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Original Contribution

PRESENCE AND DIFFERENTIATION OF *CAMPYLOBACTER* SPP. DURING PROCESSING OF DUCKS FOR FOIE GRAS LIVER IN BULGARIA

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ABSTRACT

The aim of this study was to investigate the presence of *Campylobacter* spp. during the slaughter processing of moulard ducks in Bulgaria. The prevalence of *Campylobacter* was determined from live bird to prepackaged carcasses and cuts for 4 ducks flocks.

Intestinal content and skin of the live ducks was positive in 72,5% and 12,5% of the samples, respectively. After scalding and hot wax *Campylobacter* was isolated in 12,5% of the samples of skin surfaces. The breast meat with skin (magret) was contaminated in 7,5%. The chilled liver showed 12,5% positive samples. *C. jejuni* was the most commonly found species (81%) followed by *C. coli* (19%). *C. jejuni subsp. jejuni* predominated subspecies *C. jejuni subsp. doylei* in 85,3% and 14,7% respectively.

Key words: Slaughterhouse, magret, liver, ducks, *C. jejuni*, *C. coli*

INTRODUCTION

Fatty liver production is the process of force-feeding (cramming) ducks, which are normally at 10 weeks of age, for a period of 14 days. During this period the weight of the liver will increase from an initial weight of about 40g to a final weight of between 300-700g. Moulard ducks are one of the most popular Foie Gras birds used for fatty liver production. Poultry and poultry products are of great importance as a source of food borne pathogens like *Campylobacter* spp. Particularly, thermophilic *Campylobacter* (*C. jejuni* and *C. coli*) are considered as e major cause of human *Campylobacter* gastroenteritis and consumption of undercooked poultry is a leading cause of infections. (1; 2; 3)

Poultry entered the slaughterhouses are carrier of *Campylobacter* as in their intestinal tract, as well onto their skin and feather (4; 5; 6; 7). This create premise of wide bacterial spead during plant processing. At the slaughterhouse there is a hazard of bacterial contamination of poultry meat, as a different technological steps influence onto increasing or decreasing of microbial contamination (8; 9). Some investigators noted scalding, defeathering and evisceration equipments as a point with significant hazards of *Campylobacter* cross contamination (9; 10; 11).

Plant processing of Foie gras ducks differ from standard slaughter processing of waterfowl poultry, as important point is strong liver protection and lots of handy operation by workers. The aim of this study was to investigate *Campylobacter* carrier in Foie Gras ducks at age of slaughter as well prevalence of *Campylobacter* spp. contamination during plant processing.

MATERIAL AND METHODS

From 4 different Moulard ducks flocks were collected samples after transport and during processing at slaughterhouse. Ducks were at the age of slaughter (84 days) after 2 week period of force feeding for fatty liver generation.

For investigation of *Campylobacter* spp. carrier after transport 10 birds of each flock were marked and swab samples of skin and feather surfaces were taken off. During the slaughtering process the caecum of the same birds were removed after evisceration.

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For investigation of *Campylobacter* spp. prevalence during the slaughtering process from the same birds were collected swab samples of skin surfaces after feather picking (after hot wax removing) and 25 g samples of breast meat with skin (magret) and liver (foie gras) after chilling.
Samples were worked out in campyloacter enrichment broth (Merck) with selective supplement (Merck) and over selective agar media (Merck) containing selective supplement (Merck).
Incubation was at 37°C and 42°C for 48h at microaerophilic condition achieved by using commercial gas-generating kits. Pure cultures of presumptive *Campylobacter* spp. were biochemicaly tested for oxidase and catalase production, hyppurat and indoxylacetate hydrolysis and by API Campy ® (Bio Mérieux, 20800).

RESULTS

Samples from skin and feather of the live birds after transport were in 12,5% *Campylobacter* positive, as all obtained isolates were identified as *C. jejuni* (Table 1).

Table 1 *Carrier of Campylobacter by Moulard ducks after transport*

Samples	n	***Campylobacter positive***	***C. jejuni***, (%)	***C. coli,*** (%)
Skin and feather surfaces	40	5 (12,5%)	100%	-

In the intestinal tract (cecal content) of the same birds *Campylobacter* spp. was detected in 72,5% of the samples and isolates of *C. jejuni* (72,4%) predominated *C. coli* (27,6%). Ducks flocks differed in their intestinal carriers of *Campylobacter* spp. and we found 60%, 80%, 90% and 60% positive samples in respectively flocks from I to IV. (Table 2).

Table 2 *Prevalence and species of Campylobacter in liver, skin and meat samples during processing of Moulard ducks*

Samples	n	***Campylobacter* positive** n (%)	***C. jejuni*** n (%)	***C. coli*** n (%)
Caecal content	40	**29 (72,5%)**	21 (72,4%)	8 (27,6%)
Skin surfaces	40	**5 (12,5%)**	5 (100%)	-
Breast meat with skin (magret)	40	**3 (7,5%)**	3 (100%)	-
Liver	40	**5 (12,5%)**	5 (100%)	-

During the slaughtering process in samples of skin surfaces, magret and liver *Campylobacter* positive were 12,5%, 7,5% and 12,5% respectively. All obtained *Campylobacter* isolates were identified as *C. jejuni* (Table 2). Subspecies differentiation demonstrated that *C. jejuni ssp. jejuni* was 85,3% and *C. jejuni ssp. doylei* 14,7% of *C. jejuni* isolates.
Skin surface after hot wax removing was free of *Campylobacter* in flocks I and IV. Fatty liver contains *Campylobacter* in 12,5% and magret in 7,5% of the samples.
During the processing the highest number *Campylobacter* positive samples were detected in flock III where carrier of these microorganisms were also in high percent. Inded of 60% positive samples flock I was with lov level of detected campylobacteria during plant processing of the birds (Table 3).

DISCUSSION

The obtained results for *Campylobacter* showed relatively high percentage of carriage (72,5%) in intestinal tract in fatten ducks and relatively low percentage of carriage on skin and feathers (12,5%) after transportation. Tsai & Hsiang (2005) isolated *Campylobacter* in 43,5% of investigated birds and in 92% of all

Table 3 *Prevalence of Campylobacter spp. in liver, skin and meat samples from 4 diferent flocks of ducks for liver during processing*

Samples	**Flocks (batches)**			
	I	**II**	**III**	**IV**
	n (%)	n (%)	n (%)	n (%)
Caecal content	6 (60%)	8 (80%)	9 (90%)	6 (60%)
Skin surfaces	-	3 (30%)	2 (20%)	-
Breast meat with skin (magret)	-	-	2 (20%)	1 (10%)
Liver	1(10%)	1 (10%)	2 (20%)	1 (10%)

investigated farms. Defferentation by species showed that 94,8% were *C. jejuni*, and hardly 5,2% - *C. coli*. Fallacara et al., (2001) found out *C. jejuni* in 50% of all samples of wild ducks. Autors did not found significant differences in bacteria carriage between different kinds of birds. Uradzinsk et al., 1987, defined *Campylobacter* carriage in different kind of birds for slaughtering and found *Campylobacter* positive samples in 86% of all ducks, 60,7% of all broilers, 58% of all geese и 14% turkeys.

During the survey, after picking the feathers and following hot wax, we found *Campylobacter* in two flocks (II и III) in 30% и 20% respectively. Birds showed high carriage of *Campylobacter* in intestine track 80% and 90%, respectively. That high "start" contamination in the beginning of the slaughtering process determines the contamination in birds carcasses after scalding and defeathering. According to Slavik et al. (1994) at scalding at 56°C temperature of the water, appears the biggest reduction effect, but nevertheless *Campylobacter* presence on birds skin surface remains significant ($\log_{10}$ 3.39). Our results showed that after scalding at 64°C of water temperature and following defeathering and hot wax (60°C) 12.5% of all samples were *Campylobacter* positive. All survived bacteria, including *Campylobacter* remain on bird carcass and therefore are prerequisite for spreading contamination to other birds in following slaughter processing.

Szteyn & Uradzinsk, 1994, proved *Campylobacter* contamination in 32,7% of samples of duck carcasses before disemboweling and after disemboweling in 23,6% of birds *Campylobacter* are detected in birds body cavities. According to Kasrazaden & Genigeorgis *Campylobacter* are isolated in 6.7% from duck skin during slaughtering process. In that certain slaughtering house the disemboweling is hand made in order to ensure maximum preservation of the liver from mechanical injuries.

A large number of scientists emphasize that workers in the slaughtering house contribute to bird cross-contamination (12; 13). The results from our study confirm that assumption. *Campylobacter* were investigated in 7,5% and 12,5% of samples, respectively in breast meat (magret) and liver during the processing from the workers.

A large number of scientists investigated that the presence of *Campylobacter* spp. in liver differs between 34,5% and 36% (14; 15; 16). Our results showed significantly lower percentage of *Campylobacter* positive samples, which is most probably due to the changes of liver structure because of fattening.

The results from the study of fatty liver showed that the most commonly isolated *Campylobacter* spp. was *C. jejuni* followed by *C. coli.* If differentiating *C. jejuni* in sub-species, C. *jejuni subsp. jejuni* appeared in 69,3% of the samples, followed by *C. jejuni subsp. doylei in* 30,7% of samples. Atabay et al., (1997) proved *C. jejuni subsp. jejuni* in all of investigated ducks in the farm. Carbita et al., 1992, determined carriage of thermofilic *Campylobacter* in 40,5% of the ducks, as *C. jejuni* predominated *C. coli*. Ridsdale el al., 1998, isolated *C. jejuni subsp. jejuni*, *C .coli* and *C. upsaliensis* in caecal content, while from the carcasses only *C.jejuni subsp. jejuni* and *C.coli*. are isolated.

CONCLUSION

Study results showed that Foie Gras ducks are carriers of *Campylobacter* spp., but at the same time the specific slaughtering process seems to restrict bacteria spreading and following contamination of cut products, such as fatty liver and magret that are of greatest significance in this type of poultry production. Low percentage of *Campylobacter* detection in fatty liver is probably related to the increase of fat content in the liver and further unsuitable conditions for bacteria growth. *C. jejuni* subsp. *jejuni* is predominately isolated thermophilic *Campylobacter* spp. during slaughter processing of ducks for foie gras liver.

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