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Influence of Subtherapeutic Levels of Oxytetracycline on *Salmonella typhimurium* in Swine, Calves, and Chickens

D. G. EVANGELISTI, A. R. ENGLISH, A. E. GIRARD,* J. E. LYNCH, AND I. A. SOLOMONS

Medical Research Laboratories, Pfizer Central Research, Groton, Connecticut 06340

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Subtherapeutic levels of oxytetracycline in animal feeds have been evaluated to determine their influence on the relative quantity, prevalence, shedding, and antibiotic susceptibility of *Salmonella typhimurium* in swine, calves, and chickens, when compared with nonmedicated controls. The medicated groups were fed rations containing oxytetracycline commencing 5 days prior to oral inoculation with *S. typhimurium* and continuing through a 28-day post-inoculation period. Colonization of *S. typhimurium* occurred in all three animal species as evidenced by clinical signs of infection and/or colony counts in feces measured on seven separate occasions over the 28-day observation period. The accumulated data demonstrate that the subtherapeutic use of oxytetracycline did not bring about any increases in the quantity, prevalence, or shedding of *S. typhimurium* in swine, calves, and chickens. In fact, the medication generally brought about a decrease in the percentage of animals carrying *S. typhimurium* during the study period. In contrast to results in swine and calves, there was a significant occurrence of *S. typhimurium* resistance to oxytetracycline in chickens. Resistant colonies were isolated from chickens sporadically but never on more than two consecutive test periods. These isolates were also resistant to streptomycin, but not to the other six antibiotics tested. The population of resistant *S. typhimurium* isolated from medicated chickens was no larger than that of susceptible *S. typhimurium* isolated from the nonmedicated animals. It is concluded that no evidence has been obtained which would relate the continuous low-level feeding of oxytetracycline for a 4-week period to an increased incidence of disease in animals or as a hazard to humans.

Surveys in Japan (4) and in England (5, 8) have shown a high incidence of transferable drug resistance among resistant strains of *Escherichia coli* isolated from domestic animals. These surveys were interpreted as indicating an association between the antibacterial drugs fed to animals and the isolation of strains of *E. coli* capable of transferring resistance to other enteric organisms, especially the salmonellae. Such observations aroused general concern over the feeding of subtherapeutic levels of antibiotics to animals primarily because of a possible hazard to human health. This potential health hazard of transferable drug resistance was considered in depth by the Swann Committee in the United Kingdom and the Antibiotic Task Force of the Food and Drug Administration (FDA) in the United States.

The report of the FDA Task Force on the "Use of Antibiotics in Animal Feeds" (7) led to the publication in the Federal Register (2) of "Statements of Policy and Interpretation Regarding Animal Drugs and Medicated Feeds." In this publication it is stated that: "A feed-use drug used on a continuing basis which significantly increases the numbers of salmonella [sic] in the animal would logically affect the the numbers of salmonella [sic] organisms on the animal derived food products. Therefore, the Commissioner concludes that a significant increase in salmonella [sic] organisms in animals would constitute an increased hazard to human health."

The studies reported here were undertaken to ascertain whether or not the continuous feeding of oxytetracycline at subtherapeutic levels results in an increase in the relative quantity, prevalence, and duration of shedding of *Salmonella typhimurium* in swine, calves, and chickens over that observed in nonmedicated controls. In addition, the susceptibility of *S. typhimurium* to oxytetracycline and several

other antimicrobial agents used in human clinical medicine was determined before and during the course of these studies.

MATERIALS AND METHODS

Organism(s). The culture of *S. typhimurium* used was obtained from the American Cyanamid Company and identified as strain 289. We have assigned the number 58D013 to the culture. It was recovered from swine, calves, and chickens before being used in the experiments with these food-producing animals. In swine, the isolate was administered orally and recovered from the feces. The fecal isolate was then passed by intravenous injection and reisolated from the liver 24 h later. In a calf, the swine liver isolate of *S. typhimurium* was introduced intravenously and recovered from the liver after 48 h. *S. typhimurium* 58D013 was inoculated via cardiac puncture into a broiler chicken and reisolated from the liver after 6 h. All strains (the original and animal isolates) produced an acid butt with H_2S and an alkaline slant in triple sugar iron agar (TSI, Difco), were urease negative, agglutinated with *Salmonella* group B antiserum (BBL), and were susceptible to oxytetracycline, neomycin, kanamycin, gentamicin, ampicillin, cephalothin, streptomycin, and chloramphenicol.

Preliminary studies were performed to determine the appropriate method for preparing inocula (in saline or as a slurry in feed, etc.), and each strain was titrated in vivo to insure that a sufficient number of organisms were introduced to facilitate colonization of the intestines. Optimum results were obtained when animals were fasted 5 to 6 h before receiving the *Salmonella* challenge. Swine and calves were fed 0.5 lb (approximately 226 g) of basal feed to which was added a 40-ml suspension of *S. typhimurium* containing, as determined by plate count, $\sim 1.1 \times 10^{11}$ to 1.4×10^{11} organisms. The chickens were given the inoculum by delivering a slurry of *S. typhimurium* suspension in feed into the gullet. A plate count of the inoculum revealed that each chicken received $\sim 2.92 \times 10^{11}$ organisms.

Animals. All animals were purchased from commercial sources. At the start of the experiment, the Hampshire-Yorkshire cross swine weighed an average of 9.6 kg, whereas the Holstein calves weighed an average of 85.5 kg, and the chickens, at 8 days of age, averaged 0.798 kg.

All animals used in these studies were previously determined to be *Salmonella*-free. The animals were lotted into groups of 10 by weight (chickens), weight and sex (swine), or weight and source (calves). Each lotted group of animals was housed in a separate room. Handlers were required to follow rigid procedures established to prevent cross-contamination between animal groups. Since isolation of the swine and calf groups was complete, no environmental controls were necessary. Environmental controls were included in the chicken studies as complete isolation was not possible. Group isolation, however, did not prevent interaction of animals within the group. Housing was thus as near as possible to actual swine-, cattle-, and chicken-raising conditions. Feed and water were available on an ad libitum basis.

Experimental diets. Groups designated T-1 were fed a ration of nonmedicated feed throughout the experimental period while the T-2 groups were fed the same feed containing oxytetracycline, commencing 5 days prior to *S. typhimurium* inoculation and throughout the remainder of the experimental period. No other medication was administered over the experimental period. Finished feeds were bioassayed for oxytetracycline content at the start and termination of each study by established procedures (3). The levels of oxytetracycline in each animal feed are presented in Table 1.

Clinical records. Clinical records were maintained over the course of the study for both medicated and nonmedicated groups. All occurrences of symptoms of disease were recorded.

Pretest *Salmonella* screening. Animal feces and the environment were tested prior to treatment for the presence of naturally-occurring *Salmonella* by taking samples from the feed to be used (medicated or nonmedicated), the water sources, and feces of all animals. One sample each was taken from the feed and water sources. Fecal samples were taken from all animals on two separate days. The 1-g samples of feed, 1-g samples of feces (0.2 g for chickens) and 1-ml samples of water were added respectively to 9.0 ml of tetrathionate broth (BBL) and incubated at 37 C for 48 h. A loopful of broth was then streaked on brilliant green sulfa agar (BGSA, Difco) and incubated for 48 h (read at 24 and 48 h). Colonies suspected of being *Salmonella* were inoculated into TSI agar slants and urea broth (Difco). Growth on the TSI slant was used as the source of antigen for serological testing. *Salmonella* group B-specific antiserum (BBL) was employed to determine if the culture was a member of the group. The experiments were started only when negative results were obtained from all screenings.

Quantitation of *S. typhimurium* from inoculated animals. Seven fecal specimens were collected from each animal over the 28-day post-inoculation period and were processed as soon as possible. One gram of each sample was placed in 9 ml (1:10 dilution) of brilliant green broth (Difco) and serially diluted in brilliant green broth in increments of 10. Dupli-

TABLE 1. *Levels of oxytetracycline in each animal feed*

Animal	Nonmedicated feed (basal)	Amt of oxytetracycline-hydrochloride added to feed (g/t)
Swine	Pig grower ration	150
Calf	Rough cattle finisher ration	101.01[a]
Chicken	Chick starter ration	200

[a] Calculated to provide a level equivalent to 350 mg/head per day at the start of the experiment.

cate samples of 0.1 ml of each dilution were spread on BGSA plates. A 0.1-ml sample of each dilution was also spread on BGSA containing oxytetracycline (25 mg/ml). Plates were incubated at 37 C for 48 h. Only non-lactose-fermenting colonies (red color) were counted. Five colonies suspected of being *Salmonella* were picked and further identified using the scheme described above. However, as the counts decreased with time, there were many instances where there were fewer than 5 colonies available for identification. The total *Salmonella* count per gram (dry weight) was recorded after verifying that colonies were group B *Salmonella*. Samples containing less than 100 *Salmonella* organisms per gram (wet weight) of feces would not be detected by this procedure and thus a zero count would equal less than 100.

Antibiotic susceptibility. Each culture identified as a group B *Salmonella* was tested for antibiotic susceptibility by the standardized disc susceptibility test as published in the Federal Register (1) using the following commercial (BBL) antibiotic discs: ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg, streptomycin (10 μg), oxytetracycline (30 μg).

Incidence of pretest oxytetracycline-resistant E. coli. *E. coli* are defined here as large, lactose-fermenting, metallic sheen colonies which develop on eosin methylene blue agar (EMB, Difco). The IMViC series was not used to verify identification. After lotting but prior to the start of treatment, a fecal sample (1 g) was taken from each animal and placed in 9 ml of saline and homogenized. The sample was further serially diluted by 10-fold increments and duplicate portions of 0.1 ml of the 10^{-2}, 10^{-4}, and 10^{-6} dilutions were placed on EMB agar and on EMB agar plates containing 50 μg of oxytetracycline per ml. The plates were incubated at 37 C for 48 h. Only the large, lactose-fermenting, metallic sheen colonies were counted. The percentage of *E. coli* resistant to oxytetracycline (count on EMB plus antibiotic/count on EMB $\times$ 100) was determined for each animal. The percentages obtained from each animal were totaled and an average percentage of oxytetracycline-resistant *E. coli* was calculated.

Statistical methods. The statistical methods employed were uniformly applied to the analysis of the swine, calf, and chicken data. The details of the statistical methods are described in Results.

RESULTS

Swine. The oxytetracycline-resistant *E. coli* examined before the start of the experiment represented an average of 5.4% of the total *E. coli* population in the infected controls and 0.08% in the medicated animals.

The viable *Salmonella* counts from the fecal samples are presented in Table 2. It is apparent that *S. typhimurium* colonized the swine gut in both the medicated and nonmedicated groups. Logarithms of the colony counts obtained over the seven sampling periods were averaged for each animal, and this measure of quantity was compared with a two-sample t test. This comparison showed that the quantity of *Salmonella* in the medicated group was not significantly different from those in the nonmedicated controls.

The medicated group exhibited more *Salmonella*-free fecal samples, 30/70 (10 swine/group $\times$ 7 samplings = 70 samples), compared to 17/70 for the control group. A statistical analysis of prevalence was performed by examining the overall gross patterns of shedding. A score was assigned to each animal using the number of days (out of 7) on which shedding occurred. For instance, animal 18 had a score of 4, animal 36 had a score of 3, and animal 16 (in whom reinfection may have occurred on day 14) had a score of 4. Two-sample t tests were then run on these scores and no statistical significance emerged. The 95% confidence interval for the mean differences between the medicated group and the controls is -3.1 to 0.5, which indicates that in a larger sample the medicated group could be expected to have, on the average, anywhere from 3.1 fewer incidences of shedding per animal to 0.5 greater occasions over the seven sampling periods used in this experiment.

The percentage of swine shedding *Salmonella* as a function of time is plotted in Fig. 1A, which shows a decrease in both groups with time. The rate of decrease in shedding appeared to be somewhat faster in the oxytetracycline group. Since all animals were inoculated initially, the statistical analysis dealt with the rate at which *S. typhimurium* disappeared. A rate of change in the probability of shedding per day was computed across all 28 days of the trial for each animal. These rates were then compared with a two-sample t test. There was no significant difference, and a 95% confidence interval on the mean difference suggests that the difference in probability of shedding per day lies between 3% less to 0.6% more for the oxytetracycline-treated group compared to the nonmedicated controls.

Susceptibility tests were performed with 168 isolates from the treated group (T-2) over the seven sampling periods during the 28-day post-inoculation period. The only instance of resistance was with one isolate (pig 11), which was found to be resistant to oxytetracycline, as defined in the Federal Register (1). There were no changes in the susceptibility of *S. typhimurium* to the other antibacterial agents tested. This resistant colony (Table 2) was isolated from a 10^{-2} dilution of sample spread on a medium containing oxytetracycline. No resist-

TABLE 2. *Salmonella typhimurium counts per gram[a] of swine feces*

Swine no.	*S. typhimurium* (counts/g of swine feces)						
	2[b]	4	7	10	14	21	28
Group T-1[c]							
1	4.19×10^7	1.90×10^6	9.17×10^3	5.99×10^4	4.04×10^3	8.02×10^3	2.50×10^2
4	2.45×10^7	4.59×10^6	2.25×10^4	0	0	0	0
9	2.76×10^6	1.38×10^5	1.96×10^5	4.81×10^4	1.69×10^4	5.59×10^3	2.36×10^2
12	5.34×10^4	3.39×10^4	2.24×10^3	0	1.39×10^3	0	1.86×10^2
18	3.38×10^5	2.84×10^3	1.64×10^4	1.00×10^4	0	0	0
23	2.60×10^6	7.14×10^3	4.07×10^4	1.85×10^3	2.25×10^3	9.96×10^5	0
27	2.50×10^5	1.25×10^5	4.43×10^4	4.81×10^4	2.25×10^4	2.25×10^4	7.07×10^3
29	3.10×10^5	6.96×10^3	3.46×10^4	2.48×10^4	2.67×10^4	1.54×10^3	1.20×10^5
36	3.38×10^5	1.14×10^4	0	0	0	0	3.32×10^4
37	9.77×10^6	7.77×10^4	2.02×10^3	0	1.13×10^3	0	0
Group T-2[d]							
2	3.29×10^4	0	0	0	0	0	0
7	2.13×10^5	2.58×10^5	1.06×10^4	6.85×10^4	2.26×10^4	1.07×10^6	0
11	2.56×10^7	6.25×10^4	1.75×10^4[e]	1.49×10^4	6.42×10^4	1.67×10^4	1.47×10^5
14	5.03×10^5	1.49×10^4	0	0	0	0	0
16	9.38×10^6	5.22×10^4	6.35×10^3	0	1.86×10^2	0	0
20	5.28×10^7	1.40×10^4	0	0	0	0	0
24	1.50×10^5	0	3.24×10^3	0	0	0	0
30	5.87×10^6	9.09×10^4	7.69×10^4	1.29×10^4	1.21×10^5	0	3.19×10^6
31	1.55×10^5	3.33×10^3	1.25×10^3	9.09×10^3	8.07×10^2	0	2.70×10^3
38	3.74×10^7	2.71×10^4	3.88×10^2	0	0	0	4.41×10^3

[a] Dry weight.
[b] Days post-inoculation.
[c] T-1, Control nonmedicated group.
[d] T-2, Oxytetracycline-medicated group.
[e] Resistant colony (one colony isolated from plate containing oxytetracycline at 10^{-2} dilution; see text for discussion).

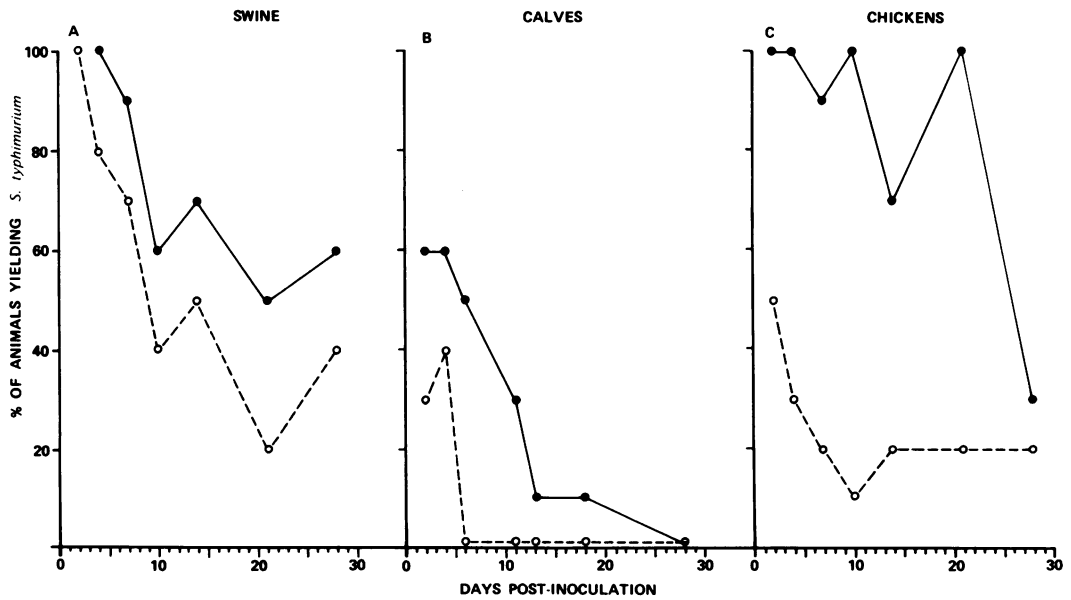

FIG. 1. *Percentage of animals shedding S. typhimurium. Symbols: ●, nonmedicated controls; ○, oxytetracycline treated.*

ant colonies were isolated from antibiotic-free medium. The resistant count then was ~1 × 10^2 or approximately 1% of the total *S. typhimurium* population. These results demonstrate that the subtherapeutic level of oxytetracycline did not cause a significant increase in resistant *S. typhimurium*. No resistance was detected in the 232 *S. typhimurium* isolates recovered from the nonmedicated group.

Clinical signs of disease, body temperature, and diarrhea scores are plotted in Fig. 2A. The observations demonstrate that clinical signs of infection were established in swine. In fact, the animals were on the borderline of a severe illness which would normally be treated with therapeutic doses of the antibiotic. The mediated group did not demonstrate a reduction in temperature elevation or a significant difference in the severity of diarrhea when compared with the nonmedicated group.

Calves. During the first post-inoculation week, disease symptoms were so severe that there was concern for the survival of the animals. By the second week the animals had a generally healthy appearance. It seemed then that the challenge was near the lethal dose and the illness was acute in nature, since recovery and disappearance of *S. typhimurium* were rapid and complete. The fecal counts observed during the course of this study were not as high as anticipated, but a higher challenge may have been fatal. In fact, one animal in the nonmedicated group died on the sixth post-inoculation day. The necropsy report indicated the presence of *Pasteurella hemolytica* and *S. typhimurium*. *S. typhimurium* was cultured from the liver, spleen, lung, a lymph node, and the ileocecal junction. *P. hemolytica* was isolated from the lungs. Gross lesions were present in the lung, probably due to pasteurellosis with the additional stress of the *S. typhimurium* challenge.

The oxytetracycline-resistant *E. coli* examined before the start of the experiment represented an average of 65.6% of the total *E. coli* population in the infected controls and 59.7% in the medicated animals.

The viable *Salmonella* counts obtained from the fecal samples are shown in Table 3. It is clear that the *S. typhimurium* population in

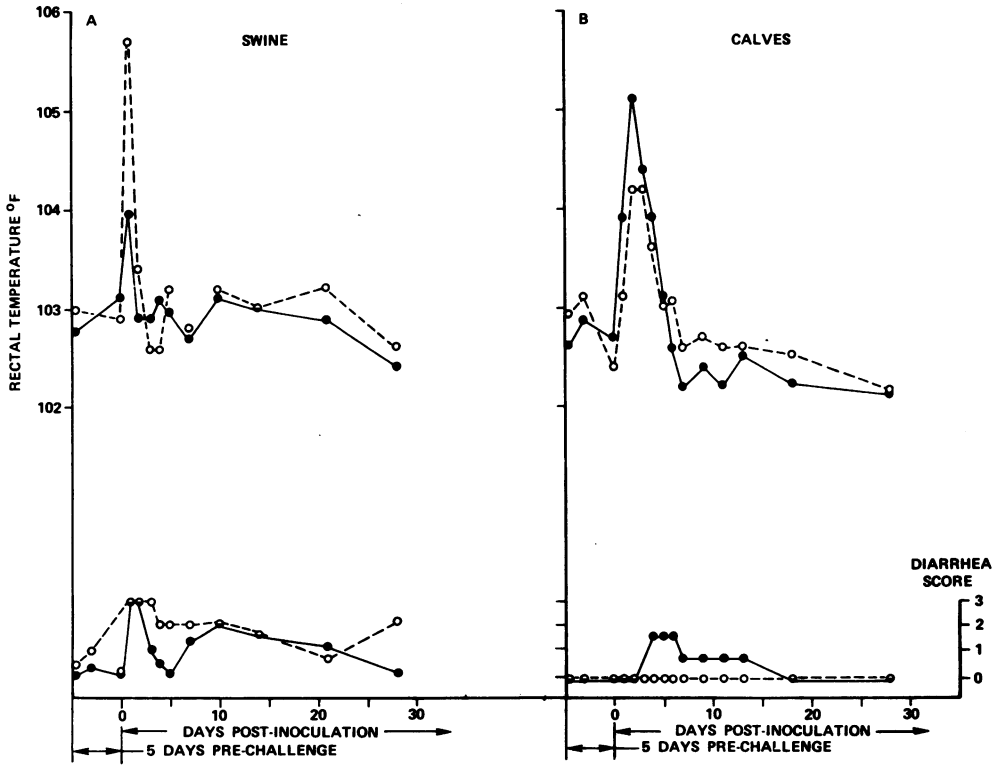

FIG. 2. *Temperature and diarrhea measurements (median values). Symbols: ●, nonmedicated controls; ○, oxytetracycline treated. Diarrhea was scored according to stool consistency with 0 as normal and 3 being a very loose consistency.*

TABLE 3. *Salmonella typhimurium counts per gram[a] of calf feces*

Calf no.	*S. typhimurium* (counts/g of calf feces)						
	2[b]	4	6	11	13	18	28
Group T-1[c]							
12	8.89×10^6	2.60×10^7	6.96×10^7[d]				
26	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0
31	2.16×10^2	0	0	0	0	0	0
34	2.43×10^2	4.96×10^7	5.67×10^4	0	0	0	0
36	5.46×10^5	6.90×10^6	8.32×10^5	8.60×10^3	6.93×10^3	4.79×10^3	0
39	1.69×10^4	3.37×10^4	7.39×10^3	3.94×10^2	0	0	0
41	0	0	0	0	0	0	0
42	2.93×10^4	8.16×10^7	5.00×10^5	2.29×10^2	0	0	0
44	0	1.31×10^4	0	0	0	0	0
Group T-2[e]							
16	0	0	0	0	0	0	0
21	2.33×10^2	3.75×10^2	0	0	0	0	0
22	0	7.14×10^3	0	0	0	0	0
28	2.55×10^3	2.16×10^2	0	0	0	0	0
30	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0
47	3.96×10^3	2.13×10^2	0	0	0	0	0

[a] Dry weight.
[b] Days post-inoculation.
[c] T-1, Control nonmedicated group.
[d] Died on day 6.
[e] T-2, Oxytetracycline-medicated group.

both the medicated and nonmedicated groups decreased with time. A comparison of logarithms of the colony counts with a two-sample *t* test showed that the quantity of *Salmonella* in the medicated group was significantly less than that in the nonmedicated controls, having a value of $P < 0.05$.

The medicated group exhibited many more *Salmonella*-free cultures (63/70) over the duration of the experiment than did the nonmedicated controls (44/66). A statistical analysis of the prevalence of *S. typhimurium* in the medicated versus the nonmedicated group yielded no significant difference. None of the *S. typhimurium* isolates from either group was found to be resistant to oxytetracycline or to the other antimicrobial agents used in susceptibility testing.

The percentage of calves shedding *S. typhimurium* as a function of time is plotted in Fig. 1A. The shedding of test organism was found to decrease in both groups with time. Although the rate of decrease in shedding was faster in the oxytetracycline group, statistical analysis of the rate of change in probability of shedding yielded no significant difference. A 95% confidence interval on the mean difference suggests that the difference in probability of shedding per day lies between 1.6% less to 0.08% more for the oxytetracycline-treated group compared to the nonmedicated controls.

Rectal temperature measurements and diarrhea scores are plotted in Fig. 2B. These observations demonstrate that clinical signs of infection were established in the nonmedicated calves and, as noted previously, the challenge was a near-lethal one. The oxytetracycline-medicated group showed some reduction in the magnitude of temperature rise and better control of diarrhea compared with nonmedicated controls.

Chickens. The oxytetracycline-resistant *E. coli* examined before the start of the experiment represented an average of 30.0% of the total *E. coli* population in the infected controls and 29.8% in the medicated animals.

The viable *Salmonella* counts obtained from the fecal samples are shown in Table 4. A comparison between the two groups demonstrates the beneficial effects of this subtherapeutic dosage regimen of oxytetracycline. The quantity of *S. typhimurium* was considerably

TABLE 4. *Salmonella typhimurium counts per gram[a] of chicken feces*

Chicken no.	*S. typhimurium* (counts/g of chicken feces)						
	2[b]	4	7	10	14	21	28
Group T-1[c]							
7	1.56×10^5	3.13×10^5	1.36×10^5	2.92×10^4	7.69×10^2	1.17×10^4	0
8	4.02×10^7	4.52×10^5	3.63×10^4	1.57×10^4	3.52×10^4	2.42×10^3	0
14	1.21×10^5	2.24×10^6	1.88×10^6	2.50×10^4	1.64×10^3	1.22×10^4	0
19	2.18×10^4	1.06×10^4	4.37×10^2	2.89×10^2	0	1.32×10^4	0
31	1.03×10^7	7.79×10^5	6.61×10^5	1.94×10^5	1.87×10^3	3.33×10^4	0
34	3.06×10^5	1.36×10^5	5.21×10^3	7.61×10^3	0	3.39×10^3	0
37	3.47×10^6	5.56×10^4	4.38×10^3	4.97×10^3	1.52×10^4	1.23×10^3	2.55×10^4
56	1.99×10^6	3.15×10^4	7.43×10^3	8.85×10^2	2.18×10^4	1.00×10^4	1.65×10^3
73	3.47×10^5	1.88×10^4	0	4.82×10^3	0	2.08×10^4	5.08×10^2
81	2.92×10^6	7.21×10^3	3.25×10^2	8.57×10^2	2.08×10^3	5.47×10^3	0
Environmental group:							
18	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0
Group T-2[d]							
17	4.20×10^{2e}	0	0	0	0	0	5.18×10^{3e}
20	0	3.03×10^3	0	0	0	5.91×10^{3e}	2.34×10^{3e}
38	1.02×10^3	0	0	0	0	0	0
47	2.34×10^3	0	0	0	0	0	0
48	0	0	0	0	0	5.31×10^{2e}	0
51	1.70×10^3	0	0	0	2.65×10^2	0	0
78	1.45×10^4	5.75×10^3	7.25×10^3	3.21×10^{2e}	2.73×10^3	0	0
79	0	1.88×10^{6e}	1.44×10^{5e}	0	0	0	0
80	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0

[a] Dry weight.
[b] Days post-inoculation.
[c] T-1, Control nonmedicated group.
[d] T-2, Oxytetracycline medicated group.
[e] Resistant colony (see text for discussion).

less in the medicated group than in the control group. This observation was shown to be a statistically significant decrease, having a value of $P < 0.001$. The medicated group also yielded (Table 4) many more *Salmonella*-free cultures (53/70) over the duration of the experiment than did the nonmedicated controls (11/70). This finding was also shown to be statistically significant, having a value of $P < 0.001$.

The percentage of chickens shedding *S. typhimurium* as a function of time is plotted in Fig. 1C. All chickens in the nonmedicated group were shedding *S. typhimurium* on day 21; a dramatic decline was observed only on the final day of the experiment. In contrast, only five animals of the medicated group were shedding *S. typhimurium* on post-inoculation day 2, demonstrating that oxytetracycline was able to decrease the extent of colonization. Although the percentage of animals shedding during a sampling period was fairly constant, shedding by a given animal was not (i.e., animal 51 shed *S. typhimurium* on day 2 and 14, but not on days 4, 7, 10, 21, and 28). This suggests the possibility of some degree of cross-infection. The rate of decrease in shedding was significantly more rapid in the oxytetracycline group than in the nonmedicated controls. This difference is statistically significant, having a value of $P < 0.001$.

Although drug-resistant *S. typhimurium* were detected in the fecal sample of medicated animals (21 resistant colonies of a total of 32 recovered from the medicated group), they were isolated only sporadically, and never on more than two consecutive test periods. The resistance developed by *S. typhimurium* was, with but one exception (ampicillin), limited to both oxytetracycline and streptomycin. The ampicillin resistance was detected only on day 2 in animal 17; one colony of the two recovered from this animal on that day was shown to be

resistant to ampicillin only. On day 10, one of the two colonies of *S. typhimurium* isolated from animal 78 was shown to be resistant to oxytetracycline and streptomycin. The resistant population then would be about 1.6×10^2 for that animal. All the other notations of resistance in Table 4 represent whole populations which were resistant to oxytetracycline and streptomycin (19/19 colonies isolated from the animals). There was no change in the susceptibility of the resistant *S. typhimurium* to the other antibacterial agents tested. Although there was development of resistance in the oxytetracycline-medicated animals, there was no increase in the quantity, prevalence, or shedding of *S. typhimurium* within this group. Since the animals within the group were allowed to interact with one another, there was a strong possibility for cross-infection to take place. Extensive cross-infection within the medicated group with resistant *S. typhimurium* was not observed.

DISCUSSION

A considerable amount of time was expended on attempting to colonize *Salmonella* in the intestine of the host species without killing the animals. Initial studies were done with *S. choleraesuis* in swine. An inoculum of 10^{11} cells/ml was introduced orally via stomach tube or in a small quantity of feed. This inoculum, however, proved to be unsatisfactory as it brought about an acute, sometimes fulminating infection in the young swine, without evidence of intestinal colonization. Lower numbers of *S. choleraesuis* failed to colonize or develop evidence of clinical disease.

Clinical signs of disease were apparent in swine and calves when using *S. typhimurium* at a concentration of approximately 10^{11} cells/ml. Temperature elevation and/or diarrhea of varying degree were evident in both medicated and nonmedicated groups. The viable counts of *S. typhimurium* obtained from all the swine and calves provided evidence that the organism successfully colonized the intestines. No clinical signs of disease were observed in the two groups of chickens, even though colonization by *S. typhimurium* was also successful utilizing an inoculum of $\sim 2.9 \times 10^{11}$ per chicken.

The challenges used in these model studies were intentionally much larger than would be found under practical field conditions. Animals with symptoms of disease, as observed in the swine and calf experiments, would normally be treated with larger doses of antibiotic (therapeutic rather than subtherapeutic drug levels).

The numbers of *S. typhimurium* in medicated swine, calves, and chickens did not significantly increase over those observed in the nonmedicated controls. On the contrary, the quantity of *S. typhimurium* isolated from the medicated calves and chickens was significantly less than that of their nonmedicated counterparts. The degree of prevalence and shedding of *S. typhimurium* was not increased in the medicated swine and calves; these factors were significantly decreased in the medicated chickens. If one assumes that an increase in the animal *Salmonella* reservoir is a hazard to man, these data suggest that the use of subtherapeutic levels of oxytetracycline actually reduce the potential hazards of *S. typhimurium* to human and animal health.

Except for chickens, there was no change in the susceptibility of *S. typhimurium* in the medicated animals. Walton (9) and later Smith (6) reported that in vivo transfer of resistance can be demonstrated by administering large numbers of donor organisms to young (1-day old) chickens for several days to insure a very high proportion of R-factor-containing *E. coli* in the microflora followed by oral inoculation of a large number of *S. typhimurium* cells. That resistant *S. typhimurium* were found in our studies is not particularly surprising, since the birds were started on medicated feed at 8 days of age and inoculated orally 5 days later with 300 billion cells of *S. typhimurium*. It is likely that a highly resistant enteric flora was established in these young birds, as opposed to the older animals used in the calf and swine experiment. Although resistant strains evolved in our medicated chicks, no massive increase in the quantity, prevalence, or shedding of *S. typhimurium* was observed. This result was striking, since selective antibiotic pressure, theoretically, should have favored an increase of the resistant strains. Extensive transfer from animal to animal (seeding) within the medicated group was not observed. These data indicate that the resistant *Salmonella* had no survival advantage over the susceptible strain.

Accepting that food animals are usually fed antibiotic for more than 28 days, we feel that the results would not have been significantly different had we extended the experimental period, since all treated and nontreated animals of all species demonstrated a general decline in shedding *Salmonella* as a function of time. The medicated animals included in these experiments were fed antibiotic throughout the studies although there is usually a withdrawal period prior to marketing. The inclusion of a withdrawal period conceivably

could have removed any differences observed between the treated and nonmedicated groups, particularly with regard to antibiotic resistance, because of the reduction of antibiotic selective pressure.

A significant increase in the reservoir of *Salmonella* in food animals is speculated to constitute an increased risk to human health. The results reported here indicate there was no significant increase in the quantity, prevalence, and shedding of *S. typhimurium* in the medicated group compared to nonmedicated inoculated controls. Although only one strain was employed, the data generally demonstrate that the subtherapeutic use of oxytetracycline actually decreases the quantity, prevalence, and shedding of *S. typhimurium* in animals. No evidence has been obtained which would associate the continuous low-level feeding of oxytetracycline with an increased incidence of salmonellosis in animals or humans.

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