JOURNAL
OF THE
Tennessee Academy of Science

VOLUME XXXI OCTOBER, 1956 No. 4

THE NEED FOR ANTIBIOTIC FREE DIETS IN THE EXPERIMENTAL INFECTION OF ANIMALS*

RICHARD T. ROSS, D. FRANK HOLTMAN, AND
ROBERT F. GILFILLAN
Department of Bacteriology, University of Tennessee, Knoxville, Tennessee

Biological research frequently involves animal experimentation, and the commercial feeds now available for the maintenance of such animals are almost invariably fortified with some form of antibiotic residue. While small amounts of these residues have been shown to stimulate growth, they may well be detrimental to the investigation concerned with experimental infection of laboratory animals.

Biologists who use feeds containing antibiotics during the course of animal experimentation, may find the observations made in our laboratory to be of special interest.

Some of our initial experiments on the host-parasite relationship of the chick and *Salmonella pullorum* (1955 a, b) resulted in failure to produce infection even with massive injections of a virulent strain of this bacterium. Upon our discovery that the starter mash supplied the chicks contained small amounts of aureomycin and penicillin, it was assumed that these antibiotics were responsible for the failure to induce disease in the host. Hence, it was decided to compare the infectivity of the organism for chicks maintained on an antibiotic-free diet with those supplied the antibiotic-fortified mash.

MATERIALS AND METHODS

All chicks used in this experiment were one to three day old White Leghorn cockerels. Ration containing antibiotic was obtained from a local mill. This feed contained 8 grams of aureomycin and 2 grams of penicillin per ton, or approximately 11 micrograms of antibiotic per gram. Control chicks were maintained on an identical ration from which the antibiotics had been withheld. Food and water were available *ad libitum*

*Supported by National Science Foundation Grant No. G-939.

to both experimental and control groups throughout the investigation. Strain 3522/51 of *S. pullorum* obtained from the Communicable Disease Center, Chamblee, Georgia, was used as the test organism. Inocula were prepared from saline suspensions of 24 hour broth cultures. These were administered to the chicks either orally or intraperitoneally. Numbers of survivors were recorded at intervals of 12 hours.

Results

Survival time of chicks inoculated with 10^3 organisms is recorded in Table 1. This inoculum was 100 per cent fatal for chicks maintained on an antibiotic-free diet. The average hour

Table 1

Survival time of chicks infected with *Salmonella pullorum*

Groups of Chicks	Total Deaths	Average Hour of Death
Antibiotic-free diet:		
(a) Normal	0/50	---
(b) Infected 10^3 cells orally	50/50	68.5
(c) Infected 10^3 cells IP	50/50	62.3
Antibiotic-fortified diet:		
(d) Infected 10^3 cells orally	5/50	12.3
(e) Infected 10^3 cells IP	3/50	7.9

of death of the birds infected orally was 68.5 as compared to 62.3 for those infected intraperitoneally. Post mortem examination, including culture on differential media, revealed a wide distribution of *S. pullorum* throughout the host.

A total of 8 deaths was observed in the 100 chicks infected with the test organism and maintained on the antibiotic-fortified diet. However, none of the microorganisms recovered from these chicks could be identified as *S. pullorum*. Furthermore these birds succumbed within 8 to 12 hours after introduction of the pathogen so it is probable that the deaths were due to factors other than the experimental infection.

Subsequent experiments using inocula containing up to 10^9 organisms produced results similar to those described. Infected chicks supplied the antibiotic-free mash died rapidly, whereas no deaths, attributable to infections with *S. pullorum*, were observed in the birds receiving the antibiotic-fortified diet. There was, however, a noticeable degradation of the general vitality of chicks receiving 10^7 organisms, or more. This effect gradually disappeared and the birds eventually recovered.

Discussion

The use of antibiotics in feed has been reported to interfere in various ways with biological research. Miller *et al* (1955) found marked changes in the intestinal microflora of mice with the feeding of streptomycin. The animals actually became highly susceptible to very small numbers of *Salmonella enteritidis*, which multiplied readily and replaced the streptomycin-sensitive bacteria normally present in the intestine of the mouse. Slanetz (1953) has attained evidence that prolonged feeding of antibiotic residues interferes with antibody production, which could lead to the animal, on this type of diet, becoming increasingly susceptible to disease.

While results such as those described evidently apply when certain antibiotics as well as certain species or strains of infectious agents are used, it is apparent that they do not apply in all host-parasite relationships. Our own findings illustrate the necessity of withholding such antibiotics as aureomycin and penicillin from the diets of chicks during experimental investigations concerned with pullorum disease, not because they enhance the infection, but because they prevent its typical pattern of development. Conversely, these results suggest that the inclusion of antibiotics in feeds for the prevention of spontaneous *S. pullorum* infections may be highly desirable whenever chicks are to be subjected to nutritional or physiologic study.

While the results discussed here apply specifically to the chick and S. *pullorum*, it can be conjectured that problems similar to ours may arise when antibiotic-fortified feeds are employed in other host-parasite relationships. Hence, it is important that the investigator make himself aware of the composition of feeds employed in his animal research.

Summary

Small amounts of aureomycin and penicillin in chick starter mash were found to interfere markedly with the laboratory infection of chicks with *S. pullorum*, CDC 3522/51.

References

Miller, C. P., Bohnoff, M., and Drake, B. I. 1955. The effect of antibiotic therapy on the susceptibility of mice to salmonella infection. Bact. Proceed., p. 103.

Ross, R. T., Holtman, D. Frank, and Gilfillan, R. F. 1955. a. The effect of *Salmonella pullorum* infection on amino acids of the chick. J. Bact., 70:272-275.

Ross, R. T., Holtman, D. Frank, and Gilfillan, R. F. 1955. b. The effect of the introduction of amino acids into chicks infected with *Salmonella pullorum*. J. Bact., 70:276-278.

Slanetz, C. A. 1953. Interference of antibiotics with antibody production. Antibiot. and Chemother., 3:629.