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**Prevalence of Thermotolerant Campylobacter in Pheasants  
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Prevalence of thermotolerant *campylobacter* in pheasants (*phasianus colchicus*)

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## Abstract

This study was undertaken with the aim to evaluate the prevalence of thermotolerant *Campylobacter* spp. in living pheasants in Italy. To achieve this goal, a total of 240 living pheasants, equally shared between female and male birds, were examined. Thermotolerant *Campylobacter* spp. was isolated in 104 out of 204 (43.3%) living pheasants analysed. *C. coli* (100%) and *C. jejuni* (13.5%) were identified by PCR. Adult pheasants showed a significantly higher prevalence value ( $P < 0.05$ ) than younger pheasants.

## Introduction

Thermotolerant *Campylobacter* spp., primarily *Campylobacter jejuni* and *Campylobacter coli*, are among the most common bacteria causing acute human gastroenteritis in industrialized countries (Friedman *et al.*, 2000). Both species colonize the intestinal mucosa of most warm-blooded animals, including food-producing species and humans (Newell, 2001). However, the favoured environment appears to be the intestinal tract of several avian species where *Campylobacter* spp. colonizes as a commensal organism (Newell & Fearnley, 2003).

Current scientific knowledge on the presence of *Campylobacter* spp. in pheasants (*Phasianus colchicus*) is scarce and fragmentary, although this microorganism has been reported in pheasant in Germany (Atanassova & Ring, 1999), Russia (Stern *et al.*, 2004), and Czech Republic (Nebola *et al.*, 2007).

To address this lack of information, the present study was undertaken with the aim to evaluate the prevalence of these microorganisms in commercially reared pheasants in southern Italy.

## Materials and methods

A total of 240 living pheasants were examined from a game birds farm located in the province of Napoli (Southern Italy). 120 birds were examined with an age ranged between 0 and 1 month (young group). Subsequently, the same flock (further 120 pheasants) was examined when the birds exceeded 1 month of age (adult group). These sample sizes (n = 120

pheasants) were calculated using the formula proposed by Thrusfield (1995) for a large (theoretically 'infinite') population using the following values: expected prevalence (8%), confidence interval (CI; 95%) and desired absolute precision (5%).

With respect to sex, they were 120 male (60 young and 60 adult individuals) and 120 female (60 young and 60 adult individuals) birds. The pheasants were apparently in healthy body conditions.

Cloacal swabs were collected from all 240 pheasants, stored in Amies Transport Medium (Oxoid) at 4 °C, transported to the laboratory and analysed within 1 h of collection. Samples were inoculated into *Campylobacter* selective enrichment broth (Oxoid) and incubated at 42 °C for 48 h under microaerobic conditions provided by CampyGen (Oxoid). Subsequently, each sample was streaked onto *Campylobacter* blood-free selective agar (CCDA; Oxoid). After incubation at 42 °C for 48 h under microaerobic conditions, the plates were examined for typical *Campylobacter* colonies. Suspected colonies were subcultured on sheep blood agar (SBA; Oxoid) and finally incubated for 24 h at 42 °C.

Under phase contrast microscopy, colonies comprising curved or spiral motile rods were presumptively identified as *Campylobacter* spp. and submitted to a multiplex polymerase chain reaction (PCR).

DNA was extracted from isolated colonies on SBA using the PrepMan sample reagent (Applied Biosystems, Foster City, USA), following the manufacturer's protocol. The specific detection of the *Campylobacter* genus was based on PCR amplification of the *cadF* gene using oligonucleotide primers *cadF2B*, 5'-TTG AAG GTA ATT TAG ATA TG-3' and *cadR1B*, 5'-CTA ATA CCT AAA GTT GAA AC-3' as described by Konkel *et al.* (1999). All DNA extracts were also examined for the presence of *C. jejuni* and *C. coli* species using oligonucleotide primers C-1, 5'-CAA ATA AAG TTA GAG GTA GAA TGT-3', C-4, 5'-GGA TAA GCA TAG CTA GCT GAT-3' and COL1, 5'-ATG AAA AAA TAT TTA GTT

TTT GCA-3', COL2, 5' - ATT TTA TTA TTT GTA GCA GCG-3', respectively, as described by Winters *et al.* (1997) and Gonzalez *et al.* (1997). The PCR conditions were as described by Cloak & Fratamico (2002) and the products were separated by electrophoresis on 1.5% agarose gels (Gibco-BRL), stained with ethidium bromide and visualized under UV light. PCR amplified without the DNA was used as negative control, whereas two reference *Campylobacter* strains, *C. jejuni* ATCC 29428 and *C. coli* ATCC 33559, obtained from LGC Promochem, were used as positive controls (LGC Promochem, Teddington, UK).

The pheasant data (age and sex, all categorical) were analysed by univariate (Pearson's chi-square test for independence) statistical analysis using the *Campylobacter* spp. status (positive/negative) as dependent variable. Statistical analysis was performed using SPSS 13 for Windows.

## Results and Discussion

Thermotolerant *Campylobacter* spp. were isolated from 104/240 (43.3%; 95% CI = 37.0-49.9%) of the cloacal swabs examined (Table 1). Regarding the age, young birds showed a prevalence of 3.3% (95% CI = 1.1-8.8%), whereas pheasants older than 1 month showed a prevalence of 83.3% (95% CI = 75.2-89.3%); this difference was statistically significant ( $P = 0.001$ ). We can explain this finding taking into account that *Campylobacter* spp. infection in birds is typically observed from the second to the fourth week of life (Newell & Fearnley, 2003; Shreeve *et al.*, 2000). In contrast, there was no significant difference related to sex ( $P = 0.602$ ). The results of the chi-square test are summarized in Table 1.

As proved by PCR, 100% of the strains were identified as *C. coli* (104/104), and 14 out of the 104 (13.5%) positive samples were also positive to *C. jejuni*.

The findings of the present survey demonstrate the occurrence of *Campylobacter* spp. in living pheasants in Southern Italy with a prevalence of 43.3%. *C. coli* were the predominant species identified, in line with Nebola *et al.* (2007) who isolated *C. coli* with a prevalence higher than *C. jejuni* in pheasants from Czech Republic.

Comparative data on the prevalence of campylobacteriosis in pheasant are limited although this microorganism has been reported in pheasants from Germany (Atanassova & Ring, 1999), Russia (Stern *et al.*, 2004), and Czech Republic (Nebola *et al.*, 2007) with a prevalence of 25.9%, 26.7%, and 70.2%, respectively. Only one study is available from Italy by Soncini *et al.* (2006) who reported negative results from pheasant neck skins.

In conclusion, our results demonstrated that *Campylobacter* spp. may be found in the intestines of apparently healthy pheasants. Thus, this avian species may be considered as a potential carrier of *Campylobacter* spp. for humans and animals, since pheasants are frequently used for repopulation of protected areas (e.g. National Park, Natural Reserve) as well as game reserves.



## References

- Atanassova, V. & Ring, C. (1999). Prevalence of *Campylobacter* spp. in poultry and poultry meat in Germany. *International Journal of Food Microbiology*, 51, 187-190.
- Cloak, O.M. & Fratamico, P.M. (2002). A multiplex polymerase chain reaction for the differentiation of *Campylobacter jejuni* and *Campylobacter coli* from a swine processing facility and characterization of isolates by pulsed-field gel electrophoresis and antibiotic resistance profiles. *Journal of Food Protection*, 65, 266–273.
- Friedman, C.R., Neimann, J., Wegener, H.C. & Tauxe, R.V. (2000). Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In I. Nachamkin, & M.J. Blaser. (2000). *Campylobacter* 2nd edn (pp.121–138). Washington: ASM Press.
- Gonzalez, I., Grant, K.A., Richardson, P.T., Park, S.F. & Collins, M.D. (1997). Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the *ceuE* gene encoding a putative virulence determinant. *Journal of Clinical Microbiology*, 35, 759–763.
- Konkel, M.E., Gray, S.A., Kim, B.J., Garvis, S.G. & Yoon, J. (1999). Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cadF* virulence gene and its product. *Journal of Clinical Microbiology*, 37, 510–517.
- Nebola, M., Borilova, G. & Steinhäuserová, I. (2007). Prevalence of *Campylobacter* subtypes in pheasants (*Phasianus colchicus* spp. *torquatus*) in the Czech Republic. *Veterinarni Medicina*, 52, 496-501.
- Newell, D.G. (2001). Animal models of *Campylobacter jejuni* colonization and disease and the lessons to be learned from similar *Helicobacter pylori* models. *Symposium series* (Society for Applied Microbiology), 30, 57S-67S.

- Newell, D.G. & Fearnley, C. (2003). Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology*, *69*, 4343–4351.
- Shreeve, J.E., Toszeghy, M., Pattison, M. & Newell, D.G. (2000). Sequential spread of *Campylobacter* infection in a multipen broiler house. *Avian Diseases*, *44*, 983-988.
- Soncini, G., Valnegri, V.L., Vercellotti, L., Colombo, F., Valle, D., Franzoni, M. & Bersani, C. (2006). Investigation of *Campylobacter* in reared game birds. *Journal of Food Protection*, *69*, 3021-3024.
- Stern, N.J., Bannov, V.A., Svetoch, E.A., Mitsevich, E.V., Mitsevich, I.P., Volozhantsev, N.V., Gusev, V.V. & Perelygin, V.V. (2004). Distribution and characterization of *Campylobacter* spp. from Russian poultry. *Journal of Food Protection*, *67*, 239-245.
- Thrusfield, M. (1995). Surveys. In M. Thrusfield. (1995). *Veterinary Epidemiology* 2nd edn (pp.178–198). Oxford: Blackwell Science.
- Winters, D.K., O'Leary, A.E. & Slavik M.F. (1997). Rapid PCR with nested primers for direct detection of *Campylobacter jejuni* in chicken washes. *Molecular and Cellular Probes*, *11*, 267–271.

**Table 1.** Results and statistical analysis (Pearson's chi-squared test for independence) of pheasants tested, related to sex and age.

<b>Pheasant data</b>	<b>No. of pheasant tested</b>	<b>No. of positive pheasant</b>	<b>%</b>	<b>95% CI</b>	<b>P*</b>
<b>Sex</b>					
Male	120	54	45.0	36.0-54.3	0.602
Female	120	50	41.7	32.9-51.0	
<b>Age group</b>					
Young (< 1 month)	120	4	3.3	1.1-8.8	0.001
Adult (> 1 month)	120	100	83.3	75.2-89.3	
<b>Total</b>	240	104	43.3	37.0-49.9	

CI, confidence interval.

\*Chi-square.