Typing of *Campylobacter jejuni* and *C. coli* isolated from laying hens during the production cycle

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ABSTRACT

*C. jejuni* and *C. coli* isolated on three layer farms during the production cycle were typed by PCR-RFLP flagellin gene profiling. Three typical restriction patterns were detected for *C. coli* (H, O, R), 5 for *C. jejuni* (I, P, Q, T, S) and 8 were common for both. The D profile was present in all three flocks. C fla-type was detected on farms A and B. The results of the study suggest that some genotypes tend to prevail and persist more than others on farms and in certain geographic areas.

Key Words: *C. jejuni*, *C. coli*, PCR-RFLP typing, Laying hens.

INTRODUCTION

*Campylobacter coli* and *C. jejuni* are recognized as two of the main causes for human foodborne poisoning. These bacteria are often transmitted through poultry. Several serotypes and biotypes can be distinguished using phenotypical methods such as serotyping and biotyping (Lior, 1984) although these methods are difficult to perform and sometimes fail to identify the strains. The availability of molecular typing techniques has spurred new epidemiological investigations into this field. A fla PCR-RFLP, which is one of the most common gene profiling techniques, has proved to be helpful in discriminating *Campylobacter* strains during field studies (Petersen and Newell, 2001). In the United States, PCR-RFLP is the method of choice for human campylobacteriosis inspection and surveillance plans. Surveys based on *Campylobacter* molecular features have shown...
that a wide range of bacterial types exist on layer farms. In this investigation, the dynamic behaviour of \textit{C. coli} and \textit{C. jejuni} strains isolated during the table egg production cycle on layer farms was studied by A flagellin PCR-RFLP.

\textbf{Material and methods}

Investigation were performed on three table egg production layer farms in the Apulia region. On all three farms, only one group of animals was housed in the sheds. There was no contact among the farms and the laying hens had been bought from different northern or southern Italian suppliers. Faecal samples were obtained with cloacal swabs, starting from the day the birds were housed and then collected every three months over the course of one year. Overall 48 cloacal swabs were obtained from farm A and 24 from farms B and C since the bird capacity of farm A was double that of farms B and C. The swabs were obtained from randomly chosen subjects. A total of 6 sampling sessions were carried out on farm A and 5 on farms B and C. All the samples were immediately put into tubes containing a selective enrichment broth (\textit{Campylobacter enrichment broth}, OXOID) and sent to the laboratory under refrigerated conditions within a few hours. Methods described elsewhere (Camarda et al., 2000) were used to isolate \textit{Campylobacter} strains. Species identification was done using Multiplex PCR (Denis et al., 1999). Each isolated \textit{Campylobacter} strain was cultured in Brucella Broth (Biolife) supplemented with 20\% glycerol and kept in cryovials at -80\°C until the next tests were performed.

Molecular typing: flagellin PCR-RFLP. Overall 130 of the 183 isolated Campylobacter strains were genotyped following the standard methods provided by Campynet European Network for the harmonisation and standardisation of molecular typing methods for Campylobacter (http://campynet.vetinst.dk). Each RFLP profile was assigned a numerical value on the grounds of the obtained restriction patterns.

\textbf{Results and discussion}

Bacteriological analyses of specimens from newly arrived chicks were always negative in agreement with previous studies carried out on hatching chicks (Di Modugno et al., 1997). Starting from the second sampling the analyses were positive and positivity persisted throughout the egg production cycle. A total of 183 Campylobacter strains were isolated, broken down as follows: 83 on farm A, 41 on farm B, and 59 on farm C. Campylobacter coli was the most common species on the three farms (60.66\% of the isolated strains) while detection of \textit{C. jejuni} was less frequent (39.34\%). Molecular typing (PCR-RFLP) distinguished 16 restriction profiles. Three patterns (H, O, R) were characteristic of \textit{C. coli}, S (I, P, Q, T, S) were exclusively detected in \textit{C. jejuni}, while 8 (A, B, C, D, E, F, L, M) were present in both species. Some of these restriction patterns were detected only on one farm while others were common to more than one. In the \textit{C. coli} strains isolated on farms A and B, C fla-type prevailed over the others while \textit{C. coli} F type was often found on farm C. In the \textit{C. jejuni} strains, B, S and D were the most frequently detected fla-types on farms A, B and C, respectively. The trends measured during the production cycle indicated that some molecular types tended to prevail and persist longer than others. The D type, detected both in \textit{C. jejuni} and \textit{C. coli} strains, was always present in all the tested groups during the production cycle. Fla-type C was also always present on farms A and B. Some molecular types (H, O, P, Q, R, S, T) were only detected once and then never again.

\textbf{Conclusions}

Evaluation of the degree of contamination of the flocks broadly confirmed our expectations. Contamination of the birds increased proportionally to their age (Di Modugno et al., 1997) through to the day of slaughtering when the contamination rates were almost 100\%. All the newborn chicks were Campylobacter - free and this was in agreement with previous studies that reported that thermophilic Campylobacter was consistently absent in newly hatched chicks and during the perinatal phase (Di Modugno et al., 1997). Examination of the obtained fla A profiles depicted the prevalence of some molecular types on each farm. These types were present in the groups during the production cycle with different rates. Other strains with a dif-
different molecular type joined the prevailing Campylobacter population during the breeding cycle and this is consistent with the fact that different Campylobacter molecular types can coexist in a same group of animals (Camarda et al., 2000). It is likely that the egg production cycle, which may last up to 18, months, favours colonization with different genotypes. Moreover, the occurrence of new molecular types in the infecting Campylobacter population may be connected with the flagellin gene mutations detected by enzymatic restriction. The use of PCR-RFLP to study multiple farms over a relatively long period showed that specific genotypes (C, D) were present and persisted in all the flocks tested during the production cycle. These findings suggest that some genotypes may prevail over others is certain geographical regions. Epidemiological studies carried out in Great Britain (Shreeve et al., 2002) seem to support this assumption. It is likely that the capacity of some Campylobacter donal groups to infect animals and the environment may be related to their ability to reach and contaminate poultry products. Some Campylobacter jejuni genotypes that infect the broiler gut during the production cycle are also detected on the carcasses after slaughtering. Further studies will focus on whether there is a relation between the genetic patterns of these strains and the ones often associated with human illness.

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