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Time Required to Protect the Intestinal Tract of Chicks against *Salmonella enterica* serovar Enteritidis using Competitive Exclusion

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ABSTRACT

Competitive exclusion (CE) has been designed to accelerate the colonization of the alimentary tract of young commercial birds and it has been also used to repopulate the digestive tract after antibiotic therapy. The method has been successfully adopted as a means to prevent enteric salmonellosis. The present study was carried out to evaluate if CE is able to prevent this kind of infection. Newly hatched chicks were given a CE culture and at different intervals of time birds infected with *Salmonella* Enteritidis were placed together with the group of treated birds. CE culture was prepared from feces of adult laying hens incubated overnight at 37C under aerobic conditions. Birds were killed 4 and 8 days after challenge and viable counts of *Salmonella* Enteritidis were assessed in the cecal contents. The results showed that *Salmonella* infection was reduced even if CE culture administration was concomitant with the inclusion of the infected bird in the group.

INTRODUCTION

Newly hatched free-range birds hatch and live together with their parents. This contact allows that microorganisms excreted by the parents colonize the gut of young birds. The acquired intestinal microbiota helps the birds to grow up healthy and acts as a defense mechanism against harmful microorganisms. The processes of installation of microorganisms in the intestinal tract and gut colonization last up to six weeks (Spencer & Garcia, 1995). In the modern poultry industry, birds are hatched in artificial incubators, which are submitted to a rigorous process of cleaning and disinfection. Thus, chicks hatch in a sterile ambient and have no contact with the parents. There will be a delay in the formation of the gut microflora. Furthermore, vertically transmitted infectious pathogens like *Salmonella* Enteritidis (Lister, 1988) reach the alimentary tract before the microflora is even mature. Nurmi & Rantala (1973) prepared a culture of feces of adult birds and administered it to newly hatched broiler chicks; the procedure was successful to protect against *Salmonella* Infantis infection. The so-called "Nurmi concept" or "Competitive Exclusion (CE)" has been adopted in Sweden since 1981 as part of the national program to control *Salmonella* infection (Wierup *et al.*, 1992). The detection of *Salmonella* in broilers has decreased to lower than 1% in carcasses, feces and in the environment since then (Wierup *et al.*, 1992). CE is recommended worldwide by several researchers (Corrier *et al.*, 1994; Rambousek *et al.*, 1995; Mead, 2000; Davies & Breslin, 2003).

The control of *Salmonella* in commercial poultry has become a matter of concern since outbreaks of human Salmonellosis caused by *Salmonella* Enteritidis (SE) were reported worldwide and the main source of infection in the outbreaks were meat and eggs of chickens

(Rodrigue *et al.*, 1990; Barrow, 2000). The adoption of CE is one of the measures suggested to prevent SE dissemination among birds (Mead, 2000). CE cultures should be given to newly hatched chicks as soon as possible, since SE acquired through vertical transmission will spread out easily among the birds in the beginning of life (Soncini *et al.*, 2000; Oliveira *et al.*, 2000). After feeding procedures are started, 48 hours are necessary for the intestinal flora to be established. This period might be reduced to 2 hours when the birds are treated with CE culture, although complete colonization might last up to 32 hours (Soerjadi *et al.*, 1981). Starvic (1985) considers that protection is adequate within 6 hours. CE was effective in preventing contact infection when the culture was administered to newly hatched chickens 24 hours before challenge (Oliveira *et al.*, 2000). However, protection might be obtained earlier according to Almeida *et al.* (2002). In view of the lack of information, *in ovo* administration of CE culture has been tried as a means to accelerate the installation of the intestinal microflora. However, the procedure was unsuccessful, since the product was deleterious to the embryo (Meijerhof & Hulet, 1997). Therefore, this study was carried out in order to assess the period of time needed to protect chicks against SE. Newly hatched chicks were given a broth culture of feces of adult birds orally and were then challenged with SE, by introducing an infected bird in each group of birds at different intervals of time after CE treatment.

MATERIALS AND METHODS

Bacteria

It was used a spontaneous mutant of *S.* Enteritidis phage type 4 resistant to both nalidixic acid and spectinomycin (SE nal/spec^r). Cultures were prepared in nutrient broth (Oxoid CM67) and incubated overnight using a shaking water bath (100 strokes/min) at 37°C.

Competitive Exclusion culture

Fresh feces were obtained from adults birds reared in the poultry facilities of Faculdade de Ciências Agrárias e Veterinárias (FCAV-UNESP) and inoculated in nutrient broth (1:10 w/v). The culture was incubated aerobically at 37°C for 24 hours, without shaking and then tested for the absence of *Salmonella.*

Birds

Newly hatched chicks were obtained from a commercial broiler hatchery. Groups of six birds were housed together in a box separated from other groups. Feed with no additives and drinking water were provided *ad libitum*. A heat source was provided. Swabs from the cloacae were assessed for the absence of *Salmonella*.

Experimental Procedure

Birds in groups 1, 2, 3 and 4 were given 0.1 mL of the fecal culture (CE) orally. Birds in group 5 did not receive CE culture (control group). At 0, 6, 12 and 24 hours after CE treatment, birds were challenged, i.e., two birds infected with SE were placed with each group. There were four replicates per group (Table 1).

Table 1 - Experimental design.

Groups	Replicates	Treatment	Challenge (Hours)
1	4	CE	0
2	4	CE	6
3	4	CE	12
4	4	CE	24
5	4	-	0

Number of birds per replicate = 6; CE = Competitive Exclusion.

Infected birds had been inoculated with 0.1 mL broth culture of SENal^{r}Spec^r containing 1.2 x 10^5 CFU.

Three and five days after the challenge, three birds from each group and one infected bird were sacrificed to assess the viable counts of SENal^{r}Spec^r in the cecal contents.

Bacterial counting

Decimal dilutions of cecal contents were made in PBS, pH 7.4 and 0.1mL aliquots were cultured on Brilliant Green agar containing nalidixic acid (100µg/mL) and spectinomycin (100µg/mL). Plates were incubated at 42°C for 24 hours.

RESULTS AND DISCUSSION

The modern practices in the poultry industry use artificial incubation and hatching. The colonization of the enteric tract of newly hatched chicks by desirable microorganisms is delayed compared to chicks hatched in contact with adult birds. Therefore, the alimentary tract can be easily colonized by pathogenic bacteria (Fowler & Mead, 1989). Under natural conditions, microorganisms that are first established usually remain for the rest of the life in the alimentary tract of the birds (Savage, 1987; Miles, 1993). Therefore, gut colonization in the beginning of life would promote a natural barrier impeding the colonization and multiplication of *Salmonella* and other pathogenic bacteria in the alimentary tract (Fowler & Mead, 1989; Oliveira *et al.*, 2000).

The risk of *Salmonella* infection in young birds is still high even if a Competitive Exclusion product is administered. Some serotypes, like *Salmonella* Enteritidis, can be transmitted vertically (Gast, 1997; Berchieri Jr., 2000) and spread rapidly among young birds afterwards (Soncini *et al.,* 2000; Oliveira *et al.,* 2000). Despite having contact with birds infected with *Salmonella*, other birds might be protected by CE techniques. According to Oliveira *et al., (*2000), colonization of the intestinal tract is fast in CE techniques and help to prevent infection by contact among young birds. Besides, there are indications that the desired effect can be achieved even after infection, as reported by Ziprin *et al*., (1993) in birds treated 72 hours after experimental infection with *Salmonella* Typhimurium. Thus, the concept of Nurmi has been recommended worldwide as part of *Salmonella* control programs in birds (Day, 1992), although it is still not clear how long it takes to effectively protect teh birds. Therefore, this work was carried out to assess the protection of newly hatched chicks in contact with birds infected with *Salmonella* Enteritidis. This design was chosen because chicks infected with *Salmonella* Enteritidis have been reported as the main source of infection to poultry flocks (Zancan *et al.,* 2000; Gama *et al*., 2003).

All the birds in the control group were rapidly infected, showing that the dissemination occurs almost immediately in the beginning of life, as previously shown by Soncini *et al*., (2000) and Oliveira *et al*., (2000). Viable counts of SE in the cecal contents of control birds were high (Table 2). Conversely, most birds from the treated groups showed no SE counts and some birds had quite few organisms. These data corroborate previous studies that reported beneficial effects of CE given to birds at the same time of the challenge with *Salmonella* or after it (Ziprin *et al.,* 1993). Protection has been reported as early as two hours (Soerjadi *et al*., 1981) and at six hours (Starvic 1985) after treatment. However, in the present study, protection was adequate even if treatment and challenge were performed simultaneously ($p<0.05$). Similar findings have been reported by Almeida *et al.,* (2002) using a similar approach.

In conclusion, according to the experimental conditions used in this study, CE culture may prevent *Salmonella* Enteritidis infection through contact between young birds just after its administration.

Table 2 - Number (Log_{10}) of viable cells of *S. Enteritidis* Nal Spec (SE Nal Spec) in the cecal contents of birdsytreated with fecal culture (CE) and challenged at four different periods of time by contact with SE-infected birds.

Groups	Viable number of SE Nal Spec (Log_{10}) per gram of cecal contents	
	4 days post contact	8 days post contact
1	<2 (<2 - <2)	2.53 (<2 - 3.89)
2	2.34 (<2 - 3.52)	<2 (<2 - <2)
3	<2 (<2 - <2)	<2 (<2 - <2)
4	2.52 (<2 - 4.31)	<2 (<2 - <2)
5	4.92 (2.22 - 7.62)	5.55 (3.15-7.95)

< 2 = Log_{10} median count per gram from 24 birds (range in parenthesis).

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