ACC TAT ACT TTA TCG TCG TC	315 bp	Doi and Arakawa 2007
	rmtB	GCT TTC TGC GGG CGA TGT AA		
		ATG CAA TGC CGC GCT CGT AT	173 bp	
Streptomycin	*strA*	TTGAATCGAACTAATA		
		TCAACCCCAAGTCAGAGG	806 bp	Zhang et al. 2014
	strB	ATGTTCATGCCGCCTGTTTTT		
		CTAGTATGACGTCTGTCGC	837 bp	
	aadA			
	(aadA1 or aadA2)	GCAGCGCAATGACATTCTTG		
		ATCCTTCGGCGCGATTTTG	282 bp	Costa et al. 2008
Kanamycin	*aphA1*	ATGGGCTCGCGATAATGTC		
		CTCACCGAGGCAGTTCCAT	600 bp	Maynard et al. 2004
	aphA2	GAACAAGATGGATTGCACGCGCT		
		CTTCAGCAATATCACGG	680 bp	
Gentamycin	*ant(2'')-Ia*			
/Kanamycin	*(aadB)*	CCA TGA TGG ATA CTT TCT CG		
		GAG GAG TTG GAC TAT GGA TT	208 bp	Karczmarczyk et al. 2011
Quinolone	*qnrA*	CAGCAAGAGGATTTCTCACG		
		AATCCGGCAGCACTATTACTC	630 bp	Ciesielczuk et al. 2013
	qnrB	GGCTGTCAGTTCTATGATCG		
		GAGCAACGATGCCTGGTAG	488 bp	
	qnrC	GCAGAATTCAGGGGTGTGAT		
		AACTGCTCCAAAAGCTGCTC	118 bp	
	qnrD	CGAGATCAATTTACGGGGAATA		
		AACAAGCTGAAGCGCCTG	581 bp	
	qnrS	GCAAGTTCATTGAACAGGGT		
		TCTAAACCGTCGAGTTCGGCG	428 bp	
	qepA	GCAGGTCCAGCAGCGGGTAG		
		CTTCCTGCCCGAGTATCGTG	218 bp	
	aac(6')-Ib-cr	TTGGAAGCGGGGACGGAM		
		ACACGGCTGGACCATA	260 bp	
	oqxAB	CCGCACCGATAAATTAGTCC		
		GGCGAGGTTTTGATAGTGGA	313 bp	

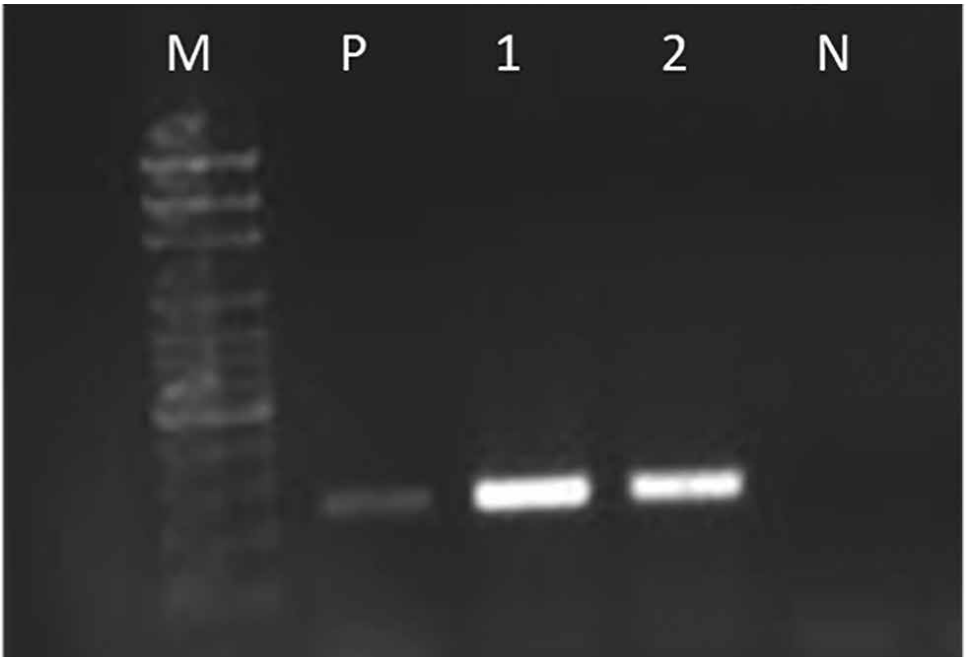

Fig. 1. Results of PCR.

M: Marker P: Positive Control (284bp) 1, 2: Positive specimens N: Negative control

Table 2. Antibiotic susceptibility test.

Antibiotic	Isolate 1 S/I/R	Isolate 2 S/I/R	MIC
Amikacin	S	S	≤ 4
Amoxicillin-clavulanate	S	S	≤ 2/2
Ampicillin	S	S	≤ 2
Aztreonam	S	S	≤ 1
Ertapenem	S	S	≤ 0.25
Gentamicin	S	S	2
Imipenem	S	S	2
Colistin	S	S	≤ 1
Meropenem	S	S	≤ 0.13
Netilmicin	S	S	2
Piperacillin	S	S	≤ 4
Piperacillin-tazobactam	S	S	≤ 4/4
Cefepime	S	S	≤ 1
Ceftazidime	S	S	≤ 0.5
Ceftriaxone	S	S	≤ 0.5
Cefuroxime	S	S	4
Ciprofloxacin	R	R	1
Tigecycline	S	S	1
Trimethoprim-sulfamethoxazole	S	S	≤ 1/19

S - susceptible; I - intermediate; R - resistant

The most common genus was *Capillaria* spp. Seven (23.3%) hedgehogs were co-infected with two or three kinds of parasites. The distribution of species, numbers and percentage of parasites are presented in Table 3.

Table 3. Prevalence of the parasite genus and its distribution in 30 hedgehog faeces samples.

Genus of parasites	Positive samples (n = 30)	
	Numbers	(%)
Samples infected by one species		
Capillaria spp.	12	40
Crenosoma spp.	0	0
Eimeria spp.	0	0
Acanthocephala spp.	0	0
Co-infected samples		
Capillaria spp. + *Crenosoma* spp.	3	10
Capillaria spp. + *Eimeria* spp.	2	6.6
Crenosoma spp + *Acanthocephala* spp.	1	3.3
Capillaria spp. + *Acanthocephala* spp. + *Eimeria* spp.	1	3.3
Total number and prevalence of parasite genus		
Capillaria spp.	18	60
Crenosoma spp.	4	13.3
Eimeria spp.	3	10
Acanthocephala spp.	2	6.6

Discussion

Hedgehogs in cities lead an active life at night and during the day. People take injured or sick hedgehogs found on the street to veterinary clinics to save them. During handling, they may be exposed to many zoonotic pathogens from hedgehogs. The main zoonotic disease associated with hedgehogs is salmonellosis. Although affected hedgehogs may experience anorexia, diarrhoea, and weight loss, approximately 28% are asymptomatic carriers (Riley and Chomel 2005). Salmonellosis is the most common zoonotic bacterial disease in both domestic and wild hedgehogs, and *S.* Enteritidis and *S.* Typhimurium are the most common serovars (Ruszkowski et al. 2021). Hedgehogs shed *Salmonella* agents with their faeces and contaminate the areas they roam, especially grass. Transmission to humans other than through direct contact with hedgehogs can occur by contact with grass contaminated with infectious agents or through cats or dogs that come into contact with such grass (Ruszkowski et al. 2021).

Keeble and Koterwas (2020) reported the prevalence of *Salmonella* infection in hedgehogs as 3–4% from faecal samples or rectal swabs. Meanwhile they pointed out that in feeding areas shared with humans and birds (natural picnic areas), the prevalence increased to 71%, possibly due to cross-contamination. The rate of *Salmonella* carriage among European hedgehogs in Norfolk, England, was reported as 19% (1 *S.* Typhimurium, 13 *S.* Enteritidis) (Keymer et al. 1991).

Lawson et al. (2018) investigated various tissue samples (liver and small intestine contents) from 170 wild hedgehogs and faecal samples from 208 wild hedgehogs for *Salmonella* in the United Kingdom, and compared them with those found in humans. They isolated *S.* Enteritidis from 27% of the tissue samples and 3% of the faeces and indicated that *Salmonella* spp. may have originated from a common population. They also emphasised

that hedgehogs can serve as a source of *Salmonella* spp. and reservoir hosts for humans. Krawczyk et al. (2015) detected *Salmonella* from 10% (9/90) of hedgehog faeces in the Netherlands, and they determined that three of them were *S.* Enteritidis, which is the most common pathogenic serotype in humans. Rautio et al. (2016) found the prevalence of *S.* Enteritidis to be 57% in 37 dead Finnish hedgehogs. A study in Denmark reported that the prevalence of *S.* Enteritidis strains isolated from European hedgehogs was 25%, and the strains belonged to the same clonal lineages as strains isolated from infected humans (Nauerby et al. 2000).

When some human outbreaks were investigated in Norway in 2000, it was suggested that hedgehogs might be the primary source of infection of specific *S.* Typhimurium clones of human clinical importance in some geographic regions (Heir et al. 2002). Handeland et al. (2002) investigated the faecal carrier of *Salmonella* in 320 wild hedgehogs collected from four regions in Norway. During an epidemic of salmonellosis in humans one year later, samples were negative in two areas where they were collected, while *S.* Typhimurium (4,5,12:i:1,2) was isolated in 39% and 41% of hedgehogs in two other regions. Likewise, they reported that the causative agent in salmonellosis epidemic in humans was *S.* Typhimurium (4,5,12:i:1,2). They noted that the prevalence (71%) was higher in animals sampled near gardens and feeding stations than animals in gardens, parks, roadsides and groves (25%).

Kagambega et al. (2013) reported the prevalence of eight *Salmonella* serotypes in Burkina Faso as 96% and emphasised that hedgehogs are carriers of many *Salmonella* serotypes in livestock. In a study conducted in Chile, the presence of *Salmonella* was investigated in the faeces of 200 hedgehogs kept as exotic animals. *Salmonella enterica* subsp. *enterica* serotypes Muenchen (2), Infantis (2) and IV43:z4,z23:-(1) were found in 5 animals, and the authors emphasised that exotic domestic animals could potentially harm public health (Perez et al. 2021). In one of 26 hedgehogs studied in Catalonia, Spain, *Salmonella enterica* subsp. *enterica* serovar Kottbus was isolated, and a pattern of antibiotic resistance was determined before starting any treatment. The strain has been reported to be resistant to ampicillin, streptomycin, tetracycline, sulphamethoxazole, trimethoprim/sulphamethoxazole, and nalidixic acid (Molina-López et al. 2015).

This study determined the *Salmonella* carriage prevalence of 6.66% in wild hedgehogs in Türkiye using both culture and PCR. Two *Salmonella* isolates were defined as *S.* Typhimurium*,* whose antigenic formula was 4,5,12:i:1,2. Despite the low number of samples taken, it was pleasing that in our city, which has the densest population in Türkiye, the *Salmonella* carrier detected in the free-living hedgehogs in the parks frequented extensively by people is low compared to other countries.

Kagambega et al. (2013) reported that strains isolated from hedgehogs had streptomycin resistance genotypically. In the same study, they determined the presence of multiple antibiotic resistances against ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline, frequently used in veterinary medicine. Another study reported multi-drug resistance against ampicillin, streptomycin, sulphonamide, tetracycline, trimethoprim-sulphamethoxazole, and nalidixic acid in *Salmonella* isolates from hedgehogs (Molina-López et al. 2015). Although high rates of multi-antibiotic resistance were detected in many countries, no phenotypic and genotypic resistance development was detected against antibiotics frequently used in veterinary medicine in a study conducted in England. The researchers attributed the absence of antimicrobial resistance (AMR) to their low exposure to high antibiotic-use places such as animal farms or clinical settings (Lawson et al. 2018).

In this study, two *S.* Typhimurium isolated from hedgehogs were resistant only to ciprofloxacin out of 19 tested antibiotics. In addition, aminoglycoside and quinolone resistance genes were not detected in the strains. This discrepancy between phenotypic

and genotypic tests may be due to other genes causing resistance or a different enzymatic pathway outside the investigated mechanisms. The fact that two *Salmonella* isolated hedgehogs live in the same area and the two isolates are resistant to the same antibiotic is remarkable in terms of demonstrating the importance of *Salmonella* contagion.

The first report on the helminths of hedgehogs in Türkiye was by Merdivenci (1965). The detected species were *Gongylonema neoplasticum*, *Spirura rytipleurites seurati*, *Physaloptera clausa*, *C. striatum*, *Capillaria erinacei* (syn. *Aonchotheca erinacei*), *Hepaticola soricicola* and *Prosthorynchus* spp. After a long period later, a study by Çırak et al. (2010) identified helminths in *post mortem* examinations and found the species and their prevalences to be 72.2% for *P. clausa*, 55.5% for *C. striatum*, 55.5% for *A. erinacei*, 55.5% for *Hymenolepis erinacei*, 50% for *Nephridiorhynchus major* and 22.2% for *Eucoleus aerophilus* (syn. *Capillaria aerophila*) and *N. major* were the parasites reported for the first time in Türkiye by researchers.

Capillaria spp. were the most common species in hedgehogs, and their prevalence according to the countries was: 33% and 39.5% in two studies in Germany (Epe et al. 2004; Raue et al. 2017), 35% in Serbia (Pavlovic and Savic 2017), 36.8% in Greece (Liatis et al. 2017), 13% in Iran (Naem et al. 2015), 61% in the UK (Gaglio et al. 2010). In this study, the prevalence of *Capillaria* spp. (60%) was higher than in Germany, Serbia, Greece and Iran but lower than in the UK.

The prevalence of *Crenosoma striatum* reported by researchers varied from 3.4% to 71.4% in different countries, such as 3.4% in Poland (Mizgajska-Wiktor et al. 2010), 20% in Serbia (Pavlovic and Savic 2017); 27.4% and 26.6% in Germany (Epe et al. 2004; Raue et al. 2017), 47.3% in Greece (Liatis et al. 2017), 61% in Iran (Naem et al. 2015), 71% in the UK (Gaglio et al. 2010). In this study, the percentage of infection with *Crenosoma* spp. was 13.3%, i.e. lower than in Serbia, Germany, Greece, Iran, and the UK but higher than in Poland. The prevalence of *Acanthocephala* spp. was 69% in Sicily, 23.5% in Sardinia, 30% in Iran, and 15.7% in Greece, which was higher than in this study (6.6%), and 1.35% in the UK, which was lower than this study (Poglayen et al. 2003; Gaglio et al. 2010; Naem et al. 2015; Liatis et al. 2017). Percentage of oocysts of the coccidiosis agent was reported to be 15.7% in Greece and 14.2% in Germany, which is higher than in this study (10%), but in another study in Germany, the rate was reported to be 5.7%, i.e. lower than this study (Epe et al. 2004; Liatis et al. 2017; Raue et al. 2017). Parasitological results of this study indicate a high percentage of parasites (63.3%) in hedgehogs in Istanbul.

Zoonotic pathogens survive for a long time (up to several months) in soil and water and are taken up by free-living helminths during this time (Lemunier et al. 2005; Perkins and Fenton 2006; Gourabathini et al. 2008). Because the foodborne pathogen *Salmonella* is intolerant to low pH, certain environments in the vertebrate host (such as the high acidity of the stomach) may prevent settling unless ingested with food. Helminths can allow infection to evade the primary immune defence and reach the optimum site for subsequent occurrence (Lacharme-Lora et al. 2009; Foka et al. 2019). Brosschot et al. (2021) reported that helminth infection aids the initial colonization of *Salmonella*, but *S.* Typhimurium does not require helminths to survive in the small intestine.

In conclusion, this is the first study on *Salmonella* and parasites of hedgehogs in Istanbul city, providing basic data for further studies. *Salmonella* Typhimurium is known to cause serious infections in humans and animals. For this reason, more comprehensive studies are needed in terms of *Salmonella* and other zoonotic agents that can be transmitted from wild hedgehogs to humans and other animals in our daily lives. This study also shows that hedgehogs are susceptible to many parasites and can be used as an indicator for showing the potential risk of hedgehogs as a reservoir for these parasites in areas located near human settlements.

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References

Anderson TC, Marsden-Haug N, Morris JF, Culpepper W, Bessette N, Adams JK 2017: Multistate outbreak of human *Salmonella* Typhimurium infections linked to pet hedgehogs–United States, 2011–2013. Zoonoses Public Health **64**: 290-298

Beck W 2007: Endoparasites of the hedgehog (in German). Wien Klin Wochenschr **119**: 40-44

Bexton S, Couper D 2019: Veterinary care of free-living hedgehogs. In Practice **41**: 420-432

Brosschot TP, Lawrence KM, Moeller BE, Kennedy MH, FitzPatrick RD, Gauthier CM, Reynolds LA 2021: Impaired host resistance to *Salmonella* during helminth co-infection is restored by anthelmintic treatment prior to bacterial challenge. Plos Neglect Trop D **15**: e0009052

Centers for Disease Control and Prevention (CDC) 2019: Outbreak of *Salmonella* infections linked to pet hedgehogs. Atlanta (GA, USA)

Ciesielczuk H, Hornsey M, Choi V, Woodford N, Wareham D 2013: Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. J Med Microbiol **62**: 1823-1827

Costa D, Poeta P, Saenz Y, Coelho AC, Matos M, Vinue L, Rodrigues J, Torres C 2008: Prevalence of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from healthy pets. Vet Microbiol **127**: 97-105

Çırak VY, Senlik B, Aydogdu A, Selver M, Akyol V 2010: Helminth parasites found in hedgehogs (*Erinaceus concolor*) from Türkiye. Prev Vet Med **97**: 64-66

Doi Y, Arakawa Y 2007: 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin Infect Dis **45**: 88-94

Epe C, Coati N, Schneider T 2004: Results of parasitological examinations of faecal samples from horses, ruminants, pigs, dogs, cats, hedgehogs and rabbits between 1998 and 2002. Deut Tierarztl Woch **111**: 243-247

Eyigör A, Goncagül G, Carli KT 2007: A PCR-ELISA for the detection of *Salmonella* from chicken intestine. J Biol Environ Sci **1**: 45-49

Foka EIS, Mayaka TB, Agyingi L, Yondo J, Mbida M 2019: Prevalence and coincidence of helminth and *Salmonella* infection in a cohort of hospital patients in Cameroon. Eur J Pharm Med Res **6**: 200-204

Gaglio G, Allen S, Bowden L, Bryant M, Morgan ER 2010: Parasites of European hedgehogs *Erinaceus europaeus* in Britain: epidemiological study and coprological test evaluation. Eur J Wildlife Res **56**: 839-844

Girişgin AO, Şenlik B, Aydın L, Cirak VY 2015: Ectoparasites of hedgehogs (*Erinaceus concolor*) from Türkiye. Berl Munch Tierarztl **128**: 315-318

Gourabathini P, Brandl MT, Redding KS, Gunderson JH, Berk SG 2008: Interactions between foodborne pathogens and protozoa isolated from lettuce and spinach. Appl Environ Microb **74**: 2518-2525

Goz Y, Yilmaz AB, Aydin A, Dicle Y 2016: Ticks and fleas infestation on east hedgehogs (*Erinaceus concolor*) in Van province, eastern region of Türkiye. J Arthropod-Borne Di **10**: 50-54

Handeland K, Refsum T, Johansen BS, Holstad G, Knutsen G, Solberg I 2002: Prevalence of *Salmonella* Typhimurium infection in Norwegian hedgehog populations associated with two human disease outbreaks. Epidemiol Infect **128**: 523-527

Hasseltvedt V, Bergesen E, Blinkenberg J, Digranes A, Heir E, Iversen BG, Tveit I 2000: Transmission of salmonellosis through hedgehogs in Norway. Eurosurveillance **4**: 1522

Heir E, Lindstedt BA, Nygård I, Vardund T, Hasseltvedt V, Kapperud G 2002: Molecular epidemiology of *Salmonella* Typhimurium isolates from human sporadic and outbreak cases. Epidemiol Infect **128**: 373-382

Hoff C, Nichols M, Gollarza L, Scheftel J, Adams J, Tagg KA, Francois Watkins L, Poissant T, Stapleton GS, Morningstar-Shaw B, Signs K 2022: Multistate outbreak of *Salmonella* Typhimurium linked to pet hedgehogs, United States, 2018–2019. Zoonoses Public Health **69**: 167-174

Hofmannova L, Hauptman K, Huclova K, Kvetonova D, Sak B, Kvac M 2016: *Cryptosporidium erinacei* and *C. parvum* in a group of overwintering hedgehogs. Eur J Protistol **56**: 15-20

Hofmannova L, Jurankova J 2019: Survey of *Toxoplasma gondii* and *Trichinella* spp. in hedgehogs living in proximity to urban areas in the Czech Republic. Parasitol Res **118**: 711-714

Hutterer R 2005: Order Erinaceomorpha. In: Wilson DE, Reeder DM (Eds): Mammal species of the world: A taxonomic and geographic reference. Johns Hopkins University Press, Baltimore, pp. 220-311

Ichimi R, Yoshino A, Higashigawa M 2018: *Salmonella* Stanley bacteremia transmitted from a pet hedgehog. Pediatr Int **60**: 606-607

Kagambega A, Lienemann T, Aulu L, Traoré AS, Barro N, Siitonen A 2013: Prevalence and characterisation of *Salmonella* Enterica from the faeces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human *Salmonella* isolates. BMC Microbiol **13**: 1-9

Kahya S, Eyigör A, Çarli KT 2013: Comparison of different sample types for *Salmonella* detection from chicken layer breeder flocks. J Res Vet Med **32**: 19-24

Karczmarczyk M, Martins M, McCusker M, Mattar S, Amaral L, Leonard N 2011: Characterisation of antimicrobial resistance in *Salmonella* Enterica food and animal isolates from Colombia: identification of a qnrB19-mediated quinolone resistance marker in two novel serovars. FEMS Microbiol Lett **313**: 10-19

Kazemi-Darabadi S, Akbari G, Ebrahimi E, Zangisheh M 2018: Computed tomographic anatomy and topography of the lower respiratory system of the southern white-breasted hedgehog (*Erinaceus concolor*). Iran J Vet Surg **13**: 26-33

Keeble E, Koterwas B 2020: Selected emerging diseases of pet hedgehogs. Vet Clin N Am-Exotic **23**: 443-458

Keymer IF, Gibson EA, Reynolds DJ 1991: Zoonoses and other findings in hedgehogs (*Erinaceus europaeus*): a survey of mortality and review of the literature. Vet Rec **128**: 245-249

Kottwitz LB, Scheffer MC, Dalla-Costa LM, de SS Farah SM, Moscalewski WS, Magnani M 2011: Molecular characterisation and resistance profile of *Salmonella* Enteritidis PT4 and PT9 strains isolated in Brazil. J Med Microbiol **60**: 1026-1031

Krawczyk AI, van Leeuwen AD, Jacobs-Reitsma W, Wijnands LM, Bouw E, Jahfari S, van Hoek AHAM, van der Giessen JWB, Roelfsema JH, Kroes M, Kleve I, Dullemont Y, Sprong H, de Bruin, A 2015: Presence of zoonotic agents in engorged ticks and hedgehog faeces from *Erinaceus europaeus* in (sub) urban areas. Parasit Vectors **8**: 1-6

Lacharme-Lora L, Perkins SE, Humphrey TJ, Hudson PJ, Salisbury V 2009: Use of bioluminescent bacterial biosensors to investigate the role of free-living helminths as reservoirs and vectors of *Salmonella*. Env Microbiol Rep **1**: 198-207

Lawson B, Franklinos LH, Fernandez JRR, Wend-Hansen C, Nair S, Macgregor SK 2018: *Salmonella* Enteritidis ST183: emerging and endemic biotypes affecting western European hedgehogs (*Erinaceus europaeus*) and people in Great Britain. Sci Rep-UK **8**: 1-11

Lemunier M, Francou C, Rousseaux S, Houot S, Dantigny P, Piveteau P, Guzzo J 2005: Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts. Appl Environ Microb **71**: 5779-5786

Liatis TK, Monastiridis AA, Birlis P, Prousali S, Diakou A 2017: Endoparasites of wild mammals sheltered in wildlife hospitals and rehabilitation centres in Greece. Front Vet **4**: 220

Maynard C, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, Masson L 2004: Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal *Escherichia coli* isolates of animal and human origin. J Clin Microbiol **42**: 5444-5452

Merdivenci A 1965: Türkiyenin helmintolojik cografyası (in Turkish, Helminthological geography of Türkiye). In: Unat EK, Yasarol S, Merdivenci A (Eds): Türkiyenin Parazitolojik Cografyası (in Turkish, Parasitological Geography of Türkiye). Ege Üniversitesi Matbaası, Izmir, pp. 55-113

Mizgajska-Wiktor H, Jarosz W, Pilacinska B, Dziemian S 2010: Helminths of hedgehogs, *Erinaceus europaeus* and *E. roumanicus* from Poznan region, Poland - coprological study. Ann Parasitol **56**: 329-332

Molina-López RA, Vidal A, Obón E, Martín M, Darwich L 2015: Multidrug-resistant *Salmonella enterica* serovar Typhimurium monophasic variant 4, 12: i:-I isolated from asymptomatic wildlife in a Catalonian wildlife rehabilitation center, Spain. J Wildlife Dis **51**: 759-763

Monecke S, Gavier-Widen D, Mattsson R, Rangstrup-Christensen L, Lazaris A, Coleman DC, Ehricht R 2013: Detection of mecC-positive *Staphylococcus aureus* (CC130-MRSA-XI) in diseased European hedgehogs (*Erinaceus europaeus*) in Sweden. Plos One **8**: e66166

Mullineaux E, Keeble E 2016: BSAVA manual of wildlife casualties. British Small Animal Veterinary Association, UK

Naem S, Pourreza B, Gorgani-Firouzjaee T 2015: The European hedgehog *Erinaceus europaeus* is a reservoir for helminth parasites in Iran. Vet Res **6**: 149-153

Nauerby B, Pedersen K, Dietz HH, Madsen M 2000: Comparison of Danish isolates of *Salmonella enterica* serovar Enteritidis PT9a and PT11 from hedgehogs (*Erinaceus europaeus)* and humans by plasmid profiling and pulsed-field gel electrophoresis. J Clin Microbiol **38**: 3631-3635

Orkun Ö, Çakmak A, Nalbantoğlu S, Karaer Z 2019: Molecular detection of a novel *Babesia* sp. and pathogenic spotted fever group rickettsiae in ticks collected from hedgehogs in Turkey: *Haemaphysalis erinacei*, a novel candidate vector for the genus *Babesia*. Infect Genet Evol **69**: 190-198

Özen A 2010: Some biological, ecological and behavioural features of *Erinaceus Concolor Martin*, 1838 (Mammalia: Insectivora) in Türkiye. Gazi Univ J Sci **19**: 91-97

Pavlovic I, Savic B 2017: Helminth fauna of the northern white-breasted hedgehog (*Erinaceus roumanicus*) in Serbia. J Parasit Dis **41**: 605-606

Perez S, Barreto M, Retamal P 2021: Detection of antimicrobial-resistant *Salmonella enterica* strains in samples of ground hedgehogs (*Atelerix albiventris*) reared as pets in the urban area of Santiago, Chile. Austral J Vet Sci **53**: 133-137

Perkins SE, Fenton A 2006: Helminths as vectors of pathogens in vertebrate hosts: A theoretical approach. Int J Parasitol **36**: 887-894

Poglayen G, Giannetto S, Scala A, Garippa G, Capelli G, Scaravelli D, Brianti E, Reeve NJ 2003: Helminths are found in hedgehogs (*Erinaceus europaeus*) in three areas of Italy. Vet Rec **152**: 22-24

Polley L 2005: Navigation parasite webs and parasite-flow emerging and remerging parasitic zoonoses of wildlife origin. Int J Parasitol **35**: 1279-1294

Quinn PJ, Carter ME, Markey BK, Carter GR 1999: Clinical Veterinary Microbiology. 3rd edn. Harcourt Publishers, London, 381 p.

Raue K, Heuer L, Bohm C, Wolken S, Epe C, Strube C 2017: 10-year parasitological examination results (2003 to 2012) of faecal samples from horses, ruminants, pigs, dogs, cats, rabbits and hedgehogs. Parasitol Res **116**: 3315-3330

Rautio A, Isomursu M, Valtonen A, Hirvelä-Koski V, Kunnasranta M 2016: Mortality, diseases and diet of European hedgehogs (*Erinaceus europaeus*) in an urban environment in Finland. Mammal Res **61**: 161-169

Resmi Gazete 2015: List of the Wild Animals Protected by the Republic of Türkiye Ministry of Agriculture and Forestry. Available at: https://www.resmigazete.gov.tr/eskiler/2015/04/20150429-5.htm. Accessed December 19, 2021

Riley PY, Chomel BB 2005: Hedgehog zoonoses. Emerg Infect Dis **11**: 1-5

Ruszkowski JJ, Hetman M, Turlewicz-Podbielska H, Pomorska-Mól M 2021: Hedgehogs as a potential source of zoonotic pathogens - A review and an update of knowledge. Animal **11**: 1754

Saenz Y, Brinas L, Domínguez E, Ruiz J, Zarazaga M, Vila J, Torres C 2004: Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. Antimicrob Agents Ch **48**: 3996-4001

Sykes L, Durrant J 2005: The Natural Hedgehog. Gaia Books, London, 108 p.

Taylor MA, Coop RL, Wall RL 2007: Veterinary parasitology. 3rd ed. Blackwell publishing ltd, Oxford, p: 900

Youssefi MR, Rahimi MT, Halajian A, Moosapour AA, Nikzad R, Nikzad M, Ebrahimpour S 2013: Helminth parasites of eastern European hedgehog (*Erinaceus concolor*) in northern Iran. IJP **8**: 645

Zhang FY, Huo SY, Li YR, Xie R, Wu X., Chen LG 2014: A survey of the frequency of aminoglycoside antibiotic-resistant genotypes and phenotypes in *Escherichia coli* in broilers with septicaemia in Hebei, China. Brit Poultry Sci **55**: 305-310