ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 11 (8): 536-540, 2012
ISSN 1682-8356

Effects of Trans-Cinnamaldehyde on *Campylobacter* and Sperm Viability in Chicken Semen after *In vitro* Storage†

G.Q. Liu[1], A.M. Donoghue[2], J.R. Moyle[2], I. Reyes-Herrera[3], P.J. Blore[3], R.K. Bramwell[3], D.E. Yoho[3], K. Venkitanarayanan[4] and D.J. Donoghue[3]
[1]College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, P.R. China 225009
[2]Poultry Production and Product Safety Research Unit, Agricultural Research Service, USDA, Fayetteville, Arkansas 72701, USA
[3]Poultry Science Department, University of Arkansas, Fayetteville, Arkansas 72701, USA
[4]Department of Animal Science, University of Connecticut, Storrs, CT 06269, USA

Abstract: *Campylobacter* is one of the leading causes of bacterial human acute gastroenteritis. These microorganisms are highly prevalent in poultry semen and may contribute to vertical transmission of the pathogen between the breeder hen and offspring. Unfortunately, strategies to reduce or eliminate these pathogens in poultry semen negatively impact sperm viability. Many plant essential oils have been reported to exhibit antimicrobial activity against bacteria, fungi and viruses. The objective of our study was to examine the efficacy of trans-cinnamaldehyde, the main component in cinnamon oil, to reduce *Campylobacter* concentrations in chicken semen. Semen was collected from roosters, pooled and diluted with semen extender, then divided into treatments: negative control (no *Campylobacter*, no trans-cinnamaldehyde), positive control (inoculated with *Campylobacter*, no trans-cinnamaldehyde) or treatments containing concentrations of 0.24, 0.12, 0.06, 0.03 or 0.015% trans-cinnamaldehyde. Treatment groups receiving *Campylobacter* were then immediately inoculated with ~10^5 cfu/mL of a wild-type *Campylobacter jejuni* and held at 4°C or 23°C for 2 h. Semen was stored at 4°C for an additional 24 h and assessed for *Campylobacter* concentrations and sperm viability at 2, 6 and 24 h utilizing SYBR 14/Propidium iodide live/dead stain and fluorescent microscopy. The study was replicated eight times. After 2h at 23°C a 2 log reduction in *Campylobacter* counts were observed in the 0.12 and 0.24% trans-cinnamaldehyde treatment groups compared to positive controls. In the 4°C treatments, no differences were observed between treatments and controls after 2 h. Samples evaluated after 24 h incubation *in vitro* at 4°C, showed significant reductions of *Campylobacter* counts in the 0.06, 0.03 or 0.015% trans-cinnamaldehyde treatments groups, while the 0.12 and 0.24% groups eliminated detectable *Campylobacter* counts. Sperm viability remained at 80% or above for all treatment groups. Trans-cinnamaldehyde reduced *Campylobacter* in semen, without detrimentally affecting sperm viability and might provide a practical solution to eliminate *Campylobacter* in poultry semen after *in vitro* storage.

Key words: *Campylobacter*, trans-cinnamaldehyde, chicken, sperm

INTRODUCTION

Campylobacter, a Gram-negative bacterium, is one of the leading bacterial causes of human acute gastroenteritis in many countries, especially in developed countries (Centers for Disease Control and Prevention, 2007; Workman *et al*., 2006). *Campylobacter* is commonly found in the gastrointestinal tract of most wild and domestic animals (Wagenaar *et al*., 2006), however, epidemiological evidence indicates that consumption or handling of raw or undercooked contaminated poultry products are the most common sources of human *Campylobacter* infections (Corry and Attabay, 2001; Lee and Newell, 2006; Wong *et al*., 2007). Due to the ubiquitous nature of the organism, there are multiple potential sources for flock infection, leading to 75-90% *Campylobacter* prevalence among poultry flocks in the United States (Stern *et al*., 2001). Numerous studies have also documented the incidence and prevalence of this pathogen in the reproductive tract of both male and female poultry (Buhr *et al*., 2002; Hiett *et al*., 2002a; Cox *et al*., 2002a, 2002c, 2005a, 2005b; Cole *et al*., 2004, 2006), in poultry semen (Hiett *et al*., 2003; Donoghue *et al*., 2004; Buhr *et al*., 2005; Cox *et al*., 2002a, 2002b, 2005a; Cole *et al*., 2006, Vizzier-Thaxton *et al*., 2006), hatcheries and eggs (Hiett *et al*., 2002b; Byrd *et al*., 2007) indicating that vertical transmission of the organism (from breeders to their offspring) can play an important role in the transmission and incidence of *Campylobacter* in poultry flocks (Cox *et al*., 2002c; Wagenaar *et al*., 2006). Unfortunately, studies have shown that strategies such as addition of antibiotics or iron chelators to conventional poultry semen diluents, or

Corresponding Author: A.M. Donoghue, USDA/ARS/PPPSRU O-303, Poultry Science Center, University of Arkansas, 1260 W. Maple St. Fayetteville, AR 72701, USA

changes in oxygen or temperatures conditions during storage, have not been completely effective in the reduction or elimination of *Campylobacter* from poultry semen (Cole *et al*., 2004, 2006; Donoghue *et al*., 2004). A potential strategy to control *Campylobacter* in semen is use of the active components of plant-derived essential oils. Trans-cinnamaldehyde is a natural compound that is the principal component in cinnamon oil (*Cinnamomum verum)*. Trans-cinnamaldehyde has been reported to possess antimicrobial activity towards a wide range of pathogens, including *Clostridium botulinum* (Bowles and Miller, 1993), *Clostridium perfringens* (Si *et al*., 2009), *Staphylococcus aureus* (Bowles *et al*., 1995), *E. coli* O157:H7, *Campylobacter jejuni*, *Listeria monocytogenes* and *Salmonella enterica* (Friedman *et al*., 2002; Kollanoor Johny *et al*., 2008, 2010).

Recently it was demonstrated that trans-cinnamaldehyde was effective in reducing *Salmonella Enteritidis* and *Campylobacter jejuni* in chicken cecal contents and could potentially be used to control these pathogens in chickens through the drinking water on farms (Kollanoor Johny *et al*., 2008; 2010). Thus, the objective of the present study was to evaluate the potential efficacy of trans-cinnamaldehyde in reducing *Campylobacter* concentrations from chicken semen after *in vitro* storage.

MATERIALS AND METHODS

Oil preparation: Trans-cinnamaldehyde (Sigma-Aldrich, St. Louis, MO) was dissolved (0.48%, vol:vol) in Field Ready Green Extender (IMV International Corp, North Maple Grove, MN) with 30% 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich, St. Louis, MO) before adding to chicken semen. Concentrations of trans-cinnamaldehyde were selected based on its effect on chicken sperm viability (data not shown) and its activity against *Campylobacter jejuni* (Friedman *et al*., 2002).

Sample preparation: Thirty 48 week old roosters were individually caged, fed standard diets *ad libitum* and kept under a 14L: 10D photoperiod during the experiment. The study was replicated 8 times. In each trial, semen samples from the roosters were collected by abdominal massage and aspirated into sterile test tubes. Pooled semen were diluted 1:1 (vol:vol) with Field Ready Green semen extender. Then, 400 ul semen aliquoted into 500 ul control or treatments containing 0.24, 0.12, 0.06, 0.03 or 0.015% trans-cinnamaldehyde (diluted in Green Extender). The negative control (no *Campylobacter*) or the positive control (inoculated with *Campylobacter*) groups received no trans-cinnamaldehyde. Each treatment group, except for the negative control, was then inoculated with 10^5 cfu/ml of a wild-type *C. jejuni* isolate (previously collected from chicken semen) in 100 ul of *Campylobacter* Enrichment Broth (CEB). The negative control received 100 ul of CEB alone. Conventionally, poultry semen is collected and inseminated within a short period and thus it is kept at room temperature (23°C). Alternatively, if a longer period will occur before insemination, the semen is kept at refrigeration temperature (4°C). To simulate both possibilities, samples were evaluated at room temperature (23°C) after 2 h or at 4°C for 2, 6 and 24 h with agitation (150 rpm; Thurston *et al*., 1998).

Enumeration of bacteria and sperm viability: After each *in vitro* storage interval, a sample was taken from each treatment group, serially diluted with Butterfield's phosphate diluent (BPD, 1:10) and plated on duplicate Campy Line agar plates for enumeration (CLA; Line, 2001). The plates were then incubated for 48 h at 42°C in a microaerophilic environment (5% O_2, 10% CO_2 and 85% N_2). After incubation, characteristic colonies were confirmed as *Campylobacter* by observation of typical cellular morphology using a phase contrast microscope and with a commercial latex agglutination test kit (Pan Bio Inc., Columbia, MD) specific for *C. jejuni, C. coli* and *C. laridis*. The colonies on each CLA plate were counted on a Leico Darkfield plate colony counter (Leica Inc., Buffalo, NY) and the direct counts were converted to $\log_{10}$ colony-forming units per milliliter of extender. At each time point, sperm viability was assessed for each treatment group utilizing SYBR 14/Propidium iodide live/dead kit and fluorescent microscopy according to the method of Donoghue *et al*. (1995).

Statistical analysis: Data were analyzed using the GLM procedure of SAS (SAS Institute, 2002). The number of *Campylobacter* colonies was logarithmically transformed ($\log_{10}$ cfu/ml) before analysis to achieve homogeneity of variance. Sperm viability data expressed as percentages were arc sine transformed before analysis. A probability of $p<0.05$ was required for statistical significance. The data in Table 1 and 2 are shown as arithmetic means for clarity of presentation.

Table 1: Effect of trans-cinnamaldehyde on sperm viability (%) in chicken semen after *in vitro* storage at 4° or 23°C[1, 2]

	Temperature			
	23°C	4°C		
Treatment	2 h	2 h	6 h	24 h
Negative control	95[a]	95[a]	95[a]	95[a]
Positive control	93[a]	95[a]	94[a]	91[b]
Trans-cinnamaldehyde (0.24%)	84[b]	93[a]	88[c]	80[d]
Trans-cinnamaldehyde (0.12%)	92[a]	95[a]	91[b]	81[d]
Trans-cinnamaldehyde (0.06%)	94[a]	95[a]	94[a]	88[c]
Trans-cinnamaldehyde (0.03%)	94[a]	95[a]	94[a]	90[b]
Trans-cinnamaldehyde (0.015%)	94[a]	95[a]	94[a]	90[b]

[a-c]Means with no common superscript within columns differ significantly ($p<0.05$).

[1]All data were arc sine transformed for statistical analysis. For clarity of presentation, arithmetic means are presented.

[2]In 8 separate trials, sperm viability of each treatment was assessed utilizing SYBR 14/Propidium iodide live/dead kit

Table 2: Effect of Trans-cinnamaldehyde on *Campylobacter* in chicken semen[1, 2]

	Temperature			
	23°C	4°C		
Treatment	2 h	2 h	6 h	24 h
Positive control	$5.22x10^{5\,a}$	$6.59x10^{5\,a}$	$8.03x10^{5\,a}$	$5.80x10^{5\,a}$
Trans-cinnamaldehyde (0.24%)	$8.96x10^{3\,b}$	$2.47x10^{5\,a}$	$9.03x10^{3\,d}$	$<10^{e}$
Trans-cinnamaldehyde (0.12%)	$9.61x10^{3\,b}$	$4.32x10^{5\,a}$	$3.54x10^{4\,c}$	$<10^{e}$
Trans-cinnamaldehyde (0.06%)	$2.65x10^{4\,a,b}$	$4.51x10^{5\,a}$	$1.07x10^{5\,b}$	$6.06x10^{2\,d}$
Trans-cinnamaldehyde (0.03%)	$1.41x10^{5\,a}$	$3.18x10^{5\,a}$	$2.52x10^{5\,a,b}$	$1.14x10^{4\,c}$
Trans-cinnamaldehyde (0.015%)	$4.41x10^{5\,a}$	$4.02x10^{5\,a}$	$4.28x10^{5\,a}$	$1.66x10^{5\,b}$

[a-e]Means with no common superscript within columns differ significantly ($p<0.05$).

[1]All data were log_{10} transformed for statistical analysis. For clarity of presentation, arithmetic means are presented.

[2]In 8 separate trials, 0.4 mL of diluted semen was added to Field Ready Green Extender containing different concentrations of Trans-cinnamaldehyde and then inoculated with 0.1 mL wild-type *C. jejuni* semen isolate averaging 10^6 cfu/mL. Each treatment group was incubated at 4°C or 23°C for 24 hr with agitation (150 rpm)

RESULTS

The effect of trans-cinnamaldehyde on sperm viability is depicted in Table 1. At 23°C, the 0.24% trans-cinnamaldehyde group significantly reduced sperm viability after 2 hrs storage, but the value was still above 80% which would be acceptable for artificial insemination. The other trans-cinnamaldehyde groups maintained sperm viability equal to the positive and negative controls, suggesting that trans-cinnamaldehyde at these concentrations does not negatively affect chicken spermatozoa when stored for 2 h at 23°C. At 4°C, all trans-cinnamaldehyde groups maintained sperm viability equal to the controls at 2 h of storage (Table 1). After 6 h of storage at 4°C, trans-cinnamaldehyde at 0.12% and 0.24% sperm viability was reduced but still maintained 88-91% viability whereas treatments of 0.015-0.06% were not different from control (Table 1). Sperm viability in all the trans-cinnamaldehyde groups and the positive control were lower than that of the negative control at 24 h of storage at 4°C, however, sperm viability ranged from 80% to 91%, which would still be acceptable for artificial insemination.

The effect of trans-cinnamaldehyde on *Campylobacter* in chicken semen is depicted in Table 2. Trans-cinnamaldehyde lowered *Campylobacter* counts at 2 h 23°C in the 0.12% and 0.24% groups by 2 logCFU/ml. At 2 h of storage at 4°C trans-cinnamaldehyde had no effect on reducing *Campylobacter*. However, by 6 h of storage at 4°C, *Campylobacter* was reduced in the 0.12-0.24% groups compared to control. *Campylobacter* was not detected in the 0.12% and 0.24% trans-cinnamaldehyde groups at 24 h 4°C and all other treatments were significantly lower than the control.

DISCUSSION

Campylobacter is one of the leading bacterial causes of human foodborne infections in the United States (Friedman *et al*., 2000; Centers for Disease Control and Prevention, 2007). Epidemiological evidence has emphasized the importance of poultry products as a significant source of human *Campylobacter* infection (Jacobs-Reitsma, 2000; Corry and Attabay, 2001). Studies suggest that the organism is highly prevalent in poultry semen and may contribute to vertical transmission between the breeder hen and offspring (Cox *et al*., 2002b; Cole *et al*., 2004, 2006). Unfortunately, strategies to reduce or eliminate *Campylobacter* in poultry semen, such as aeration, reduced storage temperatures and dilution with extenders containing antibiotics or iron chelators have not been completely effective because they either did not reduce *Campylobacter* levels or negatively impacted sperm viability parameters (Cole *et al*., 2004; 2006; Donoghue *et al*., 2004).

Trans-cinnamaldehyde, a major component of the bark extract of cinnamon, is a food-grade chemical approved by the Food and Drug Administration as Generally Regarded as Safe (21 CFR 182.60). Trans-cinnamaldehyde has reported antimicrobial activity towards a wide range of foodborne pathogens, including Gram-positive and Gram-negative bacteria (Bowles and Miller, 1993; Bowles *et al*., 1995; Friedman *et al*., 2002). Friedman and co-workers (2004) demonstrated that trans-cinnamladehyde can reduce pathogenic bacteria at refrigeration temperatures, which would suggest its potential for reducing *Campylobacter* in poultry semen kept temporarily under refrigeration temperatures for insemination practices.

The data obtained in this study indicated that trans-cinnamaldehyde has the potential as an antimicrobial agent for reducing *Campylobacter* in chicken semen. At 0.12% and 0.24% trans-cinnamaldehyde after 24 h of *in vitro* storage, *Campylobacter* was not detectable yet acceptable sperm viability was maintained. Follow up studies to determine the influence of trans-cinnamaldehyde on fertility are needed, however, these studies show that trans-cinnamaldehyde can effectively

reduce *Campylobacter* from semen without severely impacting sperm viability. Trans-cinnamaldehyde could be used to reduce this pathogen in semen used for artificial insemination and to further reduce vertical transmission of *Campylobacter* in chicken flocks.

ACKNOWLEDGEMENTS

Funded in part by Jiangsu Government Scholarship for Overseas Studies and by USDA, CSREES National Integrated Food Safety Program #2006-02429 to Venkitanarayanan and Donoghue and the Arkansas Bioscience Institute Program.

REFERENCES

Bowles, B.L. and A.J. Miller, 1993. Antibotulinal properties of selected aromatic and aliphatic aldehydes. J. Food Prot., 56: 788-794.

Bowles, B.L., S.K. Sackitey and A.C. Williams, 1995. Inhibitory effects of flavor compounds on *Staphylococcus aureus* WRRC B124. J. Food Saf., 15: 337-347.

Buhr, R.J., N.A. Cox, N.J. Stern, M.T. Musgrove, J.L. Wilson and K.L. Hiett, 2002, Recovery of *Campylobacter* from segments of the reproductive tract of broiler breeder hens. Avian Dis., 46: 919-924.

Buhr, R.J., M.T. Musgrove, L.J. Richardson, N.A. Cox, J.L. Wilson, J.S. Bailey, D.E. Cosby and D.V. Bourassa, 2005. Recovery of *Campylobacter jejuni* in feces and semen of caged broiler breeder roosters following three routes of inoculation. Avian Dis., 49: 577-581.

Byrd, J., R.H. Bailey, R. Wills and D. Nisbet, 2007. Recovery of *Campylobacter* from commercial broiler hatchery trayliners. Poult. Sci., 86: 26-29.

Centers for Disease Control, 2007. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food–10 states, United States, 2006. Morb. Mortal. Wkly. Rep., 56: 336-339.

Cole, K., A.M. Donoghue, P.J. Blore, J.S. Holliman, N.A. Cox, M.T. Musgrove and D.J. Donoghue, 2004. Effects of aeration and storage temperature on *Campylobacter* concentrations in poultry semen. Poult. Sci., 83: 1734-1738.

Cole, K., A.M. Donoghue, I. Reyes-Herrera, N.R. Rath and D.J. Donoghue, 2006. Efficacy of iron chelators on *Campylobacter* concentrations in turkey semen. Poult. Sci., 85: 1462-1465.

Corry, J.E.L. and H.I. Attabay, 2001. Poultry as a source of *Campylobacter* and related organisms. J. Appl. Microbiol., 90: 96S-114S.

Cox, N.A., J.L. Wilson, M.T. Musgrove, R.J. Buhr and B.P. Hudson, 2002a. Isolation of *Campylobacter* from the vas deferens of 65 week old commercial broiler breeder roosters. Poult. Sci., 80 (Suppl.):153.

Cox, N.A., N.J. Stern, J.L. Wilson, M.T. Musgrove, R.J. Buhr and K.L. Hiett, 2002b. Isolation of *Campylobacter spp*. from semen samples of commercial roosters. Avian Dis., 46: 717-720.

Cox, N.A., N.J. Stern, K.L. Hiett and M.E. Berrang, 2002c. Identification of a new source of *Campylobacter* contamination in poultry: Transmission from breeder hens to broiler chickens. Avian Dis., 46: 535-541.

Cox, N.A., C.L. Hofacre, R.J. Buhr, J.L. Wilson, J.S. Bailey, L.J. Richardson, D.E. Cosby, M.T. Musgrove, K.L. Hiett and S.M. Russell, 2005a. Attempts to isolate naturally occurring *Campylobacter*, *Salmonella* and *Clostridium perfringens* from the ductus deferens, testes and ceca of commercial broiler breeder roosters. J. Appl. Poult. Res., 14: 126-129.

Cox, N.A., J.S. Bailey, L.J. Richardson, R.J. Buhr, D.E. Cosby, J.L. Wilson, K.L. Hiett, G.R. Siragusa and D.V. Bourassa, 2005b. Presence of naturally occurring *Campylobacter* and *Salmonella* in the mature and immature ovarian follicles of late-life broiler breeder hens. Avian Dis., 49: 285-287.

Donoghue, A.M., D.L. Garner, D.J. Donoghue and L.A. Johnson, 1995. Viability assessment of turkey sperm using fluorescent staining and flow cytometry. Poult. Sci., 74: 1191-1200.

Donoghue, A.M., P.J. Blore, K. Cole, N.M. Loskutoff and D.J. Donoghue, 2004. Detection of *Campylobacter* or *Salmonella* in turkey semen and the ability of poultry semen extenders to reduce their concentrations. Poult. Sci., 83: 1728-1733.

Friedman, C.R., J. Neimann, H.C. Wegener and R.V. Tauxe, 2000. Epidemiology of *C. jejuni* infections in the United States and other industrialized nations. Pages 121-138 in Campylobacter.

Friedman, M., P.R. Henika and R.E. Mandrell, 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. J. Food Prot., 65: 1545-1560.

Friedman, M., P.R. Henika, C.E. Levin and R.E. Mandrell, 2004. Antibacterial activities of plant essential oils and their components against *Escherichi coli* O157:H7 and *Salmonella enterica* in apple juice. J. Agric. Food Chem., 52: 6042-6048.

Hiett, K.L., N.J. Stern, N.A. Cox and R.J. Buhr, 2002a. Genotype analyses of *Campylobacter* isolated from distinct segments of the reproductive tracts of broiler breeder hens. Curr. Microbiol., 45: 400-404.

Hiett, K.L., N.A. Cox and N.J. Stern, 2002b. Direct polymerase chain reaction detection of *Campylobacter spp*. in poultry hatchery samples. Avian Dis., 46: 219-223.

Hiett, K.L., G.R. Siragusa, N.A. Cox, R.J. Buhr, M.T. Musgrove, N.J. Stern and J.L. Wilson, 2003. Genotype analyses of *Campylobacter* isolated from the gastrointestinal tracts and the reproductive tracts of broiler breeder roosters. Avian Dis., 47: 406-414.

Jacobs-Reitsma, W., 2000. *Campylobacter* in the food supply. In *Campylobacter*. I. Nachamkin and M.J. Blaser, Eds. ASM Press, Washington, DC., pp: 467-481.

Kollanoor Johny, A., M.J. Darre, T.A. Hoagland, D.T. Schreiber, A.M. Donoghue, D.J. Donoghue and K. Venkitanarayanan, 2008. Antibacterial effect of trans-cinnamaldehyde on *Salmonella* enteritidis and *Campylobacter jejuni* in chicken drinking water. J. Appl. Poult. Res., 17: 490-497.

Kollanoor Johny, A., M.J. Darre, A.M. Donoghue, D.J. Donoghue and K. Venkitanarayanan, 2010. Antibacterial activity of *trans*-cinnamaldehyde, eugenol, carvacrol and thymol on *Salmonella* Enteritidis and *Campylobacter jejuni* in chicken cecal contents *in vitro*. J. Appl. Poult. Res., 19: 237-244.

Lee, M.D. and D.G. Newell, 2006. *Campylobacter* in poultry: Filling an ecological niche. Avian Dis., 50: 1-9.

Line, J.E., 2001. Development of a selective differential agar for isolation and enumeration of *Campylobacter* spp. J. Food Prot., 64: 1711-1715.

SAS Institute, 2002. SAS/STAT User's Guide: Release 9.03 edition. SAS Institute Inc., Cary, NC.

Si, W., X. Ni, J. Gong, H. Yu, R. Tsao, Y. Han and J.R. Chambers, 2009. Antimicrobial activity of essential oils and structurally related synthetic food additives towards *Clostridium perfringens*. J. Appl. Microbiol., 106: 213-220.

Stern, N.J., P. Fedorka-Cray, J.S. Bailey, N.A. Cox, S.E. Craven, K.L. Hiett, M.T. Musgrove, S. Ladely, D. Cosby and G.C. Mead, 2001. Distribution of *Campylobacter* spp. in selected U.S. poultry production and processing operations. J. Food Prot., 64: 1705-1710.

Thurston, R.J., T.R. Scott, N. Korn and D.A. Barnes, 1998. Effects of varying aeration treatment on fertilizing capacity of semen diluted with perfluorochemical emulsion and stored for twenty-four hours. Poult. Sci., 77: 1051-1055.

Vizzier-Thaxton, Y., N.A. Cox, L.J. Richardson, R.J. Buhr, C.D. McDaniel, D.E. Cosby, J.L. Wilson, D.V. Bourassa and M.B. Ard, 2006. Apparent attachment of *Campylobacter* and *Salmonella* to broiler breeder rooster spermatozoa. Poult. Sci., 85: 619-624.

Wagenaar, J.A., D.J. Mevius and A.H. Havelaar, 2006. *Campylobacter* in primary animal production and control strategies to reduce the burden of human campylobacteriosis. Rev. Sci. Tech. Off. Int. Epiz., 25: 581-594.

Wong, T.L., L. Hollis, A. Cornelius, C. Nicol, R. Cook and J.A. Hudson, 2007. Prevalence, numbers and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. J. Food Prot., 3: 566-573.

Workman, S.N., S.J. Sobers, G.E. Mathison and M.C. Lavoie, 2006. Human *Campylobacter*-associated enteritis on the Caribbean island of Barbados. Am. J. Trop. Med. Hyg., 74: 623-627.

[†]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that are suitable