zoonotic diseases

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Brief Report

Molecular Detection of Human Pathogenic Gastric *Helicobacter* Species in Wild Rabbits (*Oryctolagus cuniculus*) and Wild Quails (*Coturnix coturnix*)

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Abstract: Wildlife plays a major role in the maintenance and transmission of multihost pathogens. Several *Helicobacter* spp. have been described to have zoonotic potential; thus, human, domestic and wild animal interactions deserve more attention. In this study, the presence of the DNA of human pathogenic gastric *Helicobacter* species was determined in gastric samples collected from wild rabbits and wild quails during the national hunting campaigns in Portugal. Eleven out of the 12 wild rabbits (91.7%) and all six wild quails tested (100%) were PCR positive for one or more gastric *Helicobacter* species. In both animal species, *H. felis*, *H. bizzozeronii* and *H. salomonis* DNA were detected. In addition to these non-*Helicobacter pylori Helicobacter* spp. (NHPH), *H. pylori* DNA was also identified in gizzard samples of wild quails. These findings might indicate that wild rabbits and wild quails may act as reservoirs and contribute to the *H. pylori* and NHPH environment dissemination, causing both Public Health and One health concerns to arise.

Keywords: one health; PCR; wildlife; gastric *Helicobacter*; zoonoses

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1. Introduction

Wildlife plays a major role in the maintenance and transmission of multihost pathogens and the understanding of the role of host species in the epidemiological cycle is essential to prevent diseases caused by zoonotic pathogens [1]. Although both wild and domestic animal reservoirs can be considered important sources of emerging infectious diseases, it is the human impact in the ecological systems that commands the level of risk at the humans/animals interface upon zoonotic disease emergences episodes [2]. In addition, the number of infectious diseases of zoonotic origin has been increasing, and approximately 72% are transmitted from wildlife [3,4].

Helicobacter species are Gram-negative, motile bacteria with a helical form that colonize the gastrointestinal tract of humans and a wide range of animal species [5–8]. Gastric

Helicobacter species have been studied over the years for their association with gastrointestinal diseases and zoonotic potential [9]. These bacteria have been widely described in humans, dogs and cats [8,10,11].

Helicobacter pylori (*H. pylori*) is the most prevalent gastric pathogen in humans, infecting over half the global population and causing gastritis, gastroduodenal ulcers, gastric adenocarcinoma and mucosa associated lymphoid tissue (MALT) lymphoma [5,12,13]. Gastric non-*Helicobacter pylori Helicobacter* species (NHPH) include a range of *Helicobacter* species previously described as *Helicobacter heilmannii* type 1 (*H. suis*) and *Helicobacter heilmannii* type 2 (*H. felis*, *H. bizzozeronii*, *H. salomonis*, *H. heilmannii* and *H. ailurogastricus*) [8,14].

In addition, gastric NHPH have been observed in 0.2–6% of human gastric biopsies and have been associated with a range of gastric pathologies, especially MALT lymphoma, as well as extra digestive diseases [8,15–17]. *H. suis* is the most common NHPH in humans suffering from gastric disorders, followed by *H. salomonis*, *H. felis*, *H. heilmannii* and *H. bizzozeronii* [5,8,15]. Humans may acquire these infections through contact with animals since most of NHPH are animal associated bacteria, but only some of them are recognized as potentially zoonotic [9,11,18].

There are two reports of *Helicobacter* species in rabbits. In these studies, gastric NHPH (*H. felis* and *H. salomonis*) DNA was detected in rabbit stomach samples [19,20]. Regarding birds, enterohepatic but not gastric *Helicobacter* species are commonly detected and mainly in intestinal or faecal samples [18,21–24].

Despite humans being considered the natural reservoir for *H. pylori*, this *Helicobacter* species has been detected in other domestic and wild animals [8,11,20,24–27]. Nevertheless, to the authors' knowledge, there is a lack of studies regarding the presence of gastric *Helicobacter* species in both wild rabbits and wild birds.

From an eco-epidemiological perspective, wild animals may have an important role in the spread of several pathogens [28–31].

Hunting dogs and humans can become infected with game pathogens through direct contact with fresh carcasses and the handling or consumption of raw or undercooked meat; thus, as a result, game wardens, hunters, butchers and other wildlife professionals are at high risk. Therefore, the aim of this study was to screen different regions of wild rabbit stomachs (fundus and corpus) and wild quail gizzards for the presence of DNA from *H. pylori* and zoonotically important NHPH, mainly associated with pigs, dogs and cats.

2. Materials and Methods

2.1. Animals and Samples Collection

Wild rabbits (*Oryctolagus cuniculus*) and wild quails (*Coturnix coturnix*), shot during three national hunting campaigns held in the centre of Portugal (Coimbra district), were subjected to convenience sampling. All the sampled animals were adults. Dissection of the stomach was performed using disposable scalpels and disposable tweezers. Representative gastric tissues from the fundus and corpus of wild rabbits and from the gizzard of wild quails were collected using a sterile disposable Kruuse® (Langeskov, Denmark) Biopsy punch 8 mm per sample, rendering cross-contamination between samples unlikely, and then they were stored at −20 °C for further DNA extraction.

The animals were not slaughtered or euthanized in for this investigation and the stomach specimens were obtained as sub-products of the usual meat inspection activity that takes place throughout these campaigns. None of the acts were taken exclusively for the purpose of research, and the researchers had no influence over the campaign's planning or meat inspection methods.

2.2. DNA Extraction and PCR Conditions and Sequencing

DNA was extracted from 8 mm of gastric frozen tissue samples, using the EXTRACTME® DNA tissue Kit (BLIRT S.A., Gdansk, Poland) and according to instructions provided by the supplier.

All the samples were tested for the presence *H. pylori*, *H. felis*, *H. salomonis*, *H. bizzozeronii*, *H. heilmannii*, *H. suis* and *H. ailurogastricus* DNA through conventional PCR analysis, according to previously described protocols (Table 1).

Table 1. Primer sequences used for detection of *H. pylori* and NHPH and thermo cycling conditions.

Helicobacter Species	Primer	Sequence	Target Gene	Amplicon Size	Thermal Cycle Conditions			*Helicobacter* Species Culture Used as Positive Control
					Nr. Cycles	Temp. (C)	Time	
H. suis	BFHsuis_F1	AAA ACA MAg gCg ATC gCC CTg TA	ureA	150 bp	40	95 60 72	20 s 30 s 30 s	HS1
	BFHsuis_R1	TTT CTT CgC CAg gTT CAA AgC g	ureA					
H. heilmannii	Hh-IceA-FWQ	gTT TCC AAC CAA AAg ACT CA	iceA	135 bp	30	94 55 72	30 s 30 s 30 s	ASB1.4
	Hh-IceA-RVQ	ATT gCC TAg Agg TTg TgT Tg	iceA					
H. ailurogastricus	Ha-LpsA-FWQ	CTT gAg TAC ggC gAT gTC AAT	lpsA	136 bp	30	94 55 72	30 s 30 s 30 s	ASB7.1
	Ha-LpsA-RVQ	ggg gAA AAA TgT gCT TgA AgT	lpsA					
H. salomonis	Hsal_FQ_PAR	CTC TTA TgA gTT ggA CTT ggT gCT CAC CAA T	ureAB	91 bp	45	94 61 72	30 s 30 s 1 min	R1051
	Hsal_RQ_PAR	TTT gCC ATC TTT AAT TCC AAT gTC ggC	ureAB					
H. felis	BFHfel_F2	gCT ggT ggC ATC gAT ACg CAT	ureAB	154 bp	45	94 60 72	30 s 30 s 1 min	CS1
	BFHfel_R2	TTT TTA gAT TAg CgC gTC Cgg gA	ureAB					
H. bizzozeronii	Hbizz_FQ_PAR	CCA ACA AAT CCC CAC AgC ATT TgC CAg	ureAB	91 bp	45	94 58 72	1 min 1 min 1 min	R1053
	Hbizz_RQ_PAR	AgT CCC ATC AgC Wgg WCC TgT TCC CCC AC	ureAB					
H. pylori	BFHpyl_F1	AAA gAg CgT ggT TTT CAT ggC g	ureAB	217 bp	45	94 59 72	30 s 30 s 1 min	26695
	BFHpyl_R1	ggg TTT TAC CgC CAC CgA ATT TAA	ureAB					

Aliquots of each PCR product were electrophoresed on a 1.5% agarose gel stained with Xpert Green Safe DNA gel stain (GRISP, Porto, Portugal) and examined under UV light for the presence of specific fragments. The size of the DNA fragment was compared to the standard molecular weight, the 100 bp DNA ladder (GRISP, Porto, Portugal) and the molecular weight of the positive controls (Table 1). For the negative control, distilled water was used. For the positive controls, DNA was extracted from pure cultures of each *Helicobacter* species tested (Table 1).

To exclude false positive samples, the amplicons of each positive sample were sequenced. Bidirectional sequencing was carried out using the Sanger method at the Genomics core facility of the University of Porto's Institute of Molecular Pathology and

Immunology. MegaX Molecular Evolutionary Genetic Analysis version 10.1.8 was used for sequence editing and multiple alignments. The sequences obtained were subject to the basic local alignment search tool (BLAST) using the non-redundant nucleotide database (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 7 December 2021).

3. Results and Discussion

A total of 30 gastric samples were evaluated: 24 were collected from 12 wild rabbits and consisted of equal amounts of fundus and corpus mucosa, and six were collected from six wild quails.

Eleven out of the 12 wild rabbits (91.7%) and the six (100%) wild quails were PCR positive for one or more gastric *Helicobacter* species.

3.1. Wild Rabbits

Among the 11 PCR positive animals, the most prevalent *Helicobacter* was *H. salomonis* (9/11), followed by *H. bizzozeronii* (5/11), and then *H. felis* (2/11) (Table 2).

Table 2. Number and percentage of positive PCR results for gastric *Helicobacter* species in wild rabbits.

	Wild Rabbits (*n* = 12)	**Fundus (*n* = 12)**	**Corpus (*n* = 12)**
H. salomonis	9 (75.0%)	8 (66.7%)	8 (66.7%)
H. bizzozeronii	5 (41.7%)	1 (8.3%)	5 (41.7%)
H. felis	2 (16.7%)	1 (8.3%)	1 (8.3%)

Concerning the wild rabbit specimens, and regardless of the stomach counterpart: four animals were *H. salomonis*-positive, one was *H. felis*-positive, another was *H. bizzozeroni*-positive, four were *H. bizzozeronii* plus *H. salomonis*-positive and one was *H. felis* plus *H. salomonis*-positive (Table A1).

According to the gastric location, nine samples of each fundus and corpus location were PCR positive for at least one *Helicobacter* species (Table 2).

The bidirectional sequencing and BLAST analysis of consensus sequences obtained showed a homology of 97% to 100% with the respective species (Table A1).

Co-infections, i.e., the presence of more than one *Helicobacter* species, were detected in 41.7% of wild rabbits; the most frequent bacterial association was *H. salomonis* plus *H. bizzozeronii* (33.3%) and *H. salomonis* plus *H. felis* (8.3%). Regarding the *Helicobacter* species identified, these results are in agreement with those previously described in pets, as well as industrial and laboratory rabbits, which reported *H. felis*, *H. salomonis* and *H. bizzozeronii* DNA in the gastric *corpus* [19,20], but are here reported for the first time in free range wild rabbits.

3.2. Wild Quails

Of the six PCR positive animals, the most frequent species was *H. salomonis* (6/6), followed by *H. bizzozeronii* (2/6), and then *H. felis* (1/6) and *H. pylori* (1/6) (Table 3).

Concerning the wild quails: three were *H. salomonis*-positive, one was *H. salomonis* plus *H. bizzozeronii*-positive, one was *H. salomonis* plus *H. pylori*-positive and another was *H. salomonis* plus *H. felis* plus *H. bizzozeronii*-positive (Table A1).

The bidirectional sequencing and BLAST analysis of the consensus sequences obtained showed a homology of 97% to 100% with the respective species (Table A1).

Table 3. Number and percentage of positive PCR results for gastric *Helicobacter* species in wild quails.

	Wild Quails (*n* = 6) Gizzard
H. salomonis	6 (100%)
H. bizzozeronii	2 (33.3%)
H. felis	1 (16.7%)
H. pylori	1 (16.7%)

Co-infections were detected in 50.0% of the animals and different bacteria associations were found: *H. salomonis* plus *H. bizzozeronii* (16.7%), *H. salomonis* plus *H. pylori* (16.7%) and *H. salomonis plus H. felis* plus *H. bizzozeronii* (16.7%). These are novel results and constitute new findings that report the presence of different gastric *Helicobacter* species DNA in birds.

Previous studies reported the detection of enterohepatic *Helicobacter* in wild birds' faecal samples using PCR [21–23].

To the authors knowledge, this is the first time that gastric NHPH is detected in the fundus/corpus of free range wild rabbits and NHPH and *H. pylori* DNA in the gizzard of wild quails. This might indicate that wild rabbits and wild quails may act as reservoirs and contribute to the *H. pylori* and NHPH environment dissemination, causing both Public Health and *One health* concerns to arise. DNA extraction of the wild rabbit samples and the wild quail samples was performed on different days. Disposable sterile scalpels and tweezers were used to open the stomachs and, for each sample collection, a sterile disposable Kruuse® Biopsy punch was used, rendering cross-contamination between samples unlikely. It can, however, not be excluded that the presence of *Helicobacter* DNA in the stomach from some of the animals tested is a consequence of recent contamination from the environment, as described by [32]. Future studies using a larger sample size and including histopathological analysis of gastric tissues is, therefore, necessary to confirm these findings and to associate the presence of *Helicobacter* spp. with possible gastric alterations.

Wild animals, including leporids and birds, are known to be reservoirs for several infectious and transmissible diseases [30,33], raising concerns regarding zoonotic pathogens cross-species spillover [34]. The close contact of wild animals with domestic animals and humans is continuously increasing due to different factors, which can drive the zoonotic spillovers to become more common [29,34] and directly affect animal and human health [30,31,33,34].

Hunting dogs and humans can be exposed to game pathogens through direct contact with fresh carcasses, the handling or consumption of raw or undercooked meat or indirect contact through contaminated water or the environment; thus, game wardens, hunters, butchers and other wildlife professional duties are at high risk.

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Appendix A

Table A1. Wild rabbits and wild quails PCR positive results collection and sequencing data according to sample anatomical collection.

Animals (*n* = 18) Samples (*n* = 30)	***H. bizzozeronii***	***Accession nr./ Homology ****	***H. felis***	***Accession nr./ Homology***	***H. salomonis***	***Accession nr./ Homology***	***H. pylori***	***Accession nr./ Homology***	***H.suis***	***Accession nr./ Homology***	***H. ailuro-gastricus***	***Accession nr./ Homology***	***H. heil-mannii***	***Accession nr./ Homology***
WR1	Fundus	-		-		-		-		-		-		-
	Corpus	-		-		-		-		-		-		-
WR2	Fundus	-		+	FQ670179 99.35%	-		-		-		-		-
	Corpus	-		-		-		-		-		-		-
WR3	Fundus	-		-		-		-		-		-		-
	Corpus	+	FR871757 99.42%	-		-		-		-		-		-
WR4	Fundus	-		-		+	AJ130882 100%	-		-		-		-
	Corpus	-		-		+	AJ130882 100%	-		-		-		-
WR5	Fundus	-		-		+	AJ130882 100%	-		-		-		-
	Corpus	+	FR871757 100%			+	AJ130882 100%	-		-		-		-
WR6	Fundus	-		-		-		-		-		-		-
	Corpus	+	FR871757 95.08%	-		+	AJ130882 100%	-		-		-		-
WR7	Fundus	-		-		+	AJ130882100%	-		-		-		-
	Corpus	-		-		+	AJ130882 100%	-		-		-		-
WR8	Fundus	+	AF508003 97.11%	-		+	AJ130882 100%	-		-		-		-
	Corpus	+	FR871757 100%	-		+	AJ130882 100%	-		-		-		-
WR9	Fundus	-		-		+	AJ130882 100%	-		-		-		-
	Corpus	-		-		+	AJ130882 100%	-		-		-		-
WR10	Fundus	-		-		+	AJ130882 100%	-		-		-		-
	Corpus	-		+	JQ736360 100%	+	AJ130882 100%	-		-		-		-
WR11	Fundus	-		-		+	AJ130882 100%	-		-		-		-
	Corpus	+	AF508003 100%	-		+	AJ130882 100%	-		-		-		-

Table A1. *Cont.*

Animals (*n* = 18) Samples (*n* = 30)	***H. bizzozeronii***	***Accession nr./ Homology ****	***H. felis***	***Accession nr./ Homology***	***H. salomonis***	***Accession nr./ Homology***	***H. pylori***	***Accession nr./ Homology***	***H.suis***	***Accession nr./ Homology***	***H. ailuro-gastricus***	***Accession nr./ Homology***	***H. heil-mannii***	***Accession nr./ Homology***
WR12	Fundus	-		-		+	AJ130882 100%	-		-		-		-
	Corpus	-		-		-		-		-		-		-
Q1	Gizzard	-		-		+	AJ130882 100%	-		-		-		
Q2	Gizzard	-		-		+	AJ130882 100%	-		-		-		
Q3	Gizzard	-		-		+	AJ130882 100%	-		-		-		
Q4	Gizzard	+	FR871757 100%	-		+	AJ130882 100%	-		-		-		
Q5	Gizzard	+	FR871757 100%	+	AF116580 97.12%	+	AJ130882 100%	-		-		-		
Q6	Gizzard	-		-		+	AJ130882 100%	+	CP024947 100%	-		-		

References

1. González-Barrio, D.; Maio, E.; Vieira-Pinto, M.; Ruiz-Fons, F. European Rabbits as Reservoir for *Coxiella burn*. *Infect. Dis.* **2015**, *21*, 1055–1058. [CrossRef]
2. Mégraud, F.; Broutet, N. Review article: Have we found the source of *Helicobacter pylori*? *Aliment. Pharmacol. Ther.* **2000**, *14* (Suppl. 3), 7–12. [CrossRef]
3. Jones, K.E.; Patel, N.G.; Levy, M.A.; Storeygard, A.; Balk, D.; Gittleman, J.L.; Daszak, P. Global trends in emerging infectious diseases. *Nature* **2008**, *451*, 990–993. [CrossRef] [PubMed]
4. Vorou, R.M.; Papavassiliou, V.G.; Tsiodras, S. Emerging zoonoses and vector-borne infections affecting humans in Europe. *Epidemiol. Infect.* **2007**, *135*, 1231–1247. [CrossRef] [PubMed]
5. Ali, B.; Chloe, D.W.; Mehmet, A.; Sofie, D.B.; Annemieke, S.; Gokhan, T.; Tülin, G.G.; Freddy, H.; Fatih, K. Presence of gastric *Helicobacter* species in children suffering from gastric disorders in Southern Turkey. *Helicobacter* **2018**, *23*, e12511. [CrossRef]
6. Amorim, I.; Smet, A.; Alves, O.; Teixeira, S.; Saraiva, A.L.; Taulescu, M.; Reis, C.; Haesebrouck, F.; Gärtner, F. Presence and significance of *Helicobacter* spp. in the gastric mucosa of Portuguese dogs. *Gut Pathog.* **2015**, *7*, 12. [CrossRef]
7. Choi, Y.K.; Han, J.H.; Joo, H.S. Identification of novel *Helicobacter* species in pig stomachs by PCR and partial sequencing. *J. Clin. Microbiol.* **2001**, *39*, 3311–3315. [CrossRef]
8. Haesebrouck, F.; Pasmans, F.; Flahou, B.; Chiers, K.; Baele, M.; Meyns, T.; Decostere, A.; Ducatelle, R. Gastric helicobacters in domestic animals and nonhuman primates and their significance for human health. *Clin. Microbiol. Rev.* **2009**, *22*, 202–223. [CrossRef] [PubMed]
9. Mladenova-Hristova, I.; Grekova, O.; Patel, A. Zoonotic potential of *Helicobacter* spp. *J. Microbiol. Immunol. Infect.* **2017**, *50*, 265–269. [CrossRef]
10. De Groote, D.; Van Doorn, L.-J.; Van den Bulck, K.; Vandamme, P.; Vieth, M.; Stolte, M.; Debongnie, J.C.; Burette, A.; Haesebrouck, F.; Ducatelle, R. Detection of non-pylori *Helicobacter* species in "*Helicobacter heilmannii*"-infected humans. *Helicobacter* **2005**, *10*, 398–406. [CrossRef]
11. Flahou, B.; Haesebrouck, F.; Smet, A. Non-*Helicobacter pylori* Helicobacter Infections in Humans and Animals. In *Helicobacter pylori Research*; Springer: Tokyo, Japan, 2016; pp. 233–269. [CrossRef]
12. Guevara, B.; Cogdill, A.G. *Helicobacter pylori*: A Review of Current Diagnostic and Management Strategies. *Dig. Dis. Sci.* **2020**, *65*, 1917–1931. [CrossRef] [PubMed]
13. Lopo, I.; Libânio, D.; Pita, I.; Dinis-Ribeiro, M.; Pimentel-Nunes, P. *Helicobacter pylori* antibiotic resistance in Portugal: Systematic review and meta-analysis. *Helicobacter* **2018**, *23*, e12493. [CrossRef]
14. Joosten, M.; Lindén, S.; Rossi, M.; Tay, A.C.Y.; Skoog, E.; Padra, M.; Peters, F.; Perkins, T.; Vandamme, P.; Van Nieuwerburgh, F.; et al. Divergence between the Highly Virulent Zoonotic Pathogen *Helicobacter heilmannii* and Its Closest Relative, the Low-Virulence "*Helicobacter ailurogastricus*" sp. nov. *Infect. Immun.* **2015**, *84*, 293–306. [CrossRef]
15. Baele, M.; Pasmans, F.; Flahou, B.; Chiers, K.; Ducatelle, R.; Haesebrouck, F. Non-*Helicobacter pylori* helicobacters detected in the stomach of humans comprise several naturally occurring *Helicobacter* species in animals. *FEMS Immunol. Med. Microbiol.* **2009**, *55*, 306–313. [CrossRef] [PubMed]
16. Gorlé, N.; Bauwens, E.; Haesebrouck, F.; Smet, A.; Vandenbroucke, R.E. *Helicobacter* and the Potential Role in Neurological Disorders: There Is More Than *Helicobacter pylori*. *Front. Immunol.* **2021**, *11*, 584165. [CrossRef]
17. Ménard, A.; Smet, A. Review: Other Helicobacter species. *Helicobacter* **2019**, *24* (Suppl. 1), e12645. [CrossRef] [PubMed]
18. Ménard, A.; Péré-Védrenne, C.; Haesebrouck, F.; Flahou, B. Gastric and Enterohepatic Helicobacters other than *Helicobacter pylori*. *Helicobacter* **2014**, *19*, 59–67. [CrossRef]
19. Van den Bulck, K.; Baele, M.; Hermans, K.; Ducatelle, R.; Haesebrouck, F.; Decostere, A. First report on the occurrence of "Helicobacter heilmannii" in the stomach of rabbits. *Vet. Res. Commun.* **2005**, *29*, 271–279. [CrossRef] [PubMed]
20. Van den Bulck, K.; Decostere, A.; Baele, M.; Marechal, M.; Ducatelle, R.; Haesebrouck, F. Low frequency of *Helicobacter* species in the stomachs of experimental rabbits. *Lab. Anim.* **2006**, *40*, 282–287. [CrossRef] [PubMed]
21. García-Amado, M.A.; Sanz, V.; Martinez, L.M.; Contreras, M.; Lentino, M.; Michelangeli, F. Low occurrence of *Helicobacter* DNA in tropical wild birds, Venezuela. *J. Wildl. Dis.* **2013**, *49*, 991–995. [CrossRef] [PubMed]
22. Nebbia, P.; Tramuta, C.; Ortoffi, M.; Bert, E.; Cerruti Sola, S.; Robino, P. Identification of enteric *Helicobacter* in avian species. *Schweiz. Arch. Fur Tierheilkd.* **2007**, *149*, 403–407. [CrossRef]
23. Robino, P.; Tomassone, L.; Tramuta, C.; Rodo, M.; Giammarino, M.; Vaschetti, G.; Nebbia, P. Prevalence of *Campylobacter jejuni*, *Campylobacter coli* and enteric *Helicobacter* in domestic and free living birds in North-Western Italy. *Schweiz. Arch. Tierheilkd.* **2010**, *152*, 425–431. [CrossRef] [PubMed]
24. Solnick, J.V.; Schauer, D.B. Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. *Clin. Microbiol. Rev.* **2001**, *14*, 59–97. [CrossRef] [PubMed]
25. Cortez Nunes, F.; Letra Mateus, T.; Teixeira, S.; Barradas, P.; de Witte, C.; Haesebrouck, F.; Amorim, I.; Gärtner, F. Presence of *Helicobacter pylori* and *H. suis* DNA in Free-Range Wild Boars. *Animals* **2021**, *11*, 1269. [CrossRef]
26. Van Den Bulck Kathleen Decostere, A.; Baele, M.; Driessen, A.; Debongnie, J.; Burette, A.; Stole, M.; Ducatelle, R.; Haesebrouck, F. Identification of Non- Helicobacter pylori Spiral Organisms in Gastric Samples from Humans, Dogs, and Cats Identification of Non- *Helicobacter pylori* Spiral Organisms in Gastric Samples from Humans, Dogs, and Cats. *J. Clin. Microbiol.* **2005**, *43*, 2256–2260. [CrossRef]

27. Whary, M.T.; Fox, J.G. Natural and experimental *Helicobacter* infections. *Comp. Med.* **2004**, *54*, 128–158. [PubMed]
28. Cunningham, A.A.; Daszak, P.; Wood JL, N. One Health, emerging infectious diseases and wildlife: Two decades of progress? *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **2017**, *372*, 20160167. [CrossRef] [PubMed]
29. Gortázar, C.; Ferroglio, E.; Höfle, U.; Frölich, K.; Vicente, J. Diseases shared between wildlife and livestock: A European perspective. *Eur. J. Wildl. Res.* **2007**, *53*, 241. [CrossRef]
30. Siembieda, J.L.; Kock, R.A.; McCracken, T.A.; Newman, S.H. The role of wildlife in transboundary animal diseases. *Anim. Health Res. Rev.* **2011**, *12*, 95–111. [CrossRef]
31. Sleeman, J.M.; Richgels KL, D.; White, C.L.; Stephen, C. Integration of wildlife and environmental health into a One Health approach. *Rev. Sci. Et Tech.* **2019**, *38*, 91–102. [CrossRef]
32. Hamada, M.; Elbehiry, A.; Marzouk, E.; Moussa, I.M.; Hessain, A.M.; Alhaji, J.H.; Heme, H.A.; Zahran, R.; Abdeen, E. *Helicobacter pylori* in a poultry slaughterhouse: Prevalence, genotyping and antibiotic resistance pattern. *Saudi J. Biol. Sci.* **2018**, *25*, 1072–1078. [CrossRef]
33. Kruse, H.; Kirkemo, A.-M.; Handeland, K. Wildlife as source of zoonotic infections. *Emerg. Infect. Dis.* **2004**, *10*, 2067–2072. [CrossRef] [PubMed]
34. Plowright, R.K.; Parrish, C.R.; McCallum, H.; Hudson, P.J.; Ko, A.I.; Graham, A.L.; Lloyd-Smith, J.O. Pathways to zoonotic spillover. *Nat. Rev. Microbiol.* **2017**, *15*, 502–510. [CrossRef]