Tabella 2: Riepilogo dei controlli per Influenza Aviaria effettuati in allevamento in Emilia Romagna.
Table 2: *Results of AI serological controls in flocks of Emilia-Romagna.*

Indirizzo produttivo	Allevamenti controllati	Allevam. positivi	%	Numero controlli	Campioni esaminati	Campioni positivi	%
Polli riproduttori	83	0	0,0%	477	9.545	0	0,0%
Pollastre	164	0	0,0%	346	6.757	0	0,0%
Galline ovaiole	34	0	0,0%	43	844	0	0,0%
Altri polli	223	1	0,4%	405	7.747	8	0,1%
Tacchini riprod.	11	0	0,0%	37	716	0	0,0%
Tacchini carne	77	0	0,0%	163	2.884	0	0,0%
Faraone	20	0	0,0%	50	939	0	0,0%
Selvaggina	38	1	2,6%	104	1.909	16	0,8%
Anatidi	11	3	27,3%	68	1.215	112	9,2%
Colombi	10	0	0,0%	11	210	0	0,0%
Struzzi	24	0	0,0%	32	345	0	0,0%
Misti	16	2	12,5%	46	756	7	0,9%
TOTALE	*711*	*7*	*1,0%*	*1.782*	*33.867*	*143*	*0,4%*

COMUNICAZIONE 8

STUDY OF THE SURVIVAL RATE OF *C. jejuni* ON DIFFERENT FOOD CONTACT SURFACES

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Key Words: cross contamination, *Campylobacter jejuni*, surfaces

Studio della dinamica di sopravvivenza di *C. jejuni* su alcune superfici di lavorazione degli alimenti
Parole chiave: contaminazione crociata, *Campylobacter jejuni*, superfici

Riassunto: Per valutare la dinamica di sopravvivenza di *C.jejuni* su alcune superfici di lavorazione degli alimenti, cinque ceppi rappresentanti sia isolati ambientali che ceppi di referenza sono stati inoculati in campioni di metallo e formica. Prima dell'inoculo i ceppi sono stati sospesi in Tryptycase Soy Broth (TSB) per simulare le condizioni di sopravvivenza delle cellule su una superficie contaminata, ed in Phosphate Buffered Saline (PBS) (pH 7) per simulare le condizioni di sopravvivenza delle cellule su una superficie pulita. I risultati ottenuti dimostrano che sia la natura della superficie che le condizioni ambientali influenzano la dinamica di sopravvivenza delle cellule di *C.jejuni*.

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Introduction
Although many cases of Campylobacter enteritis have been attributed to undercooking of poultry (3), cross contamination of raw to cooked foods has been identified as a significant risk factor for *C. jejuni* infections (1,2). Cross contamination may be particularly important in relation to the high prevalence of contamination in raw poultry products and other foods and the low infectious doses that have been reported for *Campylobacter* species. The objective of this study was to assess the survival rate and persistence of *C. jejuni* under varying organic loads on stainless steel and a Formica™ laminate surface used in food preparation areas.

Materials and methods
The five *C. jejuni* strains used in the study were obtained from the U.S. Department of Agriculture-Agriculture Research Service (Athens, GA) and Qualicon Inc. (Wilmington, DE) and represent both environmental isolates and type reference strains. Stock cultures of each strain were maintained in phosphate buffered saline (PBS, pH 7.0) containing 10-16% (vol/vol) glycerol and stored at –20°C. For each strain, individual 10 ml Brucella broth cultures were prepared (48 h, 42°C) in a microaerophilic environment (10% CO_2, 85% N_2, 5% O_2) and 0,1 ml subsamples of each was transferred to 30 ml of fresh Brucella broth and incubated as previously described. The broth cultures were divided in two 15-ml aliquots and the cells from each pelleted by centrifugation (12 min. at 5 °C and 90,000×*g*), resuspended in 3 ml of sterile 0,1% peptone water (PW), pooled into a common tube (five strains), and centrifuged again as previously described. One half of the cell pellets were resuspended in 25 ml of trypticase soy broth (TSB) and the other half in 25 ml PBS (ca 10^8 CFU/ml). Sterile (121°C /15 min) 5 cm^2 samples (coupons) of stainless steel and of a Formica ™ countertop laminate, examples of typical food contact surfaces, were purchased from a local home supply store. One hundred μl of TSB and PBS *C. jejuni* suspensions were individually inoculated in the center of each coupon to yield a population of approximately 10^7 CFU/cm^2. Each inoculated coupon was transferred to a sterile petri dish, covered with a filter paper lid to facilitate drying, and incubated at 26,7°C and 60-62% RH. Three coupons per contact surface and inoculum suspension (TSB and PBS) were sampled immediately after inoculation and at 15, 30, 45, 60, 90, 120, 135, 150, 165, 180, 195, 210, 225 and 250 min of incubation. Viable *C. jejuni* cells were recovered from the coupons using a rinse procedure. Each coupon was placed in a stomacher bag containing 4,9 ml of 0,1% PW and agitated for 2 min in an IUL Instruments Masticator (model No. 0400). This initial 1:50 dilution was agitated in a vortex mixer, serially diluted in PW,

and spiral plated in duplicate on Campy Cefex Agar. The plates were incubated for 48 h at 42°C in a microaerophilic environment and colonies enumerated. At the time of plating, 1 ml of the sample PW dilution was transferred to 8 ml of Campylobacter Enrichment Broth and incubated as previously described. Three to six trials were conducted for each coupon and suspension broth type. Survivor curves (log viable *C. jejuni* per ml of rinse versus time) were plotted for each trial and best-fit linear regression lines were determined. *D*-values (min) were calculated as the negative reciprocal of the survivor curve slope obtained by regression analysis. A mean *D*-value was calculated from the individual trial *D*-values.

Results

Examination of the survivor curves for the five-strain pool of *C. jejuni* in TSB on stainless steel surfaces revealed a biphasic curve that appeared to be related to the degree of desiccation of the 0,1 ml volume of inoculum on the coupon. No significant reduction in the *C. jejuni* population was detected over the first 90-min post inoculation. Between 90 and 120 min, the liquid area of the inoculum spot began to recede which led to a significant linear (r = -0.98) decrease in the *C. jejuni* population of approximately 7 logs over the next 105 min of incubation. No viable *C. jejuni* were recovered after 195 min. The mean decimal reduction time (*D*-value) under these storage conditions was 13.1 min. In contrast, survivor curves for *C. jejuni* cells suspended in PBS and then inoculated on the stainless steel coupons had a significantly different inactivation profile. Similar to the TSB survivor data, the population of viable *C. jejuni* organisms remained constant over the first 90 min post inoculation. This was followed by a linear (r = -0.98) decrease in population of nearly 4 logs from 90 to 225 min of incubation. The rate of cell inactivation was over two fold slower (*D* = 29.5 min) when the cells were suspended in PBS compared to TSB (*D* = 13.1 min). This finding was contrary to our original hypothesis where we speculated that *C. jejuni* cells suspended in a nutritionally enriched media such as TSB (analogous to more highly contaminated food preparation surfaces containing significant organic loads) would be protected from inactivation to a greater degree than cells suspended in a nutritionally inferior saline solution (PBS, example of a clean food preparation surface). Several factors may account for these findings. First, PBS may be a better support medium than TSB for maintaining the viability of *C. jejuni* under desiccation and aerobic conditions. A second explanation may be the presence of an inhibitor in TSB that led to a greater inactivation of *C. jejuni* during storage. When inoculated onto a Formica™ countertop surface, *C. jejuni* cells suspended in TSB respond similarly to the stainless steel survivor data such that there was an initial lag phase of minimal cell death that extended out to about 165 min of incubation. This initial phase was followed by a rapid and linear (r = -0.96) decrease in *C. jejuni* population of about 6.7 logs from 165 to 225 min post inoculation. The mean *D*-value over the final 60 min of incubation was 8.7 min. The inoculum appeared to dry on the Formica™ surface at a slower rate than stainless steel. When suspended in PBS, *C. jejuni* cells that were inoculated on Formica™ surfaces were protected from the surrounding environmental conditions to a greater degree than when suspended in TSB. For example, when suspended in TSB, significant cell inactivation began between 165 and 180 min of incubation as opposed to between 195 to 210 min in PBS. Moreover, after 225 min of incubation, only a 1,4 log reduction in *C. jejuni* population was detected for cells suspended in PBS as opposed to a 6.7 log reduction after 210 min for cells suspended in TSB.

Discussion

These findings clearly demonstrate that both suspension media as well as food preparation surface composition can influence the survival and persistence of *C. jejuni*. In general, as food contact surfaces remain moist, *C. jejuni* can persist for extended periods of time. As surfaces dry, *C. jejuni* cell populations undergo a rapid decline that is dependent on the presence of organic and inorganic loads but still can be detected after approximately 180 to 210 min post contamination (3 to 3.5 h) in the case of cells suspended in TSB, to between 289 to 335 min (4.8 to 5.6 h) when suspended in PBS. Clearly there is sufficient time for *C. jejuni* cross contamination to occur between contaminated food contact surfaces and ready to eat foods that come in contact with these surfaces.

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