



Hunted game birds – Carriers of foodborne pathogens

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ABSTRACT

Game birds may carry zoonotic bacteria in their intestines and transmit them to hunters through bird handling or through the handling and consumption of contaminated meat. In this study, the prevalence of foodborne bacteria was screened from game bird faeces and mallard breast meat using PCR. The sampling occurred in southern Finland from August to December during the hunting season. Isolates were characterized by multi-locus sequence typing. Mesophilic aerobic bacteria and *Escherichia coli* counts were used to assess the microbial contamination of mallard meat. In total, 100 woodpigeon (*Columba palumbus*), 101 pheasants (*Phasianus colchicus*), 110 mallards (*Anas platyrhynchos*), and 30 teals (*Anas crecca*) were screened during the hunting season. Additionally, 100 mallard breast meat samples were collected. *Campylobacter* and *Listeria* were commonly detected in the faeces and *Listeria* on mallard meat. *L. monocytogenes* of sequence types associated with human listeriosis were frequently found in game bird faeces and on mallard meat. Good hygiene during game bird handling, storing the game bird meat frozen, and proper heat treatment are important measures to minimize the health risk for hunters and consumers.

1. Introduction

Game birds are typically hunted in the wild for food or sport. Game bird hunting has increased during the last decades (Horigan et al., 2014). Hunting is also becoming more popular in northern countries including Finland. Mallard (*Anas platyrhynchos*) and teal (*Anas crecca*) are the most commonly hunted waterfowls in Finland, with the annual hunting bag amounting to 240 000–300 000 birds (<https://riista.fi/en/>). Moreover, around 200 000 woodpigeons (*Columba palumbus*) and 50 000 pheasants (*Phasianus colchicus*) are hunted. Most of the pheasants are domestic birds hatched and reared under controlled conditions on farms before being released into the wild to be hunted. Woodpigeons, mallards, and teals are migrating birds.

Wild birds can carry zoonotic pathogens that affect animals, humans, and the environment (Smith et al., 2020). They can also carry common foodborne bacteria (e.g. *Salmonella*, *Campylobacter*, *Yersinia*, and *Listeria*) in their intestines (Najdenski et al., 2018). Game feeding, habitat, migration, and contact with farms may play a role in the spread of these zoonotic bacteria. Information is still lacking concerning the presence of

foodborne pathogenic bacteria in the faeces of game birds. They are typically asymptomatic, and therefore the pathogens can easily be disseminated to hunters and consumers unnoticed through the handling and consumption of infected game birds.

The production and consumption of game bird meat vary between countries. The consumption of game bird meat in Finland is still small compared to the UK, where the game bird industry is large (Horigan et al., 2014). Game bird meat is usually associated with healthy and ethical meat production and it supports local production (Tomasevic et al., 2020). However, it is still a rare delicacy, which is mostly supplied directly from hunters to consumers and restaurants. The slaughter process of game birds is less controlled than for farm animals and is usually performed by hunters. Microbial contamination of game bird carcasses is affected by shot location, slaughter hygiene, and the cold chain. Bacteria, including pathogens, typically enter game meat processing plants along with the birds. They can be carried on the outer surfaces and within the gastrointestinal tracts of arriving birds. Data concerning the microbial contamination of game bird carcasses is scarce, and no legal limits for microbial counts on game bird carcasses exist. There is a

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risk of sporadic foodborne infections associated with the preparation and consumption of game bird meat (Horigan et al., 2014).

A limited number of reports exist on the prevalence of foodborne pathogens in game birds. Thus, this study was performed to explore the prevalence of important foodborne pathogens (*Salmonella*, *Campylobacter*, *Yersinia*, and *Listeria*) linked to hunted game birds. We additionally assessed the microbiological quality and safety of the breast meat of hunted mallards.

2. Materials and methods

2.1. Sampling and sample preparation

We studied the presence of zoonotic foodborne pathogenic bacteria (*Salmonella*, *Campylobacter*, *Yersinia*, and *Listeria*) in the colon content of 100 woodpigeons, 101 pheasants, 110 mallards, and 30 teals hunted in southern Finland. Pheasant and mallards have been reared until approximately one month of age at two farms and then released into the wild where they were given supplementary feeding. The samples were collected between August and December during the hunting season in 2013 and 2014. Intestinal samples were collected during evisceration and transported chilled to the laboratory. A 1-g sample was taken from the colon and mixed with 9 ml of buffered peptone water (BPW, Labema, Kerava, Finland).

Additionally, we studied the presence of foodborne pathogenic bacteria and the number of mesophilic aerobic bacteria (MAB) and *Escherichia coli* (EC) on duck meat originating from 100 hunted mallards. The mallards were hunted on 8 different days during the hunting season of 2016 in southern Finland. They were delivered uneviscerated to a small game processing plant on the hunting day. The birds were sampled after plucking and evisceration before chilling. The skin side of the breast fillet (50 cm²) was swabbed with a moistened cotton swab according to Sauvala et al. (2019). Between 5 and 15 samples were collected on 8 sampling days. The samples were transported chilled to the laboratory. The swab moistened with 10 ml of BPW was mixed with 40 ml of BPW.

2.2. PCR screening and isolation of foodborne pathogenic bacteria

We screened the presence of *Campylobacter* (*rrn*), *Salmonella* (*trt*), *Yersinia* (*ail*), and *Listeria* (*mlp*) by real-time PCR based on SYBR Green according to Sauvala et al. (2019). DNA was extracted using ZR Fecal DNA MiniPred™ (Nordic BioSite Oy, Helsinki, Finland) and Chelex®100 resin (BioRad, Hercules, California) for faecal and carcass samples, respectively.

Salmonella, *Yersinia*, and *Listeria* were isolated from ON-enrichment according to Sauvala et al. (2019). *Campylobacter* was isolated from pheasant and mallard faeces according to Kovanen et al. (2019).

2.3. Bacterial counts on the breast meat of mallards

The numbers of MAB and EC were determined according to Sauvala et al. (2019). *E. coli* was isolated using Harlequin *E. coli*–agar (Labema) instead of a Chromocult plate. Detection limits for MAB and EC were 1.3 log₁₀ cfu/cm² (20 cfu/cm²) and 0.7 log₁₀ cfu/cm² (5 cfu/cm²), respectively.

2.4. Multi-locus sequence typing (MLST) of *Yersinia* and *Listeria* isolates

The DNA of *Yersinia* and *Listeria* isolates were extracted using Pure-Link Genomic DNA Mini Kit (Invitrogen, Carlsbad, USA). Whole-genome sequencing was performed using NovaSeq6000 at the Center for Genomics and Transcriptomics (CeGaT, Tuebingen, Germany). Reads were assembled into contigs using the Patric web-based service (<https://www.patricbrc.org/app/Assembly>). The sequence types (ST) of *Y. pseudotuberculosis* and *L. monocytogenes* isolates were assigned by the

web-based service of the Center for Genomic Epidemiology (<https://cge.cbs.dtu.dk/services/MLST/>). Kovanen et al. (2019) have reported on the MLST of *C. jejuni* isolates from pheasants and mallards.

2.5. Statistical analyses

Data were analysed using SPSS statistic version 25 (IBM, Armonk, NY). Non-parametric tests were used for both categorical (pathogens) and continuous variables (MAB and EC) because they were not normally distributed (Kolmogorov-Smirnov test of normality). $P < 0.05$ was statistically significant.

3. Results and discussion

In this study, we focused on the prevalence of *Salmonella*, *Campylobacter*, *Yersinia*, and *Listeria* in game birds, which are commonly associated with human foodborne diseases in the EU (EFSA and ECDC, 2019). PCR was used to screen for the presence of these pathogens in game birds. We found all the pathogens in the colon contents of hunted game birds in Finland, with varying prevalences between the bird species (Table 1).

Birds are considered a major reservoir for *Campylobacter*, which is the most common cause of human foodborne enteritis in Europe (EFSA and ECDC, 2019). It is a gastrointestinal commensal in birds, which seldom induces infection. *Campylobacter* (*rrn*) was the most frequently detected pathogen in the faecal samples of all four hunted game bird species in our study (Table 1). Earlier studies (Horigan et al., 2014) have also frequently found this pathogen in asymptomatic game birds. In a recent study from Germany, *C. jejuni* was detected in 12% of hunted ducks by PCR (Thierfelder et al., 2019). *Campylobacter* was significantly ($p < 0.05$, Fisher's exact test) more often detected in mallards (71%) and teals (73%) than in woodpigeons (27%) and pheasants (22%) in our study. One reason for this may be that they were reared in small natural ponds, which were crowded with birds. Ponds are ideal environments for *Campylobacter*, where it may easily spread between birds. *C. jejuni*, which is the most common species causing human campylobacteriosis, was the only thermotolerant *Campylobacter* species identified in hunted mallards and pheasants. The STs of *C. jejuni* isolates have recently been reported from mallards and pheasants (Kovanen et al., 2019). Most of the STs of mallards were novel; however, some were also types found in humans. Only ST 19 was found in pheasants. It is a widely distributed type associated with human campylobacteriosis. ST19 was quite recently reported in game pheasants in the UK (Seguino et al., 2018).

In addition to *Campylobacter*, *Listeria* (*mlp*) was also detected in all hunted game bird species in our study (Table 1). The prevalence varied from 14% (pheasants) to 34% (mallards). *L. monocytogenes*, which is the most important human pathogenic species, was isolated from 15 mallards, 9 pheasants, and one teal (Table 2). Most isolates (88%) belonged to serotype 1/2a found in pheasants, mallards, and teals. Serotype 1/2b was found in two mallards and serotype 4b in a pheasant. Two atypical hemolytic *Listeria innocua* isolates, which were recently confirmed to be pathogenic, were found in a mallard and a pheasant (Moura et al., 2019). *Listeria* has rarely been isolated from game birds in earlier studies (Horigan et al., 2014). However, Hellström et al. (2008) isolated *L. monocytogenes* from 36% of wild bird faecal samples. Most of the STs found in game bird faeces in our study (Table 2: ST1, 7, 20, 37, 224, 391, 400, and 451) have been identified in humans in Europe (<https://bigbdb.pasteur.fr/listeria/>). Some (ST1, 7, 37, and 451) have also been found in humans with listeriosis in Finland (Finnish Institute for Health and Welfare). The prevalence of *Listeria* in the intestinal tracts of birds may reflect the degree of contamination in their environments, which may explain the high prevalence of *Listeria* in mallards living in small contaminated ponds in our study.

Salmonella was the second most commonly reported cause of enteritis and the most common cause of foodborne outbreaks in 2018 within the EU (EFSA and ECDC, 2019). It can also be pathogenic to birds,

Table 1
Prevalence of foodborne pathogenic bacteria in the faeces of hunted game birds by PCR.

Game bird	<i>Salmonella (ttr)</i>		<i>Campylobacter (rrn)</i>		<i>Yersinia (ail)</i>		<i>Listeria (mpl)</i>	
	%	95%CI	%	95%CI	%	95%CI	%	95%CI
Woodpigeon N = 100	<1.0	0.0–3.6	26.6 ^a	17.1–35.7	<1.0 ^a	0.0–3.6	20.0	12.7–29.2
Pheasant N = 101	5.0	1.6–11.2	21.8 ^a	14.2–31.1	5.9	2.2–12.5	13.9 ^a	7.8–22.2
Mallard N = 110	3.6	1.0–9.0	70.9 ^b	61.5–79.2	10.0 ^b	5.1–17.2	33.6 ^b	24.9–43.3
Teal N = 30	3.3	0.1–17.2	73.3 ^b	54.1–87.7	<3.3	0.0–11.6	16.7	5.6–34.7

a and b differ significantly from each other at the 0.05 level using the Fisher's exact test.

Table 2
Listeria monocytogenes isolates found in game bird faeces and meat.

Source	Isolation year	Number of isolates	Serotype	Sequence types
Pheasant Faeces	2013–14	8	1/2a	7, 20, 37, 451, 585
		1	4b	1
Mallard Faeces	2013–14	13	1/2a	37, 391, 400, 451, 585
		2	1/2b	224
Teal Faeces	2013	1	1/2a	37
Breast meat	2016	13	1/2a	8, 18, 412, 451, 585

^abold STs have been found on moose and deer carcasses in Finland (Sauvala et al., 2019).

especially if birds are immunocompromised (Minias, 2020). The prevalence of *Salmonella* in healthy game birds has been reported to be low (<5%) (Horigan et al., 2014). *Salmonella (ttr)* was also rarely detected in the faeces of hunted game birds in our study. The highest prevalence (5%) was detected among pheasants (Table 1). Paulsen et al. (2008) found no *Salmonella* in hunted pheasants in the Slovak Republic. *Salmonella* has also rarely been reported in healthy migratory birds (<1%), including hunted waterfowl (Antilles et al., 2015; Grigar et al., 2017; Najdenski et al., 2018). It seems that *Salmonella* is quite rarely excreted in the faeces of healthy game birds.

Yersiniosis was the fourth most commonly reported foodborne zoonosis in 2018 within the EU (EFSA and ECDC, 2019). Finland had the highest incidence rate of 9.6 cases per 100 000 inhabitants. *Y. pseudotuberculosis* causes yersiniosis to a lesser extent than *Y. enterocolitica*, which causes most (>95%) of the cases. Birds usually carry *Yersinia* in their intestines without any symptoms but *Y. pseudotuberculosis* in particular can cause yersiniosis, a systemic infection under stressful conditions (Le Guern et al., 2016; Rouffaer et al., 2017; Stoute et al., 2016). *Yersinia (ail-positive)* was detected in pheasants (6%) and mallards (10%) in our study. We isolated *ail-positive Y. enterocolitica* from one mallard and *Y. pseudotuberculosis* serotype O:1 from three pheasants. All *Y. pseudotuberculosis* isolates belonged to ST90 (McNally scheme), which has been found in wild birds and humans in Europe (<https://enterobase.warwick.ac.uk/species/index/yersinia>). The ST of the *ail-positive Y. enterocolitica* was unknown. In a recent study, no *ail-positive Yersinia* was found in hunted free-living birds in Poland, including mallards and woodpigeons (Odyniec et al., 2020). Rouffaer et al. (2017) reported a high prevalence (31%) of non-pathogenic (*ail-negative*) *Y. enterocolitica* in sparrows in Belgium. They found *Y. pseudotuberculosis* from 4% of the sparrows. *Y. enterocolitica (ail-positive)* and *Y. pseudotuberculosis* have sporadically also been reported in migratory birds (Najdenski et al., 2018; Niskanen et al., 2003). This shows that potentially pathogenic (*ail-positive*) *Y. enterocolitica* and *Y. pseudotuberculosis* are circulating among game birds.

Foodborne pathogens may be transmitted from the intestinal tracts

of hunted game birds to the carcass and meat during evisceration. *Salmonella*, which was a quite rare (3.6%) finding in mallard faeces, was not detected on mallard breast meat in our study (Table 3). *Campylobacter*, which was a common finding (71%) in mallard faeces, was detected on only 9% of the mallard meat samples, indicating relatively good slaughter hygiene. *Yersinia* was also detected on mallard meat (3%). A high prevalence of *Listeria* (44%) was an unexpected finding in our study. *L. monocytogenes* serotype 1/2a, which was the dominant type in mallard faeces, was isolated from 13 meat samples. Sequence types ST8, 18, and 451 found on mallard meat have also been associated with human listeriosis in Finland (Finnish Institute for Health and Welfare), indicating a possible health risk for consumers during meat handling. However, the possible link between mallard meat and human listeriosis needs further studies. The prevalence of *Listeria* on the meat samples varied significantly ($P < 0.5$, Kruskal-Wallis test) between sampling days (Table 3). This study demonstrates that intestinal pathogens of hunted game birds can be found on mallard meat. It also shows that mallard meat, which can be contaminated with psychotropic *Listeria* and *Yersinia*, should not be stored at refrigerator temperatures for several days. Game meat, even when packed under vacuum, should be stored frozen to prevent the growth of these pathogens and health risks to consumers. Additionally, proper heat treatment of the meat before usage is essential to avoid human infections.

We used MAB and EC, which are commonly used indicators to assess process hygiene in the food chain, to study the surface contamination of mallard breast meat. Only MAB has legal limits on farm animal carcasses within the EU (EC 2073/2005). The median MAB and EC values on mallard breast meat were 3.5 log cfu/cm² and 0.7 log cfu/cm², respectively (Table 3). We observed a weak positive correlation ($r = 0.34$, $P < 0.01$, Spearman's rho) between the MAB and EC counts. There were clear differences between samples and some significant differences ($P < 0.05$, Kruskal-Wallis test) between the EC values and sampling days (Table 3). For some samples, MAB counts were unacceptable (>4.5 log cfu/cm²) according to the limits set for farm animals by EU legislation, indicating a hygiene problem during processing. However, the median MAB value (3.5 log cfu/cm²) on mallard meat was lower compared to the median MAB value on moose (4.0 log cfu/cm²) and deer (4.4 log cfu/cm²) carcasses recently reported by Sauvala et al. (2019), indicating good slaughter hygiene of mallards.

The median EC values (0.7 log cfu/cm²), including the maximum value (2.6 log cfu/cm²), were slightly below the values reported from muscle cuts of pigeons and partridges by El-Ghareeb et al. (2009). The relatively low EC values indicate low faecal contamination in our study, which may be one reason why *Campylobacter* and *Yersinia* were rarely detected and no *Salmonella* was detected on the mallard meat surface.

4. Conclusions

Game birds are important carriers of foodborne pathogens. In our study, mallard meat was frequently contaminated with *L. monocytogenes* of STs associated with human listeriosis. Good slaughter hygiene and hunter education are needed to avoid the contamination of bird

Table 3

Median values (\log_{10} cfu/cm²) of mesophilic aerobic bacteria (MAB) and *Escherichia coli* (EC), and PCR prevalence of *Salmonella* (S), *Campylobacter* (C), *Yersinia* (Y), and *Listeria* (L) on the breast meat of 100 mallards.

Sampling days (No. of samples)	Median values (min-max) of MAB	Median values (min-max) of EC	Number of positives			
			S (<i>ttr</i>)	C (<i>rrn</i>)	Y (<i>ail</i>)	L (<i>mpl</i>)
1 (10)	3.2 (2.3–4.6)	0.7 (0.7–2.6)	0	0	0	0 ^a
2 (15)	3.5 (2.6–5.2)	0.7 (0.7–2.6) ^{a,c,d}	0	1	0	1 ^a
3 (15)	3.3 (1.3–4.1)	0.7 (0.7–2.3)	0	0	0	9
4 (15)	3.7 (3.0–5.2)	2.1 (0.7–2.6) ^b	0	2	0	12 ^b
5 (15)	3.3 (2.9–4.2)	0.7 ^{a,c}	0	0	2	9
6 (15)	3.3 (1.9–4.4)	1.8 (0.7–2.4) ^{a,b,d}	0	0	1	3 ^a
7 (10)	4.0 (3.1–4.5)	1.8 (0.7–2.3) ^{a,b,d}	0	6	0	6
8 (5)	3.3 (2.6–3.7)	0.7 ^{a,c,d}	0	0	0	4
All samples	3.5 (1.3–5.2)	0.7 (0.7–2.6)	0	9	3	44

^a, ^b, ^c, and ^d differ significantly from each other at the 0.05 level using pairwise comparisons with the Kruskal-Wallis test adjusted by Bonferroni correction.

carcasses with intestinal bacteria. Game bird meat should be stored frozen and properly heat treated.

Declaration of competing interest

The authors declare no conflict of interest.

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