INFLUENCE OF A SUB-THERAPEUTIC LEVEL OF VIRGINIAMYCIN IN FEED ON THE INCIDENCE AND PERSISTENCE OF SALMONELLA TYPHIMURIUM IN EXPERIMENTALLY INFECTED SWINE

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Summary

Swine were infected with Salmonella typhimurium, USDA #295, and treated with virginiamycin medicated feed continuously at a level of 55 g/metric ton for approximately 2 months. The incidence and persistence of shedding of this organism in the feces and in selected tissues were determined. No significant changes in the course of the infection were detected when virginiamycin treated animals were compared to nonmedicated infected controls.

(Key Words: Sub-Therapeutic, Virginiamycin, Salmonella Typhimurium, Swine.)

Introduction

Virginiamycin is an antibiotic produced by a mutant of Streptomyces virginiae, exhibiting activity primarily against gram-positive organisms (Van Dijck, 1969). Its biological and chemical properties resemble those of mikamycin, osteogrycin, PA 114 factor, pristinamycin, streptogramin, and vernamycin. These antibiotics have in common the presence of two major factors exhibiting synergistic activity against a number of microorganisms (Vazquez, 1967). In virginiamycin, these are designated as factors M and S.

Factor M, present in the highest concentration, is a macrocyclic lactone containing an oxazole ring. Factor S, the minor component, consists of a cyclopeptide lactone ring (Vanderhaeghe and Parmentier, 1960). Both factors exhibit bacteriostatic activity separately, but in combination, they are bactericidal. Apparently, their mode of action is mediated through the inhibition of protein synthesis, specifically by inhibiting the formation of peptide chains at the level of translation (Cocito, 1969).

Virginiamycin is presently approved in the United States and in many other countries as a feed additive for growth promotion and improved feed conversion in swine and for the prophylaxis and treatment of swine dysentery (Miller and Barnhart, 1961; Miller et al., 1972). The current virginiamycin approval for the treatment and prophylaxis of swine dysentery involves treatment at 110 g/metric ton for 2 weeks, followed by 55 g/metric ton continuously up to 34.5 kg bodyweight. For feed conversion and growth promotion, virginiamycin has been approved for continuous feeding until market weight at levels of 5.5 and 11 g/metric ton, respectively.

For regulatory approval, a number of studies are now required for determining the safety of antibiotic feed additives to animals and man (Dept. of Health, Education, and Welfare, 1975). These requirements result from the recommendations of the FDA Task Force on the use of antibiotics in animal feeds (Dept. of Health, Education, and Welfare, 1972). They are based on the concern that antibiotic treatment of farm animals harboring salmonellae could result in an increase in the shedding and persistence of these organisms due to a reduction of the gram positive flora in the gut. This enhanced shedding could lead to an increased hazard in spreading the organisms both to animals and humans. As a partial fulfillment of these requirements, the following study was designed to evaluate the effect of subtherapeutic levels of virginiamycin on the prevalence, quantity, and duration of shedding of Salmonella typhimurium in swine.
Materials and Methods

Experimental Animals. Thirty pigs, 4 to 5 weeks of age, Salmonella-free, and not previously treated with antibiotics, were employed. The pigs were divided into the following five groups: Group A (10 pigs), Group B (10 pigs), Group C (three pigs), Group D (three pigs) and Group E (four pigs). All animals were individually housed in wire bottom cages.

Treatments. Pigs assigned to treatment Group A were housed in an isolation room. No antibacterial medication by any route was administered to these pigs throughout the entire treatment period. These pigs were employed as nonmedicated infected controls.

Group 13 pigs were housed in an isolation room separate from treatment Group A. Each pig in Group 13 received virginiamycin in the feed at a level of 55 g/metric ton 5 days prior to infection (day-5) with Salmonella typhimurium and throughout the entire experimental period.

Group C was employed as a nonmedicated, noninfected control group, housed in a separate building away from treatment Groups A and B. Group D was housed in the same building with Group C, and was utilized as a medicated, noninfected control group. The purpose of Groups C and D was to evaluate the extent of spreading salmonella throughout the research facility.

Group E animals housed in separate cages were interspersed between the cages housing animals in Groups A and B to determine the presence of salmonellae spreading between cages in the same room.

No other antibacterial medication was used or administered to pigs during the experimental period. Management practices were the same for all groups and precautions were taken to prevent cross contamination from one group to the other by animal caretakers. The basal diet employed for this study was of commercial origin.

Fecal samples were obtained from each of the 30 pigs on days 12 and 6 prior to medicatin and on day 0, just prior to artificial exposure of Groups A and B to the test strain of Salmonella typhimurium. These samples were used to ascertain the naturally occurring incidence of Salmonella spp., and the baseline incidence of resistant organisms in these animals.

Samples of the diet were also tested for the presence of Salmonella spp. prior to starting the study to ensure the absence of contaminating organisms which could influence the results. Double enrichment techniques were employed for this purpose. These techniques are described later under Bacteriology.

Inoculation of Test Organism and Fecal Sampling. Salmonella typhimurium strain USDA #295 was used to challenge pigs in Groups A and B. Prior to this, the sensitivity pattern of the test strain to commonly used antibiotics was determined.

Each pig in Groups A and B was inoculated orally by the use of a syringe and tubing with 13 to 15 ml of a 24-hr culture of the test strain. The inoculum concentration was determined by plate count to be $3 \times 10^9$ microorganisms per milliliter.

Fecal samples were taken from each pig from all Groups at 2, 4, 7, 10, 14, 21 and 28 days postinoculation, and thereafter at weekly intervals until two consecutive samples tested negative to the Salmonella spp. test strain, or until study termination at 59 days.

Clinical Records. Clinical records were maintained during the course of the study. All occurrences of disease symptoms, regardless of their nature, were recorded daily. Attempts were made to isolate infecting salmonella from all dead animals. In addition, records were maintained at regular intervals relating to body weights, mortality, feed consumption, body temperatures and scour scores.

Bacteriology. To qualitatively establish the resistance patterns of naturally occurring coliforms in the pigs, fecal samples were collected 12 days prior to initiating the study. Only fresh fecal specimens were collected, within 1 to 2 hr of elimination. One gram samples (based on wet weight) were scraped from the bottom of the cages, placed into sampling cups and then homogenized in 9 ml of sterile saline. Dilutions of $10^{-3}$ up to $10^{-6}$ were plated in duplicate on the surface of brilliant green bile agar (.1 ml of the inoculum per plate). Plates were incubated for 17 to 19 hr at 37 C. After incubation, 20 randomly selected colonies of suspected coliforms were isolated from plates containing 100 to 800 colonies. These isolates were then tested for sensitivity to the following antibiotics: neomycin, streptomycin, nitrofurantoin, chlorotetracycline, chloramphenicol and sulfathiazole. Susceptibility testing was performed in accordance with the standardized disc susceptibility method (Code of Federal Regulations, Part 460).

To ascertain the presence of naturally occurring Salmonella spp. in the pigs, fecal sam-
ple were collected on three occasions (−12, −5, and 0 days) prior to infection. One-gram samples were inoculated into 10 ml of selenite broth and incubated at 37 C for 36 to 52 hours. A loopful of broth was then streaked in triplicate onto plates containing brilliant green sulfa agar and incubated for 24 hr at 37 C. All colonies suspected of being Salmonella spp. were picked and inoculated into triple sugar iron (TSI) agar tubes and incubated at 37 C for 24 hours. Serological typing was employed to identify the species of salmonellae.

For the recovery of inoculated Salmonella typhimurium, fecal specimens were collected at various intervals, as previously described. These were processed immediately after collection. A 1-g sample was homogenized in 9 ml of selenite broth, and 10-fold dilutions were carried out in the same broth, up to $10^6$. Each dilution was plated in duplicate on the surface of brilliant green sulfa agar in a volume of .1 milliliter. After spreading the inoculum over the surface of the agar, plates were incubated at 37 C for 24 hours. Total salmonellae counts per gram of feces were recorded for each fecal sample. When Salmonella typhimurium was not recovered, an enrichment procedure was used for the isolation of salmonellae. Fecal samples were homogenized in selenite broth, incubated at 37 C for 36 to 52 hr, and a loopful of the incubated broth plated on brilliant green sulfa agar. The latter procedure was designed to ensure the qualitative recovery of low numbers of infecting Salmonella typhimurium. Five colonies of Salmonella typhimurium from each fecal sample were selected and inoculated into TSI agar tubes and incubated at 37 C for 24 hours. Salmonella typhimurium were identified serologically. Flagellar serum against Salmonella typhimurium was utilized. A loopful of growth from TSI agar slants was transferred to trypticase soy broth and incubated for 24 hr at 37 C. The broth cultures were then diluted with an equal volume of saline containing 6% formalin. To .5 ml formalized broth in small test tubes (12 x 75 mm), .5 ml of the diluted flagellar (i) serum aganst Salmonella typhimurium (BBL) was added and the tubes were then placed in a water bath at 50 C. Results were read and recorded after 1 hr of incubation. Isolated colonies identified as Salmonella typhimurium were maintained on trypticase soy agar slants.

Results and Discussion

A summary of the results of resistance studies on coliforms isolated from fecal samples prior to starting the study are found in table 1. Of the organisms isolated, the greatest percentage of resistance was found to occur against chloramphenicol, streptomycin, neomycin and sulfathiazole. All isolates were multiply resistant to a combination of antibiotics, the most common resistance patterns being chloramphenicol:streptomycin:sulfathiazole and chloramphenicol : streptomycin : neomycin : sulfathiazole. This strongly implicates the existence of R factors as a source of the multiple resistance.

The sensitivity pattern of the Salmonella typhimurium strain (USDA #295) employed to challenge the pigs in Groups A and B was determined. The organism was found resistant to chloramphenicol, erythromycin and streptomycin. It exhibited borderline resistance to tetracycline and penicillin. Expectedly, the organism was resistant to virginiamycin, which exhibits activity against gram positive organisms only.

Clinical symptoms observed after challenge with the test organism were variable and inconsistent. Three out of 10 pigs in Group A and five out of 10 pigs in Group B exhibited increased body temperatures on the second day after exposure to the test organism. These elevated temperatures persisted for 2 to 3 days before returning to normal. Body temperatures for pigs in the remaining groups were within normal range with the exception of two pigs...
TABLE 1. RESISTANCE PATTERNS OF ISOLATED COLIFORMS FROM PIGS PRIOR TO TREATMENT WITH VIRGINIAMYCIN

<table>
<thead>
<tr>
<th>Pattern of resistance</th>
<th>Percent resistant</th>
<th>Pattern of resistance</th>
<th>Percentage resistant</th>
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<tbody>
<tr>
<td>ST, S</td>
<td>4.3</td>
<td>S, CTC, ST</td>
<td>.2</td>
</tr>
<tr>
<td>N, CTC, ST</td>
<td>2.8</td>
<td>CTC, ST, S, C</td>
<td>.3</td>
</tr>
<tr>
<td>CTC, ST</td>
<td>6.0</td>
<td>N, S, ST</td>
<td>2.8</td>
</tr>
<tr>
<td>N, ST</td>
<td>.3</td>
<td>N, CTC, F/M, ST</td>
<td>.5</td>
</tr>
<tr>
<td>CTC, ST, S</td>
<td>31.3</td>
<td>CTC, ST, N, S</td>
<td>47.0</td>
</tr>
<tr>
<td>ST</td>
<td>.3</td>
<td>N, S, CTC</td>
<td>.3</td>
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<tr>
<td>CTC, F/M, ST, N, S</td>
<td>3.5</td>
<td>S, CTC</td>
<td>.2</td>
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<tr>
<td>...</td>
<td>...</td>
<td>CTC, F/M, ST</td>
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aST—Streptomycin; S—Sulfathiazole; CTC—Chlortetracycline; N—Neomycin; F/M—Nitrofurantoin. Sensitive and resistant organisms were defined according to the standardized disc susceptibility method (Code of Federal Regulations).

bResults obtained from 20 random isolates of coliforms from each plate containing a dilution resulting in 100 to 800 colonies per plate.

from Group C which exhibited increased temperatures 3 days prior to infection of Groups A and B. This was not considered to be salmonellae related. Diarrhea was noticed in about 50% of the pigs from Groups A and B after infection with salmonella test strain. With exception of one pig from Group C, all remaining animals were normal. Regarding weight gains, all animals gained normally, except for two pigs from Group A and one from Group B.

Attempts to isolate salmonellae from the diet employed in this study were negative. Similarly, no salmonellae organisms were detected in any of the animals during the pre-infection sampling periods. Attempts to isolate salmonellae from any of the animals in Groups C, D and E, utilizing double enrichment techniques proved fruitless. These results suggest the absence of spread of the infection within the facility and in the rooms in which Groups A and B were housed.

Rate of Shedding Results. The rate of shedding of salmonellae from Groups A and B was determined from direct colonic counting on fecal samples. When actual counts could not be developed, enrichment procedures were followed to determine positive or negative responses.

Given actual counts as one quantitative index of shedding, a positive enrichment as a qualitative index of potential shedding and the negative enrichment response, it was difficult to establish a meaningful mathematical technique for estimating a quantitative average response per group. The number of positive responses in each group at each time interval was expressed as a percentage of 10 infected animals per group. In addition, the number of pigs from which an actual colonic count was detected was expressed as a percentage of 10 animals for each group at each time. Plots of these criteria are presented in figure 1. Group comparisons utilizing the chi-square test resulted in no statistically significant difference.

Persistence and Shedding Results. Another measurable parameter was persistence of infection. This was best monitored by evaluating the number of animals shedding at various sampling periods and the necropsy time of individual pigs in the treated and nontreated groups. No statistically significant differences could be detected in the persistence of salmonellae shedding between the virginiamycin treated and nontreated groups.

Isolation of salmonellae from Tissues. Liver, spleen, lymph node and colon were sampled from all of the animals in each group after necropsy and analyzed for the presence of salmonellae by direct plating and double enrichment methods. Salmonella colonies could not be isolated by direct plating methods from any of the animals. However, utilizing double enrichment techniques, Salmonella colonies were isolated from the liver, spleen, lymph node and colon of one animal from the nonmedicated, infected group (Group A). Colonies of salmonellae were also isolated from colon samples obtained from three additional pigs of the same group. In Group B (infected, medicated), salmonellae colonies were isolated only from colon samples.
obtained from four pigs. No salmonellae organisms were isolated from any of the pigs in Groups C, D and E, reconfirming the absence of spread of the infection within the facility and rooms housing infected animals. There were no measurable statistical differences between groups.

In this study, pigs infected with *Salmonella typhimurium* and treated with virginiamycin medicated feed at levels of 55 g/metric ton resulted in no significant changes in the course of the infection when compared with infected, nonmedicated control animals. No significant differences in the incidence, rate and persistence of shedding could be detected through virginiamycin treatment. In addition, no significant differences could be detected in the recovery of salmonellae organisms from selected tissues as a consequence of virginiamycin treatment. These studies confirm that use of virginiamycin as a feed additive antibiotic in pigs results in no significant changes in the incidence and persistence of *Salmonella typhimurium* infections.

**Literature Cited**


Code of Federal Regulations. Title 21, FDA, US Gov-
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