Acta vet. scand. 1991, *32*, 491–498.

The Effect of Lactic Acid Sprays on *Campylobacter jejuni* Inoculated onto Poultry Carcasses

By *Kofitsyo S. Cudjoe* and *Georg Kapperud*

Department of Food Hygiene, Norwegian College of Veterinary Medicine, and
Department of Bacteriology, National Institute of Public Health, Oslo, Norway.

Cudjoe, K. S. and G. Kapperud: The effect of lactic acid sprays on *Campylobacter jejunum* inoculated on poultry carcasses. Acta vet. scand. 1991, 32, 491–498. – Spraying poultry carcasses with 1 % lactic acid 10 min after inoculation with *Campylobacter jejuni,* resulted in a significant reduction in the number of the bacteria after 4 h at 4°C. Some of the inoculated cells, however, survived for at least 144 h. Spraying 10 min after inoculation with 2 % lactic acid, totally eliminated all inoculated *C. jejuni* within 24 h. On the other hand, spraying 24 h after inoculation, with either 1 % or 2 % lactic acid did not eliminate all the bacteria. Inoculated *C. jejuni* on poultry carcasses not sprayed with lactic acid, survived at 4°C throughout the sampling period (up to 144 h) and showed little tendency to decrease in number even when the carcasses started to deteriorate. Resident campylobacters on poultry carcasses were significantly reduced by the lactic acid treatment. Frozen and thawed chickens appeared to show a graying of the skins immediately after spraying with lactic acid, slightly stronger with 2 % lactic acid, but the colour reverted to normal after 24 h. We were not able to observe any colour change on the fresh broiler chickens after lactic acid treatment. Our results indicated that lactic acid had a significant bactericidal effect on *C. jejuni* on both naturally and artificially contaminated poultry carcasses. This effect, however, became manifest only several hours after acid treatment.

lactic acid decontamination.

Introduction

Campylobacter jejuni is regarded as an important cause of acute human enteritis (*Skirrow* 1977, *Blaser et al.* 1979). Epidemiological and bacteriological evidence indicate that foods of animal origin, especially poultry, are the most important sources of infection (*Blaser et al.* 1984). Poultry intended for human consumption is frequently contaminated with *C. jejuni* belonging to the same serotypes and biotypes as those responsible for human campylobacteriosis (*Rosef et al.* 1984, *Stern et al.* 1984, *Hood et al.* 1988). Case-control studies have incriminated chicken consumption as a predominant risk factor associated with *C. jejuni* enteritis (*Anon.* 1984, *Deming et al.* 1987.). During highly automated slaughtering processes widespread contamination of carcasses, equipment, working surfaces, processing water, and the hands of operators takes place (*Hartog et al.* 1983, *Oosterom et al.* 1983, *Genigeorgis et al.* 1986, *Kasrazadeh & Genigeorgis* 1987, *Clark & Bueschkens* 1988).

Terminal decontamination using lactic acid has been proposed to reduce the final contamination of carcasses (*Snijders et al.* 1984). In earlier studies (*Snijders et al.* 1984, *Cudjoe* 1988), lactic acid had a strong decontaminative effect on the total viable and coliform counts on carcasses. *Stern et al.* (1985), examining broiler chickens immediately after lactic acid treatment, observed

only a limited effect on the resident *C. jejuni* flora. The possibility that the bactericidal effect of lactic acid could become evident in the course of time, was not addressed. We have sprayed poultry carcasses artificially contaminated with *C. jejuni* as well as naturally contaminated ones with lactic acid and monitored the survival of the bacteria for up to 6 days.

Materials and methods

Preparation of standardized C. jejuni inoculum

A strain of *C. jejuni* biotype II (*Lior* 1984) isolated previously from poultry (*Cudjoe et al.* unpublished data) was kept frozen at −70°C, resuscitated and screened for purity by plating on blood agar 2 times. A loopful of the fresh bacterial culture was inoculated into each of 10 tubes containing 10 ml of Preston enrichment broth without antibiotics (CM 67; SR 48; and SR 84; Oxoid Ltd., Basingstoke, Hampshire, England). Blood agar plates and tubes containing the *C. jejuni* cultures were incubated for 24 h at 42°C under microaerobic conditions, achieved in anaerobic jars without catalyst, using gas generating sachets (BR 38; Oxoid Ltd.). The broth cultures were then centrifuged at 1500 × g for 15 min at 25°C and the supernatants aspirated. The pellets were washed once with 0.1 % sterile peptone water (Difco Laboratories, Detroit, MI), centrifuged as before, and the final cell sediments pooled and resuspended in 2.5 ml of 0.1 % sterile peptone water at 37°C. The bacterial density was adjusted to an absorbance of 1.38 at 620 nm, which corresponded to about 10^6 cfu/ml. Before inoculation of carcasses, the bacterial density of the inoculum was checked by plating 10-fold serial dilutions in duplicate on Preston agar (CM 689, SR 48, and SR 117; Oxoid, Ltd.) supplemented with 1.8 % Bacto agar (Difco) to achieve a total agar concentration of 3 %.

Inoculation and spraying

Twenty ready-for-the-shop broiler chickens from a breeder farm which had previously been examined for *Campylobacter* and found to be negative, were randomly selected and used for this study. To ascertain that the carcasses were not naturally contaminated with campylobacters, the sternal skin surface of the selected carcasses were swabbed. The swabs were plated directly on Preston agar, and placed in Preston enrichment broth with antibiotics (SR 117, Oxoid Ltd.) for 24 h before plating 0.1 ml on Preston agar. All incubations were performed as described earlier. The carcasses were then frozen at −20°C for 2 weeks for practical reasons, thawed at refrigeration temperature and a 60 cm^2 area marked on the sternal skin surface of each. Into this marked skin area, 1.0 ml of the standardized *C. jejuni* culture was carefully and evenly inoculated by using a fine tipped brush to give approximately 1.7×10^4 cfu/cm^2.

After 10 min of inoculation, 5 carcasses were each sprayed with 100 ml of sterile distilled water, 5 with 100 ml of 1 % lactic acid (E. Merck, Darmstadt, Germany), 5 with 100 ml of 2 % lactic acid, and 5 were not sprayed. Spraying was performed uniformly over the delineated area using a 1 l capacity domestic spray gun. During spraying, each carcass was held in a vertical position about 30 cm from the spray gun, and excess acid or water was allowed to drain off. The spray gun was calibrated to deliver approximately 2 ml per ejection in a fine mist. To maintain stable conditions and avoid dehydration, carcasses were stored in pairs in sterile metal boxes (65 × 22 × 22 cm) with lids on at 4°C with sampling at 0, 2, 4, 24, 48 and 72 h (Experiment 1). A subjective visual color assessment was made on each carcass after spraying and again at each sampling time.

In a 2nd trial (Experiment 2) 8 carcasses were inoculated as above, and 2 sprayed with distilled water, 2 with 1 % lactic acid, 2 with 2 % lactic acid, with the last 2 not sprayed. Sampling was after 0, 48, 72, 96, 120 and 144 h.

In a 3rd trial (Experiment 3) 4 carcasses were inoculated and sprayed with 1 % lactic acid as above. Two were resprayed with 1 % lactic acid 24 h later and the other 2 after 48 h. Sampling was after 0, 24, 48 and 72 h after respraying.

Finally in Experiment 4, broiler chickens not previously held frozen were inoculated as above with 1.7×10^3 cfu/cm^2 and stored at 4°C. After 24 h 2 were sprayed with 1 % and 2 with 2 % lactic acid as above. Eight carcasses determined as naturally contaminated after swabbing their sternal skin surface immediately after slaughter and plating the swabs directly on Preston agar (see above), were also sprayed 4 with 1 % and 4 with 2 % lactic acid. Sampling was after 0, 24 and 48 h after spraying.

Recovery and enumeration

At the specified sampling periods, 10 cm^2 from each delineated skin area was aseptically excised, weighed, and homogenized in a Colworth 400 Stomacher (A. J. Seward, London, England) with the appropriate volume (1:10 w/v) of 0.1 % peptone water. Further 10-fold serial dilutions were made in Preston enrichment broth without antibiotics, which were incubated microaerobically at 37°C for 2 h, to resuscitate sublethally injured cells (*Humphery* 1986). Aliquots of 0.1 ml of the dilutions were then spread plated onto 3 % Preston agar in duplicate. Finally, 40 µl of the reconstituted antibiotics (SR 117, Oxoid Ltd.) were added to the tubes containing 1 ml of homogenate in 9 ml of Preston enrichment broth, and further enriched for 24 h microaerobically at 42°C before plating 0.1 ml on Preston agar. All plates were incubated microaerobically at 42°C and read after 48–60 h. Enumerated colonies were examined by phase contrast microscopy and further characterized by their ability to show catalase and oxidase activity.

Statistical analyses

Numerical data was transformed to logarithmic values and the mean log counts (cfu/cm^2) calculated. Student's *t*-test was used to compare mean log counts from the different treatments at 5 % significance level.

Results

Spraying poultry carcasses with 1 % or 2 % lactic acid 10 min after inoculation with *C. jejuni* biotype II, resulted in noticeable reductions in numbers during subsequent storage at 4°C (Table 1). When the carcasses were sprayed with 2 % lactic acid, significant reductions were achieved 2 h after spraying, and no campylobacters could be detected after 24 h at 4°C either by direct plating or by enrichment. On the other hand, although 1 % lactic acid significantly reduced the level of the initial inoculum 4 h after spraying, survivors could still be detected. On carcasses not subjected to spraying, there was no significant reductions in numbers while on carcasses sprayed with distilled water numbers declined by less than a log cycle. When the inoculated carcasses were resprayed (Experiment 3) with 1 % lactic acid after 24 or 48 h storage at 4°C, *C. jejuni* was only detected after enrichment (results not shown).

When fresh broiler chickens were inoculated and stored at 4°C for 24 h before spraying with either 1 % or 2 % lactic acid, the decontaminating effect of the lactic acid treatment was much reduced (Table 2). However, no campylobacters were detected from any of the naturally contaminated carcasses

Table 1. The effect of spraying previously frozen broiler chickens stored at 4°C with 1 % and 2 % lactic acid, 10 min after inoculation with *C. jejuni* biotype II.

Time (h)[a]	Number of *C. jejuni* (mean log cfu[c]/cm^2 ± 1 SD)			
	Lactic acid			
	2 %	1 %	Distilled water	Not sprayed
Experiment 1:				
0	4.07±0.06	3.98±0.20	4.30±0.25	4.14±0.16
2	2.95±0.85	3.61±0.56	4.07±0.15	4.32±0.06
4	2.05±1.68	3.08±0.54	4.11±0.13	4.30±0.10
24	nd[b]	2.64±1.36	3.95±0.31	4.17±0.26
48	nd	1.13±1.49	3.88±0.43	4.09±0.56
72	nd	0.84±1.07	3.48±0.52	3.91±0.30
Experiment 2:				
0	4.01±0.03	4.09±0.03	4.37±0.34	4.21±0.12
48	nd	1.65±0.00	3.47±0.19	4.31±0.07
72	nd	1.65±0.00	3.74±0.04	3.94±0.01
96	nd	0.83±0.01	4.33±0.03	4.40±0.08
120	nd	0.83±0.01	3.90±0.05	3.88±0.42
144	nd	0.83±0.01	3.82±0.01	4.37±0.00

[a] Time in hours after spraying when samples were taken and examined, except at time zero, when samples were taken before spraying. [b] nd: *C. jejuni* not detected. [c] cfu = Colony forming units.

sprayed with 2 % lactic acid or from 3 of the 4 sprayed with 1 %. On the other sprayed with 1 % lactic acid, numbers remained nearly constant.

Frozen and thawed chickens appeared to show a graying of the skins immediately after spraying with lactic acid, slightly stronger with 2 % lactic acid, but the colour reverted to normal after 24 h. We were not able to observe any colour change on the fresh broiler chickens after lactic acid treatment.

Table 2. The effect lactic acid treatment on inoculated *C. jejuni* biotype II and naturally occurring campylobacters on fresh broiler chickens stored at 4°C.

Time (h)[a]	Number of *C. jejuni* (mean log cfu/cm^2 ± 1 SD)			
	Artificially contaminated		Naturally contaminated	
	2 % Lactic acid	1 % Lactic acid	2 % Lactic acid	1 % Lactic acid
Experiment 4:				
0	4.06±0.05	4.09±0.01	1.57±0.07	1.43±0.04
24	3.06±0.19	3.84±0.03	nd[b]	0.95
48	2.20±0.24	3.68±0.09	nd	1.22

See Table 1 for legends.

Discussion

The ability of organic acids to inhibit bacterial growth on meat surface and eventually kill the bacteria, has been reported by several workers (*Mountney & O'Malley* 1965, *Belmuller et al.* 1973, *Itoh et al.* 1976, *Baird-Parker* 1980, *Stern et al.* 1985, *Cuk et al.* 1987). *Juven et al.* (1988) noted that the addition of ascorbic and isoascorbic acid to meat samples experimentally inoculated with *C. jejuni,* caused significant increase in the death rate of the bacteria. *Stern et al.* (1985) observed significant reductions in the number of *C. jejuni* after 5 and 10 min exposure to 1 % lactic acid at 5 and 50°C in vitro. However, when broiler chickens naturally contaminated with *C. jejuni* were immersed in 1 % lactic acid, no significant reductions were obtained immediately after the acid treatment. They attributed this observation to the short exposure time and the possible protective effect provided by the chicken carcasses. In contrast to the work of *Stern et al.* (1985) we found that 1 % and 2 % lactic acid sprays significantly reduced the survival of inoculated *C. jejuni* on poultry carcasses as well as naturally contaminated carcasses. After the carcasses had been stored at 4°C for 24 h, drastic reductions and a total die-off of the bacteria was achieved for 1 % and 2 % lactic acid, respectively. In order to give the bacteria ample time to adhere to the skin surface before spraying and to simulate the natural situation, we allowed inoculum (1.7×10^3 cfu/cm^2) to establish for 24 h at 4°C on fresh broiler chicken before spraying with lactic acid. Furthermore naturally contaminated carcasses were similarly sprayed. Again, our results confirmed the previous conclusion that lactic acid significantly reduces campylobacter numbers on poultry carcasses. What is however interesting, is the fact that we were not able to eliminate all inoculated *C. jejuni* with the 2 % lactic acid, while none of the naturally contaminating campylobacters survived the 2 % lactic acid treatment. Our results indicate that lactic acid has a significant bactericidal effect on *C. jejuni* on poultry carcasses. This effect, however, became manifest only several hours after the acid treatment. Our observation differs from *Stern et al.* (1985) who observed only a limited effect some few seconds after lactic acid treatment. It is obvious from this and earlier studies (*Cudjoe* 1988) that the effect of lactic acid is also time dependent. This became apparent when the lethal effect of 1 % lactic acid diminished probably due to diffusion of the acid into the carcasses after 48 h (Table 1). The remaining *C. jejuni* cells survived for 144 h on the carcasses with no significant reduction in numbers. However, respraying poultry carcasses with 1 % lactic acid after 24 or 48 h, appeared to totally eliminate and/or sublethally injure the surviving cells, since the campylobacters still present required enrichment to become detectable. Respraying, however, is not economically feasible and also not practically applicable under present circumstances.

Contrary to what *Stern et al.* (1985) reported, we observed no colour change in lactic acid sprayed fresh broiler chickens. However, spraying frozen and thawed carcasses with lactic acid, a slight graying of the skins of the carcasses was observed immediately after spraying, but this disappeared, and the colour of all carcasses reverted to normal after 24 h. The degree of subcutaneous fat cover, the mode of applying the acid onto the carcasses, the time elapsed after spraying and/or the subjective evaluation of colour development could account for the differences in results.

The bacteriostatic and bactericidal effect of weak organic acids is related to the amount of undissociated molecules present (*Macris*

1975, *Salmond et al.* 1984). It is the undissociated molecule of lipophilic organic acids that is responsible for antimicrobial activity, since they are readily soluble into cell membranes and thereby inhibit or kill microorganisms by interfering with the permeability of the microbial cell membrane, causing uncoupling of both substrate transport and oxidative phosphorylation from the electron transport system (*Macris* 1975, *Crammer & Prestegard* 1977).

It is worth noting that the impact of the lactic acid spray pressure in our study is considerably less than what could be achieved under slaughterhouse conditions, where a higher mechanical pressure would direct the acid sprays to all crevices and flushing out weakly adhered cells. This could be particularly important where cross contamination has occurred along the slaughterline. Furthermore, the number of bacterial cells inoculated on the sternal skin surface were far greater than are likely to be seen, even in grossly contaminated carcasses. It is likely, therefore, that spraying with lactic acid could be of use in controlling *C. jejuni* contamination of finished poultry carcasses. Yet lactic acid sprays could never replace standard hygienic practice at slaughter. Its unrestrained use as a terminal decontaminant could mask bad hygiene both at slaughter and at the farm level. The role of lactic acid in the prevention and control of pathogenic bacteria on carcasses should never be used to replace other efforts to reduce *C. jejuni* and other pathogenic bacteria at the farm level or the slaughterhouse.

Acknowledgements

We gratefully acknowledge the partial financial support provided by the Norwegian Agricultural Research Council towards the completion of this work. Per Einar Granum contributed valuable suggestions during final stages of the work.

References

Anonymous: Surveillance of the flow of *Salmonella* and *Campylobacter* in a community. Communicable Disease Control Section, Seatle-King County Department of Public Health. Report to U.S. Food and Drug Administration. Washington 1984.

Baird-Parker AC: Organic acids, In: Silliker JH (ed.): Microbial ecology of foods. Academic Press, Inc., New York 1980, vol 1, pp. 126–135.

Belmuller GW, Carpenter JA, Reynolds AE: Reduction of bacteria on pork carcasses. J. Food Sci. 1973, *38*, 261–263.

Blaser MJ, Berkowitz ID, LaForce FM, Cravens J, Reller LB, Wang WL: Campylobacter enteritis: clinical and epidemiological features. Ann. intern. Med. 1979, *91*, 179–185.

Blaser MJ, Taylor DN, Feldman RA: Epidemiology of *Campylobacter* infections. In: Butzler (ed.): *Campylobacter* infection in man and animals. CRC Press, Boca Raton, FL. 1984, pp. 143–161.

Clark AG, Bueschkens DH: Horizontal spread of human and poultry-derived strains of *Campylobacter jejuni* among broiler chicks held in incubators and shipping boxes. J. Food Protect. 1988, *51*, 438–441.

Crammer JA, Prestegard JH: NMR studies of pH-induced transport of carboxylic acids across phospholipid vesicle membranes. Biochem. Biophys. Res. Commun. 1977, *75*, 295–301.

Cudjoe KS: The effect of lactic acid sprays on the keeping qualities of meat during storage. Int. J. Food Microbiol. 1988, *7*, 1–7.

Cuk Z, Annan-Prah A, Janc M, Zajc-Salter J: Youghurt: an unlikely source of *Campylobacter jejuni/coli.* J. appl. Bact. 1987, *63*, 201–205.

Deming MS, Tauxe RV, Blake PA, Fowler SE, Jones TS, Lockamy EA, Patton CM, Sikes RO: Campylobacter enteritis at a university: transmission from eating chicken and from cats. Amer. J. Epidem. 1987, *126*, 526–534.

Genigeorgis C, Hassuneh M, Collins P: Campylobacter jejuni infection on poultry farms and its effect on poultry meat contamination du-

ring slaughtering. J. Food Protect. 1986, *49,* 895–903.

Hartog BJ, DeWild GJA, de Boer E: Poultry as source of *Campylobacter jejuni.* Arch. Lebensmitt. Hyg. 1983, *34,* 116–122.

Hood AM, Pearson AD, Shahamat M: The extent of surface contamination of retailed chickens with *Campylobacter jejuni* serogroups. Epidem. Infect. 1988, *100,* 17–25.

Humphery TJ: Techniques for the optimum recovery of cold injured *Campylobacter jejuni* from milk and water. J. Appl. Bact. 1986, *61,* 125–132.

Itoh KA, Chen JK, Lerke PA, Seeger ML, Unverferth JA: Effect of acid and salt concentration in fresh-pack pickles on the growth of *Clostridium botulinum* spores. Appl. environ. Microbiol. 1976, *32,* 121–124.

Juven BJ, Kanner J, Weisslowicz H, Harel S: Effect of ascorbic and isoascorbic acids on the survival of *Campylobacter jejuni* in poultry meat. J. Food Protect. 1988, *51,* 436–437.

Kasrazadeh M, Genigeorgis C: Origin and prevalence of *Campylobacter jejuni* in ducks and duck meat at the farm and processing plant level. J. Food Protect. 1987, *50,* 321–326.

Lior M: New, extended biotyping scheme for *Campylobacter jejuni, Campylobacter coli,* and *"Campylobacter laridis".* J. clin. Microbiol. 1984, *20,* 636–640.

Macris BJ: Mechanisms of benzoic acid uptake by *Saccharomyces cerevisae.* Appl. Microbiol. 1975, *30,* 503–506.

Mountney GJ, O'Malley J: Acids as poultry meat preservatives. Poultry Sci. 1965, *44,* 252.

Oosterom J, Noterman S, Karman H, Engel GB: Origin and prevalence of *Campylobacter jejuni* in poultry processing. J. Food Protect. 1983, *46,* 339–344.

Rosef O, Gondrosen B, Kapperud G: Campylobacter jejuni and *Campylobacter coli* as surface contaminants of fresh and frozen poultry carcasses. Int. J. Food Microbiol. 1984, *1,* 205–215.

Salmond CV, Kroll RH, Booth IR: The effect of food preservatives of pH homeostasis in *Escherichia coli.* J. gen. Microbiol. 1984, *130,* 2845–2850.

Skirrow MB: Campylobacter enteritis: a "new" disease. Br. med. J. 1977, *2,* 9–11.

Snijders JMA, van Logtestijn TG, Mossel DAA, Smulders FTM: Conditions for the use of lactic acid as a decontaminant in the meat industry. Proc. Europ. Meet Res. Work 1984, *30,* 232–233.

Stern NJ, Gree SS, Thaker N, Krout DJ, Chiu J: Recovery of *Campylobacter jejuni* from fresh and frozen meat and poultry collected at slaughter. J. Food Protect. 1984, *47,* 372–374.

Stern NJ, Rothernberg PJ, Stone JM: Enumeration and reduction of *Campylobacter jejuni* in poultry and red meats. J. Food Protect. 1985, *48,* 606–610.

Sammendrag

Effekten av melkesyre på Campylobacter jejuni inokulert på fjørfeslakt.

Artikkelen omtaler effekten av melkesyre på *Campylobacter jejuni* på overflaten av fjørfeslakt. Resultatene viste at spraying av slaktene med 1 % melkesyre 10 min etter inokulasjon med *C. jejuni* ga en signifikant reduksjon av antallet bakterier etter 4 timer ved 4°C. Noen av de inokulerte bakteriene viste seg imidlertid å kunne overleve minst 144 t. Bruk av 2 % melkesyre så ut til å eliminere alle *C. jejuni* innen 24 t. Hvis melkesyrebehandlingen derimot ble utført 24 t etter inokulering, ble det ikke observert noen tilsvarende eliminasjon av bakteriene hverken med 1 % eller 2 % melkesyre. På slakt som ikke ble behandlet med melkesyre, overlevde *C. jejuni* gjennom hele forsøksperioden (144 t) ved 4°C. I løpet av denne tiden viste bakterien bare liten tendens til reduksjon i antall selv om slaktene viste tegn på begynnende forråtnelse. Fjørfeslakt som var naturlig infisert med *Campylobacter,* viste en signifikant reduksjon av antall *Campylobacter*-bakterier etter melkesyrebehandling. Melkesyrebehandlingen førte til en grå misfargning av de frosne slaktene, særlig ved bruk av 2 % melkesyre, men denne fargen forsvant etter 24 timer. Ferske fjørfeslakt viste ingen fargeforandring etter melkesyrebehandling. Resultatene viser

at melkesyre har en signifikant baktericid effekt overfor *C. jejuni* på både naturlig og kunstig infisert fjørfeslakt. Denne effekten viste seg imidlertid først mange timer etter behandling.

(Received October 6, 1990; accepted February 15, 1991).

Reprints may be requested from: K. S. Cudjoe, Department of Food Hygiene, The Norwegian College of Veterinary Medicine, P. O. Box 8146, Dep. N-0033 Oslo 1, Norway.