ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Araştırma Makalesi *Research Article* DOI: 10.5281/zenodo.10894467

The Prevalence of *Helicobacter spp*. in Licensed Experimental Animal Facilities in Aegean Region, Turkey*

Ege Bölgesindeki Ruhsatlı Deney Hayvanı Tesislerinde *Helicobacter spp.* Prevalansı*

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ABSTRACT

In this study, it was aimed to investigate the presence of *Helicobacter* spp. and *H. hepaticus*, *H. bilis*, *H. muridarum*, *H. rodentium* and *H. typhlonius* species in mice, rats and gerbils in the Aegean Region. Colon and stool samples were collected from a total of 200 animals, 10 mice, 10 rats and 10 gerbils, from separate cages randomly selected between the ages of 15- 22 weeks from each of the 11 licensed experimental animal facilities. From the DNA obtained, *Helicobacter* spp. 16S ribosomal RNA gene was determined by PCR method, and positive samples were determined by multiplex PCR. The prevalence of *Helicobacter* spp. in mice was 90.91 %. According to the species-based PCR results of the positive samples, the most common species was *H. rodentium* with a prevalence of 90.91 %. In the study in which *H. bilis* and *H. muridarum* species were not detected in any facility, it was determined that *H. typhlonius* was the second most common species with a prevalence rate of 72.73 %, followed by *H. hepaticus* with a prevalence of 27.27 %. The prevalence of *Helicobacter* spp. in rats was 87.5 %. According to the PCR results of the positive samples, the most common species was *H. rodentium* with a prevalence of 87.5 %. In the study where *H. bilis*, *H. hepaticus* and *H. muridarum* species were not detected in any facility, *H. typhlonius* was the second common species with a prevalence of 12.5 % in rats. On the other hand, only *H. rodentium* and *H. typhlonius* were detected in the colon samples taken from a single facility containing gerbils. It was determined that *Helicobacter* spp., which causes infections in experimental animals, especially subclinical, is quite common in the study area. It is recommended that the Experimental Animal facilities moniterize for this agent.

Keywords: Gerbil, *Helicobacter* spp., mice, rat.

ÖZ

Bu çalışmayla Ege Bölgesindeki fare, rat ve gerbillerde *Helicobacter* spp. ve *H. hepaticus*, *H. bilis*, *H. muridarum*, *H. rodentium* ve *H. typhlonius* türlerinin varlığının araştırılması amaçlanmıştır. Ruhsatlı 11 adet deney hayvanı tesisinin her birinden 15-22 haftalık yaşlar arasından rastgele seçilen ayrı kafeslerden 10 adet fare, 10 adet rat ve 10 adet de gerbil olmak üzere toplam 200 hayvandan kolon ve dışkı örnekleri toplanmıştır. Elde edilen DNA'lardan *Helicobacter* spp.,16S ribozomal RNA geni kullanılarak PCR yöntemi ile pozitif örneklerin tür tayini ise multiplex PCR ile belirlenmiştir. Farelerde *Helicobacter* spp. prevalansı % 90.91 olarak gerçekleşmiştir. Pozitif örneklerin tür bazındaki PCR sonuçlarına göre en yaygın tür % 90.91 prevalansla *H. rodentium* olmuştur. *H. bilis* ve *H. muridarum* türlerinin hiçbir tesiste tespit edilmediği çalışmada *H. typhlonius* % 72.73 prevalans oranıyla ikinci yaygın tür olurken onu % 27.27'lik prevalansla *H. hepaticus*'un takip ettiği tespit edilmiştir. Ratlarda *Helicobacter* spp. prevalansı % 87.5 olarak gerçekleşmiştir. Pozitif örneklerin PCR sonuçlarına göre en yaygın tür % 87.5 prevalansla *H. rodentium* olmuştur. *H. bilis*, *H. hepaticus* ve *H. muridarum* türlerinin hiçbir tesiste tespit edilmediği çalışmada *H. typhlonius* % 12.5 prevalans oranıyla ikinci yaygın türdür. Gerbil bulunduran tek tesisten alınan kolon örneklerinde ise sadece *H. rodentium* ve *H. typhlonius* tespit edilmiştir. Çalışmaya konu olan bölgede, özellikle subklinik olmak üzere deney hayvanlarındaki enfeksiyonlara neden olan *Helicobacter* spp.'nin oldukça yaygın olduğu belirlenmiştir. Deney Hayvanı tesislerinin bu ajan yönünden takibinin yapılması önerilmektedir.

Anahtar Kelimeler: Fare, Gerbil, *Helicobacter* spp., sıçan.

Statement (This research was summarized from Necdet İlker İÇİL's PhD thesis and was supported by Aydın Adnan Menderes University Scientific Research Projects Unit (Project No: VTF21029).*

Geliş Tarihi/Received 13.01.2024
Kabul Tarihi/Accepted 11.03.2024
Yayın Tarihi/Publication Date 29.03.2024

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Cite this article: İçil, N. İ., Erbaş G. (2024). The Prevalence of *Helicobacter* spp. in Licensed Experimental Animal Facilities in Aegean Region, Turkey. *Journal of Laboratory Animal Science and Practices*, *4*(1), 33-43.

Introduction

Environmental and genetic factors and their interactions with each other may affect the suitability of animals to be used for experimental purposes in research. Since the emergence of infectious agents in facilities that produce or use laboratory animals not only directly affects scientific research projects due to experimental variability, but also affects animal welfare, the need to take into account the microbiological quality of these facilities has arisen (Mähler et al., 2014). Ensuring the reproducibility of research, which is one of the scientific requirements, is a widely accepted approach that requires laboratory animals that are free from diseases and other conditions that could affect experimental results (Matos-Rodrigues et al., 2020). In this context, it is recommended that every institution establishes a laboratory animal 'Health Monitoring' program integrated into any quality assurance system (Mähler et al., 2014; Bracken et al., 2017). In routine screenings of the 'Health Monitoring' program recommended by the European Federation of Laboratory Animal Science Societies (FELASA); *Helicobacter* spp. is among the infectious agents recommended to be monitored every 3 months for laboratory animals (Mähler et al., 2014).

Helicobacter-associated disease is not clinically observable in most immunocompetent rodents. However, when immunodeficient or transgenic mice with immune system abnormalities are infected experimentally or naturally, subclinical inflammation develops up to the morbidity stage (Whary and Fox, 2006). Although uncertainty in clinical presentation is limited to mono-infected animals, infections with more than one species can produce pathological lesions. For example, it is emphasized that mixed infection with *H. rodentium* may potentiate disease caused by more pathogenic species such as *H. hepaticus* or *H. bilis*, with the assumption that *H. rodentium* may be an acceptable contaminant in most conventional mouse colonies (Myles et al., 2003).

It is known that some enterohepatic strains of *Helicobacter* spp., which can cause clinical symptoms as well as a subclinical course in laboratory rodents, are associated with reduced reproductive performance, rectal prolapse, inflammatory bowel disease and typhlocolithis. *H. hepaticus* and *H. bilis* can also cause hepatitis and hepatocarcinoma in mice. These factors cause microbiological quality to negatively affect not only experimental quality but also production and breeding dynamics (Whary and Fox, 2006).

The first study on the prevalence of the microorganism in question was conducted by Shames et al. (1995) in 1995, solely for the screening of *H. hepaticus*. In this study, twenty-eight different strains or stocks, a total of 160 mice from 4 facilities, were used and it was reported that *H. hepaticus* was detected in two of the four facilities, therefore the prevalance is 50% realized. The incidence was determined as 100% in one of the positive facilities and 52% in the other. In a facility that tested negative for *H. hepaticus*, *H. bilis* was detected with an incidence of 55% (Shames et al., 1995). Another data showing the presence of naturally acquired *Helicobacter* infections in all commonly used laboratory rodent species and the majority of the frequently isolated species from samples taken from infected mice were reported by Taylor et al. (2007).

Another study revealing the prevalence of *Helicobacter* spp. compared to other bacterial agents screened was Pritchett-Corning et al. (2009) was conducted in a wide geography using a very high number of animals. According to the results of the study on the European and North American prevalence of infectious agents seen in laboratory mice and rats, the most common bacterial agent in mice was *Helicobacter* spp. with an average rate of 16.08%, and the most common species in this genus was *H. hepaticus* with 12.37%. Although the rat results of the same study differed proportionally from the mouse results, they did not change in terms of the most common bacterial agent (Pritchett-Corning et al., 2009). In a study conducted by Goto et al. (2000), the prevalence of *H. hepaticus* was 25.5%, the prevalence of *H. bilis* was 2.1%, and the prevalence of *H. rodentium* was 23.4%. It was found together with *H. hepaticus* and *H. rodentium* in 47 mice (5.7%) from six colonies.

Since it avoids euthanasia using feces for sampling, it will provide a reliable and easy method to achieve the 3Rs (refinement, reduction, replacement) in screening tests and clinical research (Neubert et al., 2022). Various studies have been conducted regarding the differences between sampling sites in terms of bacterial detection. In a study by Nilsson et al. (2004) in a study; *Helicobacter* DNA was detected at a rate of 85.7% in the fecal samples of 9 mouse strains housed in 4 different facilities, and this rate was the highest rate compared to other tissues and samples. In the first study on this subject conducted by Shames et al. (1995), it was reported that colon culture results had a 100% compatibility with cultivating fecal filtrate. Another

study in which the effect of colon and stool samples on the results was found to be insignificant was Beckwith et al. (1997). In one of the recent study that included a comparison of sampling, it was reported that fecal samples may be negative for *Helicobacter* in some cases where the intestines are positive (Cao et al., 2020). Another study reporting results in favor of the colon between two sampling sites was Matos-Rodrigues et al. (2020). In this study, in terms of *Helicobacter* spp., which was 59.6% in fecal samples, was reported as 70.1% in colon samples.

It is stated that the PCR method is the most sensitive and reliable tool to detect *Helicobacter* spp. infection due to the difficulty of culturing *Helicobacter* spp., histopathological diagnosis not being sensitive or specific, and serological methods lacking specificity in mixed infections. (Whary and Fox, 2006). In this study, *Helicobacter* spp. was detected locally in mice, rats and gerbils. In addition to determining its presence, it is planned to identify and verify a sampling method suitable for routine screening. PCR was used as the detection method in the study.

Materials and Methods

Colon and stool samples were collected from a total of 200 animals, 10 mice, 10 rats and 10 gerbils, from separate cages randomly selected between the ages of 15-22 weeks from each of the 11 licensed experimental animal facilities (110 mice, 80 rats and 10 gerbils are kept in only one facility). Since only 8 of the eleven facilities kept and used rats, the number of samples representing rats was limited to 80. These animals were placed in individual cages by prior consultation with the facility manager, thus ensuring that the fecal samples belonged to these animals. Samples taken from these animals formed the sample pool. By combining the colon and fecal samples of the animals taken from the facilities, 1 rat colon and fecal sample, 1 mouse colon and fecal sample, and a gerbil colon and fecal sample set representing each facility were created (Table 1). Mice, rats and gerbils were euthanized with CO_2 before taking the colon sample, and a colon sample of approximately 1 cm in length was taken from each animal under aseptic conditions in the Bacteriology Department of Bornova Veterinary Control Institute. Thus, the study was conducted with a total of 40 samples, including 20 colon samples and 20 stool samples related to these samples. Stool and intestinal sections were kept at -20°C until analysis.

Table 1. Sampling chart

Tablo 1. Örnekleme tablosu

Sampling facility	Animal species and numbers sampled					
	Mice		Rat		Gerbil	
	Feces	Colon	Feces	Colon	Feces	Colon
1st	10	10	-	-	-	-
2nd	10	10	10	10	-	-
3th	10	10	10	10	-	-
4th	10	10	10	10	-	-
5th	10	10	-	-	-	-
6th	10	10	10	10	-	-
7th	10	10	10	10	-	-
8th	10	10	10	10	-	-
9th	10	10	10	10	10	10
10th	10	10	-	-	-	-
11th	10	10	10	10	-	-
Total number of animals	110	110	80	80	10	10
Number of samples obtained	11	11	8	8	1	1
Total number of samples	40					

In the study conducted with the approval of Bornova Veterinary Control Institute Local Ethics Committee 2021/453425.

DNA isolation: DNA isolation was performed according to the method of Shames et al. (1995). Briefly, each sample set was suspended in 10 ml of PBS and vortexed. The mixture was centrifuged for 10" at 6000 rpm and the supernatant was filtered through a 0.8 µm filter. The resulting filtrate was centrifuged at 13,500 rpm 5' and DNA extraction from the pellet was purified using the "QiagenStool Mini Kit" (QIAGEN Inc., Valencia, CA) according to the protocol.

PCR: It was performed as a genus-specific and species-specific multiplex. *H. pylori* ATCC 43504 strain obtained from Hacettepe University, Faculty of Medicine, Department of Clinical Microbiology was used for the genus-specific 16S ribosomal RNA gene positive control.

For *Helicobacter* spp., the sequences H276f: 5'-TATGACGGGTATCCGGC-3' and H676r: 5'-ATTCCACCTACCTCTCCCA-3', generated from the 16S ribosomal RNA gene (16S rRNA), were used (Riley et al., 1996; Beckwith et al., 1997). In multiplex PCR studies, species-specific 16S rRNA primer sequences listed in Table 2 were used for each *Helicobacter* species (Feng et al., 2005).

The PCR process was carried out in a total volume of 50 µl by mixing 25 µl of HotStarTaq Master mix (QIAGEN Inc., USA), 1 µl of template DNA, 1 µl of each primer sequence of 100 µM (200 µM for multiplex) and 22 µl of distilled water. Colon DNA concentration was used as (single 1.25 µg / ml, multiplex 5 µg / ml), and fecal DNA concentration was used as (single5 µg / ml, multiplex14 µg / ml) (Feng et al., 2005). Conditioning (45 cycles): heating at 94°C for 30", denaturation at 94°C for 2", annealing at 53°C for 2" and extension at 72°C for 30" was applied.

10 µl of 50 µl PCR products created in 0.2 mL tubes were taken with the help of a pipette and mixed with 3 µl of 6x loading dye solution. The entire mixture was taken and loaded into the well in the appropriate position in the 1% agarose gel, and it was run for 40 minutes for Helicobacter spp. and 60 minutes for multiplex PCR at 80V 500A. The gel was placed in the chamber of the transilluminator device connected to the computer. After photographing under UV light, band lengths were evaluated separately for each PCR.

Statistical analysis for sampling comparisons; Frequency analyzes and Chi-square test were applied to test significance between groups. Minitab 19 statistics program was used for this process. (Minitab, LLC. 2021)

The study was conducted in Bornova Veterinary Control Institute and Aydın Adnan Menderes University, Faculty of Veterinary Medicine Microbiology Laboratories.

Table 2. Primer sequences of species (Feng et al., 2004)

Tablo 2. Türlerin primer dizileri (Feng et al., 2004)

Species		Primer sequences	Lenght (bp)
H. rodentium	*1201f*	TTGTGAAATGGAGCAAATCTTAAAAACT	191
	1375r	TAGCCAGTTTGGCATTCC	
H. typhlonius	*163f.*	AGGGACTCTTAAATATGCTCCTAGAGT	122
	262r	ATTCATCGTGTTTGAATGCGTCAA	
H. bilis	*p17f*	ATGGAACAGATAAAGATTTTAAAGCAACTTCAG	435
	p17r	CTATGCAAGTTGTGCGTTAAGCAT	
H. hepaticus	*p25f*	ATGGGTAAGAAAATAGCAAAAAGATTGCAA	705
	p25 r	CTATTTCATATCCATAAGCTCTTGAGAATC	
H. muridarum	*p30f*	ATGACAAAAAAATATTCTTTCACAAAACTATTCATTGGT	807
	p30r	TTTATTTTAGATTCCATTTAACTGCTAAATCATCAATAGT	

f:forward; r:reverse.

Results

Mice Results

In the study conducted on the samples obtained; *Helicobacter* spp. was detected in mice in all except one facility. According to this result, the prevalence was determined as 90.91%. According to multiplex PCR results, the most common species was *H. rodentium* with a prevalence of 90.91%. In the study, in which *H. bilis* and *H. muridarum* species were not detected in any facility, it was determined that *H. typhlonius* was the second most common species with a prevalence rate of 72.73%, followed by *H. hepaticus* with a prevalence of 27.27% (Table 3).

According to the multiplex PCR results (Figure 1), it was determined that *H. rodentium* was present in all facilities where *Helicobacter* spp. was detected. It was determined that 2 of the facilities were infected with *H. hepaticus* in addition to *H. rodentium*, and 7 of them were mixed with *H. typhlonius* in addition to *H. rodentium*. It was determined that one facility was infected with more than two species. Considering these findings, the resulting rates are as seen in table 3. Accordingly, the rate of facilities with

mixed infections with two species was determined as 90%, and the rate of facilities with mixed infections with more than two species was determined as 10% (Table 4).

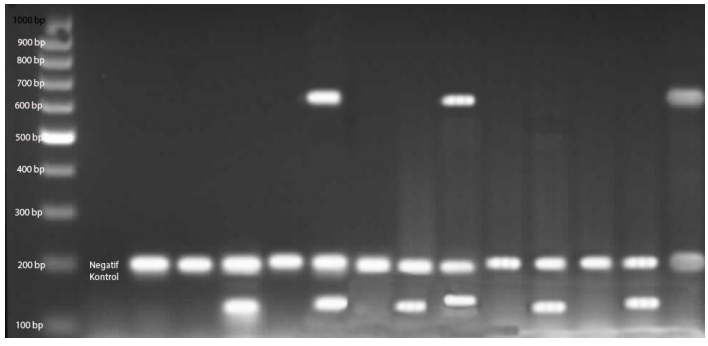

Figure 1. Multiplex PCR performed with bacterial DNA of three *Helicobacter* strains

Şekil 1. Üç *Helicobacter* suşunun bakteriyel DNA'sı ile gerçekleştirilen multipleks PCR

Rats Results

Helicobacter spp. was not detected in 1 of the 8 facilities where the study was conducted, and *H. rodentium* was found in all of the facilities where *Helicobacter* spp. was detected. According to this result, the prevalence was 87.5%. According to the results of Multiplex PCR (Figure 1) using the DNA of *Helicobacter* spp. positive samples, the most common species was *H. rodentium* with a prevalence of 87.5%. In the study, where *H. bilis*, *H. hepaticus* and *H. muridarum* species were not detected in any facility, *H. typhlonius* was the second common species with a prevalence rate of 12.5% (Table 3).

The number of facilities monoinfected with *H. rodentium* alone was determined as six. It was determined that one facility was mixed infected with *H. typhlonius* in addition to *H. rodentium*. There are no breeding facilities infected with more than two species in rat colonies (Table 4).

Gerbils Results

Helicobacter spp. was detected in the sample taken from the facility that kept gerbils. As a result of multiplex PCR for species-based discrimination using the DNA of the *Helicobacter* spp. positive sample obtained, only *H. rodentium* and *H. typhlonius* were detected in the colon samples.

Sampling Site Comparisons

When the results obtained from all animal species subject to the study are compared on the basis of sampling location (Feces - Colon); While it was observed that *H. hepaticus* was found at a higher rate in the colon samples with 11.11% versus 5.56%, the proportion of *H. typhlonius* was higher in the stool sample with 50 % versus 44.44 %. However, when these results were compared, it was determined that the difference between the two sample results was statistically insignificant (p>0,05), (Table 5).

Table 3. Helicobacter spp. in facilities and distribution rates by species

Tablo 3. Tesislerdeki Helicobacter spp. ve türlere göre dağılım oranları

	Positive %					
	Mice (n=110)		Rats (n=80)		Gerbils (n=10)	
Number of Facilities	11		8		1	
	Feces	Colon	Feces	Colon	Feces	Colon
Helicobacter spp.	90.91	90.91	87.50	87.50	0.00	100.00
H. rodentium	90.91	81.82	87.50	87.50	0.00	100.00
H. typhlonius	72.73	63.64	12.50	12.50	0.00	100.00
H. bilis	0.00	0.00	0.00	0.00	0.00	0.00
H. hepaticus	9.09	18.18	0.00	0.00	0.00	0.00
H. muridarum	0.00	0.00	0.00	0.00	0.00	0.00

Table 4. Mixed infection rates in facilities

Tablo 4. Tesislerdeki karışık enfeksiyon oranları

		Mice		Rats	
		Number of Facilities	Ratio%	Number of Facilities	Ratio%
Monoinfected		-	-	6	85.7
Polyinfected	Two species	9	90	1	14.3
	More than two species	1	10	-	-
Total		10	100	7	100

Table 5. Sampling site comparisons.

Tablo 5. Örnekleme alanı karşılaştırmaları.

		Helicobacter spp.	*H. rodentium*	*H. typhlonius*	*H. bilis*	*H. hepaticus*	*H. muridarum*
Fecal sample	Pozitive	18	17	9	0	1	0
	Negative	1	1	9	0	17	0
	Total	19	18	18	0	18	0
	Proportion %	94,70	94,44	50,00	0,00	5,56	0,00
Colon sample	Pozitive	18	17	8	0	2	0
	Negative	1	1	10	0	16	0
	Total	19	18	18	0	18	0
	Proportion %	94,70	94,44	44,44	0,00	11,11	0,00
	P value	-	-	0,49	-	0,54	-

Discussion

In clinical signs; The data we obtained regarding the clinical signs supports the data regarding the clinical disease reported by Solnick and Schauer (2001). Because these researchers emphasize that there are very few examples of *Helicobacter* species infection of an immunocompetent, natural host causing clinical disease. However, there are also study results in the literature showing that *H. typhlonius* and *H. hepaticus*, especially mixed infections, can cause clinical symptoms. These mentioned species and their mixed infection rates and findings are discussed below.

In the study conducted by Bohr et al. (2006), it was stated that 27 of a total of 40 mice strains, 37 inbred and 3 outbred, housed in an SPF facility, carried a single *Helicobacter* species, while 8 mice strains were infected by at least two different *Helicobacter* species. In the presented study, more than one species was detected in all samples taken from ten facilities. This data shows a proportionally higher rate than the results of the study conducted by Bohr et al. (2006). It is thought that the difference between the obtained rates may be due to the fact that the compared study is SPF. Another study on mixed infections was conducted by Taylor et al. (2007). The study, whose material consisted of samples taken from research institutes in the United States, Canada, Europe, Australia and Asia, showed that 6% of the institutes had at least three *Helicobacter* spp. showed that 29% were colonized with two *Helicobacter* species and 47% were colonized with a single *Helicobacter* species. In the presented study, the infection rate with more than two species, 10%, was similar to the value found in this study, but the infection rate with two species (90%) was higher to that of Taylor et al. (2007) reported.

It was determined that 8 of the 11 mice colonies and one of the 8 rat colonies where the study was conducted were mixed infected with *H. typhlonius*, which is stated to cause mucosal hyperplasia and related inflammation in the cecum and colon in immunodeficient mice, in addition to *H. rodentium*. It was observed that *H. typhlonius*, which recommended further research on naturally occurring gastrointestinal lesions in immunocompetent mice by Franklin et al. (2001), did not cause a clinical signs even in mixed infections.

It is stated that *H. rodentium* is not pathogenic in adult wild-type mice, but it increases IL-10 production in the cecum of *H. hepaticus*-infected mice (Franklin et al., 2001) and infections with more than one species can cause pathological lesions (Myles et al., 2004). While it is hypothesized that *H. rodentium* may be an acceptable contaminant in most conventional mouse colonies, it is emphasized that mixed infection with *H. rodentium* may potentiate disease caused by more pathogenic species such as *H. hepaticus* or *H. bilis* (Myles et al., 2003). In the present study, when it comes to the mouse colonies where sampling was done, no clinical findings were found in 3 facilities infected with *H. hepaticus* in addition to H. rodentium, and no notification regarding the clinical signs was received from the Veterinarians in charge of the facility. Therefore, our findings were incompatible with the literature data stating that mixed infections may cause the clinical signs. Additionally, Fox et al. (1994) showed that *H. hepaticus* caused the most severe clinical signs among all enterohepatic *Helicobacter* species, the prevalence of *H. hepaticus* in the mouse colonies examined in the presented study was found to be 27.27% and no clinical sign was encountered in these colonies.

Two different speculations can be developed as to the cause of these incompatibilities stated in the clinical signs. The first of these may be that the laboratory animals in the study have a highly developed immune system, and the second may be that the clinical sign developed by the mouse, which is a species that can reproduce relatively easily, is ignored as a result of focusing only on production in the facilities. In other words, it can be thought that the easy replacement of a colony that has lost its ability to reproduce eliminates the need for detailed clinical observation.

In our study, the prevalence of *Helicobacter* spp. of 90.91% in mice colonies was the highest prevalence rate found in studies on this subject. Because the highest prevalence obtained in studies on this subject in the literature was reported by Taylor et al. (2007) reported as 88%. A similar rate was reported by Bohr et al. (2006) as 87.5% in a study conducted in a facility. In a study conducted in a SPF facility by Jacoby and Lindsay (1998), and a *Helicobacter* prevalence of 10% was revealed. The first study on the prevalence of *Helicobacter* spp. reported the prevalence as 50% (Shames et al., 1995).

According to the results of the University of Missouri Animal Diagnostic and Research Laboratory for 1999-2000, the positivity rate for *Helicobacter* spp was reported as 10.54% (Franklin et al., 2001). In the study conducted by Pritchett-Corning et al. (2009), *Helicobacter* spp. was stated as the most common bacterial agent in mice with a rate of 16.08%.

When the rates obtained on a species basis were compared with previous similar studies; The 90.91% prevalence of *H. rodentium* was the highest in the literature. The rates of this species were 23.4% (Goto et al., 2000), 6% (Taylor et al., 2007), 8% (Wharry and Fox 2006), 15.11% (Franklin et al., 2001), 10% (Myles et al., 2003).

In another study conducted at the species level (Goto et al., 2000), the prevalence of *H. hepaticus* was 25.5% and the prevalence of *H. rodentium* was 23.4% on a colony basis, while the prevalence of *H. bilis*, for which no data could be obtained in the presented study, was 2.1%. was realized as. When these rates are compared with the rates obtained in the presented study, the prevalence of *H. hepaticus* was observed at a similar rate, while in *H. rodentium* it was quite high with a rate of 90.91%.

The first study conducted by Shames et al. (1995) on the prevalence of the *Helicobacter* genus was conducted only for the screening of *H. hepaticus*, and it was reported that *H. hepaticus* was detected in two of the four facilities in the study. This rate was higher than the 27.27% prevalence obtained in mice in the presented study. Another study reporting a higher prevalence rate than the *H. hepaticus* prevalence obtained in the study was conducted by Taylor et al. (2007). Among mice from 34 institutions surveyed in that study, *H. hepaticus* was the most frequently detected species, with a rate of 59%, alone or in combination with other *Helicobacter* species. The only study reported to be lower than the data obtained in the presented study was conducted by Bohr et al. (2006) with a *H. hepaticus* rate of 7.5%. According to the 2002-2003 data of the Swedish National Veterinary Institute, the prevalence of *H. hepaticus* was 42% (Johansson et al., 2006).

The prevalence of *H. typhlonius*, which was reported to be common in the fecal samples of research mice as well as in the sexual organs of three mouse species (Franklin et al., 2001; Scavizzi and Raspa; 2006), was determined to be 72.73% in the presented study. In the study conducted by Taylor et al. (2007), the rate of *H. typhlonius*, which was determined as the second most common species, was stated as 26%. Although this rate represents the highest rate among the literature data, it is significantly lower than the rate determined in the presented study. While a study conducted between 1999 and 2000 reported a rate of 4.88% for *H. typhlonius* (Franklin et al., 2001), it was reported that 17% of the fecal samples tested in the same laboratory in 2001 and 2002 were positive for *H. typhlonius* (Myles et al., 2003). In another study where a rate of 8% was determined for *Helicobacter* spp., the rate of *H. typhlonius* obtained in total samples was reported as 4% (Bohr et al., 2006).

It seems that survey studies conducted on rats are quite limited compared to those conducted on mice. When it comes to the prevalence of *Helicobacter* spp. in rats, lower rates are observed despite a wide range and relatively high prevalence, as in mice. In a study conducted with the species in question, the presence of *Helicobacter* spp. was reported to be 19% in rat cecum samples scanned using PCR (Wharry and Fox 2006). This rate is considerably lower than the rate obtained in the presented study, 87.5%. In the study, the rates obtained at the species level were *H. rodentium* with 87.5% prevalence and *H. typhlonius* with 12.5% prevalence. While these rates for *H. typhlonius* were similar to the 10% rate reported by Wharry and Fox (2006), the *H. rodentium* rate of 87.5% was found to be significantly higher than the 8% rate determined in the same study.

In a study conducted on rats by Goto et al. (2000), which included only 29.4% of *H. rodentium* positive samples, the colony prevalence was 30%. This rate was lower than the rate determined in the presented study. In the study, where a similar situation to rats was also valid for gerbils, the colony prevalence of *H. hepaticus*, which was positive at a rate of 78%, was 75%. There was a discrepancy between the data obtained in the presented study and the study data in which no genus or species-specific binding was observed except *H. hepaticus* in gerbils. Because in this study, while *H. rodentium* and *H. typhlonius* were among the species that could be detected in the gerbil colony, H. hepaticus was not detected.

When the results obtained from all animal species subject to the study are compared on the basis of sampling location (Feces - Colon); While it was observed that *H. hepaticus* was found at a higher rate in the colon samples with 11.11% versus 5.56%, the proportion of *H. typhlonius* was higher in the stool sample with 50 % versus 44.44 %. However, when these results were compared, it was determined that the difference between the two sample results was statistically insignificant p>0,05).

In a study that also included a comparison of sampling locations, it was reported by Cao et al., (2020) that fecal samples may be negative for *Helicobacter* in some cases where the intestines are positive, contrary to the finding we obtained in the presented study. Another study reporting results in favor of the colon between two sampling sites was Matos-Rodrigues et al. (2020). In this study, the *Helicobacter* spp. level was reported as 59.6% in fecal samples and 70.1% in colon samples. However, since the comparison in this study was not conducted on the same animals, the use of the given result for the purpose of evaluation in this direction will not be decisive. The conclusion made by Beckwith et al. (1997), in which the effect of colon and stool samples on the results was found to be insignificant, is parallel to the findings in the presented study. Another study in which a complete compatibility was reported between cultivating made with fecal filtrate and those made from cecum and colon scrapings was Shames et al. (1995).

In a study in which stomach, intestinal and hepatic tissue samples were evaluated to examine the distribution of *Helicobacter* spp. and the relationship of this distribution with the disease; *Helicobacter* DNA was detected at a rate of 85.7% in the fecal samples of 9 mice strains housed in 4 different facilities, and this rate was the highest rate compared to other tissues and samples (Nilsson et al. 2004). Although there was variability at the species level in the presented study, this variability was found to be statistically insignificant, confirming the suitability of fecal samples for screening and the results of Nilsson et al. (2004).

Conclusion and Recommendations

Considering the factors that require the animals to be in full health in order to obtain the closest results to reality from studies conducted on laboratory animals, and to ensure the reliability and reproducibility of the results during the experimental use phase, since the agent in question is quite common, it is recommended that experimental animal facilities should be monitored for this agent and studies should be detailed to create Helicobacter free facilities.

The data obtained in this study showed that the use of fecal samples did not have a significant effect on the results when compared to other samples to detect the agent. Therefore, it is thought that there is no harm in sampling using only feces in similar studies, as it is a more practical way.

Etik Komite Onayı: Etik kurul onayı Bornova Veteriner Kontrol Enstitüsü Yerel Etik Kurulu 'ndan (Tarih: 12.02.2021, Sayı: 453425) alınmıştır.
Yazar Katkıları: Fikir-N.İ.İ., G.E.; Tasarım- N.İ.İ., G.E.; Denetleme- N.İ.İ., G.E.; Kaynaklar- N.İ.İ., G.E. ; Veri Toplanması ve/veya İşlemesi- N.İ.İ., G.E. ; Analiz ve/ veya Yorum- N.İ.İ., G.E. ; Literatür Taraması- N.İ.İ., G.E. ; Yazıyı Yazan- N.İ.İ., G.E. ; Eleştirel İnceleme- N.İ.İ., G.E.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.
Finansal Destek: Bu araştırma Necdet İlker İÇİL'in Doktora tezinden özetlenmiş olup, Aydın Adnan Menderes Üniversitesi Bilimsel Araştırma Projeleri Birimi (Proje No: VTF21029) tarafından desteklenmiştir.

Ethics Committee Approval: Ethics committee approval was obtained from Bornova Veterinary Control Institute (Date: 12.02.2021, Number: 453425)
Author Contributions: Concept - N.İ.İ., G.E.; Design- N.İ.İ., G.E.; Supervision- N.İ.İ., G.E.; Resources- N.İ.İ., G.E.; Data Collection and/or Processing- N.İ.İ., G.E.; Analysis and/or Interpretation- N.İ.İ., G.E.; Literature Search- N.İ.İ., G.E.; Writing Manuscript- N.İ.İ., G.E.; Critical Review- N.İ.İ., G.E.
Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: This research was summarized from Necdet İlker İÇİL's PhD thesis and supported by Aydın Adnan Menderes University Scientific Research Projects Unit (Project No: VTF21029).

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