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Prevalence of Campylobacteria in the Finnish Broiler Chicken Chain from the Producer to the Consumer

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Aho, M. and J. Hirn: Prevalence of campylobacteria in the Finnish broiler chicken chain from the producer to the consumer. Acta vet. scand. 1988, 29, 451–462. – The prevalence of *Campylobacter jejuni* is 1.7 % (9/600) in the faeces of 4–5 week broiler chickens in Finland and 24 % (117/490) in the caeci of broiler chickens at slaughter. All waste waters at a processing plant, except water in a chlorinated (25 ppm) chilling tank, contained campylobacteria when a campylobacter positive flock was slaughtered. Caeci contained mean $\log_{10}$ 7.2 CFU campylobacteria/g. After chilling in a chlorinated ice-water tank there were still mean $\log_{10}$ 4.5 CFU campylobacteria/carcass. Campylobacteria were detected from 7.0 % (14/199) of deep-frozen broiler chicken carcasses at the market level. The concentration of *C. jejuni* in naturally contaminated deep-frozen broiler chicken carcasses decreased by 2 $\log_{10}$ units in 4 weeks.
All prevalence figures were lower than in other developed countries outside Scandinavia. In Finland one of the reasons for low prevalence may be the extensive use of Nurmi cultures in *Salmonella* prevention programs.

food chain; contamination; food hygiene.

Introduction

The importance of *Campylobacter jejuni* and *C. coli* as common causes of human gastrointestinal disease is well documented. *King* (1962) proposed chickens as the primary source of human infection and *Skirrow* (1977) demonstrated an association in some cases between human disease and contact with chickens harboring the organism at farms, in butcher shops and in home kitchens. This association was later documented in several epidemiological studies (*Severin* 1978, *Moulton et al.* 1982, *Harris* 1986b). There are also several reports of outbreaks in which the epidemiologically implicated or suspected vehicle of campylobacteriosis has been raw, barbecued or undercooked chicken (*Hayek & Cruickshank* 1977, *Istre et al.* 1984, *Rosenfield et al.* 1985). These outbreaks were with few exceptions of family size and without severe consequences. Only few epidemics among poultry abattoir workers have been reported (*Christenson et al.* 1983, *Soto et al.* 1986). In Finland *Pönka et al.* (1984) reported in an epidemiological study that 43 % of 524 campylobacter enteritis outpatients during the years 1978–81 had contacted domestic campylobacteria, 59 % of the patients had had contact with an animal and 28 % had eaten poultry meat within a week before illness.
The prevalence of campylobacteria in chickens at slaughter has been examined in several studies (Table 1) and appears to be considerably lower in Scandinavia than in other developed countries. Previous studies (*Geni-*

Table 1. Prevalence of campylobacteria in chickens at slaughter in different studies.

Region Country	Positive/ all samples	%	Type of sample	Reference
Scandinavia				
Finland	10/100	10 %	caecal contents	*Hänninen & Raevuori* 1981
Sweden	18/50	36 %	faeces	*Svedhem & Kaijser* 1981
	6/100	6 %	faeces	
Denmark	223/396	56 %	caecal contents	*Jørgensen* 1982
Norway	10/100[a]	10 %	pooled faeces	*Rosef & Kapperud* 1982
Sweden	8/16[a]	50 %	pooled caecal contents	*Engvall et al.* 1986
Other developed countries				
U.K.	114/117	68 %	caecal contents	*Bruce et al.* 1977
Italy	100/100	100 %	intestinal contents	*Comi et al.* 1984
F.R.G.	98/120	82 %	caecal contents	*Altmeyer et al.* 1985
The Netherlands	80/93	86 %	faeces	*Bänffer* 1985
U.S.A.	117/247	47 %	intestinal contents	*Harris et al.* 1986a
Developing countries				
South Africa	26/30	87 %	faeces	*Richardson & Koornhof* 1979
Brazil	108/168	64 %	faeces	*Levi & Ricciardi* 1982
Zaire	14/36	38 %	intestinal contents	*Damme & Lauwers* 1983
Chile	110/200	55 %	faeces	*Figueroa et al.* 1983
	25/26	96 %	cloacal swab	
Peru	136/160	85 %	cloacal swab	*Grados et al.* 1983

[a] positive flocks/all flocks examined

georgis et al. 1986, *Hoop & Ehrsam* 1987) indicate that prevalence of campylobacteria in chicken increases with age. Some flocks may remain for long uninfected, but when the infection begins it spreads rapidly throughout the whole flock. *Doyle* (1984) suspected that climatic change may be one reason for increased isolation of *C. jejuni* from the feaces of laying hens. Vertical transmission is unlike to occur; *Lindblom et al.* (1986) found that environmental samples from the broiler chicken grow-out houses were negative, and only very few samples were positive at a time when most birds were campylobacter positive. By contrast *Montrose et al.* (1985) succeeded in infecting specific pathogen free (SPF) chickens by maintaining them on artificially contaminated litter. House flies have also been shown to be carriers of campylobacteria (*Rosef & Kapperud* 1983, *Wright* 1983) and *Shane et al.* (1985) succeeded in infecting SPF chickens by allowing contaminated house flies to transmit campylobacteria. *Shanker et al.* (1986) found that the role of broiler chicken eggs in the transmission of *C. jejuni* to the grow-out flocks is minimal.

Variation between the results of studies concerning the prevalence of campylobacteria in broiler chickens sold fresh (Table 2) or deep-frozen (Table 3) is large, but again the results from Scandinavia appear to be at a lower level than in other developed countries. Although only a few quantitative studies are available, there is general agreement that fresh chicken carcasses may contain enough campylobacteria to cause infection in humans.

Table 2. Prevalence of campylobacteria on the surface and in the meat of fresh broiler chickens in different studies.

Region Country	Source	Positive/ all samples	%	Type of sample	Reference
Scandinavia					
Denmark	P[a]	76/224	34 %	neck	*Jørgensen* 1982
Norway	P	48/348	14 %	swab surface	*Rosef et al.* 1984
	P	151/313	48 %	swab surface	
Other developed countries					
Australia	P	18/40	45 %	carcass	*Shanker et al.* 1982
U.K.	M[b]	30/38	88 %	surface	*Dawkins et al.* 1984
F.R.G.	P	26/30	87 %	liver	*Altmeyer et al.* 1985
	P	0/50		meat	
	P	145/180	81 %	surface	
Israel	M	53/127	42 %	breast, heart, neck, wing	*Rogol et al.* 1985
	P	39/51	77 %	mixed parts	
U.S.A.	M	192/862	22 %	carcass	*Harris et al.* 1986a
Switzerland	P	206/660	31 %	skin	*Hoop & Ehrsam* 1987
Developing countries					
Brazil	P	179/227	79 %	carcass	*Levi & Ricciardi* 1982
Chile	P	21/25	84 %	surface	*Figueroa et al.* 1983

[a] sampling carried out at the processing plant
[b] sampling carried out at retail markets

Table 3. Prevalence of campylobacteria on the surface and in the meat of deep-frozen broiler chickens in different studies.

Region Country	Source	Positive/ all samples	%	Type of sample	Reference
Scandinavia					
Denmark	M[a]	9/23	39 %	melt-water	*Rosef & Bjorland* 1981
Sweden	M	6/10		part of carcass	*Svedhem et al.* 1981
Other developed countries					
The Netherlands	M		4 %	liver	*Hartog & Boer* 1982
	M		36 %	carcass	
Hungary	M	16/30	53 %	carcass	*Marjai et al.* 1982
U.K.	M	18/60	30 %	inside of carcass	*Dawkins et al.* 1984
	M	23/56	41 %	giblets	
	M	10/45	22 %	melt-water	
U.K. Scotland	M	177/198	89 %	giblets	*Fricker* 1984
U.S.A.	P[b]	6/40	15 %	liver	*Stern et al.* 1984
	P	0/40		deboned meat	

[a] sampling carried out at retail markets
[b] sampling carried out at the processing plant

During commercial processing intestinal bacteria contaminate the carcasses and widespread cross-contamination may take place (*Oosterom et al.* 1983). According to *Wempe et al.* (1983) the plucking machine and chilling tank were areas of major cross-contamination. *Luechtefeld & Wang* (1981) reported that 34 % of turkey carcasses which had been chilled in chlorinated (20–50 ppm) ice water were still positive for campylobacteria.

The present investigation was undertaken in 1985 to assess the prevalence of campylobacteria in the Finnish broiler chicken chain from the farm to the consumer and to make quantitative estimates of the contamination. Deep-frozen broiler chicken carcasses were chosen for the investigation because most broiler chicken carcasses are sold deep-frozen in Finland, although nowadays increasingly large numbers are sold fresh.

Materials and methods

Transport and culture media

SIFF medium for transport of faecal and caecal samples was prepared as described by *Sandven et al.* (1982). Transport media were portioned 10 ml in screw-capped plastic test tubes and autoclaved for 20 min at 120°C.

Skirrow broth and agar were prepared as described by *Skirrow* (1977) and *Blaser et al.* (1979) and modified by *Hänninen* (1981). The modification was addition 2 mg/l of amphotericin B (E. R. Squibb & Sons Ltd., Liverpool, U.K.) to the media.

Alkaline tryptose broth (ATB) and brucella agar (ATB-agar) were prepared as described by *Wesley et al.* (1983).

Broths were portioned 90 ml in 200 ml flasks and agars 25 ml on ∅ 9 cm Petri dishes with nodules. After inoculation, broths were incubated for 20 h and agars for 44 h at 43°C under a microaerophilic atmosphere achieved by evacuating gas boxes to −800 mbar and then filling them with a gas mixture containing 5 % O_2, 10 % CO_2 and 85 % N_2. Evacuation and filling was carried out twice. Campylobacteria were recognized by examinating wet mounts by microscopy for curved rods exhibiting darting motility, by catalase and oxidase tests and by biotyping as described by *Skirrow & Benjamin* (1980) with the modification of *Lior* (1984) to the H_2S test.

Prevalence of campylobacteria in 4–5 week broiler chickens

600 cloacal swabs were taken from 60 flocks at 55 different farms in southern Finland. Swabs were transferred immediately to SIFF medium for 1 d transport. In the laboratory, whole transport medium with the swab was enriched in Skirrow broth. Two loopfulls of incubated broth were plated on Skirrow agars.

Prevalence of campylobacteria in broiler chickens at slaughter

490 duplicate caecal swabs from 49 flocks grown out at 31 different farms in southern Finland were taken during the slaughtering process at 2 processing plants. Swabs were transferred immediately to SIFF medium for 1 d transport. In the laboratory 1 swab with whole transport medium was enriched in Skirrow broth and the other with whole transport medium in ATB. Two loopfulls of incubated broths were plated on Skirrow and on ATB agars, respectively.

Cross-contamination in the processing plant

Duplicate waste water samples (200 ml) from 9 points at a processing plant (scald tank, plucking machine, evisceration machine, washing after evisceration, removing of lungs, last washing before chilling, first screw chilling tank, second screw chilling tank and third chilling tank, chlorinated

with 25 ppm Cl_2), 5 whole caeci and 5 carcasses ready for packaging were taken when a campylobacter positive (proven at the age of 4–5 weeks) flock entered the processing plant. 0.2 ml of 10 % sodium thiosulfate was added to the water samples from the third screw chilling tank. The capacity of the processing plant was 5 500 birds/h. Slaughtering was at maximal operation during sampling. Sampling was repeated three times. After 3 h chilled transport to the laboratory, quantitative examination of both waste water and caecum samples was carried out on Skirrow agars. Samples were diluted in 0.1 % peptone water (BBL Microbiology Systems, Cockeysville, Md., U.S.A.) to give 10 fold dilutions. In addition, 1 and 10 ml of the waste water samples were enriched in Skirrow broth and after incubation 2 loopfulls of broth were plated on Skirrow agars.
Quantitative examination of campylobacter surface contamination was carried out from 3 carcasses (1 bird from each of 3 samplings) by rinsing and maceration. Whole carcasses were put into plastic bags and 225 ml 1 % buffered peptone water (BBL) was added. The carcasses were macerated for 5 min by hand. The rinsing liquids were diluted in 0.1 % peptone water (BBL) to give 10 fold dilutions and plated on Skirrow agar.

Survival of campylobacteria in naturally contaminated chicken carcasses
14 broiler chicken carcasses (4–5 from each sampling) were deep-frozen at –18°C and stored up to 9 weeks. Thawing was carried out in a refrigerator at 6°C for 20 h. Quantitative examination of campylobacteria on the surface of the carcasses was carried out weekly with the rinsing and maceration method as described above. In addition, 100 ml of the rinsing liquid was pre-enriched for 5 h at 43°C under a microaerophilic atmosphere. 10 ml of the pre-enriched liquid was enriched both in Skirrow broth and in ATB. After incubation, 2 loopfulls of the broths were plated both on Skirrow and on ATB-agar, respectively.

Prevalence of campylobacteria in deep-frozen market broiler chicken carcasses
199 deep-frozen broiler chickens slaughtered at 3 different processing plants were purchased from retail markets in Helsinki. Thawing of the carcasses was carried out as described above. Qualitative investigation of campylobacter surface contamination was carried out by the rinsing and maceration method. 100 ml of the rinsing liquid was pre-enriched for 5 h at 43°C under a microaerophilic atmosphere. 10 ml of the pre-enriched liquid was enriched and plated as described above.

Results

The prevalence of campylobacteria in faeces of 4–5 week broiler chickens was 1.7 % (9/600). Isolations were from 3 flocks (Fig. 1). 8 of the isolates were *C. jejuni* biotype 1 and 1 was *C. jejuni* biotype 2.
The prevalence of campylobacteria in caeci of broiler chickens at slaughter was 24 % (117/490) (Fig. 1). These isolates were from 13 flocks and 89 were *C. jejuni* biotype 1 and 28 *C. jejuni* biotype 2. 78 % were isolated with the ATB method and 85 % with the Skirrow method. Contaminants were frequently observed on Skirrow agar. All the examples of *C. jejuni* biotype 2 were isolated from the carcasses supplied by a single processing plant. Only 12/28 *C. jejuni* biotype 2 were isolated with the ATB method. One ATB method isolate was H_2S negative whereas the corresponding Skirrow method isolate was H_2S positive.
The prevalence of campylobacteria on the

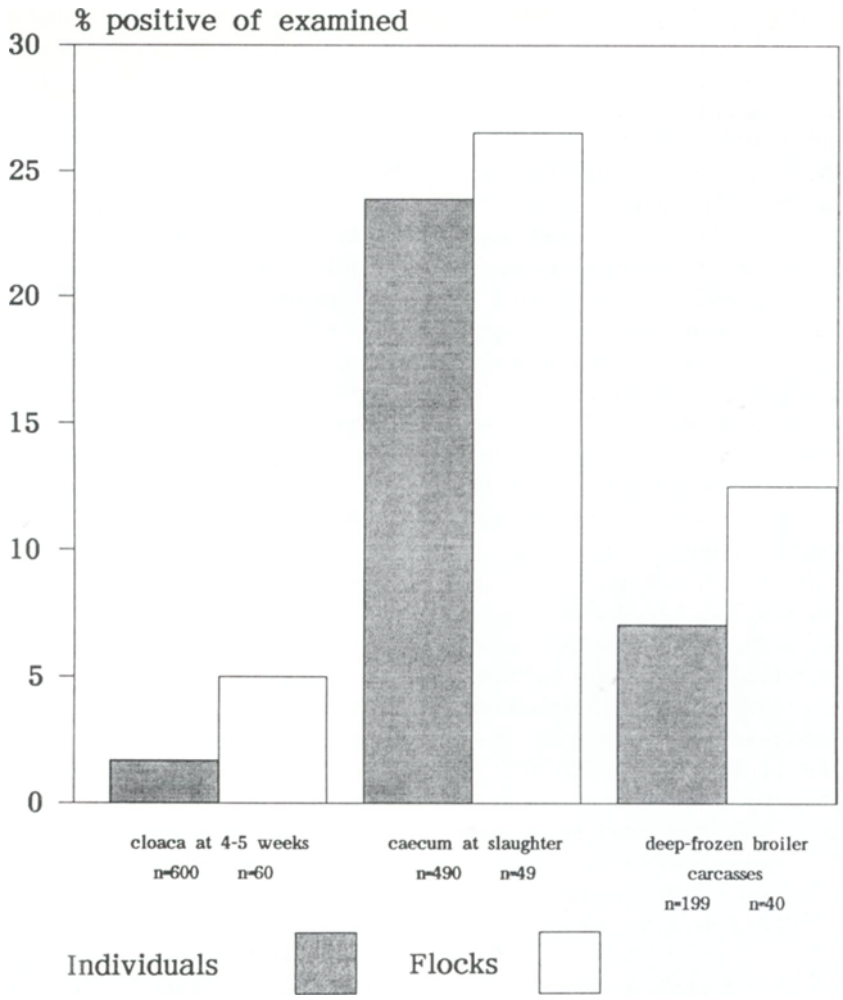
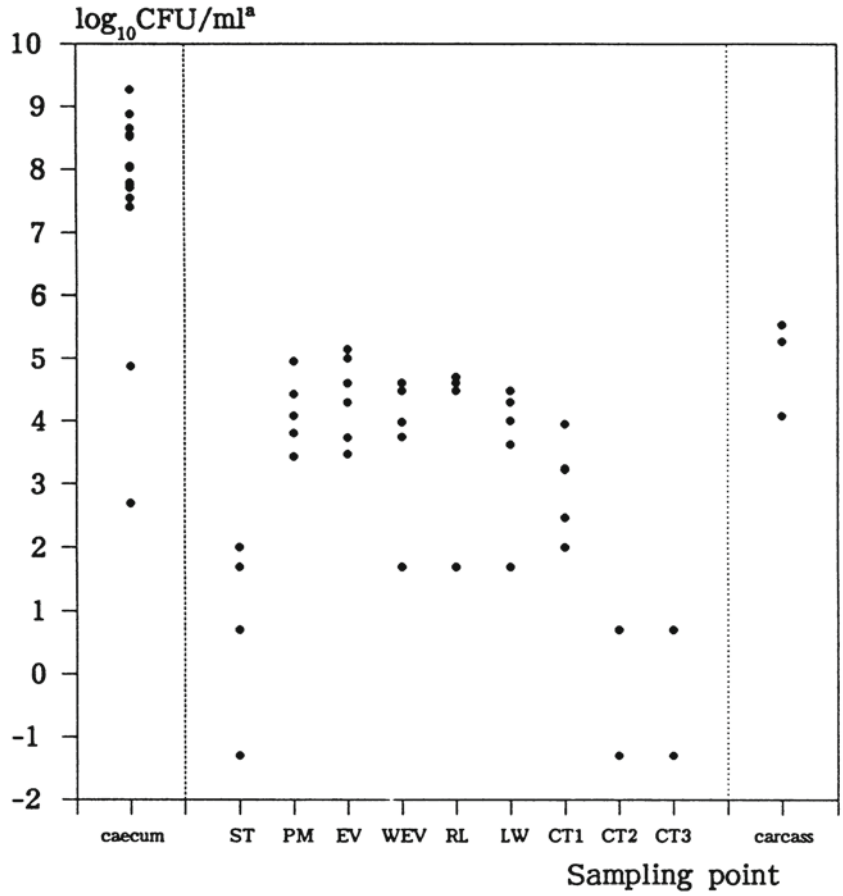


Figure 1. Prevalence of *Campylobacter jejuni* in broiler chickens and broiler chicken carcasses in Finland. Percentage of positive individuals and flocks out of all the individuals and flocks examined at the age of 4–5 weeks (cloacal swabs), at slaughter (caecal contents) and at the market level (deep-frozen broiler chickens, surface contamination investigated by the rinsing and maceration method). In the case of samples taken at the market level "flock" means 5 carcasses bought at a time and obtained from the same processing plant. See text for methods.

surface of deep-frozen broiler chicken carcasses at the market level was 7,0 % (14/199) (Fig. 1). Six of the isolates were *C. jejuni* biotype 1 and 8 were *C. jejuni* biotype 2. 2/14 were isolated with the ATB method and 13/14 with the Skirrow method. All the *C. jejuni* biotype 2 isolates were supplied by the same processing plant as described above.

Results of the quantitative examination of



[a] Units change between squares.

Figure 2. Concentration of campylobacteria in caeci (log_{10}CFU/g), in duplicate waste water samples (log_{10}CFU/ml) and on the surface of broiler carcasses ready for packaging (log_{10}CFU/carcass) when a campylobacter positive flock (age 4–5 weeks) entered the processing plant. Three different flocks were analysed. For methods see text.

Sampling points (*n=number of samples*):

Units are in log_{10}CFU/g in the first block.
caecal contents (n=15)

Units are in log_{10}CFU/ml in the second block.
ST=scald tank (*n=6*), PM=plucking machine (*n=6*), EV=evisceration (*n=6*), WEV= washing after evisceration (*n=6*), RL=remocing of lugns (*n=6*), LW=last washing before chilling (*n=6*), CT1=first chilling tank (*n=6*),CT2=second chilling tank (*n=6*), CT3=third chilling tank, chlorinated (25 ppm) (*n=6*)

Units are in log_{10}CFU/carcass in the third block.
whole carcass (*n=3*)

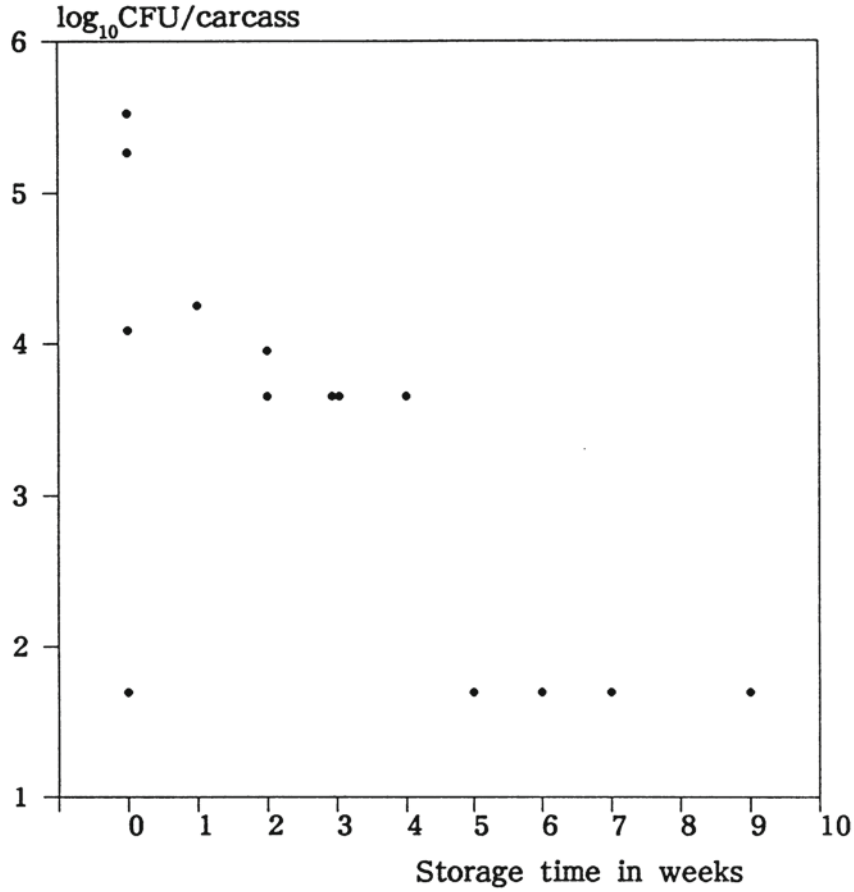


Figure 3. 14 broiler chicken carcasses from three flocks shown to be campylobacter positive (at the age of 4–5 weeks) were stored at –18°C for up to 9 weeks and examined weekly (except after 8 weeks) for campylobacteria by the rinsing and maceration method. Thawing was done in a refrigerator for 20 h. Quantitative examination was carried out from the rinsing liquid on Skirrow agars. Qualitative examination was carried out by pre-enriching rinsing liquid for 5 h at 43°C under a microaerophilic atmosphere, by enriching the pre-enriched broth both on Skirrow broth and ATB and finally by plating the enriched broth on Skirrow and ATB agars, respectively. See text for details of the method. Qualitative results (1.70 $\log_{10}$CFU/carcass) are shown in the figure only if quantitative results are lacking.

campylobacteria in caeci, in waste waters and in washing waters and screw chilling tank waters during slaughtering of a campylobacteria positive flock (proven at the age of 4–5 weeks) and on the surface of carcasses after processing are shown in Fig. 2.

Results of the quantitative and qualitative investigation of the survival campylobacteria on the surface of naturally contaminated broiler chicken carcasses at –18°C are shown in Fig. 3.

Discussion

Prevalence studies confirm that contamination of broiler chickens with campylobacteria is at a lower level in Finland than in other developed countries outside Scandinavia. Although seasonal variation could not be detected in this material it may represent the maximum, because the studies were carried out mostly in summer and autumn, at the time when campylobacteriosis is most prevalent among humans (*Pönka et al.* 1984). The widespread use of Nurmi cultures in preventing *Salmonella* infections among broiler chickens in Finland (*Nurmi & Rantala* 1973) may be one explanation for this low prevalence (*Soerjadi-Liem et al.* 1983).

From the methodological point of wiev cloacal swabs are not a very efficient way of estimating prevalence. Campylobacteria are very sensitive to environmental conditions, secretion of campylobacteria may be intermittent and sampling could not be fully supervised at the farms. The sensitivity of our method is about 100 CFU campylobacteria/sample after 1 d transport at room temperature (unpublished).

According to *Rosef et al.* (1984) and *Juven & Rogol* (1986) there is a wide variety of serotypes among isolates from single flocks of broiler chickens. Enrichment and plating methods may vary in their sensitivity of isolating different serotypes. Our few isolations of *C. jejuni* biotype 2 with the ATB method appear to support this assumption.

All waste water samples at the processing plant contained campylobacteria. Cross-contamination therefore occurs at the latest in the first 2 chilling tanks, which were not chlorinated. After chilling in the chlorinated screw chilling tank at 4°C there were still mean $\log_{10}$ 4.5 CFU campylobacteria on the surface of fresh broiler chicken carcasses. Greater decrease in contamination could possibly be obtained by increasing the concentration of chlorine to 50–300 ppm (*Luechtefeld & Wang* 1981) or by adding 0,1–1,0 % acetic acid either to the scald tank (*Okrend et al.* 1986) or to the final chilling tank. Increase in the pH of the scald water has also been suggested (*Hudson & Mead* 1987). These methods may possibly have an effect on the organoleptic quality of the carcasses. Cross-contamination during chilling can be avoided by chilling carcasses in an air chamber possibly also spraying carcasses with lactic acid.

Our studies on the survival of campylobacteria on the surface of deep-frozen broiler chicken carcasses agree rather well with previous studies both with artificial (*Hänninen* 1981) and natural contamination (*Rosef et al.* 1984). The concentration of campylobacteria on the surface of the carcasses decreased in four weeks by 2 $\log_{10}$ units, after which campylobacteria could be detected only by sensitive methods.

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Sammanfattning

Prevalens av campylobakterier i broiler kedjen från producenten till konsumenten i Finland.

Prevalensen av *Campylobacter jejuni* i Finland är 1.7 % (9/600) i avföring av 4–5 veckor gamla broilers och 24 % (117/490) i caeci vid slakten. Alla avfallsvattenprov, med ett undantag av en klorerad avkylningstank (25 ppm), i produktionsinrättning innehöll campylobakterier när en campylobakterpositiv flock slaktades. Caeci innehöll $\log_{10}$ 7.2 CFU campylobakterier/g (medelvärde). Efter avkylning i en klorerad isvattentank kunde $\log_{10}$ 4.5 CFU campylobakterier isoleras från en kropp (medelvärde). På handelsnivå isolerades campylobakterier ur 7.0 % (14/199) av djupfrysta broilers. Förekomsten av campylobakterier hos naturligt kontaminerade djupfrysta broilers efter fyra veckors förvaring i –18°C var 2 $\log_{10}$ enheter lägre än hos kropparna strax efter slaktningen.

Alla prevalensvärden är mindre än i andra utvecklade länder med undantag av de skandinaviska länderna, kanske för att Nurmi kulturer användes allmänt för att bekämpa *Salmonella* i Finland.

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