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Occurrence of Thermotolerant *Campylobacter* spp. on Eggshells: a Missing Link for Food-Borne Infections?[▽]

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We analyzed the prevalence of thermotolerant *Campylobacter* spp. compared that of to *Salmonella* spp. in raw yolk and on eggshells. A total of 2,710 eggs were investigated for each bacterium. Viable bacteria were found in 4.1% (*Campylobacter* spp.) and 1.1% (*Salmonella* spp.) of the eggshell samples, whereas the egg yolk samples were negative for both bacteria.

Thermotolerant *Campylobacter* spp. are one of the most important causes of food-borne disease in Germany. In 2008, the European Food Safety Authority (EFSA) registered 190,566 cases of human campylobacteriosis from 24 countries and 131,468 cases of human salmonellosis from 28 European countries (4). During the last 5 years, the number of *Salmonella* infections in Europe continuously and significantly decreased, while the number of *Campylobacter* cases remained on a high level. One important reservoir of thermotolerant *Campylobacter* spp. is poultry, mainly broilers and laying hens (4). Therefore, chicken meat is one source of food-borne campylobacteriosis, either directly by eating undercooked chicken or via cross-contamination of other ready-to-eat products. In comparison to the knowledge about the contamination rate of chicken meat, there are only a few data available about the prevalence of thermotolerant *Campylobacter* spp. on the eggshell or in the egg content itself. This transmission route is well described for *Salmonella* spp. but not for thermotolerant *Campylobacter* spp. Therefore, the aim of our study was to collect data about the prevalence of thermotolerant *Campylobacter* spp. in raw yolk and in parallel on the eggshells. These data can be used for a risk assessment concerning possible human food-borne campylobacteriosis via cross-contamination of ready-to-eat food or via eating undercooked eggs or food produced with raw egg.

During the years 2009 and 2010, 2,710 eggs were investigated for the presence of thermotolerant *Campylobacter* spp. and the same number of eggs was investigated for the presence of *Salmonella* spp. by using cultural and molecular methods. For the surveillance program, 271 samples with two egg packages per sample, each containing 10 eggs, were taken from the retail level, mostly from self-service supermarkets and shops. Forty-five percent of the eggs originated from laying hens kept in deep litter (barn eggs), 18% were labeled as free-range eggs, 21% came from hens kept in battery cages, and 16% were taken unsorted directly from farms. Ten eggs were pooled and investigated for the presence of thermotolerant *Campylobacter* spp. and 10 eggs for the presence of *Salmonella* spp. The eggs were cracked aseptically by using a sterile spoon to crack the eggshell on one end, and then the shell was broken by hand (using sterile gloves) in two parts to separate eggshell and egg content. The egg yolk was separated from the egg white by using a sterile syringe. Then, the eggshell and the raw egg yolk were investigated separately. For the detection of thermotolerant *Campylobacter* spp., both parts of the sample were transferred to 225 ml of Preston selective bouillon (OXOID, Germany) and incubated at 42°C under microaerobic conditions with 9.0% CO_2 in a microaerobic cabinet. After 48 h, screening for the presence of *Campylobacter* spp. was done with the commercial VIDAS enzyme-linked fluorescence immunoassay (ELFA) kit (bioMérieux, France); samples screened as positive were filtered through a 0.65-μm filter (Sartorius, Germany) and subcultured on blood agar (Merck, Germany) without any additives. After an incubation of 48 h at 42°C under microaerobic conditions with 9.0% CO_2, the colonies were confirmed and differentiated with a real-time PCR system described previously (9). The detection of *Salmonella* spp. was done in accordance with ISO 6579:2002 (5).

Thermotolerant *Campylobacter* spp. were isolated from 11 (4.1%) eggshell samples but not from any of the egg yolk samples. Eight isolates were confirmed as *Campylobacter coli* and three isolates as *Campylobacter jejuni*. Four positive samples were sold as free-range eggs and seven positive samples as barn eggs. *Salmonella* Enteritidis was found in three eggshell samples (1.1%) but not in any of the egg yolk samples. Two positive samples originated from hens kept in battery cages and one sample from hens kept in deep litter.

In its annual zoonotic report for the year 2008, EFSA reported data about the prevalence of this human pathogen in fresh meat, meat products, milk, and dairy products but not data concerning eggs or egg products (4). Adesiyun et al. found a contamination rate of 1.1% for thermotolerant *Campylobacter* spp. in the egg content of table eggs in Trinidad (1). In the study of Jones and Musgrove, 0.5% of the restricted shells eggs investigated were positive for thermotolerant *Campylobacter* spp. (6), and Sato and Sashihara (11) found in their study that between 27.9 and 36% of unpasteurized liquid egg samples were positive. This very high contamination rate could result from contamination during the production process, especially when the eggs are cracked. Therefore, one contaminated egg could be enough to contaminate a complete lot of unpasteur-

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ized liquid egg. The results of our study show that *Campylobacter* spp. can be found regularly on the eggshells of table eggs. The isolation rate in our study was much higher than the prevalence of thermotolerant *Campylobacter* spp. in bovine meat (0.3%), pig meat (0.5%), milk (2.3%), and milk products (1.2%) reported by EFSA for the year 2008 (4). The contamination rate was also found to be much higher (4.1%) than the contamination rate of eggshells for *Salmonella* spp. investigated in the same period (1.1%). Therefore, the contamination rate concerning *Salmonella* spp. is similar to isolation rates described in other studies (1, 2) and in the annual zoonosis report of the EFSA (4). Unlike Adesiyun et al. (1), we found thermotolerant *Campylobacter* spp. only on the eggshell and not in the egg content. But like the situation with broiler meat, it can be expected that the risk for food-borne human campylobacteriosis is much higher via cross-contamination of ready-to-eat products than via the consumption of raw or undercooked poultry meat (3, 7, 8). Like the situation with *Salmonella* spp., it can be presumed that contamination of the egg content is based mostly on transmission of bacteria from the eggshell to the egg content during the cracking of the eggshell. Outside of laboratory conditions, e.g., in a normal kitchen during cooking, it is nearly impossible to crack eggs aseptically. Therefore, a contaminated eggshell always creates the risk of cross-contaminating the egg yolk with pathogens and of initiating food-borne infections by producing ready-to-eat food with raw or undercooked egg content. The other possibility is cross-contamination from the eggshell to other ready-to-eat products which do not contain the egg content itself. During the cooking or production process, the risk of cross-contamination, described very well for raw poultry meat, can be excluded only by special and very strict hygienic management. Therefore, contamination of the eggshell should be much more important for a human food-borne infection than the presumed very low contamination rate and short viability of thermotolerant *Campylobacter* spp. (10). The collected data can give only initial evidence of the possible *Campylobacter* prevalence of eggs at the retail level. In the next years, more studies and data are necessary for a well-founded risk assessment concerning the role of eggs and egg products in the infective chain of human campylobacteriosis.

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