Baseline Survey on the Prevalence of *Campylobacter* in Broiler Intestines and on Broiler Carcasses in Israel

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ABSTRACT

A prevalence survey was conducted to establish baseline and comparable data on the prevalence of *Campylobacter* spp. in broiler intestines and on broiler carcasses in Israel. Fifty five slaughter batches were sampled over a 14-month period from January 2015 until February 2016. *Campylobacter* spp. were detected in the caeca samples from 49 (89.1%, 95% CI (Confidence Interval) 80.9-97.3) of the 55 slaughter batches and on 35 (63.6%, 95% CI 50.9-76.4) of the 55 carcasses. The sampled farms represent 7.6% of the broiler farms in Israel. Of the *Campylobacter* isolates from both caeca and carcass samples, 40% were identified as *C. coli*, 33% as *C. jejuni* and 27% as both *C. coli* and *C. jejuni*. In order to reduce the incidence rate of campylobacteriosis in the human population, mitigation strategies must focus on reduction of the *Campylobacter* spp. concentration in primary production and contamination reduction during processing.

Keywords: Campylobacter; Broiler; Survey; Israel.

INTRODUCTION

Campylobacter spp. is one of the major causes of bacterial gastroenteritis in humans worldwide (1-3). The incidence of campylobacteriosis has increased over the last 10 years in both developed and developing countries causing a significant disease burden (1, 2). Poultry is an important reservoir and source of human campylobacteriosis (2). Domestic poultry are frequently infected with *Campylobacter*, primarily *Campylobacter jejuni* and *Campylobacter coli* (4). *Campylobacter* is rarely detected in young broilers less than 2-3 weeks of age under commercial production conditions (4, 5). Once a broiler flock is infected with *Campylobacter*, the infection spreads rapidly throughout the flock and the bacteria colonize the gastrointestinal tract especially the ceca, in great numbers (5, 6). This eventually results in contaminated carcasses during processing, which can transmit this pathogen to humans (6).

Campylobacteriosis in humans is a notifiable disease in Israel. The incidence rate of campylobacteriosis in Israel has increased according to both the passive and the active surveillance systems. The national passive surveillance system of the Department of Epidemiology at the Israeli Ministry of Health reported an increase in the annual incidence rate during 1999-2010 from 31.04 to 90.99 cases/100,000 population (7). The active surveillance system of the Israeli Center for Disease Control is a sentinel laboratory based surveillance network established in 1997. The active surveillance system that monitor 47.9% of the total Israeli population, reported an increase from 65.7 in 1999 to 101.7 cases/100,000 population in 2012 (8). During these years there was an approximately 40% increase in poultry meat consumption in Israel. In 2015 poultry meat, which accounts for 71% of the total meat consumption in Israel, was 58 kilograms of ready to cook meat/capita – the highest among the OECD countries (9). Important trend that may have contributed to the increase in campylobacteriosis incidence is the poultry meat sales transition from mainly frozen to mainly chilled products during the last two decades (7).

According to the European Food Safety Authority and the European Center for Disease Prevention and Control 2014 report, campylobacteriosis is was the most commonly reported zoonosis with an increase in confirmed human cases in the European Union (EU) since 2008. The EU notification rate was 71.0 per 100,000 population in 2014 (10).

In Israel, the ratio between *C. jejuni* and *C. coli* isolates from humans in 2014 was 3.4 (11). A sample of human source *C. jejuni* and *C. coli* isolates are routinely tested for antibiotic resistance in the reference laboratory at the Israeli central *Campylobacter* laboratory. The 2014 report of the Israeli Ministry of Health presents a disturbing trend of increased *Campylobacter* resistance to antibiotics. In 2014 93% of the *C. jejuni* isolates and 98% of the *C. coli* isolates were resistant to ciprofloxacin (quinolones), 53% and 94% respectively were resistant to tetracycline, and 1% and 8% respectively were resistant to erythromycin (macrolides) (11).

A European Union-wide baseline survey on *Campylobacter* in broiler batches and on broiler carcasses was carried out in 2008. *Campylobacter* was detected in pooled caecal contents of broilers and on broiler carcasses in all participating countries. At Community level the prevalence of *Campylobacter*-colonized broiler batches was 71.2% and that of *Campylobacter*-contaminated broiler carcasses was 75.8%. The Member State prevalence varied from 2.0% to 100.0% and from 4.9% to 100.0%, for caecal contents and carcasses, respectively (12).

A study published in 1986, examined Israeli broiler flocks from hatching to slaughter. Twelve out of 16 flocks (75%, 95% CI 54-96%) were positive for *Campylobacter* spp. at slaughter. Out of 146 isolates in this study, 84% were *C. jejuni* and 16% *C. coli*. In a positive flock the majority of birds (~80%) were infected (5).

The objective of the survey was to determine the current prevalence of *Campylobacter* spp. in the caeca and on the carcasses of broiler slaughter batches in Israel by sampling over a 12-month period.

MATERIALS AND METHODS

Sampling plan

Thirty four poultry slaughterhouses that operated under the supervision of the Israeli Veterinary services in 2015 were studied. The sampling plan was that each slaughterhouse would be sampled twice in a 12-month period (total of 68 slaughter batches). The sampling was distributed randomly and evenly throughout the year (5-6 slaughter batch per month). With expected prevalence of 75% and confidence level of 95%, sample size of 68 slaughter batches would give a prevalence estimate with an accuracy of 90%.

Sample collection

Sample collection was done by the veterinarians of the Poultry Health Laboratories of the Israeli Egg and Poultry Board. The samplers used disposable gowns and gloves. Using sterile scissors and tweezers, 10 pairs of intact and full caeca were collected at random at evisceration from one slaughter batch. The caeca were placed in two sterile containers and the containers were put into a plastic bag. A single carcass with neck skin was collected at random from the same slaughter batch. The carcass was collected post-chilling and pre-processing. The carcass was placed in a thick plastic bag. Samples were transported to the laboratories with minimum delay in two separate chilled boxes, one for the caeca and one for the carcass. The sampler received from the slaughterhouse the details of the slaughter batch farm.

Microbiological methods

The culture of caeca and carcass samples for *Campylobacter* spp. was performed by the two Poultry Health Laboratories of the Egg and Poultry Board. The samples were tested within 80 hours of collection. All lab procedures were carried out in a biological hood. The methods used for the detection of *Campylobacter* spp. in the samples were in accordance with the European Union Commission Decision 2007/516/EC and ISO 10272-1:2006.

Caeca – *campylobacter* detection

Ten caeca per slaughter batch were placed on a sterile tray and their outer surface was disinfected. The caeca were opened with sterile scissors and tweezers and a sample of the content of each caeca was streaked directly onto two selective solid media: one blood-containing medium (PD063, Hy Labs, Israel) and one blood-free charcoal medium (PD077, Hy Labs, Israel). The samples were dispersed on the plates using quad loop sphere. Cultures were incubated in a microaerobic atmosphere (84% N2/10% CO2/6% O2) at 41.5±1°C for 44±4 hours. Positive and negative controls were tested on each day of testing. One colony from each positive caeca culture was stained by Gram stain and examined under a microscope for typical *Campylobacter* cell morphology. Two

isolates from each positive sample were sent for *Campylobacter* spp .typing at the Israeli central *Campylobacter* laboratory.

Carcass – *Campylobacter* detection

27 g of neck skin was removed aseptically from the carcass using sterile instruments and added to 243 ml buffered peptone. After 3 minutes of vigorous shaking, 10 ml of the carcass suspension was inoculated into 90 ml of Campylobacter enrichment broth (BP399, Hy Labs, Israel) and incubated microaerobically at 36.0±1˚C for 4-6 hours followed by 41.5±1˚C for 44±4 hours. Samples of broth culture were then plated onto selective media as described for caeca culture above.

RESULTS

A total of 55 samples were collected over 14-month period from January 2015 to February 2016. No sampling was done during December 2015 and January 2016 due to the workload in the bacteriology departments of the Poultry Health Laboratories. December 2015 sampling was postponed to February 2016. 15 samples were carried out during months January to March 2015, 11 during April to-June 2015, 14 during July-September 2015 and 15 during October 2015 to February 2016.

The desirable sample size of 68 slaughter batches was not met. Only 29 of the 34 slaughterhouses in the plan participated in the survey. Each slaughterhouse was sampled 1-3 times (6 slaughterhouses were sampled once, 20 twice, and 3 three times). Of the five slaughterhouses that did not participate in the survey, one was closed, one slaughterhouse did not cooperate, and 3 were small slaughter houses which worked mainly at night or slaughtered only turkeys at the time of the planned sampling date.

Prevalence of *Campylobacter*

The 55 slaughter batches sampled in the survey came from 53 broiler farms. The sampled farms represent 7.6% of the broiler farms in Israel. Fig. 1 shows the distribution of broiler farms in Israel and the location of the sampled farms.

Campylobacter spp. were detected in the caeca samples from 49 (89.1%, 95% CI (Confidence Interval) 80.9-97.3) of the 55 slaughter batches and on 35 (63.6%, 95% CI 50.9-76.4) of the 55 carcasses. Two flocks were sampled twice in different slaughterhouses. One of the flocks came out negative in both caeca and carcass samples from both of the slaughterhouses. The other came out positive in caeca samples from both of the slaughterhouses and positive in one of the slaughterhouses for the carcass samples. The 6 slaughter

Figure 1: The distribution of broiler farms in Israel and the location of the farms sampled in the study (the sampling was done in the slaughterhouse).

Table 1: detection of *Campylobacter* spp. in slaughter batches, in caeca and broiler carcasses.

		Carcass		Total
		Positive	Negative	
Caeca	Positive	35	14	49
	Negative	0	6	6
	Total	35	20	55

batches that came out negative in the caeca samples were also negative in the carcasses samples (Table 1). Fourteen (29%) of the 49 caeca-positive batches were negative in the corresponding carcass sample. Of the *Campylobacter* isolates from both caeca and carcass samples, 40% were identified as *C. coli*, 33% as *C. jejuni* and 27% as both *C. coli* and *C. jejuni*.

DISCUSSION

In order to establish baseline and comparable data, a prevalence survey was conducted. The prevalence of *Campylobacter* on broiler carcasses in the survey was 63.6%. The 95% confidence interval for the prevalence estimate was 50.9-76.4%. This prevalence is similar to the European Union community level (12). The fact that all caeca-negative batches were also carcass-negative is encouraging in terms of cross-contamination between slaughter batches. Still, a lot has to be done to reduce the contamination of carcasses from the intestinal content.

Since the elimination of *Campylobacter* spp. from poultry production seems very difficult currently, mitigation strategies must focus on reduction of the *Campylobacter* spp. concentration in primary production and further contamination reduction during processing. No single measure is sufficient for the reduction of *Campylobacter* concentration, but a combination of measures in both production levels is requied (13, 14).

The distribution of *Campylobacter* spp. in the survey was different from the distribution of isolates from human source and previous data from poultry (5). The ratio between *C. jejuni* and *C. coli* isolates in the survey was 0.9. This finding is different from that expected. The *Campylobacter* isolates were not tested for antibiotic resistance. Since the majority of infections in humans are attributed to poultry (2) we assume that the data from human isolates reflects the rate of antibiotic resistance in poultry.

The sample size was too small to evaluate seasonality, geographical effects or risk factors. We recommend performing a larger quantitative survey that will allow us to determine the level of contamination on broiler carcasses. In a quantitative survey done in the UK, 27.3% of the broiler carcasses were found to be highly contaminated with *Campylobacter* (≥1,000 c.f.u./g) (15). This information is important for the evaluation of mitigation measures. Additional data on the prevalence of *Campylobacter* spp. in other livestock in Israel, especially cattle, is needed in order to operate effectively to reduce the incidence rate of campylobacteriosis in the human population.

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