

Microbiological quality of chicken meat: *Campylobacter* relevant public health problem

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Abstract

Campylobacter is undoubtedly an important public health problem that European legislation (Directive 2003/99 / EEC) establishes as zoonotic agent to be monitored, with mandatory notification of the number of isolates, registration of cases, and the provision of information to Member States. However, despite its high prevalence, so far they have not established measures for its reduction or the obligation to implement control plans. Unlike *Salmonella*, which, most likely, thanks to community prevalence reduction targets and national control plans, still, for years, a marked downward trend in the number of cases in the EU. Consumption of contaminated chicken meat, mainly by *Campylobacter jejuni* is the leading cause of human campylobacteriosis in Europe, so it EFSA considered necessary to establish measures to reduce *Campylobacter* in chicken flocks. According to the European Food Safety Agency (EFSA), a real incidence of 9 million annual cases of campylobacteriosis in the EU is estimated. The average prevalence of *Campylobacter* in broiler flocks at Community level is 71.2%, with 75% in the case of Romania, it is therefore a major problem. A lot of birds that come to slaughter excrete the bacteria in their feces and is very difficult to prevent, during processing, contamination of carcasses and abattoir equipment occurs. Therefore it is important to reduce the bacterial load in birds before arrival at the slaughterhouse. As the reports of the EFSA and the European Center for Disease Prevention and Control (ECDC), based on the results of monitoring activities of zoonoses (diseases transmitted naturally from vertebrate animals to man diseases), carried out in 2015 in 32 European countries (28 Member States and four non-Member States), campylobacteriosis is the most commonly reported zoonosis. In view of this trend, the European Union is preparing a draft regulation that will come to amend the current legislation for the control of *Campylobacter*. Those rules shall establish a process hygiene criterion for *Campylobacter* in broiler channels to control possible contamination with this bacteria during the slaughter stage. This draft European regulation also establishes a contamination value above which corrective action is required to maintain hygiene, according to food legislation (20% of chicken carcasses with a level of *Campylobacter* ≥ 1000 cfu / g, for 2025). The European Commission considers that this approach should be implemented gradually and tighter over time.

Keywords: *Campylobacter*, public health, EFSA, chicken meat

Introduction

Given the important role of the species in the genus *Campylobacter*, the World Health Organization has developed over time a partnership with various stakeholders, to develop general policies to promote food safety. These include specific recommendations and technical expertise throughout the entire food chain, from production up to (1.2).

The who reports sporadic episodes of recorded food Community rules, however, believes that their incidence is due to the fact that these micro-organisms and, thanks to its properties, the difficulty (7.8).

Specialty reports mentioning another important aspect which argues that it is almost impossible to determine with certainty all sources of contamination with microorganisms. Wide distribution of the species in the genus *Campylobacter*, it hinders the development of effective control strategies along the food chain. However, in countries that have adopted specific measures

to reduce the prevalence of *Campylobacter* in poultry, it was observed a reduction in the number of episodes in humans food with Community rules (1, 3)

Campylobacter infections are the most common cause of bacterial gastroenteritei in industrialized countries. Annual incidence varies between countries and is growing in several of these (2,3).

Material and Methods

Research has been carried out within the framework of a slaughter of birds in Romania. The samples were processed in the laboratory of Microbiology and Food Control Laboratory, Faculty of Veterinary Medicine of Iași.

Research was conducted during the period 2016-2017, and had as objectives:

- isolation of *Campylobacter spp.* strains from broiler cecocolic junction;
- identification of the isolated strains on the basis of cultural, biochemical;
- to establish the incidence of *Campylobacter* species isolation from birds and poultry carcasses.

Assessment of species *Campylobacter spp.* has been of programs ANSVSA:S.R. ISO 10272/2007.

During the period 2016-2017 have been sampled and analysed 25 samples of cecumuri from broiler, and 25 samples of carcasses of broilers.

At the present time are being offered more possibilities for isolation of *Campylobacter* species. Insulation standard distributed by 2015, OIE Manual recommends mCCDA environment but may also be used in other alternative means of containment. Corry et al. (2003) environments and uses selective groups for *Campylobacter* selective medium: with blood (Agar Preston Agar, Agar, Skirrow was Butzler, Campy-cefex) and selective medium with charcoal (mCCDA agar agar, Karmali, CAT) plus various antibiotics: cephalosporins (usually cefoperazonă), combination of Vancomycin and trimethoprim but also some antimicrotice such as cicloheximida oramfoteracina B. The purpose of these add-ons for antimicrobial is to inhibit as much of the microflora of contamination and confined the species *Campylobacter jejuni* and *Campylobacter coli*.

In the case of our study, for the bacteriological examination were required:

1. Materials: culture media and reagents: broth medium: liquid Bolton broth Brucella, solid media: Agar agar modified (mCCD coal cefoperazonă deoxicolat; with agar blood agar Karmali; Colombia;
2. Oxidase reagent strips for:-1-4-Tetramethyl-Phenylenediamine dihidroclorică
 - hippurate hydrolysis reagent for detecting-sodium hipurat solution;
 - ninhydrin solution 3.5%-mixture of ninhydrin, acetone and butanol;
 - Indoxyl acetate-blend of indoxyl acetate and acetone impregnated in filter paper discs.
3. Sterile Petri plates and glassware: glass and plastic bottles, test tubes and pipettes, Erlanmayer, Pasteur pipettes, chance of sowing.
4. Microbiology lab-specific equipment: automatic adjustable pipetor anaerostat Cystocell, optical microscopes, microscope ML4 phase-contrast (required to observe mobility characteristic bacteria of the genus *Campylobacter*).
5. Bag systems with gas generators

Sampling cecums from broiler chickens was done in under the 'program of surveillance, prevention and control of animal diseases, those transmissible between animals and humans, animal protection and environmental protection' established by ANSVSA according to the EU rules into national law.



Fig.1. Cecums bird collected, washed and antiseptizate

Samples were collected at random for each lot of animals culled, 25 were taken from 25 cecumuri, broiler, chosen on the cutting line while abiding by the rules on traceability. Cecums taken must be intact, neîntepate and filled with content. Cecums are subject to antiseptisiei with alcohol followed by drying (Fig. 1.).

After cutting the apex, the intestinal content cecal junction is made using a Pasteur pipette, sterile. Carcasses from which the samples are taken from the cecumuri must not succeed in succession line cutting and sampling should be avoided in the first part of the sacrificed.

Results and Discussions

Campylobacter there are major food safety perspective when they colonize the digestive tract to a flock of birds, becoming a carrier. The possibility of contamination of the meat after slaughtering the birds represent a risk to human health. Because of these risks, imposed biosecurity measures to prevent the introduction of species of *Campylobacter* in poultry or halls to avoid dissemination of these microorganisms in the environment or to other flocks.

Growth medium is always slow, the colonies being observed after a long incubation. agar Karmali, after a 48-hour incubation, bacterial culture has had a development.

Typical of *Campylobacter jejuni* colonies had a flattened appearance, with irregular edges, with shades of grey, sometimes with metallic luster and that invades as a surface film geloses. Smears made from typical colonies on selective, were stained by Gram's method. In the optical microscope examination, observed gram-negativ incubation, easy-looking bracket or a comma with dimensions of 1-2 μm .

To identify the species of *Campylobacter jejuni* and *Campylobacter coli* differentiation has been made the test of hipuratului hydrolysis of Na as campilobacterii strains analyzed, who left positive test hipuratului Na hydrolysis have the species was *Campylobacter jejuni* were negative and the strains of *Campylobacter coli*.

As a result of microbiological analyses have identified 23 (92%) of samples positive for *Campylobacter*. The intestinal content of positive cecums have been isolated from 40%) 10 (strains of *Campylobacter coli*, a number of 8 samples (32%) strains of *Campylobacter jejuni* and 5 samples (20%) strains of *Campylobacter lari* (Table 1.).

Table 1. The presence of *Campylobacter* in broilers cecums

Investigated samples	Positive samples		Isolated strains															
			<i>Campylobacter coli</i>		<i>Campylobacter jejuni</i>		<i>Campylobacter lari</i>											
25	Nr.	%	Nr.	%	Nr.	%	Nr.	%										
	3	12	2	6.7	9	33.3	1	3.3	0	0	4	13.3	8	26.7	3	10	5	20

Of the 25 samples of carcasses collected from slaughterhouse and subjected to investigation in 20 samples detected *Campylobacter* spp. and 5 samples were free of campilobacterii.

Campylobacter coli was detected in 13 samples subjected to investigation in 6 samples detected *Campylobacter jejuni* in a single sample. detected *Campylobacter lari*.

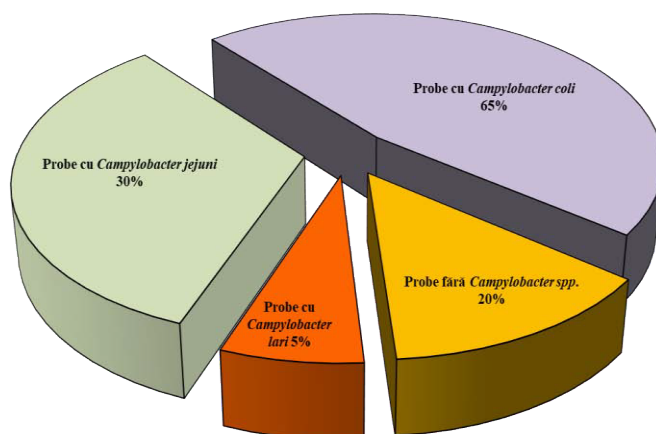


Fig.2. The share occupied by *Campylobacter* spp. in the 25 samples analysed

Salmonellosis in recent years have been the main cause of food toxiifections, statistically it has been complicated by the evolution of campylobacteriosis, particularly those of the genus *Campylobacter jejuni* and *Campylobacter coli*, responsible for more than 8 million cases annually in the EU to the people. Food Standards Agency (F.S.A.) of the United Kingdom ordered the imposition of control over trade in the commercial network with possible infected birds as well as the publication of the results of supervision, but along with campylobacteriozelor companies poultry supply agency (5.6).

Some studies show that a percentage of 4% to 13% of positive cases occur after packing, what allows to assume that there is a cross-contamination in both process and the finished product handling by workers. From the data collected could ascertain that infection season has a greater frequency in the summer months.

The leading manufacturers of broiler tries to improve biosecurity measures during natural movements finished products processing or by vaccination. Research at the University of Liverpool have shown that vaccinating poultry can be done, just that the immune response does not develop fast enough, the slaughter at about 6 weeks. Another way to reduce bowel colonisation was the use of essential oils, probiotics or bacteriophages.

So far there have been made targets of reducing his *Campylobacter*, unlike *Salmonella*, which thanks to the EU's objectives of reducing prevalence and national plans was a clear trend of decrease in the number of cases in the U. S (6, 7).

If it has them in consideration both sanitation solutions chemical ban after evisceration and that consumer marketing of frozen birds represent a rather low percentage in relation to fresh or chilled meat, it can be said that it will It remains a problem for the population campylobacteriosis.

Conclusion

1. Microbiological investigations were conducted in accordance with the guidance provided in the 'program of surveillance, prevention and control of animal diseases, those transmissible between animals and humans, animal protection and environmental protection' established by ANSVSA and on the isolation and identification of *Campylobacter* in broilers.
2. The results of the cecums confirms that the birds are carrying campylobacterii constituting a significant source of contamination during evisceration the carcasses and heighten the risk of toxiifections in humans food following handling and consumption of contaminated meat and insufficiently processed.
3. The largest share is taken by the *Campylobacter coli* samples analyzed, harvested both from cecums level and at the level of animal carcasses.
4. In view of this trend, the European Union shall prepare a draft regulation to amend the legislation will come into force for control of *Campylobacter*.
5. This regulation establishes the european project also worth over contamination requiring corrective action to maintain hygiene, in accordance with the law of food (20% of chicken carcasses with *Campylobacter* ≥ 1000 cfu/g, for 2025).

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