

No contamination of the inside of turkey hatching eggs following contamination of their shell surface by *Campylobacter jejuni*.

C. MAGRAS (1), M. LAROCHE (1), T. AUBINEAU (1), J.M. WATIER (2), M. FEDERIGHI (1)

(1)UMR ENVN / INRA 1014, Ecole Nationale Vétérinaire, BP 40706, 44307 NANTES cedex 3, France

(2)GRELIER, S.A. Elevage Avicole de la Bohardière, BP 1, 49290 Saint Laurent de la Plaine, France

Campylobacter sp. is one of the most frequent bacterial causes of human enteritis with *C. jejuni* most implicated. *C. jejuni* is often found in the digestive tract of chickens. Although vertical transmission of *C. jejuni* from breeder flocks to the broilers farms via the egg is suggested, the contamination of egg contents in the hen's reproductive tract during egg development seems to be a rare event. However the contamination of the egg contents after contamination of the eggshell can occur (Doyle 1984; Sahin et al., 2003). In the turkey food chain, *C. jejuni* has frequently been isolated. Nevertheless little information is available about its detection in turkey eggs (Acuff et al., 1982). The purpose of the study was to improve our knowledge of the survival of *C. jejuni* in the different parts of the turkey egg during the commercial processing of one day old turkey poult .

A total of 2 hundred turkey eggs were collected from the same commercial turkey breeder flock (50 to 58 weeks of age). To determine the contamination by *Campylobacter* of the flock, 30 individual faecal samples were collected then cultured (6 pooled of 5). Eggs were allocated to 4 groups (10 eggs/group, 5 repetitions) (table 1). The detection of *Campylobacter* on the eggshell and into the egg (shell membranes, yolk, albumen, liver, intestines) was examined twice during the commercial processing event: 3h after collection and/or contamination; 26 days after contamination. Eggs were contaminated by immersion in an average 8 log CFU/ml *C. jejuni* NCTC 11168 suspension. For all the samples, following an enrichment step (Preston broth), detection of *Campylobacter* was attempted using culture on 2 selective agars (Butzler and Karmali) at 42°C for 5 days in microaerophilic conditions. Suspect colonies with *Campylobacter*-like morphology were further identified as *C. jejuni*, *C. coli* using a multiplex PCR (van de Giessen et al., 1998).

Results are summarised in table 2. A total of 40 isolates of *Campylobacter*-like morphology were obtained. On the basis of identification with multiplex-PCR, *C. jejuni* was the only species recovered. The turkey breeder flock was tested positive for *C. jejuni* (3/6 pools). However *Campylobacter* was not detected from any of the 50 eggshell surface suspensions and 50 egg yolk suspensions of freshly laid eggs (Co). Following artificial contamination of eggshell, *C. jejuni* was isolated on the shell surface for 2 hours after contamination (29/50), and was also detected in the suspension of yolk and albumen of 8 eggs (8/100). No campylobacters were detected on shell of eggs disinfected (0/50). On incubation day 25, *Campylobacter* was not isolated from any of the liver and intestine content suspensions from 50 newly-hatched turkeys. Furthermore *Campylobacter* was not detected from any of the 50 shell surface suspensions and 50 shell-shell membranes suspensions.

Table 1 One experimental protocol (5 repetitions)

Time	Operation	Group
To	Collection	Co, C, D, I
	Contamination	C, D, I
To+1h	breeder farm Disinfection	D
To+3h	Sampling	Co, C, D
	Storage 18°C, 18h	
	transport, 3h	
	commercial Storage 18°C, 1d.	
	hatchery Incubation, 37°C, 25d.	
To+26d.	Sampling	I

Table 2 Number of *Campylobacter jejuni*-positive samples (NT=not tested, SM; shell membranes)

group	Shell (S)	Yolk	Albumen
Co	0/50	0/50	0/50
C	29/50	NT	5/50
D	0/50	NT	3/50
	S	S+	liver+
		SM	intestines
I	0/50	0/50	0/50

Viable *Campylobacter* has not been isolated from the internal contents of eggs laid by *Campylobacter*-shedding hens (Acuff et al., 1982, Sahin et al., 2003), but some studies showed that *Campylobacter*-positive chicken eggs are the result of egg surface contamination (Doyle 1984). So only an artificial contamination of eggshell could test the survival of *Campylobacter* in turkey eggs during commercial hatchery processing. The data of the present study confirm that freshly laid turkey eggs and newly-hatched turkey poults do not contain viable *C. jejuni*. Furthermore *C. jejuni* was able to survive on the turkey egg shell surface but its viability on the shell surface was maintained for only two hours after contamination. *C. jejuni* was not recovered for long enough during the commercial hatchery processing. Many other investigators have indicated a short period of survivability of *Campylobacter* on the eggshell (Doyle 1984, Clark and Bueschkens, 1986; Sahin et al., 2003). Although *C. jejuni* could penetrate through the eggshell of turkey eggs, the organism has not been recovered from inside the eggs when they were kept for one day at 18°C then incubated for 25 days. The survivability of *Campylobacter* inside the egg during hatchery processing appears not to be possible (Acuff et al., 1982; Doyle 1984; Clark and Bueschkens, 1986; Sahin et al., 2003). It is likely that the high susceptibility of *Campylobacter* to environmental conditions (storage at 18°C; dessication; 37°C) combined with the high pH and the presence of bactericidal compounds are reasons for its inability to colonize the digestive tract of hatched turkey poults (Clark & Bueschkens, 1986; Sahin et al., 2003).

The absence of *C. jejuni* in the digestive tract of newly hatched turkey poults supports the notion that *Campylobacter* contamination of turkey eggs is unlikely to result in turkeys infected with *Campylobacter*.

Acknowledgements

We would like to thank A. Rossero and F. Jugiau for technical assistance.

References

- ACUFF, G. R., VANDERZANT, C., GARDNER F.A. & GOLAN, F.A. (1982) Examination of turkey eggs, poults and brooder house facilities for *Campylobacter jejuni*. *Journal of Food Protection*, **45**: 1279-1281.
- CLARK, A.G. & BUESCHKENS, D.H. (1986) Survival and growth of *Campylobacter jejuni* in egg yolk and albumen. *Journal of Food Protection*, **49**: 135-141.
- DOYLE, M.P. (1984) Association of *Campylobacter jejuni* with laying hens and eggs. *Applied and Environmental Microbiology*, **47**: 533-536.
- SAHIN, O., KOBALKA, P. & ZHANG, Q. (2003) Detection and survival of *Campylobacter* in chicken eggs. *Journal of Applied Microbiology*, **95**: 1070-1079.
- VAN DE GIESSEN, A.W., TILBURG, J.J., RITMEESTER, W.S. & VAN DER PLAS, J. (1998) Reduction of *Campylobacter* infections in broiler flocks by application of hygiene measures. *Epidemiology and Infection*, **121**: 57-66