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Prevalence of *Campylobacter jejuni, Campylobacter coli* and enteric *Helicobacter* in domestic and free living birds in North-Western Italy

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Summary -In order to investigate the prevalence of some thermophilic *Campylobacter* (*C. jejuni* and *C. coli*) and enteric *Helicobacter* (*H. pullorum and H. canadensis)* in domestic and wild birds, a total of 278 bird caecal samples were analyzed over a 2 year period in North-Western Italy. Samples were collected from poultry raised in intensive farming at the slaughterhouse (n=102, group A) and in small scale rural farms (n=60, group B) as well as from wild birds (n=116, group C). PCR amplifications were carried out on DNA extracted from caecal samples. Molecular assays targeted the *hipO* gene for *C. jejuni*, the *asp* gene for *C. coli* and the 16S rRNA gene of *H. pullorum/H. canadensis*. To differentiate *H. pullorum* from *H. canadensis*, PCR products were subjected to an *Apa*LI digestion assay. Prevalence of thermophilic *Campylobacter* and enteric *Helicobacter* was significantly different among groups ($p<0.0001$). *Campylobacter* infections were detected in all three bird groups (78.4% group A, 18.3% group B and 38.8% group C, respectively), *Helicobacter*

infections were only detected in poultry, with *H. pullorum* infecting 68.6% of group A and 21.7% of group B birds. *H. canadensis* was detected in Guinea fowls (group A) and for the first time in pheasants (group B). Mixed infections by enteric *Campylobacter* and *Helicobacter* were shown in 53.9% of group A and in 5.0% of group B. Our results show that both microorganisms commonly infect poultry, especially intensive farming animals. Only hooded crows among the wild bird group (group C), proved to be highly sensitive to *Campylobacter* infection.

Keywords: *Campylobacter*, *Helicobacter*, poultry, wild birds

Introduction

The Epsilonbacteria class consists of a distinct phylogenetic group within the Proteobacteria, and includes two closely related genera: *Campylobacter* and *Helicobacter* (On, 2001). These bacteria are known as human and⁄or animal pathogens, and some species are also considered to be zoonotic agents. In the last few years, interest in thermophilic *Campylobacter* and enteric *Helicobacter* has grown. In particular, *C. jejuni* and *C. coli* are among the most frequently identified bacterial causes of human gastroenteritis in industrialized countries (Westrell et al., 2009). *H. pullorum* was isolated from patients with enteritis, hepatic disease and septicaemia (Stanley et al., 1994; Tee et al., 2001) while *H. canadensis*, originally misdiagnosed as *H. pullorum*, is one of the new enteropathogens isolated from humans (Fox et al., 2000; Solnick and Schauer, 2001). Wild birds, notably a natural reservoir of enteric bacteria, are frequently mentioned as a possible source of infection for both humans and farm animals (Waldenström et al., 2002; 2003; Colles et al., 2008) due to faecal contamination of drinking water sources and agricultural crops (Jones, 2001; Azevedo et al., 2008).

Campylobacter and *Helicobacter* species are fastidious bacteria. Moreover, traditional isolation methods used in their identification are complex and do not allow growth of some species. Interpretation of phenotypic test results, based on biochemical profiling, can be problematic due to inconsistencies in phenotype within species (On, 1996). Several methodological approaches are available for performing *Campylobacter* species typing and subspecies from isolate strains, while only few reports have described the direct application of polymerase chain reaction (PCR) on stools obtained from human and animal subjects (Houng et al., 2001; LaGier et al., 2004; Al Amri et al., 2007).

Likewise, analysis of enteric *Helicobacter* is complex. Species-specific PCR methods are used for the detection of individual species. These techniques are not always able to distinguish between closely related species such as *H. pullorum* and *H. canadensis.*

The aim of our study was to investigate by direct molecular identification the occurrence of infections by some thermophilic *Campylobacter* (*C. jejuni* and *C. coli*) and enteric *Helicobacter* (*H. pullorum and H. canadensis)* in domestic and free living birds. Among wild birds, we investigated the hooded crow (*Corvus corone cornix*), an omnivorous species that is considered responsible for the transmission of zoonotic agents between rural and wild areas (Cooper, 2007).

Animals, Material and Methods

Sample collection

Field work was conducted during a 2 year period, from April 2006 to April 2008, in North-Western Italy. A total of 278 bird caeca were analyzed.

Overall, 102 samples were collected in a slaughterhouse (group A) and originated from four categories of poultry: 33 broiler chickens, 30 growing cockerels, 14 laying hens (*Gallus gallus* var. *domestica*) and 25 Guinea fowls (*Numida meleagris*). These animals belonged to seven Italian flocks in intensive farming, except for 16 Guinea fowls of French origin, and had previously been screened for *Helicobacter* detection (Nebbia et al., 2007). Birds from four small scale rural farms were also examined (group B; n=60). This sample was made up of broiler chickens (*G. gallus var. domestica*; n=37), domestic pigeons (*Columba livia* var. *domestica*; n=14) and pheasants (*Phasianus colchicus*; n=9). Caeca of domestic birds (groups A and B) were collected immediately after evisceration, individually packed in plastic bags and rapidly delivered to our laboratory for individual testing.

Finally, 116 caeca were collected from wild birds (group C). Most of the samples were obtained from hooded crows (*Corvus corone cornix*; n=78), captured by Larsen traps within a local control plan of pests (DL 333/1998) and killed immediately before evisceration. Carcasses of other wild birds included mallards (*Anas platyrhynchos*; n=10), house-sparrows (*Passer domesticus italiae*; n=37) and feral pigeons (*C. livia var. domestica*; n=13) which had been found dead. They were later dissected in the laboratory to collect caeca.

Approximately 25 mg of the mucosal layer of caeca from every sampled animal was stored in sterile vials at −20 °C until DNA extraction.

DNA extraction

Genomic DNA was extracted from caeca using a commercial kit (DNeasy Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Each enteric tissue extract was amplified with universal primers for the 16S rRNA gene to demonstrate the presence of amplifiable DNA (Gramley et al., 1999). All samples were positive and are included in this study.

Identification of thermophilic *Campylobacter*

Two genes were amplified for the detection of *C. jejuni* and *C. coli* directly from animal tissues, the *hipO* for *C. jejuni* (hippuricase gene, 735 bp fragment) and the *asp* for *C. coli* (aspartokinase gene, 500 bp). These genes were both described by Linton et al. (1997) and used in the multiplex PCR by Al Amri et al. (2007) on stools. Briefly, PCR amplification was carried out on a final volume of 50 µl, consisting of 25 µl multiplex master mix (Qiagen, Hilden, Germany), 0.3 µl *asp* primer (0.18 µM), 1 µl *hipO* primer (2 µM) (Sigma Genosys), 4.5 µl eluted DNA and sterile water. The PCR conditions were as follows: an initial denaturation at 95 °C for 15 min, followed by 30 cycles consisting of denaturation 94 °C (1 min), annealing at 49 °C (1 min), extension at 72 °C (1 min), and final extension at 72 °C for 7 min. The amplified products were separated by electrophoresis in 2% agarose gel (Sigma Chemical Co, St. Louis, MO), stained with ethidium bromide solution (1 µg/ml) and observed at UV-trans-illuminator (Geldoc 2000, Biorad, Milano, Italy). Product size was checked by utilizing a molecular weight DNA marker (100 bp ladder, Fermentas, Glen Burnie, MD).

Identification of enteric *Helicobacter*

To identify *H. pullorum*, a fragment of 447 bp in the 16S rRNA gene was amplified by PCR according to Stanley et al. (1994), and PCR products were analyzed by electrophoresis, as described above. In order to differentiate *H. pullorum* from *H. canadensis*, PCR products were purified with the QiAquick PCR purification Kit (Qiagen) and subjected to an *Apa*LI (Fermentas) digestion assay derived by Fox et al. (2000), with 10 U of enzyme in the appropriate buffer at 37 °C for 2 hours. *H. canadensis* was identified thanks to an *ApaLI* site at position 1040 of the 16S rRNA gene, which is absent in *H. pullorum*. In this amplified fragment, the *ApaLI* site allows the digestion into two fragments of 409 bp and 38 bp. Digestion reactions were assessed by electrophoresis in 2.5% agarose gel (Certified^{TM} Low Range Ultra Agarose, Bio-rad Laboratories) and stained with ethidium bromide.

In each bird group, infection prevalence of bacteria was calculated with 95% confidence intervals. Fisher Exact Test was used to study the association among categorical variables, a two tailed significance level $\alpha = 0.05$ was adopted. Data were analyzed by SAS software (SAS Institute, 1999).

Results

Campylobacter was detected in the three bird groups, while *Helicobacter* was not infecting wild birds. Indeed, *C. jejuni* and *C. coli* were detected in 78.4% group A animals, 18.3% group B and 38.8% group C, while H*elicobacter* was detected in 68.6% group A and 21.7% group B birds. The infection prevalence was significantly different among groups for both bacteria ($p<0.0001$).

Regarding intensive farming birds (group A, Tab. 1), mixed infections by *C. coli* and *C. jejuni* were particularly abundant in *G. gallus* species, which showed no mono-infections by *C. coli*. These birds

were also positive to *H. pullorum*, but not to *H. canadensis.* On the other hand, *H. canadensis* was detected in 52.0% of Guinea fowls, all of French origin. These animals were also significantly more infected by *C. coli* than the Italian Guinea fowls ($p<0.01$; data not shown). Mixed infection by *Campylobacter* and *Helicobacter* spp. was detected in 53.9% of group A birds.

In small scale rural farms (group B, Tab. 2), pigeons were not found to be infected by the investigated bacteria. Pheasants showed co-infections by *C. jejuni*/*C. coli* (44.4%), while broiler chickens had a low prevalence of *Campylobacter* (18.9%), with both mixed and mono-infections. *H. pullorum* was detected in pheasants and broilers. Pheasants also showed infection by *H. canadensis* (22.2%) and mixed infection by *Campylobacter* and *Helicobacter* (5.0%).

Campylobacter prevalence was significantly different ($p<0.0001$) among wild bird species (group C, Tab. 3). In fact, 53.8% of hooded crows was positive, while only one mallard and three feral pigeons were found infected. Moreover, only crows presented mixed *C. jejuni/C. coli* infections.

Discussion

Our results show that *Campylobacter* and *Helicobacter* are commonly observed in poultry species in North-Western Italy, especially in animals reared in intensive farming. *Campylobacter* prevalence in group A birds was approximately at the same level as reported in Italy by EFSA (82.8%; Westrell et al., 2009), while it was consistently lower in our family rural flocks (group B; 18.3%). *Helicobacter* prevalence was also high in intensive farming flocks, in accordance with other studies in Europe. In particular, a prevalence of *H. pullorum* of 100% was observed in Italy (Zanoni et al., 2007), 60% in United Kingdom (Atabay et al., 1998), 33.6% in Belgium (Ceelen et al., 2006). These data confirm that intensive farming may encourage the spreading of germs and oro-fecal transmission, due to unfavourable factors such as high animal density, stress, drug therapy and unvaried diet.

For small scale rural poultry farming, domestic pigeons were negative to both *Campylobacter* and *Helicobacter.* This result could be due to the small sample size, however, it is confirmed by other surveys on *Campylobacter*, that show a low risk of infection for consumers. Indeed, *C. jejuni* was detected in 3.44% meat samples and 5.26% pigeon neck skin in Italy (Soncini et al., 2006), 13% intestinal contents in Japan (Ito et al., 1988), while no positive samples were observed in pigeon carcasses from retail markets in Egypt (Abd el Aziz et al., 2002).

Pheasants were found to be infected by both microorganisms. In previous studies in Italy, no *Campylobacter* spp. was detected in pheasants neck skins (Soncini et al., 2006), while a prevalence of 43.3% was reported in living birds, with a higher prevalence of *C. coli* than *C. jejuni* (Dipineto et al., 2008). In both Germany (Atanassova and Ring, 1999) and Russia (Stern et al., 2004) the prevalence was 26%, while in the Czech Republic it was 70.2% (Nebola et al., 2007). Since

pheasants are frequently used for repopulation of protected areas and game reserves, this avian species may be considered a potential source of *Campylobacter* spp. for humans and other animals. No reports on the presence of *H. pullorum* and *H. canadensis* in pheasants are available.

We identified *H. canadensis* in domestic birds, in particular poultry. These results are very interesting owing to the few studies on this enteric species whose epidemiology and zoonotic potential are scarcely known. Nonetheless, it is evident that *H. canadensis* is distributed in nature, since it was reported in humans in Canada (Fox et al., 2000) and Australia (Tee et al., 2001), wild geese in Sweden (Waldenström et al., 2003), swines in Denmark and The Netherlands (Inglis et al., 2006), Guinea fowls (Nebbia et al., 2007) and pheasants (this study) in Italy.

As far as wild birds are concerned, *Campylobacter* prevalence was high, even though the majority of positive samples is represented by hooded crows. *Campylobacter* prevalence is highly influenced by feeding habits and it was found to be high in opportunistic feeders and in most ground-foraging guilds (Ito et al., 1988; Waldenström et al., 2002). Crows are omnivorous scavengers of garbage on refuse dumps. Indeed we collected them near human residences and farms, thus their role as possible reservoirs of *Campylobacter* infection for humans and domestic animals cannot be excluded. Thermophilic *Campylobacter* in crows were reported in 89.8% of samples in Norway (Kapperud and Rosef, 1983), 62.6% in Japan (Maruyama et al., 1990), and 17% in Italy (Ferrazzi et al., 2007). Besides the small sample size, our results highlight the presence of *Campylobacter* infection in mallards and feral pigeons. Sparrows proved to be negative for *Campylobacter* DNA, a result that is in contrast with other studies (Chuma et al., 2000; Rodrigues et al., 1998).

In our study, no wild birds were positive to *Helicobacter*. In the literature there is a paucity of data on the occurrence of *Helicobacter* in wild birds. This genus was isolated for the first time in 1994 from house sparrows, gulls and terns in an estuarine environment in Massachusetts, USA (Seymour et al., 1994), and was reported in wild geese in Sweden (Waldenström et al., 2003).

In conclusion, this study confirms that poultry is commonly colonized with *C. jejuni, C. coli* and enteric *Helicobacter*, especially in intensive farming. Among wild birds, only hooded crows proved to be highly sensitive to campylobacter infection, probably due to their interaction with free-ranging chickens. The epidemiological role of birds in the spread of enteric *Helicobacter* is still unclear and further studies are necessary to establish the importance of wild populations as Helicobacter carriers.

Acknowledgements

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References

Abd el-Aziz A.S., Elmossalami M.K., el-Neklawy E.: Bacteriological characteristics of dressed young pigeon (squabs) *Columba livia domesticus*. Die Nahrung. 2002, 46: 51-53.

Al Amri A., Senok A.C., Ismaeel A.Y., Al-Mahmeed A.E., Botta G.A.: Multiplex PCR for direct identification of *Campylobacter* spp. in human and chicken stools. J. Clin. Microbiol. 2007, 56: 1350-1355.

Atabay H.I., Corry J.E.L., On S.L.W.: Identification of unusual *Campylobacter*-like isolates from poultry products as *Helicobacter pullorum*. J. Appl. Microbiol. 1998, 84: 1017-1024.

Atanassova V. and Ring C.: Prevalence of *Campylobacter* spp. in poultry and poultry meat in Germany. Int. J. Food Microbiol. 1999, 51: 187-190.

Azevedo N.F., Almeida C., Fernandes I., Cerqueira L., Dias S., Keevil C.W., Vieira M.J.: Survival of gastric and enterohepatic *Helicobacter* spp. in water: implications for transmission. J. Appl. Environ. Microbiol. 2008, 74: 1805-1811.

Ceelen L.M., Decostere A., Van den Bulck K., On S.L.W., Baele M., Ducatelle R., Haesebrouck F.: *Helicobacter pullorum* in chickens, Belgium. Emerg. Infect. Dis. 2006, 12: 263-267.

Chuma T., Hashimoto S., Okamoto K.: Detection of thermophilic *Campylobacter* from sparrow by multiplex PCR: the role of sparrow as a source of contamination of broilers with *Campylobacter*. J. Vet. Med. Sci. 2000, 62: 1291-1295.

Colles F.M., Dingle K.E., Cody A.J., Maiden M.C.: Comparison of *Campylobacter* populations in wild geese with those in starlings and free-range poultry on the same farm. Appl. Environ. Microbiol. 2008, 74: 3583-3590.

Cooper J.E.: House crows and the spread of pathogens. Vet Rec. 2007, 161: 280.

Dipineto L., Gargiulo A., De Luca Bossa L.M., Rinaldi L., Borrelli L., Santaniello A., Menna L.F., Fioretti A.: Prevalence of thermotolerant *Campylobacter* in pheasants (Phasianus colchicus). Avian Pathol. 2008, 37: 507-508.

Ferrazzi V., Moreno Martin A., Lelli D., Gallazzi D., Grilli G.: Microbiological and serological monitoring in hooded crow (*Corvus corone cornix*) in the Lombardia Region, Italy. Ital. J. Anim. Sci. 2007, 6: 309-312.

Fox J.G., Chien C.C., Dewhirst F.E., Paster B.J., Shen Z., Melito P.L., Woodward D.L., Rodgers F.G.: *Helicobacter canadensis* sp. nov. isolated from humans with diarrhea as an example of an emerging pathogen. J. Clin. Microbiol. 2000, 38: 2546-2549.

Gramley W.A., Asghar A., Frierson H.F., Powell S.M.: Detection of *Helicobacter pylori* DNA in fecal samples from infected individuals. J. Clin. Microbiol. 1999, 37: 2236-2240.

Houng H.S., Sethabutr O., Nirdnoy W., Katz D.E., Pang L.W.: Development of a ceuE-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. Diagn. Microbiol. Infect. Dis. 2001, 40: 11-19.

Inglis G.D., Conville M., de Jong A.: Atypical *Helicobacter canadensis* Strains Associated with Swine. Appl. Environ. Microbiol. 2006, 72: 4464–4471.

Ito K., Kubokura Y., Kaneko K., Totake Y., Ogawa M.: Occurrence of *Campylobacter jejuni* in free-living wild birds from Japan. J. Wildl. Dis. 1988, 24: 467-470.

Jones K.: Campylobacters in water, sewage and the environment. J. Appl. Microbiol. 2001, 90: 68S-79S.

Kapperud G. and Rosef O.: Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni, Yersinia* spp., and *Salmonella* spp. in Norway. Appl. Environ. Microbiol. 1983, 45: 375-380.

LaGier M.J., Joseph L.A., Passaretti T.V., Musser K.A., Cirino N.M.: A real-time multiplex PCR assay for rapid detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli.* Mol. Cell. Probes. 2004, 18: 275-282.

Linton D., Lawson A.J., Owen R.J., Stanley J.: PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J. Clin. Microbiol. 1997, 35: 2568-2572.

Maruyama S., Tanaka T., Katsube Y., Nakanishi H., Nukina M.: Prevalence of thermophilic campylobacters in crows (*Corvus levaillantii, Corvus corone*) and serogroups of the isolates. Jpn. J. Vet. Sci. 1990, 52: 1237-1244.

Nebbia P., Tramuta C., Ortoffi M., Bert E., Cerruti Sola S., Robino P.: Identification of enteric Helicobacter in avian species. Schweiz. Arch. Tierheilk. 2007, 149: 403-407.

Nebola M., Borilova G., Steinhauserova I.: Prevalence of *Campylobacter* subtypes in pheasants (*Phasianus colchicus* spp. *torquatus*) in the Czech Republic. Vet. Med. – Czech. 2007, 52: 496-501.

On S.L.: Identification methods for campylobacters, helicobacters, and related organisms. Clin. Microbiol. Rev. 1996, 9: 405–422.

On S.L.: Taxonomy of *Campylobacter, Arcobacter, Helicobacter* and related bacteria: current status, future prospects and immediate concerns. J. Appl. Microbiol. 2001, 90: 1S–15S.

Rodrigues J., Sousa D., Penha-Goncalves A.: Occurrence and characterization of *Campylobacter* spp. in the sparrow, rat and pigeon. Rev. Port. Ciencias Vet. 1998, 93: 74–77.

SAS Institute Inc., SAS OnlineDoc®, Version 8, Cary, NC, USA: SAS Institute Inc., 1999.

Seymour C., Lewis R.G., Kim M., Gagnon D.F., Fox J.G., Dewhirst F.E., Paster B.J.: Isolation of *Helicobacter* strains from wild bird and swine feces. Appl. Environ. Microbiol. 1994, 60: 1025–1028.

Solnick J.V. and Schauer D.B.: Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. Clin. Microbiol. Rev. 2001, 14: 59-97.

Soncini G., Valnegri V.L., Vercellotti L., Colombo F., Valle D., Franzoni M., Bersani C.: Investigation of *Campylobacter* in reared game birds. J. Food Protect. 2006, 69: 3021-3024.

Stanley J., Linton D., Burnens A.P., Dewhirst F.E., On S.L.W., Porter A., Owen R.J., Costas M.: *Helicobacter pullorum* sp. *nov*. - genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. Microbiol. 1994, 140: 3441-3449.

Stern N.J., Bannov V.A., Svetoch E.A., Mitsevich E.V., Mitsevich I.P., Volozhantsev N.V., Gusev V.V., Perelygin V.V.: Distribution and characterization of *Campylobacter* spp. from Russian poultry. J. Food Protect. 2004, 67: 239-245.

Tee W., Montgomery J., Dyall-Smith M.: Bacteremia caused by a *Helicobacter pullorum*–Like Organism. Clin. Infect. Dis. 2001, 33: 1789-1791.

Waldenström J., Broman T., Carlsson I., Hasselquist D., Achterberg R.P., Wagenaar J.A., Olsen B.: Prevalence of *Campylobacter jejuni, C. lari* and *C. coli* in different ecological guilds and taxa of migrating birds. Appl. Environ. Microbiol. 2002, 68: 5911-5917.

Waldenström J., On S.L., Ottvall R., Hasselquist D., Harrington C.S., Olsen B.: Avian reservoirs and zoonotic potential of the emerging human pathogen *Helicobacter canadensis*. Appl. Environ. Microbiol. 2003, 69: 7523–7526.

Westrell T., Ciampa N., Boelaert F., Helwigh B., Korsgaard H., Chríel M., Ammon A., Mäkelä P.: Zoonotic infections in Europe in 2007: a summary of the EFSA-ECDC annual report. Eurosurveillance, 14. 20 January 2009, *www.efsa.europa.eu*

Zanoni R.G., Rossi M., Giacomucci D., Sanguinetti V., Manfreda G.: Occurrence and antibiotic susceptibility of *Helicobacter pullorum* from broiler chickens and commercial laying hens in Italy. Int. J. Food Microbiol. 2007, 116: 168-173.

Table 1: Prevalence (%) of *Campylobacter* and *Helicobacter* DNA in intensive farming birds (group A) and 95% confidence interval.

	Group A host species				
	Broilers (n=33)	**Growing cockerels (n=30)**	**Laying hens (n=14)**	**Guinea fowls (n=25)**	**Total (n=102)**
mono-infected *C. jejuni*	36.4% (20.4-54.9)	16.7% (5.6-34.7)	0% (0.0-23.2)	16.0% (4.5-36.1)	20.6% (13.2-29.7)
mono-infected *C. coli*	0% (0.0-10.6)	0% (0.0-11.6)	0% (0.0-23.2)	24.0% (9.4-45.1)	5.9% (2.2-12.4)
co-infected *C. jejuni/C. coli*	54.5% (36.3-71.9)	60.0% (40.6-77.3)	85.7% (57.2-98.2)	20.0% (6.8-40.7)	51.9% (41.8-53.1)
mono-infected *H. pullorum*	42.4% (25.6-60.8)	83.3% (65.3-94.4)	92.9% (66.1-99.8)	20.0% (6.8-40.7)	55.9% (45.7-62.0)
mono-infected *H. canadensis*	0% (0.0-10.6)	0% (0.0-11.6)	0% (0.0-23.2)	52.0% (31.3-72.2)	12.7% (7.0-20.8)
co-infected *Helicobacter*	0% (0.0-10.6)	0% (0.0-11.6)	0% (0.0-23.2)	0% (0.0-13.7)	0% (0.0-3.5)
co-infected *Campylobacter Helicobacter*	33.3% (18.0-51.8)	60.0% (40.6-77.3)	78.6% (49.2-95.3)	60.0% (38.7-78.9)	53.9% (43.8-63.8)

Table 2: Prevalence (%) of *Campylobacter* and *Helicobacter* DNA in small scale rural farm birds (group B) and 95% confidence interval.

	Group B host species			
	Broilers (n=37)	**Domestic pigeons (n=14)**	**Pheasants (n=9)**	**Total (n=60)**
mono-infected *C. jejuni*	8.1% (1.7-21.9)	0% (0.0-23.2)	0% (0.0-33.6)	5.0% (1.0-13.9)
mono-infected *C. coli*	2.7 (0.07-14.2)	0% (0.0-23.2)	0% (0.0-33.6)	1.7% (0.04-0.9)
co-infected *C. jejuni/C. coli*	8.1% (1.7-21.9)	0% (0.0-23.2)	44.4 (13.7-78.8)	11.7% (4.8-22.6)
mono-infected *H. pullorum*	24.3% (11.8-41.2)	0% (0.0-23.2)	22.2 (2.8-60.0)	18.3% (9.5-30.4)
mono-infected *H. canadensis*	0% (0.0-9.5)	0% (0.0-23.2)	22.2 (2.8-60.0)	3.3% (0.4-11.5)
co-infected *H. pullorum/H. canadensis*	0% (0.0-9.5)	0% (0.0-23.2)	0% (0.0-33.6)	0% (0.0-5.9)
co-infected *Campylobacter Helicobacter*	0% (0.0-9.5)	0% (0.0-23.2)	33.3 (7.5-70.0)	5.0% (1.0-13.9)

Table 3: Prevalence (%) of *Campylobacter* and *Helicobacter* DNA in wild birds (group C) and 95% confidence interval.

	Group C host species				
	Crows (n=78)	**Mallards (n=10)**	**Sparrows (n=15)**	**Urban pigeons (n=13)**	**Total (n=116)**
mono-infected *C. jejuni*	43.6% (32.4-55.3)	10.0% (0.25-44.5)	0% (0.0-21.8)	7.7% (0.0-52.2)	31.0% (22.8-40.3)
mono-infected *C. coli*	33.8% (0.8-10.8)	0% (0.0-30.8)	0% (0.0-21.8)	15.4% (1.9-45.4)	4.3% (1.4-9.8)
co-infected *C. jejuni/C. coli*	6.4% (2.1-14.3)	0% (0.0-30.8)	0% (0.0-21.8)	0% (0.0-52.2)	4.3% (1.4-9.8)
infected *Helicobacter*	0% (0.0-4.6)	0% (0.0-30.8)	0% (0.0-21.8)	0% (0.0-52.2)	0% (0.0-3.1)