Prevalence of thermotolerant *Campylobacter* in broiler flocks and broiler carcasses in Italy

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Summary

In accordance with European Union regulations, from 5 February until 15 December 2008, sampling and analysis activities were conducted in Italy to assess the extent of contamination caused by thermotolerant *Campylobacter* in broiler chickens farmed nationwide. The survey involved 48 poultry slaughterhouses distributed across eleven regions of Italy, where the caeca and carcasses of 393 slaughter batches were sampled. A total of 284 batches (72.3%) gave positive results for *Campylobacter* spp. as follows: 52.1% were contaminated by *C. jejuni*, 55.6% by *C. coli* and 1.1% by *C. lari*. *C. jejuni* and *C. coli* were isolated together in 37 batches (13% of positive results). *Campylobacter* spp. was isolated only from the caeca in 251 slaughter batches (63.9%) including caecal isolates of *C. jejuni* (48.2%), *C. coli* (50.6%), and *C. lari* (1.2%). Carcasses from 182 batches (46.3%) were contaminated by *C. jejuni* in 40.7% of cases, *C. coli* in 57.7% and the absence of *C. lari* from all batches examined. The contamination level observed in the carcasses ranged between 10 and 1.6×10^7 cfu/g.

Keywords

Broiler, Caeca, *Campylobacter*, Carcass, Italy, Prevalence.

Introduction

Campylobacter spp. is recognised as the principal agent of gastroenteritis in humans. In the United States, approximately 5.2 million cases of food poisoning are recorded each year, of which 2.5 million are attributed to thermotolerant *Campylobacter* (2).

The data collected in the 2007 zoonoses report of the European Food Safety Authority (EFSA) indicate that *Campylobacter* spp., with 200 507 human cases in 23 member states, is the most important source of food poisoning in Europe and the trend has increased over the past five years (9).

Chicken meat has proved to be the most common source of infection. The mean prevalence of *Campylobacter* spp. infection in this product is 26% (range from 0% to 86.5%). In broiler flocks, the mean prevalence of *Campylobacter* spp. infection is 25.2% (range 0% to 82.8%) (9).

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In 2006, EFSA reported the presence of *Campylobacter* spp. isolates from poultry which were resistant to antimicrobials, especially fluoroquinolones (94% isolates resistant to ciprofloxacin) (7).

With Directive 2003/99/EC (3), the European Union (EU) intended to improve the monitoring of zoonoses and zoonotic agents, with the aim of obtaining reliable and comparable data on their spread, as well as on resistance to antimicrobial drugs and on foodborne outbreaks. The EU, with Regulation 2160/2003 (4), has also required that member states implement control programmes every three years. Data will be used to define the microbiological criteria that are not currently included in Regulation 2073/2005/EC (5).

The absence of common surveillance monitoring protocols and sampling criteria among member states, as well as the absence of common analytical techniques for the isolation of micro-organisms between laboratories, meant that a comparison of data available was not possible.

To obtain reliable data on the prevalence of *Campylobacter* spp. in broilers, the European Commission (EC), in Decision 2007/516/EC, funded the first harmonised monitoring programme designed both to assess the prevalence and levels of *Campylobacter* spp. contamination in carcasses collected at slaughterhouses and to define the pattern of antimicrobial resistance.

The present study provides the results of laboratory investigations conducted by the national veterinary laboratories (*Istituto Zooprofilattico Sperimentale*: IZS) on national collections of carcasses and caeca samples from broiler flocks.

Materials and methods

Sampling

On the basis of sampling criteria defined in Decision 516/2007/EC (6), a sample of 384 slaughter batches was assigned to each member state.

Italy allocated to each region and autonomous province a number of batches proportional to the volume of animals slaughtered in 2005 in 118 poultry slaughterhouses, on the basis of data obtained from the Italian National Statistics Institute (*Istituto nazionale di statistica è un ente di ricerca pubblico*: ISTAT) (10). Each region and autonomous province programmed the sampling activities over 12 one-month periods. In each period, nearly 1/12th of all batches allocated was sampled, proportional to the number of animal groups slaughtered from each type of holding (conventional, free-range, organic). In compliance with the batch distribution scheme, the slaughterhouse, sampling date and slaughter batch were selected at random. When sampling was not proportional to the type of holding, samples were taken every month, selecting at random the slaughterhouse, date of sampling and slaughter batch.

The health status of the rearing facility in regard to contamination by *Campylobacter* spp. or *Salmonella* spp., if known at the time of slaughter, did not influence the choice of samples which was performed according to the criteria established.

From 10 animals selected from each slaughter batch, the visceral organs were removed to obtain the caeca and one carcass.

The selection of the 10 visceral organs from the animals of the same slaughter batch was performed as follows: three organs were sampled after the slaughter of the 25% of the animals, four were sampled after the slaughter of 50% and three after 75%.

The carcass, instead, was sampled from the overhead conveyor after the slaughter of 50% of the animals in the batch.

To ensure that the activities were conducted in a uniform and controlled manner, two operational instructions were issued. The first was written to establish the responsibilities of the agencies involved, as well as to describe the sampling criteria, sampling methods and criteria for the transportation of samples to the laboratory.

The second instruction issued was to regulate the operations connected with the admission and preparation of samples in the laboratory.

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The samples were collected and sent to the official laboratory within 24 h, together with an accompanying form which provided the relevant information to identify the site of slaughter, type of farming practice, date of collection and delivery to the laboratory and the temperature of samples.

Analysis of samples and test methods

Campylobacter spp. detection in caeca was performed in accordance with the method established by the community reference laboratory.

The protocol stipulated the provisions for the sampling of caeca and for the direct inoculum on selective media. The steps described below were performed in conformity with ISO standard 10272-1:2006.

Detection and enumeration of *Campylobacter* spp. in the carcasses were conducted in line with ISO standard 10272:2006 (Parts 1 and 2). The investigations were initiated within 80 h from sample collection.

The isolates underwent a first speciation at the IZS which performed the isolation (biochemical and/or molecular) and the results were then confirmed by the national reference laboratory by polymerase chain reaction (19).

Statistical analysis

Logistic regression was used to determine the association between the following:

- flock size and prevalence of *Campylobacter* spp. contamination
- slaughter capacity of the slaughterhouse and levels of contamination in batches and samples of caecal content
- slaughter capacity of the slaughterhouse and positive results to *Campylobacter* spp. in carcasses.

Information system for data management

An information system for the online management of zoonoses data collected from the monitoring plan was created. The system was published on the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'* (Istituto G. Caporale) website (zoonosi.izs.it/pls/izs_zoo/zoo_gestmenu.zoo_index).

The IZS units involved in the analysis activities, the Italian regions and autonomous provinces and the Ministry of Labour, Health and Social Policies, were authorised to access the information system using a password which provided different permissions depending on the level of responsibility and geographic jurisdiction of the user. The IZS units entered the data to be reported in the sampling form into the information system using the upload procedure and were authorised to view only the data pertaining to them. The Ministry and the national reference laboratory were authorised to view and download all data entered into the information system.

The information system also offered free access for the consultation of EU and national legislation, procedures for management activities in the field and in the laboratory, procedures for sampling, the possibility to print the sampling form and to view the latest version of the 'Data dictionary', as well as the exclusion criteria supplied by EFSA.

The summary reports of sampling activities performed, sorted by region, were published by the information system every month.

Results

The sampling activities were performed from 5 February to 15 December 2008. Caeca and carcasses were sampled from 393 slaughter batches (93.1% of the programmed batches) from 48 poultry slaughterhouses in nine regions of Italy. Two of the nine regions included in the programme did not collect any samples. On average, 35.7 samples were collected each month, with a minimum of 22 in February and a maximum of 47 in September (Table I).

The samples were collected from groups of animals that originated from conventional breeding (98.3%), free-range (1%) and organic (0.2%) establishments.

A batch was considered positive when *Campylobacter* spp. was isolated from caeca and/or the carcass.

Overall, 284 batches (72.3%) gave positive results for *Campylobacter* spp. The agent was isolated from caeca in 251 slaughter batches (63.9%) and from carcasses in 182 batches (46.3%). The frequency of contamination of the caecal content and carcasses in the regions of Italy and autonomous provinces is reported in detail in Tables II and III. Significant differences were observed in the rate of contamination between different regions,

Table I
Monthly distribution of sampling activities (number of sampled batches) in different regions of Italy in 2008

Region	No. of batches sampled	January	February	March	April	May	June	July	August	September	October	November	December	Slaughter-houses involved	No. of batches sampled	Percentage of samples collected*
Veneto	168	0	13	15	15	13	15	12	10	14	17	15	12	15	151	89.9%
Emilia-Romagna	93	0	5	9	12	10	10	10	10	10	10	10	4	5	100	107.5%
Lombardy	55	0	4	5	6	3	4	8	3	5	4	3	3	9	48	87.3%
Marches	33	0	0	0	4	6	2	2	2	6	4	5	2	4	33	100.0%
Abruzzo	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0%
Molise	16	0	0	0	0	1	1	4	0	4	3	2	1	1	16	100.0%
Piedmont	15	0	0	0	2	2	3	1	1	3	2	1	0	9	15	100.0%
Campania	6	0	0	1	1	1	0	1	0	1	0	0	0	2	5	83.3%
Sicily	4	0	0	0	0	0	0	4	4	4	4	6	2	2	24	600.0%
Tuscany	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0%
Sardinia	1	0	0	1	0	0	0	0	0	0	0	0	0	1	1	100.0%
Total	422	0	22	31	40	36	35	42	30	47	44	42	24	48	393	93.1%

* percentage of number of samples out of the total required by the survey

Table II
Prevalence of *Campylobacter* spp. contamination in caecal contents of broiler chickens sampled at the slaughterhouses in different regions of Italy

Region	No. of caeca	Total *Campylobacter* isolated		*C. jejuni*		*C. coli*		Other specie	
		No. positive (%)	Confidence interval (lc-up)	No. positive	Positive (%)	No. positive	Positive (%)	No. positive	Positive (%)
Veneto	151	118 (78.1)	70.9-84.0	58	49.2	64	54.2	1	0.8
Emilia Romagna	100	53 (53.0)	43.3-62.5	21	39.6	28	52.8	4	7.5
Lombardy	48	27 (56.3)	42.2-69.3	13	48.1	13	48.1	5	18.5
Marche	33	28 (84.8)	68.9-93.2	15	53.6	14	50.0	0	0.0
Abruzzo	–	–	–	–	–	–	–	–	–
Molise	16	1 (6.3)	1.5-28.7	1	100.0	0.0	0.0	0	0.0
Piedmont	15	9 (60.0)	35.4-80.2	3	33.3	5	55.6	3	33.3
Campania	5	5 (100.0)	54.1-99.6	2	40.0	3	60.0	0	0.0
Sicily	24	10 (41.7)	24.4-61.3	8	80.0	0	0.0	2	20.0
Tuscany	–	–	–	–	–	–	–	–	–
Sardinia	1	0	0.0	0	0.0	0	0.0	0	0.0
Total	393	251 (63.9)	59.0-68.5	121	48.2	127	50.6	15	6.0

lc lower confidence limit 95%
up upper confidence limit 95%
Totals in blue: averages

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Table III
Prevalence of *Campylobacter* spp. contamination in broiler chicken carcasses sampled at the slaughterhouses in different regions of Italy

Region	No. of carcasses	Total *Campylobacter* spp. isolated		*C. jejuni*		*C. coli*		Other species	
		No. positive (%)	Confidence interval (lc-up)	No. positive	Positive (%)	No. positive	Positive (%)	No. positive	Positive (%)
Veneto	151	108 (71.5)	63.8-78.1	40	37.0	70	64.8	0	0.0
Emilia Romagna	100	28 (28.0)	20.1-37.5	12	46.4	13	46.4	2	7.1
Lombardy	48	9 (18.8)	10.2-32	2	22.2	4	44.4	3	33.3
Marches	33	25 (75.8)	58.8-87.1	12	48.0	17	68.0	0	0.0
Abruzzo	–	–	–	–	–	–	–	–	–
Molise	16	1 (6.3)	1.5-28.7	0	0.0	1	100.0	0	0.0
Piedmont	15	0	0	0	0.0	0	0.0	0	0.0
Campania	5	0	0	0	0.0	0	0.0	0	0.0
Sicily	24	11 (45.8)	27.8-65.1	7	63.6	0	0.0	4	36.4
Tuscany	–	–	–	–	–	–	–	–	–
Sardinia	1	0	0.0	0	0.0	0	0.0	0	0.0
Total	393	182 (46.3)	41.4-51.3	74	40.7	105	57.7	9	4.9

lc lower confidence limit 95%
up upper confidence limit 95%
Totals in blue: averages

namely: for the caecal content, the values ranged from 6.3% in Molise to 100% in Campania, while for carcasses, the range varied from 6.3% in Molise to 75.8% in the Marche region.

A total of 97.4% of batches from conventional breeding farms gave positive results, while the batches from free-range and organic farms were all positive.

The trend of *Campylobacter* spp., *Campylobacter jejuni* and *C. coli* prevalence during the period under study is presented in Figures 1 and 2.

The contaminated carcasses show a variable concentration of *Campylobacter* spp. In 147 out of the 182 positive samples, the levels of contamination ranged between 10 and 1.6×10^7 cfu/g, with 29.9% of samples revealing values between 1 and 100 cfu/g, and 59.9% of samples having values between 101 and 10 000 cfu/g. Table IV shows the classes of frequencies considered for each level of contamination of *Campylobacter* spp. The remaining 35 samples were positive to the qualitative test but were negative to the quantitative test, with a level of contamination that varied between 0.04 and 10 cfu/g. A total of 48.3% of sampled batches were collected in slaughterhouses with a capacity of between 1 000 000 and 4 999 999: the larger number of positive batches was found in this class and the upper classes (Fig. 3). The association between flock size and prevalence of

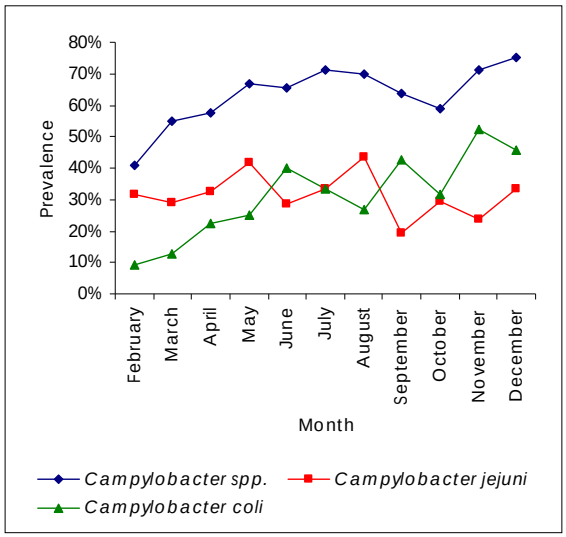


Figure 1
Prevalence of *Campylobacter* spp., *C. jejuni* and *C. coli* in the caecal contents of broiler chickens in Italy in 2008

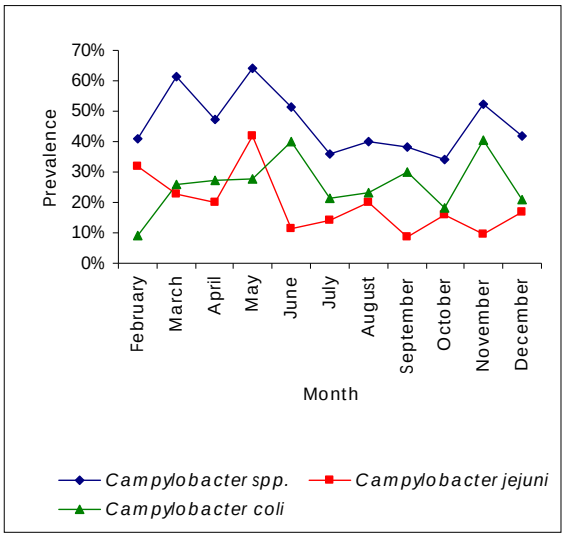


Figure 2
Prevalence of *Campylobacter* spp., *C. jejuni* and *C. coli* in broiler carcasses in Italy in 2008

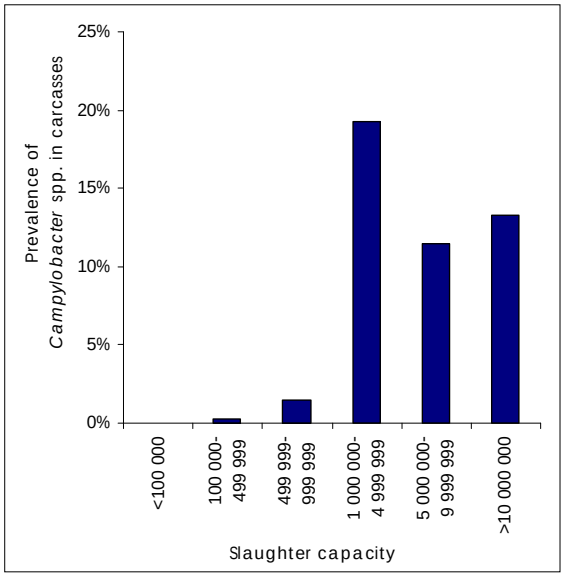


Figure 3
Slaughter capacity and *Campylobacter* spp.-positive carcasses in different batches

contamination from *Campylobacter* spp., *C. jejuni* and *C. coli* in the batches examined was analysed through logistic regression and no association of statistical significance was evidenced. In the same test, no association was found for any statistically significant correlation between the slaughter capacity and the levels of contamination from *Campylobacter* spp. in the batches and in samples of caecal content.

Table IV
Frequencies of classes of *Campylobacter* spp. contamination ranges in chicken carcasses

Classes of contamination ranges (cfu/g)	Total no. of samples	Frequency Percentage of positive samples	Cumulative percentage
1-100	44	29.9	29.9
101-1 000	58	39.5	69.4
1 001-10 000	30	20.4	89.8
10 000-1 000 000	10	6.8	96.6
100 001-1 000 000	4	2.7	99.3
>1 000 000	1	0.7	100.0

However, a statistically significant correlation (chi-square = 6.2, $p<0.01$) was observed through logistic regression between slaughter capacity and positive results to *Campylobacter* spp. in carcasses (Fig. 3).

Overall, *C. jejuni* was isolated in 148 slaughter batches (52.1% of the positive results), *C. coli* in 158 batches (55.6% of the positive results), *C. lari* in 3 batches (1.1% of the positive results). *Campylobacter* spp. isolated from 20 batches (7.0% of the positive ones) belonged to other strains or were not identified. In 37 batches (13.0% of the positives), *C. jejuni* and *C. coli* were isolated together (Table V).

In regard to caeca, *C. jejuni* was isolated in 121 slaughter batches (48.2% of the positives), *C. coli* in 127 batches (50.6% of the positive results) and *C. lari* in 3 batches (1.2% of the positives) (Table V). Different species of *Campylobacter* were isolated in 12 batches at the same time, namely: *C. jejuni* and *C. coli* in eight batches, *C. coli* and *C. lari* in two batches, *C. jejuni* and one strain of unidentified *Campylobacter* spp. in two batches.

From carcasses, *C. jejuni* was isolated in 74 batches (40.7% of the positives), *C. coli* in 105 batches (57.7% of the positives) and *C. lari* was never isolated (Table V). *C. jejuni* and *C. coli* were isolated in six batches (3.3% of the positives) at the same time.

The analysis of contamination frequency of *Campylobacter* spp. in caecal content during

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different months of the year revealed a statistically significant difference in February only; at this time, isolation was significantly lower compared to the trend of the year (p<0.05). A similar trend was recorded in February and March for *C. coli* (p<0.05). For *C. jejuni*, statistically significant differences during the period under study were not identified (p>0.05).

In carcasses, the frequency of contamination of *Campylobacter* spp. and *C. coli* were not statistically significant in the period under consideration (p>0.05); *C. jejuni* was isolated more frequently in May (p<0.05).

Discussion

The sampling schedule described in this study was the first investigation performed on a national scale with the aim of assessing the level of contamination of *Campylobacter* spp. in broiler flocks at slaughterhouses. The high rate of positive results observed confirm *Campylobacter* spp. as the most widespread zoonotic agent in broiler farms in Italy (72.3%) and in Europe (71.2%). In same survey, European prevalence for *Salmonella enteritidis/S. Typhimurium* was 9.25% and *Salmonella* spp. was 11.1% (8). Data related to the contamination of carcasses observed during this investigation confirm similar rates obtained by other studies (46.3%) that were performed in Italy (13, 14).

Despite different contamination frequencies observed in different regions of Italy, it was not possible to identify the causes as the plan was not designed with this specific objective.

Furthermore, no statistically significant association resulted between contamination frequency of lots and caecal content and farm size, slaughter capacity and age of slaughtered animals.

Most positive batches were found in establishments with a slaughter capacity of class 1 000 000 to 4 999 999 and in the upper classes.

The increasing frequency of *Campylobacter* spp. contamination in carcasses in proportion to the higher slaughter capacity, already described in literature (1, 18), could be related to the occurrence of cross-contamination, which increases with higher numbers of slaughtered animals.

During evisceration, the high concentrations of micro-organisms in the crop, colon and faeces of infected animals may lead to contamination of carcasses and the environment through both water and equipment (12, 15, 18), with the subsequent contamination of slaughter groups.

This cross-contamination between animals of the same group and among different groups is also difficult to control because of the speed of processing and slaughter.

Risk analysis has shown that a 2 log reduction in the level of *Campylobacter* spp. contamination of carcasses would result in a 30-fold reduction of human campylobacteriosis cases and that mechanisms of control applied at slaughterhouse level would be more effective than those applied on farms (11, 16, 17).

Table V
Results of isolation and identification of *Campylobacter* spp. in slaughter batches and in batches of carcasses and sampled caeca

Samples	No. examined	*Campylobacter* spp. isolation		*Campylobacter* spp.		*Campylobacter* spp. identification			
		Negative	Positive (%)	Negative	Positive (%)	*C. jejuni* (%)	*C. coli* (%)	*C. lari* (%)	Other species (%)
Slaughter* batches	393	109	284 (72.3)	–	–	148 (52.1)	158 (55.6)	3 (1.1)	20 (7.0)
Caeca	393	142	251 (63.9)	–	–	121 (48.2)	127 (50.6)	3 (1.2)	12 (4.8
Carcasses	393	211	182 (46.3)	246	147 (59.7)	74 (40.7)	105 (57.7)	0 (0.0)	9 (4.9)

* a batch was considered isolation-positive when *Campylobacter* spp. was isolated from the caecal contents and/or carcass

In relation to the species isolated, the frequency of *C. jejuni* contamination observed in caecal content (48.2%) and in carcasses (40.7%) was similar to the average contamination frequency (42.2% and 46.2% respectively) recorded in Europe (9). *C. coli* was the species most frequently present, both in caecal contents and in carcasses (57.7% and 50.6%, respectively). In Europe, similar results have been observed in Austria and Spain (9).

Conclusions

The risk factors involved in *Campylobacter* spp. infection in broilers, as well as the identification of the ecological niches that the micro-organisms utilise for survival, are not fully understood.

Sanitary measures currently applied therefore appear not to be able to fully and effectively reduce the levels of prevalence on farms.

The reduction of the prevalence of contamination in chicken meat may be obtained only by intervening in the primary phase of production and through sanitary measures designed to reduce cross-contamination during slaughter and processing.

The possibility of reducing human campylobacteriosis is dependent on proper management practices and on hygiene and biosecurity measures that are able to prevent cross contamination of raw meat products in processing plants, as well as on good hygiene practices in handling and preparing food, and of course adequate cooking of food.

Knowledge of the epidemiology of *Campylobacter* infection in animals needs to be better understood in regard to the identification and quantification of risk factors associated with the stage of breeding, transport, slaughter and processing of carcasses.

The adoption of molecular biology techniques may help to provide key information to assess epidemiological dynamics and to identify the reservoirs of infection and sources of contamination.

According to the regulation of the hygiene package, food security guarantees should be ensured by the operators of food establishments which, through systems such as hazard analysis critical control points (HACCP), should define safety criteria to manage the control of the quality of hygiene of production processes.

For food products, microbiological criteria stipulated in EC Regulation 2073/2005 provide the acceptability limits that the operators of food establishments should monitor to ensure that the management, hygiene and bio-security measures implemented are effective (5).

The absence of these criteria for some important agents of zoonoses, such as *Campylobacter* spp., can thereby represent a significant limit to the efficacy of the safety systems implemented by operators of food establishments and to the surveillance performed by the official bodies, both of which are essential to reduce the prevalence of zoonotic agents in products intended for the consumer.

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