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Mitigation strategies for *Campylobacter* spp. in broiler at pre-harvest and harvest level

Minimierungsstrategien für Campylobacter spp. beim Broiler in der Primärproduktion und bei der Fleischgewinnung

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Summary

In contrast to other foodborne zoonotic agents an elimination of *Campylobacter* spp. from animal production, especially poultry production, seems not to be feasible. Therefore mitigation strategies focus on reduction of the *Campylobacter* spp. concentration in primary production and further minimalisation during processing. In primary production biosecurity measures (incl. hygiene barriers and restricted access) are the methods applied most commonly and most effectively so far. Experimental approaches and few field trials also showed that bacteriophages, electrolyzed oxidizing water, organic acids or medium chain fatty acids (applied via drinking water) are also effective in reducing *Campylobacter* prevalence and/or concentration. However this reduction cannot be transferred in all cases to the situation in the slaughterhouse. Therefore additional measures have to be taken in account in the slaughterhouse to prevent cross-contamination. Logistic or scheduled slaughter can prevent cross-contamination but cannot further reduce *Campylobacter* concentration. Process parameters like elevated scalding temperature can contribute to such a reduction, but may also alter the product quality. Therefore no single pre- or harvest measure is sufficient for the reduction of *Campylobacter* concentration, but a combination of measures in both production levels is needed.

Keywords: thermophilic *Campylobacter* spp., bacteriophages, biosecurity, organic acids, EO water, slaughter technology, decontamination

Zusammenfassung

Im Gegensatz zu anderen lebensmittelübertragenen Zoonoseerregern ist die Eliminierung von *Campylobacter* spp. in der Primärproduktion, insbesondere in der Geflügelproduktion, nicht erreichbar. Daher sind Minimierungsstrategien zur Reduktion der *Campylobacter* spp.-Belastung in der Primärproduktion sowie die weitere Reduzierung während der Schlachtung entwickelt worden. In der Primärproduktion sind Maßnahmen zur Biosicherheit (z. B. Hygieneschleusen und Beschränkung des Zugangs) am weitesten verbreitet und am effektivsten. Laborversuche und Feldversuche haben auch gezeigt, dass Bakteriophagen, elektrolysiertes oxidierendes Wasser, organische Säuren oder mittel- und langkettige Fettsäuren (appliziert über Tränkewasser) ebenfalls die *Campylobacter*-Prävalenz und/oder -Konzentration verringern können. Diese Erfolge können aber nicht ohne Weiteres auf die Situation im Schlachthof übertragen werden. Daher müssen zusätzliche Maßnahmen im Schlachthof greifen, wie logistische oder geplante Schlachtung, die zumindest eine Kreuzkontamination verhindern können, wenn sie auch nicht zu einer Reduzierung der *Campylobacter*-Konzentration beitragen. Prozessparameter wie erhöhte Brühtemperaturen haben sich als effektiv in dieser Hinsicht erwiesen, können aber auch die Produktqualität negativ beeinflussen. Deshalb kann keine einzelne Maßnahme in der Primärproduktion oder in der Schlachtung als ausreichend betrachtet werden. Es muss vielmehr eine Kombination mehrerer Ansätze auf beiden Ebenen durchgeführt werden.

Schlüsselwörter: Thermophile *Campylobacter* spp., Bakteriophagen, Biosicherheit, organische Säuren, EO-Wasser, Schlachttechnologie, Dekontamination

Introduction

Campylobacter spp. are still the most frequent cause of bacterial foodborne infections in Germany, Europe and worldwide (EFSA, 2014a). Source attributions are not performed systematically, but from the published literature and some country specific evaluations it can be concluded, that poultry production and poultry meat is the main source of infection for humans (EFSA, 2011). Although other food animal species like pigs are not significantly differently affected by *Campylobacter* contamination on the farm level, at the retail level only poultry and poultry meat are positive for *Campylobacter* with relevant numbers (EFSA, 2014b). The reason is the different slaughter and cooling technology (cross-contamination and moist environment) and the optimal ecological niche for *Campylobacter* on poultry carcasses and - products thereof (Ellerbroek et al., 2010). In pig slaughter air cooling is regularly applied, which is of disadvantage for the survival of *Campylobacter*, whereas in poultry slaughtering spray cooling is performed, which due to moist surfaces and topography of the carcass (skin folds) is a friendly environment for *Campylobacter*.

Therefore microbiological criteria for poultry carcasses after cooling are under discussion, which aim to reduce the overall *Campylobacter* load during poultry processing and on products (Ellerbroek, 2012). To reach this goal, intervention strategies are most effective at the pre-harvest or primary production stage and/or at the harvest or slaughterhouse and processing stage (Klein, 2010; EFSA, 2011). At pre-harvest, strategies can, according to Lin (2009), be divided in three categories:

– Reduction or elimination of environmental exposure (biosecurity measures like fly nets and hygiene barriers)
– Application of agents aiming at combating *Campylobacter* colonisation and minimising the bacterial load (e. g. bacteriophages, bacteriocines)
– Improving host resistance (support the immune system, vaccines, probiotics/competitive exclusion, genetic selection of the host)

Most strategies have been shown to be effective on a laboratory scale or in in vitro experiments. However, only some of these methods have been tested in field trials and under commercial conditions. For some methods, like bacteriocine application, also unsolved legal aspects have to be considered. Therefore the aim of this review is to focus on selected strategies at pre-harvest and harvest level that are already in use (like different biosecurity measures) or that have at least been tested in field trials or larger experimental settings. Also legal aspects should be solved in principal and commercial applicability should be feasible. Therefore the application of bacteriocines, vaccination and competitive exclusion is not considered further in this study.

Pre-harvest mitigation strategies

Biosecurity measures
Biosecurity measures are the most common measures applied in primary production to combat *Campylobacter* and to minimize colonisation in poultry. Measures covered by biosecurity are often in conflict with other goals of sustainable farming, like outdoor or free range farm-ing, other animal species on the farm, green environment with a minimum of concrete surfaces, open and visitor friendly stables or even holiday on farms. However, these factors are considered risk factors for *Campylobacter* introduction and contamination of poultry (EFSA, 2011).

Hygiene barriers are present on all farms, but the practical implementation can be very different. Minimum requirements include boot dips or change of footwear, hand washing facilities and physical barriers (EFSA, 2011). Often only an optical barrier is present and dips with disinfectant are not well maintained, compromising the effectivity of measures. Still, if consequently applied, biosecurity measures are seen as the most effective measures currently available and could contribute to reduce the risk of infection up to 50% (Gibbens et al., 2001; Newell et al., 2011).

One of the main risks identified are also insects, especially flies that can be the vector for *Campylobacter* transmission to the birds (EFSA, 2011). Consequently the introduction of fly screens has been evaluated and promising results were reported especially from nordic countries (Hald et al., 2007). These protective screens have to be introduced in a very strict way in order to be effective, i. e. not only windows and doors must be protected but also other technical equipment like ventilation etc. must be included. The effectivity shown in nordic countries is not easily transferable to Middle European or South European countries, as the prevalence of *Campylobacter* as well as the occurrence of flies is different in these countries.

Basic biosecurity measures can therefore contribute to a lower *Campylobacter* load if consequently applied and restricting also the access to poultry holders by personnel or visitors.

Organic acids and MCFA
Organic acids such as formic, acetic, hydrochloric and propionic acid showed in vitro a strong synergistic activity reducing *Campylobacter* spp. at pH 4 below 1 $\log_{10}$ cfu/ml (colony forming units/ml) within one hour, and the reducing effect in combination was higher than applying the single organic acids individually (Chaveerach et al., 2002). Triglyceridic medium chain fatty acids (MCFA) and their derivatives demonstrated in emulsion in vitro a strong reducing effect on *Campylobacter jejuni* (Thormar et al., 2006; Hermans et al., 2012). Organic acids and MCFA prevented in vivo the survival of *Campylobacter* in drinking water of broiler chicken (Chaveerach et al., 2004; Hermans et al., 2012).

During a 10 h pre-slaughter feed withdrawal of naturally *Campylobacter*-colonised broiler, drinking water supplemented with 0.44% lactic acid significantly reduced the crop contamination with *Campylobacter* (62.3%) as compared with the controls (85.1%) (Byrd et al., 2001). Lactic acid also reduced the incidence of *Campylobacter* found on pre-chill carcass rinses by 14.7% compared with the controls in the same trial (Byrd et al., 2001). The MCFA derivatives monocaprin as water additive showed a *Campylobacter*-reducing effect on cloacal counts in artificially and naturally colonised broiler chicken, yet caecal counts could not be predictably reduced (Hilmarsson et al., 2006; Metcalf et al., 2011). MCFA in drinking water neither reduced nor prevented caecal *Campylobacter*-colonisation of artificially inoculated broiler chicken whereas the colonisation threshold after 24 h was raised significantly of those broilers receiving supplemented water (Hermans et al., 2012).

TABLE 1: *Effects of treatment with organic acids (OA), MCFA and EO water in broiler production on thermophilic Campylobacter*

Study design	Intervention Measure		Effects	Reference
In vivo/ field trial	OA	Lactic acid	Reduced crop counts and reduced incidence on carcasses	Byrd et al., 2001
In vitro/ in vivo	OA	Formic, acetic, hydrochloric, propionic acid	Significantly effective in drinking water, reduced transmission, limited effect on caecal contamination	Chaveerach et al., 2002; 2004
Field trial	OA and MCFA	Acetic acid, formic acid, propionic acid, sorbic acid with MCFA	Effective in drinking water, reduced caecal counts, limited effect on carcasses	Jansen et al., 2014
In vitro	MCFA	Monocaprin	Significant reductions in drinking water/feed emulsions	Thormar et al., 2006
		Caproic, caprylic, and capric acids		Hermans et al., 2010
In vivo/ field trial	MCFA	Monocaprin	Significant reduced cloacal counts, no effect on transmission	Hilmarsson et al., 2006
In vivo	MCFA	Caprylic acid	Inconsistent effect on caecal counts	Metcalf et al., 2011
In vitro/in vivo	MCFA	Caproic, caprylic, capric and lauric acid	Significantly effective in drinking water, reduction of susceptibility, no effect on caecal counts and transmission	Hermans et al., 2012
In vivo	EO water	Acid pH	Significant reduction on artificially contaminated chicken wings in10/30 min	Park et al., 2002
In vivo	EO water	Acid pH	Significant reduction on broiler carcasses in 40 min	Kim et al., 2005
In vivo	EO water	Acid pH	Significant reduction on artificially contaminated broiler carcasses in 10/15 s	Northcutt et al., 2007
Feld trial (abattoir)	EO water and OA	Neutral pH and lactic acid	Limited reduction on naturally contaminated broiler carcasses for 3 min	Rasschaert et al., 2013
Field trial (flock and abattoir)	EO water	Neutral pH	Effective in drinking water, significantly effective on broiler carcasses	Bügener et al., 2014-a

In field trials carried out by Jansen et al. (2014) a water additive based on organic acids in combination with MCFA, ammonium formate and coconut/palm kernel fatty acid distillate was applied on naturally colonised broiler chicken for three full commercial rearing periods (42 d). Results indicated that a permanent application of 0.075% blended organic acids (in ascending order: formic acid, acetic acid, propionic acid and sorbic acid) in combination with MCFA in drinking water reduced the carriage of *Campylobacter* spp. in the flock and in caecum content of broiler chicken. Moreover, the final concentration at pH 4–4.5 did not have detrimental effects on production parameters or animal welfare. But even though the introduction of *Campylobacter* spp. into the processing line could be reduced in single trials, the final contamination of the corresponding carcasses after slaughter was not significantly lowered.

These trials suggest that pre-harvest application of organic acids and MCFA to drinking water of broilers can potentially lower the caecal carriage in primary production but is not independently effective in targeting *Campylobacter* spp. in the food chain.

Electrolyzed oxidizing water
Electrolysed oxidizing (EO) water is a non-toxic sanitizer with a proven bactericidal effect widely elaborated in the food chain (Huang et al., 2008). Generated by the electrophoresis of additionally salted water, oxygen and chlorine radicals lead to disinfective, free-active chlorine and hypochlorus acid (Len et al., 2000). Depending on the ratio of catholyte (pH > 10) and anolyte (pH < 3), either alkaline, acidic or neutral EO water can be produced (Hsu, 2003, 2005). Though acidic and alkaline EO water has higher bactericidal potential, considerable corrosive effects and gas emission are disadvantages compared to neutral EO water (Len et al., 2002)

In the last decade, the efficacy of acidic EO water on major food-borne pathogens was elaborated. Fabrizio et al. (2002) indicated, that at pH 2.6 a reduction of *Salmonella* Typhimurium ($< 1 \log_{10}$ cfu/g), and of *E. coli* and coliform bacteria ($1–2 \log_{10}$ cfu/g) on broiler carcasses is possible. Especially on *Campylobacter*, acidic EO water (pH 2.5) showed a strong reducing effect of $3 \log_{10}$/g within 10 min as well as 30 min on artificially contaminated chicken wings at 4°C as well as at 23°C (Park et al., 2002). Kim et al. (2005) and Northcutt et al. (2007) also reported the effectivity of acidic EO water on *Campylobacter* on artificially contaminated broiler carcasses.

In field trials by Bügener et al. (2014a), drinking water of naturally colonised, commercial reared broiler flocks was supplemented permanently with 3% neutral electrolyzed oxidizing water (final pH 6.5–7.2) for three complete rearing periods. The addition of EO water prevented the survival of *Campylobacter* spp. in drinking water of the treated flocks, whereas the control flock water was repeatedly positive on day 35 of the rearing periods. Both, after thinning and main catching, corresponding carcasses were significantly lower contaminated. Due to the correlation of caecal content and carcass contamination (Allen et al., 2007; Reich et al., 2008), these results indicate a possible quantitative reduction in caeca of natural colonised flocks. Production parameters of the broilers were not affected negatively (Bügener et al., 2014b)

The permanent addition of neutral EO water in drinking water of broiler flocks seems therefore to reduce the carriage of *Campylobacter* spp. and appears to affect counts on carcasses. Current research focuses on the additional benefit of organic acids and EO water (Rasschaert et al., 2013) as synergistic hurdles in harvest decontamination of broiler carcasses towards a successfully synergistic large-scale arrangement. Table 1 illustrates different drinking water treatment with organic acids, MCFA and EO water and their effect on thermophilic *Campylobacter*.

Bacteriophages
Bacteriophages are viruses that target bacterial cells. Like other viruses they depend on the metabolism of their host cell, their narrow host range is restricted to one bacterial strain or species. Lytic activity of bacteriophages was discovered in the early 20th century and they were widely used in the former Soviet Union to treat bacterial infections until their use was replaced by antibiotics. Bacteriophages can be isolated from virtually every source that harbours their host bacteria. Recently they have raised new interest as therapeutics for the treat-

TABLE 2: *Effects of treatment with bacteriophages in broiler production on thermophilic Campylobacter*

Study design	Applied Phage	*Campylobacter* colonisation	Phage application		Effects on *Campylobacter* load in the broiler intestine	Reference
			Route	Age of Birds		
In vivo	NCTC 12671	*Campylobacter jejuni* C356 inoculation	Oral	15–20 d	Significant reduction	Wagenaar et al., 2005
In vivo	Cocktail NCTC 12671, 12669		Oral	39–42 d	Significant reduction	
In vivo	CP8	*Campylobacter jejuni* HPC5 inoculation	Oral	25 d	No significant reduction	Loc Carrillo et al., 2005
		Campylobacter jejuni GIIC8 inoculation			Significant reduction	
	CP34	*Campylobacter jejuni* HPC5 inoculation			Significant reduction	
In vivo	CP220	*Campylobacter jejuni* HPC5 inoculation	Oral	25 d	Significant reduction	El-Shibiny et al., 2009
		Campylobacter coli OR12 inoculation			Significant reduction	
In vivo	Cocktail phiCcolBB3 phiCcolBB37 phiCcolBB12	*Campylobacter jejuni* 2140CD1 inoculation	Oral	7 d	Significant reduction	Carvalho et al., 2010
		Campylobacter coli A11 inoculation	Feed		Significant reduction	
In vivo	NCTC 12673	*Campylobacter jejuni* 1474-06 inoculation	Oral	5 d	Significant reduction	Fischer et al., 2013
	Cocktail NCTC 12672, 12673, 12674, 12678				Significant reduction in two similar trials	
Field trial	Cocktail NCTC 12672, 12673, 12674, 12678	Natural colonisation of *Campylobacter jejuni*	Drinking water	36 d	Significant reduction	Kittler et al., 2013
Field trial	Cocktail NCTC 12672, 12673, 12674, 12678	Natural colonisation of *Campylobacter jejuni* two sequence types	Drinking water	32 d	No significant reduction	
Field trial	Cocktail NCTC 12672, 12673, 12674, 12678	Natural colonisation of *Campylobacter jejuni* two sequence types	Drinking water	31 d	Significant reduction in phage contaminated control group, no significant reduction in experimental group	

ment of multiresistant bacteria and for reducing bacterial pathogens in the food production line (Kutateladze and Adamia, 2010). Different phages and application routes have been tested for reducing *Campylobacter* in broilers in in vivo trials (Loc Carrillo et al., 2005; Wagenaar et al., 2005; El-Shibiny et al., 2009; Carvalho et al., 2010; Fischer et al., 2013; Kittler et al., 2013). The application of cocktails, consisting of more than one phage, implies the advantage of broadening the host range and reducing the risk of bacterial resistance against the applied phages (Tanji et al., 2004).

Wagenaar et al. (2005) applied a 10 $\log_{10}$ pfu (plaque forming units) dose of one phage to ten day old chickens for six days. An immediate significant *Campylobacter* reduction of 3 $\log_{10}$ cfu/g caecal content was detected. A second trial included the application of two phages in chickens nearing harvest age but interestingly just a significant 1.5 $\log_{10}$cfu/g faeces drop was observed. Loc Carrillo et al. (2005) tested phage doses of 5, 7 and 9 $\log_{10}$ pfu in 25 day old chickens. Significant drops of *Campylobacter* counts were detected for the 5 and 7 $\log_{10}$ pfu doses 24 h after phage application but not for the 9 $\log_{10}$ pfu dose, with 7 $\log_{10}$ pfu being the most effective dose (reduction up to 5.6 $\log_{10}$ cfu/g caecal content). For a poor colonizing *C. coli* strain, the 9 $\log_{10}$ pfu dose proved to be most effective (El-Shibiny et al., 2009). Administration of a three-phage-cocktail via oral gavage

and via feed was compared by Carvalho et al. (2010) in 7 day old chicks. While *Campylobacter* counts were significantly reduced in both experimental groups over the whole experimental period of seven days, application via feed was found to result in a slightly higher reduction.

Recently, first field trials were carried out by Kittler et al. (2013) using a four-phage-cocktail in naturally colonised broilers. In two of three field trials a significant reduction of *Campylobacter* of up to 3.2 $\log_{10}$ cfu/g faecal samples was achieved by applying a 7.5 $\log_{10}$ pfu dose of the cocktail to 10 000–13 500 broilers via drinking water 6–7 days before slaughter. In the non-significant trial phages were not able to propagate. Comprehensive studies on resistance of *Campylobacter* during application of this phage-cocktail were carried out by the same group, indicating that phage-resistance in *Campylobacter* is not necessarily detrimental for reduction of *Campylobacter* load in vivo (Fischer et al., 2013; Kittler et al., 2014).

These studies suggest that phages can lead to a significant reduction of *Campylobacter* in the broiler intestine resulting in a beneficial effect for public health. Phage application is easily carried out by the farmer and considered to be safe. Future studies will have to focus on suitable application protocols for commercial use of *Campylobacter* phages and on procedures controlling the

incidence of phage resistance during phage application. Table 2 summarises studies on bacteriophage application and their effect on thermophilic *Campylobacter*.

Harvest mitigation strategies

Slaughtering and processing in abattoirs and meat plants is an important step of the poultry food chain, where extensive contamination with *Campylobacter* occurs. In broiler production, meat processing is a mostly automated process with inevitable faecal contamination of the meat including *Campylobacter*, which is part of the gut flora (Mead, 2004; Reich et al., 2008). In addition, the slaughterhouse provides possibilities of cross-contamination between flocks and carcasses, of different origin (Klein et al., 2007; Reich et al., 2008).

Logistic slaughtering

Considerations for possible reduction strategies at the slaughterhouse include efforts to minimize the entry of *Campylobacter* to the slaughterhouse or at least to prevent cross-contamination between proved negative flocks and flocks with unknown or proved positive *Campylobacter* status. This could be done by logistic slaughtering, where *Campylobacter* negative flocks are slaughtered at the beginning of each slaughter day. This would reduce both, the overall prevalence for *Campylobacter* of the produced broiler meat and prevent cross-contamination. Effects on quantitative contamination are limited, because cross-contaminations between flocks usually occur on a low quantitative level (Rosenquist et al., 2003; Reich et al., 2008; Sasaki et al., 2014). "Testing and scheduling" is an alternative to this, where flocks with high loads of *Campylobacter* in the gut are identified and could be excluded from the fresh meat market. A prerequisite for these strategies is a quick and reliable testing for *Campylobacter* at the end of the fattening period. In addition, "testing and scheduling" requires a confirmed correlation between *Campylobacter* carriage of broilers and the expected contamination levels on the meat (Nauta et al., 2009a, 2009b). Quantitative risk assessments evaluated the expected advantages of logistic slaughtering and found it to be limited. Reductions in prevalence of contaminated broilers showed linear relationship to the expected human cases. Quantitative reductions on the other hand, led to exponential changes in expected cases with already small reductions in numbers of *Campylobacter* on the meat. In this regard, quantitative reduction strategies seem to be more effective (Rosenquist et al., 2003; Lindqvist and Lindblad, 2008).

Slaughter technology

Measures for reduction of *Campylobacter* at the slaughterhouse itself could be set at points known to be critical for contamination of the meat. These are scalding and defeathering and evisceration with faecal contamination. At the

TABLE 3: *Quantitative effect of different adjustable processing steps or decontamination treatments during broiler processing on Campylobacter*

Processing step	Sample	Intervention Measure		*Campylobacter* numbers	Reference
Scalding and defeathering	Whole carcass rinse after defeathering	Prevention of defecation by cloacal plugging	Plugged control	2.52 log$_{10}$ cfu/ml 3.05 log$_{10}$ cfu/ml	Musgrove et al., 1997
Scalding	Whole carcass after scalding	Scalding water temperature	53°C 53.9°C	4.5 log$_{10}$ cfu/carcass 1.7 log$_{10}$ cfu/carcass	Lehner et al., 2014
Scalding	Neck skin before chilling	Scalding water temperature	49°C 53°C 60°C	3.32 log$_{10}$ cfu/g 2.92 log$_{10}$ cfu/g 2.62 log$_{10}$ cfu/g	Wempe et al., 1983
Scalding	Chicken skin	Scalding water temperature	50°C 60°C	< 1 log$_{10}$ reduction > 2 log$_{10}$ reduction	Yang et al., 2001
Evisceration	Whole carcass rinse	Effect of visceral rupture		Average increase by 0.9 log$_{10}$ cfu/carcass	Boysen and Rosenquist, 2009
Washing	Carcass rinse	Inside outside washer (chlorinated 25 ppm)	Prewash Post wash I Post wash II Post wash III	4.69 log$_{10}$ cfu/ml 4.38 log$_{10}$ cfu/ml 4.36 log$_{10}$ cfu/ml 4.24 log$_{10}$ cfu/ml	Bashor et al., 2004
Washing	Carcass rinse	Inside outside washer (chlorinated 40 ppm)	Pre wash Post wash	1.93 log$_{10}$ cfu/ml 1.27 log$_{10}$ cfu/ml	Berrang and Bailey, 2009
Experimental after evisceration before inside outside wash	Breast skin	Spraying 15 s/30 s ASC (1000 ppm, at pH 2.39–2.67)	Reduction % increase < LOD	0.85/> 1.28 log$_{10}$ cfu/g 70/93	Purnell et al., 2014
	Neck skin		Reduction % increase < LOD	> 1.45/> 1.6 log$_{10}$ cfu/g 65/47	
	Breast skin	Spraying 15 s/30 s TSP (12 %, pH 12.4)	Reduction % increase < LOD	0.58/1.37 log$_{10}$ cfu/g 0/60	
	Neck skin		Reduction % increase < LOD	> 1.36/> 2.41 log$_{10}$ cfu/g 20/73	
	Breast skin	Spraying 15 s/30 s PAA (400 ppm PAA, 1600 ppm H2O2, 800 ppm acetic acid)	Reduction % increase < LOD	0.81/> 1.15 log$_{10}$ cfu/g 0/1	
	Neck skin		Reduction % increase < LOD	0.96/0.97 log$_{10}$ cfu/g 0/0	
	Breast skin	Spraying 15 s/30 s CD (6 ppm)	Reduction % increase < LOD	–/> 0.13 log$_{10}$ cfu/g –/10	
	Neck skin		Reduction % increase < LOD	–/–0.15 log$_{10}$ cfu/g –/0	
	Breast skin	Spraying 15 s/30 s Water only	Reduction % increase < LOD	0.03 / < 0.19 log$_{10}$ cfu/g 0/–2	
	Neck skin		Reduction % increase < LOD	0.23/0.40 log$_{10}$ cfu/g 0/0	

ASC: acidified sodium chlorite; TSP: trisodium phosphate; PAA: peroxyacetic acid; CD: chlorine dioxide; % increase < LOD: the rate of samples with numbers below the limit of detection after treatment.

scalding step, changes in the water bath temperature are an option although product quality has to be considered here. Alterations of the product quality limit the increase of temperature, sensory changes become apparent already after slight temperature changes (Lehner et al., 2014). Washing steps either after defeathering, or at the end after evisceration allow for washing off of debris from the slaughtering process or faecal contamination. The results are depending on the conditions of washing steps and can improve the overall hygiene at the slaughterhouse (Bashor et al., 2004; Lehner et al., 2014). Avoiding faecal contamination is an important part in broiler processing and cloacal plugging was tested in its ability to avoid faecal leakage. Musgrove at al. (1997) found statistically significant lower *Campylobacter* counts on plugged broilers, but the procedure was too elaborate for commercial application. Tests with mechanically induced defaecation of slaughtered birds by Northcutt et al. (2008) before entry to the scald tank on the other hand did not result in reduced *Campylobacter* counts or indicator bacteria concentration on the broilers, while an effect was seen for *Salmonella*. In addition, the process would lead to reduced entry of faecal matter to the scald tank. Table 3 illustrates slaughter processing steps where adjustments can result in the improvement of the *Campylobacter* reducing effect.

Decontamination treatments
The strategies mentioned above mainly focus on good hygiene practice and proper application of HACCP systems in prevention of faecal contamination along the slaughter line. Direct decontamination treatments are possible too and can include addition of chemicals to the washing steps like detergents or chlorine formulations, for instance in the final inside outside wash (del Rio et al. 2007; Stopforth et al. 2007). At the moment, none such treatments are approved for application on European broiler meat production. But there has been an opinion by the EFSA biohazard panel (2005) on the evaluation of the safety of chlorine dioxide (CD), acidified sodium chlorite (ASC), trisodium phosphate (TSP) and peroxyacetic acid (PAA) in washing treatments for chickens at the slaughterhouse and no safety concerns were found, but data was too limited to confirm the effectiveness of treatments. Further EFSA opinions provided guidance on efficacy and for safety evaluations of chemical decontamination substances. It includes a guide for study design and data preparation. Additionally there are still considerations about possible environmental risks or risks of development of reduced sensitivity in target organisms that might occur and data is lacking (EFSA, 2010). An EFSA opinion (2012) was published on the application of Cecure®, an aqueous solution of 1.0% of cetylpiridinium chloride and propylene glycol for poultry carcass dipping. The application seemed efficacious and safe for humans, but data on the environmental fate of the active substance was lacking and needs to be studied further (EFSA, 2012). Another EFSA opinion (2014b) focused on the evaluation of the safety and efficacy of peroxyacetic acid solutions. Efficacy was confirmed for indicator organisms and pathogens, but studies for the latter were limited. Risk evaluations mostly confirmed safety, but 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), which acts as product stabilizer, needs to be included in HACCP programs. Residues on carcasses need to be monitored. Additionally further studies are needed to assess resistance development in bacteria after treatment with PAA according to EFSA (2014b).

A recent evaluation by Purnell et al. (2014) for the effectiveness of decontamination additives in poultry meat production found the use of on-line sprays to be most promising and more favourable than dipping of carcasses. Exposure times tested were 15 s or 30 s and *Campylobacter*, *Enterobacteriaceae* and *Pseudomonas* spp. were enumerated on breast or neck skin. Significant reductions were found for all bacteria by ASC treatments for 15 s and 30 s. Trisodium phosphate was most effective in reducing numbers on neck skin after 30 s of application; effects were higher than for ASC. Overall, the reduction of *Campylobacter* after 30 s spray application was 1.37 and > 2.41 $\log_{10}$ cfu/g for TSP and >1 .28 and > 1.60 $\log_{10}$ cfu/g for ASC on breast skin and neck skin, respectively. Chlorine dioxide was the least effective agent, probably because of concentration drop during spraying. Evaluation of treatment duration led to a significant benefit of the 30 s spray versus 15 s for TSP only (Purnell et al., 2014) (Tab. 3). Physical decontamination was tested with hot steam for 10 s or water wash (80°C) for 20s followed by crust freezing, which led to *Campylobacter* reductions of ca. 3.2 and 2.9 $\log_{10}$ cfu/cm² of breast skin for steam and water, respectively. The steam treatment led to damage of the carcasses, so the hot water treatment was considered more useful, because the carcass appearance was acceptable by still achieving a similar decontamination effect (James et al., 2007). The application of ultrasound was tested by Haughton et al. (2012) on inoculated chicken skin treated for 16 minutes immersed in a sonication bath at two different intensities: high intensity (HI) at 20,000 W/L or low intensity (LI) with 20 W/L. Samples were initially inoculated with *Campylobacter* at ca. 5.0 $\log_{10}$ cfu/g. Application of HI sonication led to non-detectable *Campylobacter* counts, while LI sonication led to only minor reductions of less than one $\log_{10}$-unit, which was not significant. Musavian et al. (2014) tested the effectiveness of ultrasound (30–40 kHz) in combination with hot steam (90–94°C) on broiler carcasses online during regular slaughterhouse operations. Initial *Campylobacter* levels on breast skin were 2.35 $\log_{10}$ cfu/g and were reduced to 1.40 $\log_{10}$ cfu/g post treatment. In following trials an average reduction of 1 $\log_{10}$-unit was achieved with treatment times of 1.0–1.5 s. Sensory evaluation of broiler carcasses resulted in a fit for purchase rating.

Until now, the different chemical and physical decontamination treatments were mostly evaluated at model scale equipment, or online for a limited amount of time. Additional testing under field conditions is necessary to assess the *Campylobacter* reducing effects on a daily basis. In addition such measures should only be applied in combination with good hygiene practice and working HACCP concepts.

Conclusion

The main aim of food safety programs targeting foodborne *Campylobacter* infections must be the reduction of *Campylobacter* spp. in the final product. In case of broiler production this can only be achieved by applying mitigation strategies at both the pre- and harvest level. In primary production well established biosecurity measures are already in place but must be supplemented by more

advanced strategies like bacteriophage application or application of different organic acids or electrolyzed oxidizing water. These methods are still under development and only few real life field trials have shown their effectivity in principal. The reduction must be further achieved by measures in the slaughterhouse, beginning with measures to prevent cross-contamination like logistic or scheduled slaughter. Process parameters must be proved for effectiveness concerning *Campylobacter* reduction. So far no single parameter can be recommended, as e. g. quality parameters are also affected. Therefore measures to reduce *Campylobacter* spp. in the food chain must be applied at primary production and must be accompanied by hygienic slaughter with improved process parameters, which still have to be developed.

Conflict of Interest

The authors declare that not competing interests exist.

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