

## EVALUATION OF CE TREATMENT AGAINST CAMPYLOBACTER REGARDING THE GENETIC POULTRY STRAIN

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### Abstract

To prevent *Campylobacter* contamination of chicken, several competitive exclusion floras were administered to chicks. Collected from adult birds, caecal or mucosal floras were very often ineffective in reducing chicks colonization. As chicken seemed to be less sensitive to *Campylobacter* contamination during the first weeks of the rearing period it was decided to evaluate caecal floras collected from young chicks. In the same time we evaluated the CE treatment against *Campylobacter* regarding the genetic poultry strain (layers Isabrown and broilers JA957). On the one hand in two trials, caecal floras collected from young broilers were unable to prevent colonization of JA957 broilers gaved with 90 or 94 *Campylobacter* cfu even if colonization took place more slowly. On the other hand in two other trials, after inoculation (195 or 360 cfu) *Campylobacter* could never be detected from male Isabrown treated with CE flora issued from laying hen Isabrown. This same flora was ineffective when administered to broilers. The flora effectiveness in reducing *Campylobacter* colonization of chicken seems to be strongly dependent on the genetic poultry strain.

### INTRODUCTION

Because of the increasing human campylobacteriosis during the last years, in many countries, *Campylobacter* is considered as the main bacterial pathogen responsible for human gastro intestinal infections. Poultry meat by handling or consumption of undercooked products is strongly associated with gastro intestinal infections due to *Campylobacter*. That is why it is necessary to control contamination at the different steps of the poultry production in order to decrease the number of campylobacteriosis. Different studies pointed out cross contaminations via the slaughterhouses (Rivoal *et al.* 1999, Newell *et al.* 2001). Consequently the more poultry batches free of campylobacter could be delivered to the slaughterhouses the more we could limit the contaminations. The strict application of hygienic measures as sanitary barriers all over the rearing period seems to be one important way to prevent chicken contamination. Yet for some farmers it is quite difficult to respect this hygienic barriers and from time to time *Campylobacter* from the environment can enter the rearing house and consequently colonize the chicken. Then, others measures (such as the use of Competitive Exclusion flora) have been considered to prevent chicken colonization by *Campylobacter*. Several CE floras (caecal, mucosal) have been collected from healthy adult chicken, free of any pathogen bacteria, and were administered to young chicks. Unfortunately caecal and mucosal floras even when collected anaerobically and subcultured (Stern *et al.*, 2001) were not as efficient to prevent *Campylobacter* colonization as they did to prevent *Salmonella* colonization of chicken. In previous studies (unpublished data) we tried to prevent *Campylobacter* colonization of broilers using a mucosal CE flora collected anaerobically from the caeca of adult SPF chicken. No difference was observed in *Campylobacter* contamination of several batches of chicks

reared in isolation units, some of them being treated with mucosal flora at D0. *Campylobacter* (up to  $10^7$  cfu/g) could be detected in the caeca of birds (control as well as treated birds) sacrificed seven days after inoculation. Because of the inefficiency of floras collected from old birds, we wanted to test the efficacy of caecal floras collected from young birds (i.e. 3, 7, 10 days old) to take into account the fact that during the two first weeks of the rearing period *Campylobacter* are scarcely detected from the birds. We hypothesize that young birds are less sensitive to *Campylobacter* colonization probably due to the immature flora of the first fifteen days. In the same time we also wanted to test the possible role of the avian genetic strain in *Campylobacter* colonization resistance. So we collected caecal flora from young layers or from young broilers and administered them to birds reared in isolation units. Four trials were done.

## **MATERIAL AND METHODES**

### *Chicken*

Two different genetical strains from commercial hatcheries were chosen: a layer strain (Isabrown) and a broiler strain (JA957).

### *Isolation Unit*

"Glove box" isolaters were used for animal rearing. Feed without antibiotic and coccidiostat is delivered to birds and the drinking water is sterilized. Get in or get out of the birds, material needed for treatments or samplings, took place via a sas filled with quaternary ammonium compound. According to the trials, five to six isolation units were used.

### *Caecal flora*

Three batches of CE floras were collected anaerobically from the caeca of birds Briefly : after sacrificing the birds, the caeca were aseptically removed, placed in a sterile flask and immediately introduced in an anaerobic chamber. Then after opening the caeca, the content was poured into flask and diluted (1/10 weight/volume) in TGY broth (15% glycerol). The diluted floras were dispensed into polypropylene round bottom tubes and then stocked in a deep freezer (- 72°C) until use. In the same time, the flora was checked for the presence of *Campylobacter*.

Origin of CE treatment:

Young hen (Isabrown) caecal flora collected on D3, D7 and D10 (February 2001): trial 1-trial 4.

Chicken (broiler JA957) caecal flora collected on D3, D7 and D10 (February 2001): trial 2.

Chicken (broiler JA957) caecal flora collected on D3, D7 and D10 (September 2001): trial 3.

### *Challenge Campylobacter strain*

In the 4 trials the same *Campylobacter jejuni* strain isolated from an environmental sample of a rearing chicken house was used for the challenge.

#### ① Birds placement in isolation units

Before birds placement, swabbings of the isolation units were realised. Drinking water and feed were also sampled for *Campylobacter* detection. Furthermore on delivery day, swabbings of the transport crates were done and some chicks sacrificed for analyse. Within some hours, chicks are shared out amongst the isolation units.

② Birds treatment

As soon as the birds are placed in different isolation units every two days until D20, the CE flora was administered by oral gavage of 0.1ml to each treated bird. In the same time 0.1ml of sterile water was delivered to the others (negative and positive controls) in order to take the stress gavage into account.

③ Birds challenge

After subculture on blood agar (24-48 h, 42°C, microaerophilic atmosphere) the *Campylobacter jejuni* strain was inoculated into a Brucella broth (incubation 18 h). Then several dilutions were done to provide about  $10^2$   $10^3$  cfu to each chick except negative controls. Exact titre of the suspension given to the birds was determined by plating on blood agar and Karmali Agar. All the birds, except negative controls, were inoculated on D15.

④ Sampling (Table I)

In each trial chicken from isolation units were sacrificed and caeca removed aseptically for *Campylobacter* detection.

⑤ *Campylobacter* methodology

All the samples (caeca, droppings, feed) are diluted (1/10) into Preston broth and the swab tissues into 150 ml broth. Two selective media (Butzler n°2) and Karmali were used for direct plating (100  $\mu$ l). Furthermore *Campylobacter* numeration was realised with a spiral plater system on Karmali media (incubation 48-72 h 42° microaerophilic conditions). On the other hand an enrichment procedure in Preston Broth was applied (18-24 h). Then streaking of the enriched broth was realised onto Butzler n°2 and Karmali media.

## RESULTS AND DISCUSSION

### *Result expression*

#### *Colonization rate (Stern et al., 2001)*

The colonization rate was calculated by dividing the number of birds colonized by the organism, by the number of birds challenged with the organism.

#### *Colonization factor*

Mean log 10 cfu *Campylobacter* per g in caeca of positive birds within each group. In our study we decided that the amount of *Campylobacter*/g of caeca was 100 when *Campylobacter* could be only detected after an enrichment period and 1 *Campylobacter*/g of caeca analysed when they were not.

#### *Protection factor*

Ratio of mean log 10 of *Campylobacter* per g in caeca of positive control chicks to corresponding mean for treated birds.

#### Caecal flora

No *campylobacter* could be detected from caecal floras.

#### Challenge strain

Trial 1: 190 cfu/chick    Trial 2: 90 cfu/chick    Trial 3: 94 cfu/chick    Trial 4: 360 cfu/chick.

### Chicken (Table II)

Whatever the trials, before challenge, no *Campylobacter* was detected. Furthermore negative controls remained *Campylobacter* free all over the rearing period.

*Campylobacter* challenge succeeded in colonizing the positive controls. Two days after inoculation, all the birds of positive controls are not contaminated but within four (trial 3) or six days (others trials) the colonization rate observed is 1 and on the sixth day we noticed colonization factors ranging from 6.48 to 8.40.

When the chicken were treated with CE flora the result depend on the trial and especially on the avian commercial strain. In trial one no *Campylobacter* could be isolated in Isabrown chicken caeca (layer strain) given caecal flora collected on D3, D7 and D10 from young laying hens. Similar results were observed in trial four when young chicks Isabrown were treated with the same seven day competitive exclusion flora as in trial one. Yet on trial four this caecal flora was not effective to prevent *Campylobacter* colonization of broiler JA957. Furthermore (trial 3 and 2) administration of CE flora collected (on D3, D7 and D10) from broilers did not protect chicken of the same commercial broiler strain from *Campylobacter* contamination. Six days after inoculation all the birds were contaminated (contamination rate: 1) and colonization factors ranged from 7.13 to 8.84. Nevertheless in trial 2, contamination of treated chicken took place later compared to the contamination of positive controls. Two days after challenge, protective factor is greater than 4 when using competitive flora (id D3 and D7). The efficiency of the competitive flora seemed to be better when flora is collected from younger chicken.

In the four trials, experimental model was satisfying because *Campylobacter* quantities as low as 90 to 360 cfu administered per gavage to the fifteen days chicks (layer and broiler) were able to colonize the intestinal track of the birds reared in isolation units. Inoculation of  $10^2$  bacteria to one day chicks gave the same result in Young *and al.*, (1999) trials but our model seems to be closer to the field conditions. The colonization ability of the *Campylobacter jejuni* strain collected from the environment of a broiler house resulted in colonization of caeca up to  $10^8$  *Campylobacter* without any symptom of illness for the contaminated birds. As well as for Stern *et al.* (1990) we noticed that the ability of the *Campylobacter* strain to colonize the chicken is strongly dependent on the avian strain: in our study *Campylobacter* colonization of the layer hens Isabrown is delayed compared with broiler JA957. The efficacy of CE flora collected from young birds was different according to the trials and the chicken. In two trials when Isabrown chicken (layer strain) were treated with the CE flora collected from young laying hens, no *Campylobacter* could be isolated from the caeca of these birds sacrificed two, six and even twelve or sixteen days after inoculation but they were for positive controls. Stern (1994) noticed that efficacy of MCE treatment decreased with storage of the culture after 3 or 9 months of conservation in deep freezer (-80°). At the opposite the laying hen CE flora we used was efficient to prevent *Campylobacter* colonization of the Isabrown chicken even after 11 months storage at -72°C. Nevertheless the broiler chicken (JA957) could not be protected from *Campylobacter* contamination when treated with this flora. In the same way, Stavric *et al.* (1992) showed that layer and broiler could be protected from *E. coli* contamination but the protection was better in layers. That is why we think a genetic factor does exist. In our studies the commercial laying strain (Isabrown) provided from the hatchery was protected from *Campylobacter* contamination when a CE flora from young laying hen of the same commercial strain was used. Using CE flora from young broilers for limiting *Campylobacter* colonization of chicks JA957 was not as effective as we thought. Even if beneficial effect was observed 2 days after inoculation in one trial, this effect could not be confirmed in an other trial perhaps because of the CE flora origin. In fact, in this trial, CE were collected from chicken reared under experimental conditions different from those observed under commercial practices. In order to confirm that genetic factors are

involved in *Campylobacter* colonization of chicken, it would be interesting to test the efficacy of broiler chicken CE flora administered to layer chicks and to check several avian commercial strains using our experimental model.

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**Table I:** Caeca samples

|   |                           | Isolation units | D1<br>4 | D15         | D1<br>7 | D1<br>9 | D2<br>1 | D2<br>3 | D2<br>7 | D3<br>1 |
|---|---------------------------|-----------------|---------|-------------|---------|---------|---------|---------|---------|---------|
| <b>Trial 1</b><br><b>Chicks Isabrown</b>            | Negative controls         |                 | 5       |             | 10      |         | 5       |         | 5       | 5       |
|   | Positive controls         |                 | 5       | inoculation | 10      |         | 10      |         | 4       | 4       |
|   | treated Flora D3          |                 | 10      | inoculation | 10      |         | 10      |         | 5       | 5       |
|   | treated Flora D7          |                 | 10      | inoculation | 10      |         | 11      |         | 5       | 5       |
|   | treated Flora D10         |                 | 10      | inoculation | 10      |         | 10      |         | 5       | 5       |
| <b>Trial 2</b><br><b>Chicks JA 957</b>              | Negative controls         |                 | 5       |             | 10      |         | 10      |         | 10      | 9       |
|   | Positive controls         |                 | 5       | inoculation | 10      |         | 10      |         | 10      | 10      |
|   | treated Flora D3          |                 | 5       | inoculation | 10      |         | 10      |         | 10      | 7       |
|   | treated Flora D7          |                 | 5       | inoculation | 10      |         | 10      |         | 10      | 7       |
|   | treated Flora D10         |                 | 5       | inoculation | 10      |         | 10      |         | 10      | 10      |
| <b>Trial 3</b><br><b>Chicks JA 957</b>              | Negative controls         |                 | 5       |             | 5       | 5       | 5       | 5       |         |         |
|   | Positive controls         |                 | 5       | inoculation | 5       | 5       | 5       | 5       |         |         |
|   | treated Flora D3          |                 | 5       | inoculation | 5       | 5       | 5       | 5       |         |         |
|   | treated Flora D7          |                 | 5       | inoculation | 5       | 5       | 5       | 5       |         |         |
| <b>Trial 4</b><br><b>Chicks JA 957 and Isabrown</b> | T- (JA957 et Isabrown)    |                 | 10      |             | 20      |         | 20      |         |         |         |
|   | T+ (JA957)                |                 | 5       | inoculation | 10      |         | 12      |         |         |         |
|   | T+ (Isabrown)             |                 | 5       |             | 10      |         | 11      |         |         |         |
|   | JA957 treated Flora D7    |                 | 5       | inoculation | 10      |         | 11      |         |         |         |
|   | Isabrown treated Flora D7 |                 | 5       | inoculation | 10      |         | 10      |         |         |         |

**Table II :** Colonization rates, colonization factors and protection factors

|                 |          | TRIAL 1<br>Chick Isabrown |                             |                                 |                                 | TRIAL 2<br>Chick JA 957 |                             |                             |                                 | TRIAL 3<br>Chick JA 957 |                             |                             | TRIAL 4<br>Chick JA 957 -<br>Isabrown |             |                                      |                                  |
|-----------------|----------|---------------------------|-----------------------------|---------------------------------|---------------------------------|-------------------------|-----------------------------|-----------------------------|---------------------------------|-------------------------|-----------------------------|-----------------------------|---------------------------------------|-------------|--------------------------------------|----------------------------------|
|                 |          | T+                        | trait<br>ed<br>Flor<br>a D3 | trait<br>ed<br>Flor<br>a<br>D7* | trait<br>ed<br>Flor<br>a<br>D10 | T+                      | trait<br>ed<br>Flor<br>a D3 | trait<br>ed<br>Flor<br>a D7 | trait<br>ed<br>Flor<br>a<br>D10 | T+                      | trait<br>ed<br>Flor<br>a D3 | trait<br>ed<br>Flor<br>a D7 | JA95<br>7<br>T+                       | Isab.<br>T+ | JA95<br>7<br>trait<br>ed<br>FD7<br>* | Isab.<br>trait<br>ed<br>FD7<br>* |
| <b>DI5</b>      | cfu      | 190                       | 190                         | 190                             | 190                             | 90                      | 90                          | 90                          | 90                              | 94                      | 94                          | 94                          | 360                                   | 360         | 360                                  | 360                              |
| <b>D<br/>17</b> | CR       | 0,10                      | 0,00                        | 0,00                            | 0,00                            | 1,00                    | 0,30                        | 0,40                        | 0,80                            | 0,20                    | 0,60                        | 0,60                        | 0,60                                  | 0,00        | 0,20                                 | 0,00                             |
|                 | CF<br>PF | 0,49                      |                             |                                 |                                 | 5,94                    | 1,10                        | 1,48                        | 4,30                            | 1,06                    | 1,60                        | 0,92                        | 3,23                                  |             | 2,24                                 |                                  |
| <b>D<br/>19</b> | CR       |                           |                             |                                 |                                 |                         |                             |                             |                                 | 1,00                    | 1,00                        | 1,00                        |                                       |             |                                      |                                  |
|                 | CF<br>PF |                           |                             |                                 |                                 |                         |                             |                             |                                 | 6,98                    | 6,78                        | 5,62                        |                                       |             |                                      |                                  |
| <b>D<br/>21</b> | CR       | 1,00                      | 0,00                        | 0,00                            | 0,00                            | 1,00                    | 1,00                        | 1,00                        | 1,00                            | 1,00                    | 1,00                        | 1,00                        | 1,00                                  | 1,00        | 1,00                                 | 0,00                             |
|                 | CF<br>PF | 6,52                      |                             |                                 |                                 | 8,40                    | 8,70                        | 8,53                        | 8,50                            | 7,28                    | 7,26                        | 7,13                        | 8,18                                  | 7,96        | 8,00                                 |                                  |
| <b>D<br/>23</b> | CR       |                           |                             |                                 |                                 |                         |                             |                             |                                 | 1,00                    | 1,00                        | 1,00                        |                                       |             |                                      |                                  |
|                 | CF<br>PF |                           |                             |                                 |                                 |                         |                             |                             |                                 | 7,75                    | 7,08                        | 7,30                        |                                       |             |                                      |                                  |
| <b>D<br/>27</b> | CR       | 1,00                      | 0,00                        | 0,00                            | 0,00                            | 1,00                    | 1,00                        | 1,00                        | 1,00                            |                         |                             |                             |                                       |             |                                      |                                  |
|                 | CF<br>PF | 2,47                      |                             |                                 |                                 | 7,63                    | 8,35                        | 8,84                        | 8,82                            |                         |                             |                             |                                       |             |                                      |                                  |
| <b>D<br/>31</b> | CR       | 1,00                      | 0,00                        | 0,00                            | 0,00                            | 1,00                    | 1,00                        | 1,00                        | 1,00                            |                         |                             |                             |                                       |             |                                      |                                  |
|                 | CF<br>PF | 6,48                      |                             |                                 |                                 | 6,69                    | 8,71                        | 8,13                        | 7,92                            |                         |                             |                             |                                       |             |                                      |                                  |

\* same flora