Usefulness of a Vancomycin pretreatment when challenging chickens in order to evaluate anti-*Salmonella* preparations

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Abstract

For many years, *Salmonella* infection models have been developed in chickens with the aim to study the effects of prophylactic or therapeutic measures on the colonization of the gut. However, although the literature includes numerous challenge models, few studies investigated the infection rates among the inoculated population. We have implemented an antibiotic pretreatment of the chickens (vancomycin hydrochloride, 25 mg/bird) as an infection promoter. Indeed, vancomycin affects the normal gut microflora and releases sites for *Salmonella* at the intestinal epithelium. Two experiments were undertaken and a presence/absence cloacal swab method was used to evaluate cecal colonization. In the first experiment, birds were orally inoculated with *Salmonella* Typhimurium at 21 days of age. Three inoculum doses (3×10^3, 3×10^6, 3×10^9 cfu/bird) and an uninfected control were compared according to whether or not vancomycin had been used. Higher levels of *Salmonella* colonization (more than 70 %) were achieved in the gut by pretreating birds with vancomycin before inoculation ($p<0.05$). In the second experiment, chicks were inoculated at 7 days of age with 10^8 cfu/bird after a vancomycin pretreatment, leading to an infection rate of 87.5 %. In conclusion, vancomycin promotes efficiently the percentage of colonized birds in the challenged population, with either young animals or olders.

Keywords: *Salmonella* Typhimurium; challenge model; intestinal colonization; Vancomycin

Introduction

There exists a need for effective infection models in order to evaluate innovative non-antibiotic anti-*Salmonella* approaches in broilers. These are called "challenge models". They describe how the infection develops and persists in animals and allow studying intervention measures. The best challenge model should achieve an infection that last until slaughter age with counts of *Salmonella* as stable as possible and infection of the vast majority of birds among the challenged population (Marcq et al 2011). Although the literature includes numerous challenge models, the above characteristics are rarely obtained (Beal et al 2004; Eeckhaut et al 2008; Marcq et al 2011). In particular, few studies investigated the infection rate among the inoculated population which, if low, can lead to biases in the subsequent random slaughter in the population (e.g. for cecal quantification) if selecting birds that were not colonized efficiently from the inoculation, without any connection with the tested treatment. Hence, the objective of this work was to study the effectiveness of vancomycin to promote infection for both young and more mature broilers.

Material and methods

All *in vivo* procedures were approved by the responsible Official Care and Use Committee (protocol no. fusagx 08/04 NPC and ulg10-1019). All chicks (male, Ross breed) involved in this study were obtained from a *Salmonella*-free commercial hatchery and were confirmed to be negative for *Salmonella* spp. before the experimental inoculation. The birds had *ad libitum* access to water and feed throughout the experiments. A total of 288 chickens were used in the first part of the study. These were allocated in a 4×2 factorial completely randomized block design to study the effects of *Salmonella* inoculum dose (0, 3×10^3, 3×10^6, or 3×10^9 cfu/bird) and vancomycin pretreatment (no administration or a single administration of 25 mg/bird). Birds were orally inoculated at 21 days of age with *Salmonella* Typhimurium

CWBI-B1501 obtained from the Walloon Center of Industrial Biology (Gembloux, Belgium) whereas unchallenged chickens were administered sterile medium. Birds receiving the antibiotic treatment were orally gavaged with 25 mg of vancomycin hydrochloride (Vancocin, GlaxoSmithKline, Genval, Belgium) per 0.5 mL of deionized water 3 hours before *Salmonella* inoculation while untreated chickens received an equivalent volume of deionized water as a placebo. The results of this trial (including in addition immunological and growth performance aspects) were previously published (Marcq et al 2011). In the second trial, 40 chicks were individually challenged at 7 days of age with 10^8 cfu of the same *Salmonella* strain, using 25 mg of vancomycin per chick in order to assess the pertinence of the antibiotic pretreatment in young chicks. In both trials, a presence/absence cloacal swab method was used to evaluate cecal colonization among the inoculated population. Swabs were taken at 6, 14, and 21 days post-infection (dpi) in the first trial, and at 3 dpi in the second trial. In the first trial, *Salmonella* was enumerated in fecal samples at 6 and 22 dpi on a cage basis. In the second trial, *Salmonella* level was measured individually in cecal samples collected on 10 animals at 8, 15, and 22 dpi. All bacteriological analyses were performed as described previously (Marcq et al 2011). *Salmonella* counts data were analyzed using mixed linear models of the MIXED procedure of SAS 9.1 software (SAS Institute Inc., Cary, NC). Differences were considered significant at ($p<0.05$) following an F-test. Cloacal colonization results in the first trial were analyzed using a GENMOD procedure.

Results and discussion

In the first trial (infection at 21 days of age), swabbing revealed the effects of challenge dose and vancomycin on cloacal colonization of experimentally infected chickens. The pretreatment of challenged chickens with orally administered vancomycin increased the proportion of colonized birds ($p<0.05$). The disruption of the normal gut microflora by antibiotic administration favor colonization of chickens by *Salmonella* since the antibiotic releases sites at the intestinal epithelium (Stern 2008). Our results showed that more than 70 % of the swabs taken in the vancomycin pretreated birds were positive for *Salmonella* during the three weeks post-infection period with a slight increase over time (Figure 1.A). This colonization rate is higher than those reported by others: Vilà et al (2009) reached an infection rate of 25 % of chickens when infected at 14 days of age with 2×10^6 cfu *Salmonella* Enteritidis and Revolledo et al (2009) reached a cecal colonization rate of 30 % with chickens infected at 23 days of age with 10^7 cfu *Salmonella* Typhimurium. Furthermore, the percentages of *Salmonella*-positive swabs are often described as rapidly decreasing (Beal et al 2004; Eeckhaut et al 2008). In contrast, our results showed a percentage of *Salmonella*-positive birds that is well maintained over time, and even increasing. Several inoculum doses have also been tested in this trial to investigate if the antibiotic pretreatment allows the use of smaller inoculum doses. There was no significant interaction detected between the challenge dose and the use of vancomycin ($p>0.05$). The inoculum dose of 3×10^9 cfu/bird led to a higher proportion of birds colonized ($p<0.05$) than lower inoculum doses which led to similar proportions of colonized chickens ($p>0.05$). Counts of *Salmonella* in fecal samples were in accordance with swabbing measurements with vancomycin enhancing the level of *Salmonella* excreted at 6 dpi ($p=0.006$). However, we observed a drastic decrease in plate counting results for both vancomycin-treated and untreated groups (Figure 1.A). This clearance of the gut should be taken into account since it could limit the time post-infection during which the model would be useable. From our results, we suggest that the model should be used mainly within 1 to 2 weeks after inoculation. Indeed, at the end of the 3 weeks measurement period (22 dpi), there were 69.4 % of cecal sample negative in direct plate counting and needing enrichment and 25.0 % were negative post-enrichment. In comparison, at the first measurement date (6 dpi), only 13.9 % samples were below the detection limit in direct plate

counting and needed enrichment whereas no one was negative even after enrichment. This natural reduction of *Salmonella* counts (Beal et al 2004; Eeckhaut et al 2008) can complicate the interpretation of results if studying the *Salmonella* response to anti-bacterial products. Even if it only provides an indirect view of the cecal colonization, the monitoring of the percentage of infected birds through cloacal swabbing seems to be a relevant measure. It is less influenced by the trend through decontamination since only a portion of the birds are able to completely eliminate the pathogen. In the second trial, we evaluated the pertinence of vancomycin in younger chickens (infection at 7 days old). We recorded an infection rate as high as 90% among the infected population through inoculation of 10^8 cfu after a vancomycin pretreatment (Figure 1.B). This is better than percentages achieved by Vilà et al (2009) but similar to others (Fernandez-Rubio et al 2009). The enhancement due to vancomycin is thus less clear concerning younger chickens, which are more susceptible to *Salmonella* infection with a less established gut microflora. Nevertheless, we were unable to avoid the spontaneous elimination of *Salmonella* with a reduction in cecal counts of 1.8 log cfu per g of sample through the third week of measurements.

Conclusions

The use of vancomycin as pretreatment has several advantages. It promotes rapidly the installation of the studied pathogen in the gut of pretreated birds and it favors the excretion of the bacterium. Therefore, it facilitates the detection of an effect of the studied anti-*Salmonella* product. From this study, two efficient challenge models have been created. Their use depends on whether you want to evaluate the effects of preventive products at the beginning or throughout the rearing period, or to investigate the effects of curative strategies at the end of the grow-out period. In this last case, vancomycin revealed particularly relevant. The main limitation of these models is the time after infection during which it is possible to study the effects on cecal or fecal counts, due to the natural elimination of the challenge bacterium. A swabbing approach can cope with this problem, at least in part. The other limitation of an infection model involving the use of an antibiotic (here targeting Gram positive bacteria) is that its use is unrelevant when studying the effects of probiotics in prophylactic approaches since the antibiotic pretreatment could eliminate the probiotic bacteria.

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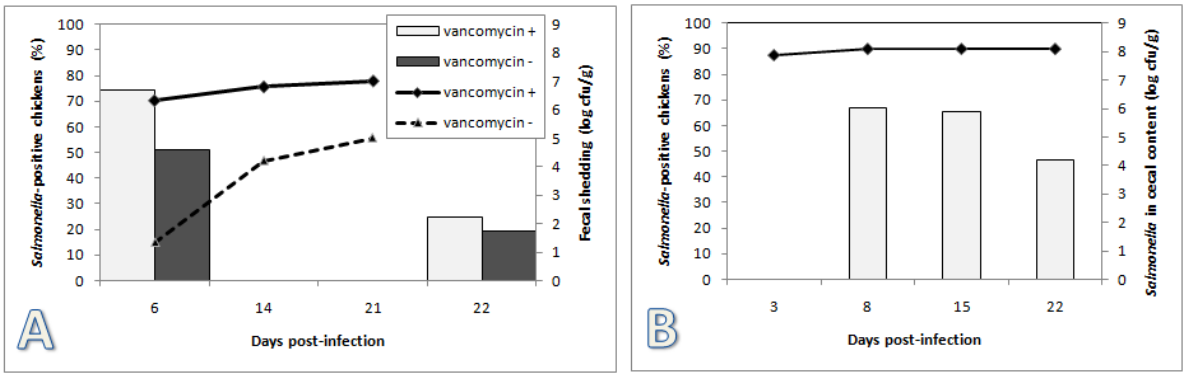

Figure 1. Evolution of the percentage of *Salmonella*-positive chickens and *Salmonella* level after (A) *Salmonella* Typhimurium challenge of 21 days old chickens with or without pretreatment with vancomycin and (B) *Salmonella* Typhimurium challenge of 7 days old chicks following a pretreatment with vancomycin. Line graphs represent the number of birds with presence of *Salmonella* /total number of birds sampled at each sampling day, expressed as a percentage. Bar graphs represent mean log cfu per gram of sample.