Prevalence of thermophilic *Campylobacter* spp. in slaughtered pigs in the Czech Republic, 2001–2003

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ABSTRACT: The prevalence of thermophilic *Campylobacter* spp. was evaluated in the caecum and on carcasses of pigs at slaughter and in the facilities of slaughterhouses in the period of 2001– 2003. During that timeframe, prevalence of *Campylobacter* spp. in both the pigs and the environment of slaughterhouses decreased. In 2001, *Campylobacter* spp. were detected in 34% of 316 samples; in 2002 there were 27% of positive findings out of the 624 samples; and in 2003, *Campylobacter* spp. were detected in 16% out of 300 samples. *Campylobacter* spp. were mostly found primarily in the caecum (292 isolates) and in smears collected from carcasses (21 isolates), while *Campylobacter* spp. were isolated only sporadically from the work surfaces of equipment in slaughterhouses. The majority of isolates were identified as *C. coli*. In 2001, 16 out of 109 strains of *Campylobacter* spp. were identified as *C. jejuni*; in 2002, 8 out of 167 strains were *C. jejuni*; and in 2003, none of 47 isolates was identified as *C. jejuni*.

Keywords: *Campylobacter jejuni*; *Campylobacter coli*; slaughter; pigs

Infections caused by thermophilic *Campylobacter* spp. in man are quite common. These microorganisms together with *Salmonella* spp. are the most common causes of diseases originating from food in the Czech Republic (Steinhauserova and Nebola, 2002). Over the last few years the number of cases of *Campylobacter* infections per year has increased, approaching the number of cases caused by *Salmonella* spp. The frequent collection of samples from patients and the improved diagnostics of pathogens may also be among the factors contributing to the increased number of reported cases. On the other hand, the routes of infection transmission to humans have not been completely explained. Farm animals may play a major role in the transmission of the disease since their intestinal system contains *Campylobacter* spp. The most important sources of these microorganisms are swine and poultry. According to some authors (Harvey et al., 1999; Moore and Madden, 2001; Moore et al., 2002) the prevalence rates of *Campylobacter* spp. may vary significantly between pig farms. Similarly, the two most common species – *C. coli* and *C. jejuni* may also be detected in pigs at different rates. Those authors generally concluded that *C. coli* was found primarily in pigs while *C. jejuni* was more dominant in poultry. Because the prevalence rates of *Campylobacter* in pigs in our country are unknown, the purpose of this study is to determine the prevalence of thermophilic *Campylobacter* in pigs at slaughter in the Czech Republic, 2001–2003.

MATERIAL AND METHODS

The prevalence of thermophilic *Campylobacter* spp. was evaluated in the caecum and on carcasses of pigs at slaughter and in the facilities of slaughterhouses in the period of 2001–2003. Samples were collected from the pigs which were reared under

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commercial conditions of recognized animal husbandry, originated from different farms and were slaughtered in different slaughterhouses in the Czech Republic.

The samples were collected as swabs from the caecum, the surface of carcasses (rectal region), and from different locations within slaughterhouses such as scalding tank, dehairing machine, conveyor-belts, meat saw and cutter. The samples were immediately transported to the laborator and cultivated the same day in accordance with the standard CSN ISO 10272. Identification of species was carried out by using the following biochemical tests: oxidase test, catalase detection test, test on hippurate hydrolysis, tests of the sensitivity to nalidixic acid and cephalotine and the test on hydrogen sulphate production on the TSI medium. Furthermore, growth tests at 30°C and 37°C were performed. The specific identification and differentiation of thermophilic *Campylobacter* spp. were performed by using polymerase chain reaction in combination with the analysis of restriction fragment-length polymorphism (PCR/RFLP). The polymorphic region of the 23S rRNA gene was amplified to yield the PCR product 491 bp long. The primers used (T1 5´-TATTCCAATACCAACATTAGT-3´ and T4 5´-CTTCGCTAATGCTAACCC-3´) are specific for thermophilic *Campylobacter* spp. while the amplification of other *Campylobacter* spp. or other bacteria does not occur. Subsequent cleavage with the restriction endonuclease *Alu*I gave unique combinations of fragments for *C. jejuni* and *C. lari,* respectively. Digestion with a second enzyme, *Tsp*509I, was used for differentiation of *C. coli* and *C. upsaliensis* (Fermer and Engvall, 1999).

RESULTS AND DISCUSSION

In 2001, 316 samples that originated from the ceacum, carcasses, and from different locations and equipment within the slaughterhouses were examined. *Campylobacter* spp. were detected in 109 (34%) of the samples. *Campylobacter* spp. were mostly found in the caecum (86 isolates) and in swabs collected from the surface of carcasses (17 isolates) while *Campylobacter* spp. were isolated only sporadically from various locations within slaughterhouses. Three were detected on the surface of conveyor-belts, two in dehairing machines and one on the surface of the cutter. No *Campylobacter* spp. was found in the scalding tank, on the surface of the meat saw or in the processing line railing (Table 1).

On the basis of biochemical testing and PCR analysis, 93 out of the 109 isolates were *C. coli* (85%) and 16 were *C. jejuni* (15%). Other thermophilic strains *Campylobacter* spp. were not found. The occurrence rates differed in individual farms and ranged between 15–50%. Of those isolates, the prevalence of *C. coli* in the caecum of pigs varied from 10–96% (data not shown).

In 2002, 624 samples were taken during 12 sample collections from pigs reared at different farms and slaughtered in different slaughterhouses. *Campylobacter* spp. were found in 167 samples (27%), 159 (53%) isolates originated in the caecum (Table 2). Four isolates came from the surface of the carcasses, three isolates were detected in the dehairing machine and one isolate was found on the conveyer-belt used in the storage of the intestines. Cultures of the scalding tank, the surfaces of

Table 1. The occurrence of *Campylobacter* spp. in slaughtered pigs and slaughterhouses in year 2001

	No. of samples	No. of strains *Campylobacter* spp.		
		Campylobacter spp. (%)	*C. coli*	*C. jejuni*
Scalding tank	15	0	0	0
Dehairing machine	18	2 (11)	2	0
Conveyor belts	20	3 (15)	3	0
Saw	6	0	0	0
Cutter	4	1 (25)	0	1
Handrail	2	0	0	0
Caecum	157	86 (55)	71	15
Carcasses	94	17 (18)	17	0
Total	316	109 (34)	93	16

Table 2. The occurrence of *Campylobacter* spp. in slaughtered pigs and slaughterhouses in year 2002

	No. of samples	No. of strains *Campylobacter* spp.		
		Campylobacter spp. (%)	*C. coli*	*C. jejuni*
Scalding tank	36	0	0	0
Dehairing machine	36	3 (8)	3	0
Conveyor belts	36	1 (3)	1	0
Saw	12	0	0	0
Cutter	12	0	0	0
Handrail	12	0	0	0
Caecum	300	159 (53)	153	6
Carcasses	180	4 (2)	3	1
Total	624	167 (27)	158 (95)	8 (5)

Table 3. The occurrence of *Campylobacter* spp. in slaughtered pigs and slaughterhouses in year 2003

	No. of samples	No. of strains *Campylobacter* spp.		
		Campylobacter spp. (%)	*C. coli*	*C. jejuni*
Scalding tank	18	0	0	0
Dehairing machine	18	0	0	0
Conveyor belts	18	0	0	0
Saw	6	0	0	0
Cutter	6	0	0	0
Handrail	6	0	0	0
Caecum	138	47 (16)	47	0
Carcasses	90	0	0	0
Total	300	47 (16)	47	0

the meat saw and cutter and the handrail proved negative.

C. coli represented 95% (158/167) of the total isolates while *C. jejuni* represented 5% (8 isolates). No other thermophilic strains of *Campylobacter* spp. were detected. The majority of *C. jejuni* (6) were isolated from the caecum of pigs and only one was found on the surface of the carcasses. The prevalence of *Campylobacter* in pigs varied in individual farms and usually ranged between 20–30%, whereas the percentage of *C. coli* isolated from the caecum varied from 2–50% (data not shown).

In 2003, 300 samples were obtained during 6 sample collection carried out in slaughterhouses from pigs reared at different farms and slaughtered in different slaughterhouses. *Campylobacter* spp. were detected in 47 cases (16%) and 100% were *C. coli* isolated from the caecum (Table 3). *Campylobacter* spp. were not detected on the surface of carcasses or on the work surfaces of slaughterhouses. The on-farm prevalence in pigs usually ranged between 20–30% (data not shown).

In our study, the prevalence of *Campylobacter* in pigs at slaughter is lower than that reported in the literature. Weijtens et al. (2000) reported that 22–98% of fecal samples from fattening pigs in the Netherlands were positive for *Campylobacter.* A similar study from the USA reported that 70–100% of pigs at slaughter (depending on the farm and the date samples were collected) were positive for *Campylobacter* (Harvey et al., 1999). Canadian authors Guevremont et al. (2004) reported that

Campylobacter were recovered from 78% of swine caecal contents at the abattoir. *Campylobacter coli* represented 96% of *Campylobacter* isolates in that study. Avrain et al. (2004) examined the prevalence of *Campylobacter* spp. in pigs slaughtered in France. After direct isolation 54% of faecal samples were positive for *Campylobacter* and of those isolates 98% were identified as *C. coli.* Similarly, the majority of isolates in our study were *C. coli.*

In 2001, 16 out of 109 isolates of *Campylobacter* were identified as *C. jejuni* (15%); in 2002 only 8 out of 167 (5%) and in 2003 none of 47 isolates was identified as *C. jejuni*. The comparison of the results obtained during the period of 2001–2003 shows that prevalence rates for *Campylobacter* spp. in both the pigs and the environment of slaughterhouses have decreased. In 2001, *Campylobacter* spp. were detected in 34% of 316 samples; in 2002 there were 27% of positive findings out of 624 samples; and in 2003, *Campylobacter* spp. were detected in 16% of 300 samples collected. One optimistic finding is that the incidence rate of *Campylobacter* spp. on the surface of carcasses has decreased. In 2001 there were 17 positive findings (18%); in 2002 only 4 samples (2%) and in 2003, *Campylobacter* cultures were negative. Similar findings also apply to samples collected from the environment of slaughterhouses: in 2001, 6 *Campylobacter* isolates were detected in dehairing machines, conveyorbelts, cutters and other facilities, while in 2002 only 4 were found in analogous samples, and in 2003 no *Campylobacter* spp. were detected. In 2001 and 2002, isolations from the caecum amounted to 55% and 53% respectively, while in 2003 the positive finding of *Campylobacter* spp. comprised 16%.

It is unknown why *Campylobacter* isolations from pigs decreased between 2001–2003. Perhaps on-farm hygiene and biosecurity have improved. The decrease in isolations from slaughterhouse equipment surfaces could possibly signify improved sanitation procedures throughout all the processing plants. On the basis of reduced prevalence of *Campylobacter* in pigs and slaughterhouses during this timeframe, we propose that the risk for *Campylobacter*-associated foodborne illness from pork probably was also reduced.

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