The prevalence of thermotolerant *Campylobacter* spp. in broiler caeca and their resistance to antibiotics

Jan Bardoň

National Reference Laboratory for Campylobacter
State Veterinary Institute
Olomouc, Czech Republic

Abstract

Campylobacteriosis is one of the most frequent alimentary infections in humans in Europe. The numbers of reported infections have exceeded those of salmonellosis for several years. These infections are caused by thermotolerant species of the genus *Campylobacter*, particularly *Campylobacter jejuni* and *Campylobacter coli*. The main sources of *Campylobacter* infections in humans are poultry and poultry meat. The State Veterinary Administration of the Czech Republic has been monitoring the prevalence of *Campylobacter* spp. in selected commodities since 2006 as a part of its zoonosis surveillance. Data collection and assessment and tests for antibiotic resistance are performed by the National Reference Laboratory for Campylobacter of the State Veterinary Institute in Olomouc. In 2012, the prevalence of thermotolerant *Campylobacter* spp. in broiler caeca in the Czech Republic reached 60%. The isolates show high levels of resistance to quinolone antibiotics.

Poultry, Campylobacter spp., alimentary infection, resistance of bacteria to antibiotics

Introduction

Together with bacteria of the genus *Salmonella*, bacteria of the genus *Campylobacter* account for a significant proportion of bacterial alimentary diseases in humans in the Czech Republic and Europe. The main sources of *Campylobacter* infections are foodstuffs and raw materials of animal origin, particularly poultry. Campylobacter infections have been gaining prominence since 2007, and are presently the most frequent cause of bacterial alimentary infections in humans. In 2012, the Czech Republic reported 18 387 cases of such infections, which indicates their relatively stable prevalence as compared with 2011, when 18 811 cases were reported. The Czech Republic, with almost 200 infections reported per 100 000 head of population, ranks among the countries with the highest prevalence of campylobacteriosis in Europe (EFSA 2012; EPIDAT 2013). Comparison of the incidence of the disease among EU member states is, however, strongly influenced by the reporting systems and levels of infection monitoring in individual countries.

The prevalence of human campylobacteriosis in Europe is monitored by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC). A joint EFSA and ECDC document shows that the most frequent bacterial alimentary zoonoses in Europe are *Campylobacter* infections. In 2010, more than 212 000 confirmed cases were reported in the EU. The numbers of human *Campylobacter* infections in Europe have been gradually growing in the last 5 years (EFSA 2012).

The results of *Campylobacter* prevalence monitoring at poultry slaughterhouses in the Czech Republic conducted in 2006 and 2007 were published by Bardoň et al. (2009). Cloacal swabs from poultry to be slaughtered were collected and examined. Ten cloacal swabs were taken from each slaughter batch (one farm) and processed as a single pooled sample. A prevalence of thermotolerant *Campylobacter* spp. in slaughtered poultry of almost 50% was demonstrated in the Czech Republic in the period monitored. In 2006, *C. jejuni* was found in 46% and *C. coli* in 3% of samples examined. In 2007, *C. jejuni* was found in 43% and *C. coli* in 2% of samples.

Address for correspondence:
Doc. MVDr. Jan Bardoň, Ph.D., MBA
National Reference Laboratory for Campylobacter
State Veterinary Institute
Jakoubka ze Stříbra 1
779 00 Olomouc, Czech Republic

Phone: +420 585 557 111
E-mail: jbardon@svuol.cz
www.maso-international.cz

The first nation-wide study of *Campylobacter* prevalence in poultry at retail sale in the Czech Republic was conducted in 2009 (Bardoň et al. 2011). Samples of chilled and frozen broiler chickens were collected in supermarkets in eight largest Czech cities. The study showed high *Campylobacter* prevalence in chilled poultry (75%), but significantly lower *Campylobacter* prevalence in frozen poultry (37%). The predominant species was *C. jejuni*, which was detected in 70% of positive findings, followed by *C. coli* (18%). In 12% of cases, samples were contaminated with both *C. jejuni* and *C. coli*.

The highest *Campylobacter* prevalence in broilers in Europe is found in Slovenia (88% and 93% when caeca and neck skin were examined), Spain (82%) and the Czech Republic (72%). A traditionally low prevalence of *Campylobacter* spp. in poultry is reported in Norway (5%), Finland (6%), Sweden (13%) and Denmark (17%) (EFSA 2012).

The results of the monitoring of thermotolerant *Campylobacter* spp. in broiler chicken caeca at Czech slaughterhouses in 2012 are presented here. The study was coordinated by the National Reference Laboratory (NRL) for Campylobacter of the State Veterinary Institute (SVI) in Olomouc. The NRL also processed the data and tested the isolates for resistance to antibiotics.

Materials and Methods

Bacteriological examination of broiler caeca

A total of 125 pooled samples of broiler (*Gallus gallus*) caeca were taken at slaughterhouses in the Czech Republic and examined between January and December 2012 in compliance with Methodological Guidelines 1/2005 of the State Veterinary Administration of the Czech Republic as amended. The caecum is the predilection site for thermotolerant *Campylobacter* in the poultry digestive tract. Veterinary inspectors collected 10 caeca from 10 randomly selected broiler carcasses from each batch at slaughter using a sterile scalpel blade and a pair of tweezers. All 10 caeca were put in one plastic bag (=1 pooled sample), which was then closed and sent to the SVI laboratory for laboratory examination. The broilers were about 35 to 40 days old. The slaughterhouses for sample collection were chosen in such a way that they covered as much of the Czech Republic as possible. Samples were taken once a month throughout the calendar year to evaluate seasonal prevalence. Laboratory analyses were performed at the State Veterinary Institute laboratories in Olomouc (NRL), Jihlava and Prague.

Qualitative tests (detection of *Campylobacter*) were conducted in accordance with the Czech standard ČSN EN ISO 10272-1 (2006) currently in effect. In isolates sent from the SVI laboratories in Prague and Jihlava to the NRL, species identification was confirmed using the Biotyper Microflex MALDI TOF (Bruker Daltonics) biological analyser, the PCR method (Ertas et al. 2002; Lund et al. 2004), or a commercial kit for real-time PCR (Taq Man *Campylobacter* spp. Kit, AB Applied Biosystems). The *C. jejuni* reference strain ATCC 33560 was used for quality control.

Test of Campylobacter resistance to antibiotics

The resistance to selected antimicrobial agents in all confirmed *C. jejuni* and *C. coli* isolates was tested using the microdilution method (McDermott et al. 2005). Plates manufactured by Trios were used for testing (Fig. 1).

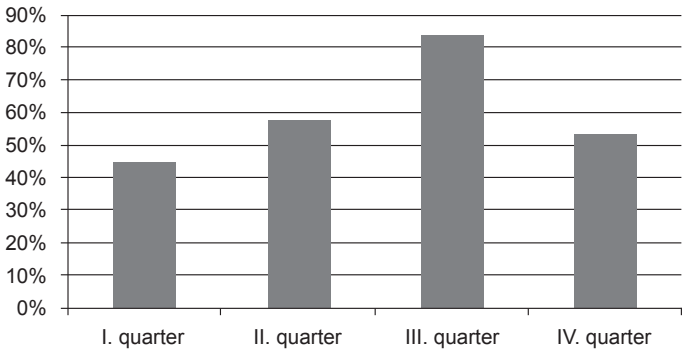

Fig. 1. Seasonal prevalence of *Campylobacter* spp.

Inoculated plates were incubated in a microaerophilic atmosphere (GENbox microaer – bioMérieux) for 48 hours at 37 °C. The resistance of isolates from all over the Czech Republic to specified antibiotics was tested in the NRL for Campylobacter at the SVI in Olomouc.

Resistance to 8 selected antibiotics, namely erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin, chloramphenicol, ampicillin and nalidixic acid, was tested. The resistance of *C. jejuni* and *C. coli* to these agents was interpreted in accordance with the values given in the recommendation of the European Reference Laboratory for Antimicrobial Resistance (EURL-AR 2011) and the 2005 report by the Comité de l'Antibiogramme de la Société Française de Microbiologie (Communique 2005). The above method was used to test a total of 39 isolates of *C. jejuni* and 17 isolates of *C. coli*. The *C. jejuni* reference strain ATCC 33560 was used as a quality control strain.

Results

The prevalence of Campylobacter spp. in broiler caeca

The results compiled by the NRL for 2012 indicate that the prevalence of thermotolerant *Campylobacter* spp. in the caeca of poultry in the Czech Republic was 60% in that year. A comparison with the results from previous years is given in Table 1.

Table 1. The prevalence of thermotolerant *Campylobacter* in the Czech Republic between 2006 and 2012 in the commodity samples monitored

Year	Material examined	Sampling location	No. of samples examined	Prevalence	References
2006	cloacal swabs of broilers	slaughterhouse	189	49%	Bardoň et al. (2009)
2007	cloacal swabs of broilers	slaughterhouse	246	45%	Bardoň et al. (2009)
2008	broiler caeca	slaughterhouse	422	61%	
	neck skin of broilers	slaughterhouse	422	70%	
2009	neck skin – chilled poultry	supermarket	120	75%	Bardoň et al. (2011)
	neck skin – frozen poultry	supermarket	120	37%	
2010	broiler caeca	slaughterhouse	134	72%	
2011	broiler caeca	slaughterhouse	145	63%	
2012	broiler caeca	slaughterhouse	125	60%	

Over the long term, the clearly dominant species among poultry in the Czech Republic is *C. jejuni* (71%). Two species, i.e. *C. jejuni* and *C. coli*, were found in 5% of positive cases.

The data in Fig. 1, which shows the seasonal prevalence of *Campylobacter* spp. in broiler caeca, indicate that the prevalence was higher in the second half of the year (and the third quarter, in particular).

Campylobacter resistance to antibiotics

Data on *C. coli* and *C. jejuni* resistance to individual antibiotics in 2012 is given in Fig. 2, which clearly demonstrates the differences in resistance to antibiotics between *C. coli* (higher resistance) and *C. jejuni*. *Campylobacter* isolates from poultry show an extremely high resistance rate to quinolone antibiotics (ciprofloxacin, nalidixic acid) and ampicillin.

Discussion

The prevalence of thermotolerant *Campylobacter* spp. in poultry for slaughter in the Czech Republic between 2006 and 2007 monitored by means of cloacal swabs taken from broilers at slaughterhouses was around 50% in individual flocks (Bardoň et al. 2009). Assessing nation-wide trends in the disease situation in poultry for slaughter in the Czech

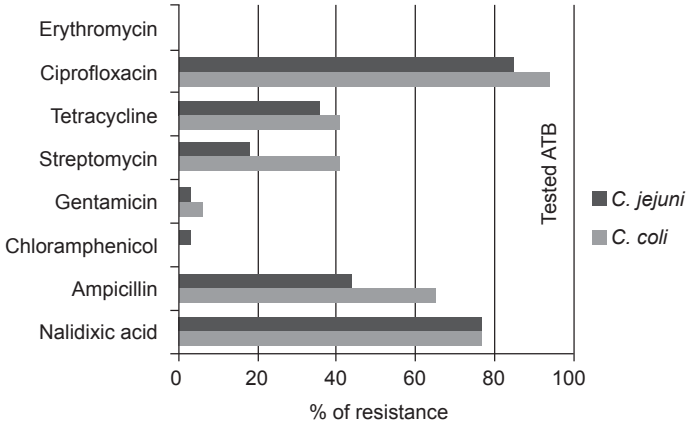

Fig. 2. Resistance of *Campylobacter* spp. to selected ATB

Republic is problematic as there are no relevant literary data on prevalence rates gathered by nation-wide monitoring in previous periods. Steinhauserová (1998) published results of ileum and liver parenchyma examinations of chickens for slaughter on selected farms in the years 1990–1991, when immunity rates of up to 63% were found. Such incidence rates correlate with our data. It is a well-known fact that the *Campylobacter* detection rate in a direct intestinal examination is higher than that detected by a cloacal swab examination.

The Czech Republic (along with Slovenia and Spain) ranks among the countries in Europe with the highest prevalence of *Campylobacter* spp. in poultry for slaughter according to EFSA data from 2010. No data exist about *Campylobacter* prevalence in broilers in England in 2010, though the incidence in that country in 2009 was 77.5%. The average prevalence in Europe in 2010 was 18.2% (reports were submitted by only 8 member states) (EFSA 2012). In view of big differences in *Campylobacter* prevalence in poultry monitored in individual countries and differences in data collection methods, neither this average nor comparisons of prevalence between individual states are objective. This is also true of comparisons of infection rates between human populations, with the Czech Republic being the country with the highest prevalence of human campylobacteriosis in the EU (EFSA 2012). The "top ranking" of the Czech Republic testifies, rather, to the high standard of infection detection and reporting methods capable of capturing a higher percentage of the disease in the human population.

Secondary contamination of broiler carcass surfaces that occurs at slaughterhouses is negatively reflected in the higher rate of positive findings in poultry at supermarkets. Bardoň et al. (2011) demonstrated 75% *Campylobacter* contamination in chilled poultry at retail in the Czech Republic.

The most frequently identified species in poultry for slaughter in the Czech Republic is *C. jejuni*. The situation is similar in the majority of EU countries (EFSA 2012). Dual contamination in individual samples by *C. jejuni* and *C. coli* was identified in 5% of positive samples. Dual contamination is manifested by a mixed culture on solid growth media. In this case, species identification by the faster MALDI TOF method fails and the PCR method identifying both species in a single sample must be used. From the epidemiologic viewpoint, it must be borne in mind that dual contamination of foods may expose the consumer to the risk of dual infection which will have a more severe clinical manifestation and be more difficult to treat with antibiotics.

Campylobacter isolates from poultry at slaughterhouses are highly resistant to quinolone antibiotics (ciprofloxacin and nalidixic acid). One of the reasons for this may be the selection pressure of fluoroquinolones, which are widely used on poultry farms. It is clear that placing this group among the "restricted use antibiotics" is merely declaratory in nature, with no real effect on the use of these antibitiotics in practice.

Conclusions

The prevalence of thermotolerant *Campylobacter* in poultry for slaughter in the Czech Republic is relatively high (60%) and impacts the unfavourable infection situation in the human population. *Campylobacter* prevalence monitored in the caeca of poultry at slaughterhouses showed a non-significant decrease from 63% in 2011 to 60% in 2012. *C. jejuni* was predominant among the strains isolated (71% positive isolates).

Campylobacter prevalence in the human food chain will require further monitoring. It would also be useful to monitor it in the young of companion animals (kittens, puppies), which, along with raw materials of animal origin, are referred to as a possible source of infection, particularly in children.

Resistance to antibiotics is on the increase. This is a global problem, and greater attention will have to be paid to it in veterinary medicine not only with respect to *Campylobacter* spp.

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