Effect of the use of antimicrobials in food-producing animals on pathogen load: Systematic review of the published literature

Prepared for

Center for Veterinary Medicine
US Food and Drug Administration
Rockville, MD

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1. Introduction and Methods

In August 2000, the Center for Veterinary Medicine (CVM) of the US Food and Drug Administration (FDA) requested Exponent's assistance with their ongoing evaluation of the potential impact of antimicrobial drugs in food-producing animals on public health. Their recently proposed "Framework for evaluating and assuring the human safety of the microbial effects of antimicrobial new animal drugs" (CVM, 1999) outlined two separate but related factors, which require evaluation. These include: 1) the quantity of antimicrobial drug resistant enteric bacteria formed in the animal's intestinal tract following exposure to the antimicrobial new animal drug (resistance); and 2) changes in the number of enteric bacteria in the animal's intestinal tract that cause human illness (pathogen load) (CVM, 1999). The following document provides a review and analysis of the published literature regarding the second aspect of the proposed framework, i.e., the effect of the use of antimicrobials in food-producing animals on pathogen load.

It has been suggested that one of the benefits associated with the use of antibiotics in food-producing animals is a potential decrease in pathogen shedding (NRC 1999). However, since most antibiotics are only effective against certain types of bacteria (e.g., gram–positive cocci), this therapy may also disturb the normal intestinal microbial ecosystem resulting in an increase in the bacteria that can cause human infections (e.g., *Salmonella*) or prolonging the duration of the carrier state of such bacteria. Animals carrying increased amounts of pathogens (pathogen load) at the time of slaughter may present an increased risk for contamination of food and resulting human illness (CVM, 1999).

In order to obtain copies of relevant articles on this topic, a number of literature searches were conducted using a variety of approaches. A total of 33 literature databases were included in the search. These included medical databases (e.g., Medline, Toxline), agricultural databases (Agricola, Agris, etc), food literature databases (Foodline, Food

Adlibra), veterinarian and zoological databases (e.g., Zoological Record Online®) and general scientific databases. Search terms included “antibiotics AND pathogen AND food ”; “animals AND food AND pathogen load”; “food-producing animals AND antibiotics” and other combinations of keywords.

Copies of the relevant articles were obtained, including those from foreign language journals and books. The references of all relevant articles were reviewed to obtain additional publications missed by the electronic search. Each article was reviewed by one or more members of the research team. Pertinent data extracted from the studies included the following:

- Animal species under study
- Antibiotic in question
- Dose of antibiotic
- Study (experimental) design
- Bacterial species evaluated
- Results

Additional data included the numbers of subjects in each group, the statistical significance of individual study results, and study limitations.

It should be noted that the nomenclature for some bacterial classification has changed since the period in which some of these papers were published. The *Salmonella* isolates that are indicated in the following papers are various serovars of *Salmonella enterica*, primarily *Salmonella enterica var. typhimurium*, commonly referred to as *Salmonella typhimurium* throughout the rest of this review. The following sections of this report describe our findings, summarize each of the reviewed studies, and present the results of our analyses of the literature.

2. Studies of swine and calves

2.1 Challenge studies

In an early study, Evangelisti et al. (1975) studied the effects of subtherapeutic levels of oxytetracycline in the feed of swine, calves, and chickens. Results for chickens will be discussed in Section 3 on poultry. Swine and calves were fed feed that contained a suspension of *Salmonella typhimurium*, All animals, feed, and the environment were determined to be negative for *Salmonella* organisms before the experiment. Oxytetracycline was added to feed five days prior to inoculation and continued throughout the experiment (28 days). Swine and calves received 150 and 101.01 g/ton of oxytetracycline, respectively.

S. typhimurium colonized the gut of the swine in both test and control groups. Clinical signs of disease such as diarrhea and elevated temperature were similar in both groups. The quantity of *Salmonella* was not significantly different between groups. Although the medicated group exhibited a lower prevalence of shedding and a faster rate of decrease in shedding, the results were not statistically significant.

Calves became severely ill within the first week post-inoculation, but recovery was quick. The challenge of *S. typhimurium* was near the lethal dose. One animal in the control group died. The quantity of *Salmonella* was significantly less in the test group compared to the control group ($p<0.05$). The prevalence of *Salmonella* in the medicated group was lower and the rate of decrease in shedding was greater than the controls, but the differences were not statistically significant. The medicated group experienced less severe diarrhea and less-pronounced increases in temperature than the control group.

The challenges used were significantly larger than the challenges that would be found in normal situations, and animals would normally be provided therapeutic instead of subtherapeutic doses of antibiotics in the event of infection. In commercial situations,

antibiotics are withdrawn for a period of time before slaughter; however, in this case antibiotics were administered until slaughter. Withdrawal of antibiotics may have removed any differences observed between test and control groups.

The quantity, prevalence, and shedding rate of *S. typhimurium* in swine and calves did not significantly increase in groups medicated with oxytetracycline compared to nonmedicated groups. In fact, the quantity of *Salmonella* was significantly lower in medicated calves.

In a follow-up study by Girard et al. (1976), sub-therapeutic levels of oxytetracycline plus neomycin were fed to swine, calves, and chickens to determine the effect on quantity, prevalence, and shedding of *S. typhimurium*. As in the previous study, results for chickens will be discussed in Section 3. The same methods as the Evangelisti et al. (1975) study were used in this experiment. Swine were fed 150 g/ton of oxytetracycline and neomycin sulfate and calves were fed 94.9 g/ton of each antibiotic.

The combination of oxytetracycline and neomycin significantly reduced the quantity, prevalence, and degree of shedding of *Salmonella* in swine. In calves, decreases in quantity and prevalence were statistically significant. It appears that the combination of oxytetracycline and neomycin was more effective in reducing *Salmonella* counts than oxytetracycline alone.

Gutzmann et al. (1976) studied the effect of chlortetracycline in feed given to swine that were experimentally infected with *Salmonella typhimurium*. Fecal samples were examined to analyze the occurrence and duration of *Salmonella* excretion. Sixty swine were split into six groups: A) no antibiotic, no exposure; B) 220.5 g/ton of chlortetracycline, no exposure; C) 110.2 g/ton of chlortetracycline, 110.2 g/ton of sulfamethazine, and 55.1 g/ton of penicillin G (CP), no exposure; D) no antibiotic, exposure; E) 220.5 g/ton of chlortetracycline, exposure; and F) 110.2 g/ton of chlortetracycline, 110.2 g/metric ton of sulfamethazine, and 55.1 g/ton of penicillin G (CP), exposure.

All infected animals developed fever on the first day of exposure and experienced diarrhea of variable severity. Group E (medicated with a high dose of chlortetracycline) had only slight diarrhea compared to group F (low doses of antibiotics) and group D (controls). Swine were categorized as colonized if fecal samples provided more than 10^5 CFU/g of bacteria for two consecutive days post-exposure. There were no significant differences in the number of chlortetracycline-resistant *Salmonella* isolated from the medicated groups (B, C, E, and F). The number of *Salmonella* decreased with time for all groups whether or not they were medicated. There was no significant increase ($p>0.05$) in the number of *Salmonella* in groups E or F. The animals in group E (high dose chlortetracycline) excreted fewer *Salmonella* than group D (controls). This difference was only significant for day 9 after exposure ($p<0.05$).

In the non-exposed group of swine given chlortetracycline, sulfamethazine, and penicillin G, *Salmonella* was isolated from two pigs. Although the groups were separated from each other, it was not possible to prevent birds from frequenting the pig pens, which may have helped spread *Salmonella* from exposed to non-exposed swine.

Necropsy results showed that group D had lesions in the alimentary tract indicating that the swine had recovered from gastroenteritis, whereas groups E and F did not present any pathological changes. No deaths occurred in the unexposed groups, but 10 occurred in the exposed groups: three in group D (non-medicated group), two in group E (multiple low dose antibiotic group), and five in group F (chlortetracycline-supplemented group). The swine that died in groups E and F were, for the most part, negative for *Salmonella* in the liver and spleen, whereas 10^2 to 10^3 CFU/g of *Salmonella* were isolated from these organs in the non-medicated and the remaining CP-medicated swine. Lymph nodes were negative for *Salmonella* in 8 of 13 swine given chlortetracycline or CP diets. By the final sampling period, *Salmonella* was no longer detected in some of the swine. The authors concluded that the use of sub-therapeutic levels of antibiotics does not increase the amount of *Salmonella* and the duration of carriage. Use of chlortetracycline at 220.5 g/ton decreased the excretion rate of *Salmonella*.

DeGeeter et al. (1976) support the earlier studies. Thirty-one swine were fed either 110 mg/kg of lincomycin or a control diet and inoculated with a nalidixic acid-resistant strain of *S. typhimurium*. The swine were followed for 56 days and no significant difference was observed in treated vs. untreated swine in the quantity, duration, and prevalence of shedding.

In a similar study by Williams et al. (1978), swine were experimentally infected with a CTC-resistant (Experiment 1) or a CTC-sensitive strain (Experiment 2) of *S. typhimurium*. Twenty-nine pigs were used for the first experiment and 32 were used for the second. Swine were fed a diet containing 110 mg/kg of chlortetracycline or a control diet from five days before oral inoculation with *S. typhimurium* until the end of the experiment (51 or 52 days post infection for Experiment 1, or 66 days after post infection for Experiment 2). Fecal samples were taken on multiple days until the end of the experiment at which point the animals were killed and liver, spleen, mesenteric lymph node, and colon content samples were examined.

One of the three samples of control feed indicated 4.2 mg/kg of CTC activity, and two of four control feed samples in Experiment 1 indicated 0.69 mg/kg of penicillin activity. The feed did not contain *Salmonella* organisms.

Clinical signs in animals that were inoculated with *S. typhimurium* included diarrhea, decreased food consumption, and depression. The test group experienced more diarrhea than the control group in Experiment 1 and less diarrhea in Experiment 2. Three animals died in the control group and two animals died in the test group in Experiment 2. For animals inoculated with the CTC-resistant *S. typhimurium*, a significantly greater quantity of organisms was shed from the test animals than controls ($p<0.05$). For animals inoculated with CTC-sensitive *S. typhimurium*, the opposite result was observed: the control group had significantly longer shedding times ($p<0.05$).

CTC enhanced the spread of *Salmonella* in test groups inoculated with resistant organisms, but CTC decreased the spread in test groups inoculated with sensitive strains. More colon contents were positive for *S. typhimurium* in test animals given resistant strains, whereas less colon contents were positive in test animals given sensitive strains, when compared to controls.

The authors concluded that feeding CTC to swine that have been infected with CTC-resistant S. typhimurium increases the quantity, duration, and prevalence of fecal shedding, whereas feeding CTC to swine inoculated with CTC-sensitive S. typhimurium decreases shedding.

Wilcock and Olander (1978) evaluated 85 weanling pigs allotted to seven test groups and two control groups. Animals received an oral challenge with *S. typhimurium* and antibiotics according to the following protocols:

Group 1	Fasted, inoculated, continuous feed medication (110 g neomycin and 110 g oxytetracycline/ton of feed) plus additional 440 g of oxytetracycline/ton of feed during diarrhea;
Group 2	Fasted, inoculated, continuous feed medication (same as above) plus additional 100 mg of nitrofurazone/liter of drinking water feed during diarrhea;
Group 3	Fasted, inoculated, continuous feed medication only;
Group 4	Fasted, inoculated, continuous feed medication only;
Group 5	Not fasted, inoculated, no medication (control group I);
Group 6	Not fasted, inoculated, no medication, electrolyte solution, feedings withheld during diarrhea;
Group 7	Not fasted, inoculated, feed medication and nitrofurazone during diarrhea;
Group 8	Not fasted, inoculated, continuous feed medication plus additional 100 mg of nitrofurazone/liter of drinking water feed during diarrhea; and
Group 9	Not fasted, not inoculated, no medication (control group II).

Each day a composite sample of feces from each pen was cultured for *Salmonella* spp. Forty-five pigs were killed 105 or 120 days after inoculation. At the time of slaughter, approximately 10 g of liver, gallbladder, ileal-mesenteric tissue, and the tip of the cecum were cultured for *Salmonella*. Most of the inoculated pigs shed *Salmonella*e for at least 120 days, regardless of the treatment regimen they received. Among the 45 pigs killed 105 or 120 days after inoculation, 39 had *S. typhimurium*. The authors concluded that antibiotics might play a role in the prevention but not in the treatment of salmonellosis.

Jacks et al.(1988) studied the influence of efrotomycin — an antibiotic produced from fermentations of *Nocardia lactamdurans* — on *S. typhimurium* inoculated pigs. The experimental animals received 16 mg of antibiotic per kg of feed. The control groups included inoculated animals that received no efrotomycin, medicated pigs that did not undergo *Salmonella* inoculation, and pigs that received neither the antibiotic nor the inoculation. The fecal sampling for *S. typhimurium* occurred on days 1, 2, 4, 6, 8, 10, 12, 14, 21, 28, 35, 42, 49, and 56. After 8 weeks (56 days), the pigs were slaughtered and tissue specimens were also tested for *S. typhimurium*.

The results indicated that the duration of shedding and the numbers of *Salmonella*e isolated from efrotomycin-medicated and non-medicated animals were similar. The authors concluded that efrotomycin did not alter the ecologic balance of the intestinal flora of swine in any way that would alter the colonization of *S. typhimurium.*

Ebner and Mathew (2000) inoculated forty-eight 50-day-old pigs with S. typhimurium. The experimental animals were given one of the following: 1) intramuscular injection with ceftiofur sodium followed by oxytetracycline supplementation in feed, 2) apramycin in feed for 14 days followed by oxytetracyline, 3) carbadox in feed followed by oxytetracycline once the pigs reached a weight of 35 kg, or 4) no antibiotics. Fecal samples were collected before inoculation and at different periods post-inoculation.

The percentage of pigs shedding *S. typhimurium* was highest at four or seven days post-inoculation for all four groups. Results at this time showed a higher number of *Salmonella* organisms but a lower percentage of resistant organisms. The authors noted that antibiotic-sensitive organisms might be replicating more quickly than antibiotic-resistant organisms. Pigs that received apramycin/oxytetracyline shed less than pigs that did not receive antibiotics. No differences were observed between groups that were given antibiotics. The authors concluded that the use of antibiotics may increase the percentages of antibiotic-resistant *S. typhimurium*, but does not result in increased shedding.

2.2 Studies not involving bacterial challenge (observational)

One of the earliest studies by Bridges, et al., (1952) looked at the effect of penicillin, streptomycin and a combination of penicillin and streptomycin on the number of bacteria in swine feces. Five groups of 10 pigs each were fed 227 mg/100 lb. feed of penicillin, 250 mg/ 100 lb. of feed of streptomycin, or a combination of both. Fecal samples were collected after a five-day pre-treatment period and periodically throughout the experiment.

Penicillin significantly increased total bacterial count and enterobacterial count, whereas streptomycin either alone or with penicillin did not significantly increase bacterial counts.

In a similar study by Bridges et al. (1953) the same antibiotics at the same concentration were used to determine the effect on the microflora of pigs. Four groups of three pigs were used in this experiment. Fecal samples were collected after a 7-day preliminary period, after which fecal samples were collected weekly until 9 samples for each pig were collected.

Penicillin increased the number of coliform bacteria, whereas streptomycin did not affect the number. In combination, penicillin and streptomycin increased the number of coliform bacteria by the same amount as penicillin alone. Neither of these antibiotics

alone or in combination affected the Staphylococcus *Staphylococcus* or *Shigella* genus. Penicillin or streptomycin alone increased the number of *Proteus* organisms, but the increase was not statistically significant. However, in combination, the antibiotics significantly increased the number of *Proteus* organisms (204 vs. 37 million per gram).

One possible reason for the increased fecal shedding of organisms in pigs that are fed penicillin, is that penicillin 1) may retard growth of organisms that compete against coliform bacteria for nutrients, 2) may reduce bacteria that is antagonistic against coliform bacteria, or 3) may alter the intestinal flora thus changing the oxidation and reduction state of the intestinal contents.

Both Bridges et al. studies (1952, 1953) concluded that penicillin increases fecal shedding. However, only the second study found that streptomycin either alone or in combination with penicillin increases fecal shedding.

Fuller et al. (1960) conducted an experimental study comparing pigs that received 10 g/ton of penicillin with those that received 3 lb/ton of Aurofac (which contains 3.6 g/lb. of chlortetracycline) and those left untreated. Fecal samples from the experimental animals were obtained at weekly intervals over a 110-day period and tested for streptococci, lactobacilli, and coliform organisms.

The results indicated that the numbers of bacteria among controls ranged between 10^5 and 10^9 for streptococci, 10^5-10^9 for lactobacilli and 10^4-10^7 for coliform organisms. The results in the chlortetracycline group did not differ markedly, while pigs that received penicillin had a transient reduction in the numbers of streptococc*i* but showed no difference in the numbers of lactobacilli and coliforms. The qualitative assessment of isolates indicated that the types of organisms were virtually the same in all study groups.

Woods et al. (1972) added antibiotics to feed to determine the effects on pathogens in the nasal passages. Three trials were completed. Trial 1 consisted of two groups of 32 pigs weighing an average of 48 pounds and 73 pounds, respectively. In trial 2, 72 pigs were

used averaging 27 pounds and, in trial 3, 170 nursing pigs averaging 11 pounds were used. Antibiotics were added to feed that was provided to swine for three weeks to determine its effects on pathogen load in the nasal tract. Swine were provided four different types of diets: 1) no antibiotic; 2) basic antibiotic consisting of 10 mg/lb chlortetracycline in the first trial and 8 mg/lb oxytetracycline plus 2 mg/lb oleandomycin in the second trial; 3) 50 mg chlortetracycline, 50 mg sulfamethazine, and 25 mg procaine penicillin; and 4) 100 mg/lb chlortetracycline.

Bacteriological sampling of the respiratory tract using nasal swabs isolated the following potential pathogens: *Bordetella bronchiseptica, Hemophilus suis, Pasteurella multicoda*, *Streptococcus equisimilis*, and *Mycoplasma* spp. Although the results are not sufficiently described, the study indicated that the addition of antibiotics apparently decreased colonization of nasal passages compared to the regular diet without antibiotics.

3. Studies of poultry

3.1 Challenge studies

A number of studies were published over the years by the Houghton Laboratory poultry research group in the United Kingdom. In the earliest study, Smith and Tucker (1975a) fed continuous diets containing 10 or 100 mg/kg of different antibiotics to forty-five chickens infected orally with a nalidixic acid-resistant strain of *S. typhimurium*. The amount of *S. typhimurium* isolated from feces was compared between the groups. The antibiotics under study included virginiamycin, bacitracin, flavomycin, nitrovin, tylosin, and sulphaquinoxaline.

The recovery frequency of *S. typhimurium* from chickens fed virginiamycin, bacitracin, flavomycin, tylosin, or 10 mg/kg of nitrovin was similar or greater than that from chickens that did not receive antibiotics. The recovery percentage of the group fed 100 mg/kg of nitrovin was much higher. In repeated experiments, excretion rates of chickens fed 10 and 100 mg/kg of virginiamycin and bacitracin were slightly higher than those of the control group. The amount and duration of excretion in the groups fed flavomycin, tylosin, and nitrovin was much greater than that of the control group.

Concentrations of nitrovin, tylosin, and flavomycin that caused the greatest increases in recovery frequencies of *S. typhimurium* were studied further. The rate, amount, and duration of *S. typhimurium* excretion in nitrovin-supplemented chickens were much higher than in control groups. From Day 26 to Day 54, the percentage of chickens that excreted greater than 50 colonies on a plate ranged from 16 to 79%, whereas for the same time period, the range for control chickens was 0 to 7%. The excretion patterns of chickens fed 10 mg/kg of flavomycin were similar to those of control chickens. Chickens fed 10 mg/kg of tylosin had higher concentrations of *S. typhimurium* on Days 19, 26, and 33 compared to controls. Each chicken was killed three weeks after no *S. typhimurium* was isolated from feces. The post-slaughter analyses revealed that *S. typhimurium* was

only isolated from the cecal contents of the nitrovin group and often at high concentrations. The rate and amount of *S. typhimurium* excreted by groups given 100 and 500 mg/kg of sulphaquinoxaline was much lower than the control groups. Even after changing to regular feed on Day 46, the amount of organisms continued to decrease in the group given 100 mg/kg. The 500 mg/kg group was not re-examined.

Another study by Smith and Tucker (1975b) used the same experimental design, but tested different antibiotics. These included ampicillin, chloramphenicol, furazolidone, neomycin, oxytetracyline, polymixin, spectinomycin, streptomycin, or a mixture of trimethoprim and sulphadiazine. Most of the antibiotics in this study were initially effective in reducing excretion amounts and rates of *S. typhimurium* and *E. coli.* However, for the most part, the effectiveness was short-lived due to the emergence of antibiotic-resistant strains of these bacteria.

A 1978 publication by Smith and Tucker reported on the results of a similar study. A total of 33 chickens infected orally with *S. typhimurium* were continuously fed diets containing the following antibiotics: 1) lincomycin and avoparcin (used for growth promotion); 2) amprolium and monensin (used for controlling coccidiosis); 3) dimetridazole (used for controlling histomoniasis); and 4) arsenicals (used for a number of reasons). These supplements were provided in 10 mg/kg and 100 mg/kg concentrations. Chickens were fed antibiotic supplements from the day of hatching and infected at 4 days of age with a nalidixic-acid resistant strain of *S. typhimurium*. In this experiment, avoparcin and lincomycin acted similarly to nitrovin and tylosin (Smith and Tucker, 1975b), in that they increased *S. typhimurium* concentrations and duration in chickens compared to controls. Both lincomycin and avoparcin in 10 mg/kg and 100 mg/kg resulted in greater excretion concentrations and durations. The 100 mg/kg dose showed the greatest effect. At slaughter, higher numbers of *S. typhimurium* were observed in the cecal contents of the antibiotic-supplemented chickens compared to controls. Removing avoparcin from the diet one week before slaughter had little effect. The feces and cecal contents of the groups fed avoparcin and lincomycin were still heavily infected. For a group that was fed a control feed for 21 days and then avoparcin-

supplemented feed for the remaining time, the excretion pattern was similar to control chickens in the first 21 days, but then the excretion amount and rate increased substantially until the pattern was similar to that of avoparcin-raised chickens. Amprolium, monensin, dimetridazole, arsenilic acid, and nitro-hydroxyphenylarsonate did not have much effect on infection. At 56 days after infection, very few chickens were excreting *S. typhimurium*.

Another experiment examined infections acquired by contact from five chickens that were inoculated in each study group. Avoparcin, lincomycin, nitrovin, tylosin, and dimetridazole increased *S. typhimurium* excretion amount and duration. *S. typhimurium* spread to more control chickens than chickens in the lincomycin group, but the infection was lighter. More chickens that were fed antibiotics that enhanced *S. typhimurium* infection continued to excrete organisms in their feces when they were killed at 56 days of age (the usual age of slaughter in commercial operations) compared to control chickens (Smith and Tucker 1978).

A similar experiment was published by Smith and Tucker in 1980. Three antibiotics that were previously studied (Smith and Tucker 1975b, 1978) were used at a single concentration: 1) bacitracin (10 mg/kg) which had been previously found to slightly promote *S. typhimurium* colonization; 2) avoparcin (10 mg/kg) which had been found to strongly promote colonization; and 3) sodium arsenilate (250 mg/kg) that had been found to inhibit colonization (Smith and Tucker, 1980). Both nalidixic acid-sensitive and resistant strains of *S. typhimurium* and nalidixic-acid resistant (nal^r) strains *of S. heidelberg*, *S. oranienburg*, *S. infantis*, and *S. senftenberg* were used to orally infect five of 33 chickens in every group at 4 days of age. None of the four diets contained antimicrobials. The antibiotic-supplemented diets were fed to the chickens continuously from the day of hatching.

Four *Salmonella* strains in addition to *S. typhimurium* were isolated more often and in higher concentrations from chickens fed avoparcin than in controls. The infections lasted much longer and a higher proportion of chickens remained heavily infected at 50 days

when the experiment ended. The infection spread more extensively among non-medicated chickens than avoparcin-supplemented chickens, but the infections were light and most did not persist until the end of the experiment. There was no difference in the rate and amount of excretion of the four *Salmonella* strains in bacitracin-supplemented chickens. The percentage of chickens that excreted *S. typhimurium* nal-sensitive strains was higher in avoparcin and bacitracin-supplemented chickens, but lower in arsenilate-supplemented chickens, compared to controls.

In earlier studies (Smith & Tucker, 1975b, 1978), certain antibiotic agents were observed to favor the colonization of *Salmonella* in the alimentary tract. A criticism was raised that only one strain, nal^r *S. typhimurium* was tested on only one type of chicken. In an additional experiment, multiple *Salmonella* organisms and types of chickens, as well as one type of turkey were tested, with similar results. Avoparcin promoted *Salmonella* colonization, sodium arsenilate usually reduced it, and bacitracin had little effect.

In the Evangelisti et al. (1975) and Girard et al. (1976) studies, chickens were inoculated through delivery of a slurry of *S. typhimurium* into the gullet. Chickens received 200 g/ton of oxytetracycline in the Evangelisti study and an additional 200 g/ton of neomycin was added in the Girard study. Chickens did not show clinical signs of infection. The quantity, prevalence, and shedding of *Salmonella* were significantly lower in medicated chickens for groups that received oxytetracycline alone or in combination with neomycin.

In a study by Jarolmen et al. (1976) sub-therapeutic levels of chlortetracycline (200 g/ton) were administered to chickens that had been orally inoculated with nalidixic acid-resistant *Salmonella* species. In Experiment A, S. enteritidis, S. infantis, or *S. typhimurium* was inoculated into seeder chicks that were then housed with contact birds to evaluate the spread of *Salmonella*. The objective of Experiment B was to determine the excretion patterns of *S. typhimurium* from chickens that were fed chlortetracycline. Fresh droppings were examined at regular intervals for 8 weeks.

Results from Experiment A indicate that recovery of *Salmonella* species from medicated seeder chicks was either similar or less than the recovery from non-medicated chicks. The percentage recovery from medicated contact chicks was much lower than the nonmedicated contact chicks. No *Salmonella* was recovered from the livers of seeder and contact chicks that were killed at the end of the experiment.

The amount of *S. typhimurium* that was recovered from droppings was much lower in medicated chickens than controls for the entire study; there was a 50-fold statistically significant reduction in the *Salmonella* count between 4 and 8 days post-inoculation in medicated chickens. At the end of Experiment B, the birds were killed. *Salmonella* was not recovered from the livers or spleens and the incidences of *Salmonella* from caecal contents were not statistically different between test and control chickens. Authors concluded that subtherapeutic use of chlortetracycline reduced the amount of *Salmonella* in droppings and reduced the spread of infection amongst chickens.

In a later study, the authors used a slightly different approach (Barrow et al. 1984). This experiment involved infecting chickens with naturally occurring *Salmonella* found in bone-meal or in a suspension of chicken feces. Many serotypes were isolated from the bone meal used to infect chickens: *S. seftenberg*, *S. Newport*, *S. derby*, *S. lexington*, *S. typhimurium*, *S. agona*, *S. anatum*, *S. schwarzengrund*, and *S. mbandaka.* Food containing bone meal and 0, 10, or 100 mg/kg of avoparcin was fed to the chickens. Direct swabs of the cloaca showed that infection of the chickens occurred quickly and increased in all groups until the bone meals were withdrawn. Avoparcin supplementation did not affect the rate of infection.

After bone-meal withdrawal, the levels of infection declined. The infection levels in groups that had received avoparcin were slower to decline than for groups that had received no antibiotic. There was a dose response relationship after bone meal was withdrawn between avoparcin and the percentage of chickens that shed *Salmonella* organisms; the percentage of infected chickens in the 100 mg/kg avoparcin-supplemented group was significantly higher than for those chickens in the 10 mg/kg group. Infection

rates in the 10 mg/kg avoparcin group were significantly higher than those of the non-supplemented group. The results of the study using suspension of feces containing *S. montevideo* demonstrated similar results.

In the most recently published study by the same group (Barrow, 1989), the authors inoculated chickens with nalidixic acid-resistant strains of *S. typhimurium, S. pullorum, S. cholerae-suis, S. dublin*, and *S. arizonae* and fed different concentrations of avoparcin in order to explain the variation in response to commercial levels of antibiotics.

In the first experiment, the birds were infected when they were four days old, but fed avoparcin-supplements from Day 1. The controls received regular feed. In one experimental group fed 0 to 40 mg/kg of avoparcin, the excretion rate showed a dose response association with increasing concentrations of avoparcin. In a second group of chickens fed concentrations of between 0 and 15 mg/kg, no significant increases in excretion rate were observed for those chickens fed 5 mg/kg of avoparcin, but statistically significant differences in excretion and caeca occurred at 7.5 mg/kg between 28 and 42 days. The increase produced by 10 mg/kg at 49 days was also significant, but any further increases in avoparcin concentration did not increase fecal excretion. In a third group, also fed 0 to 15 mg/kg of avoparcin, small increases occurred at higher concentrations (10 and 12.5 mg/kg) and at later stages in the experiment (Day 49 and Day 42, respectively). Avoparcin supplementation increased the excretion rates of *Salmonella* strains that were not usually associated with food-poisoning in the United Kingdom (*S. chlolerae-suis, S. dublin*, and *S. arizonae*). *S. pullorum* was excreted in low numbers for both avoparcin-supplemented and non-supplemented chickens.

In the second experiment, 15 chickens were fed regular feed for three weeks and then divided into three groups. The three groups were fed avoparcin at 10 mg/kg, avoparcin at 100 mg/kg, and no avoparcin, respectively. The chickens were infected with *S. typhimurium* on the same day as avoparcin supplementation began and were killed five days later to prevent development of any resistance to the antibiotic. Contents from various parts of the alimentary tract were analyzed for bacteria. No *Salmonella* bacteria

were isolated from the chickens with regular feed. Small numbers ($\log_{10}$ 2.6) of *Salmonella* were observed from the caeca and cloacae of chickens fed 10 mg/kg of avoparcin and much higher numbers ($\log_{10}$ 7.7) were isolated from chickens fed 100 mg/kg of avoparcin. *E. coli* was not affected at 10 mg/kg, but 100 mg/kg increased *E. coli* counts by at least one log in most parts of the gut compared to control chickens. Enterococcal counts were similar at 10 mg/kg to control counts, but 100 mg/kg of antibiotic eliminated streptococci from the gut with the exception of the crop. Lactobacilli counts were unaffected by either concentration of avoparcin. Anaerobes were not affected by the 10 mg/kg concentration and were significantly reduced at 100 mg/kg

It is important to note that the results published by other groups do not always agree with the Barrow, Smith, and Tucker conclusions that antibiotics in animal feed cause an increase in pathogen colonization and shedding. For example, Benazet et al. compared fecal excretion of bacteria in chicks treated with the antibiotic nosiheptide (20 g/ton of feed) to that in chicks that did not receive the treatment (Benazet et al. 1980). Nosiheptide is an antibiotic produced by *Streptomyces actuosus* belonging to the thiostrepton group. This compound is exclusively effective on gram-positive bacteria and is practically unabsorbed by the digestive tract.

In the first experiment, two groups of ten 15-day old chicks received inoculation with *S. typhimurium*. The number of *Salmonella*e per gram of feces was evaluated at 2-day intervals during the first two weeks and then on a weekly basis through the fourth week post-inoculation. The results indicated statistically significant excess in the numbers of *Salmonella* in the feces of treated birds compared to the numbers of *Salmonella* in the feces of untreated birds only on day 2 post-inoculation. However, subsequent samples and the overall results showed no appreciable differences.

In the second part of the study, the authors compared the numbers of fecal coliforms, particularly *E. coli,* in the other two groups of chicks. One group (controls) received a routine ("basal") diet without antibiotics throughout the study. The other group

(exposed) received a basal diet during the first 15 days and then the nosiheptide-supplemented feed for the following 33 days. The excretion of coliform bacteria was measured in both groups before and after the introduction of nosiheptide. The results showed no appreciable difference in the excretion of coliforms between the two groups and the authors indicated that the numbers of coliform bacteria per gram of feces were within "normal limits." However, they provided no information as to what is considered a normal range.

Gustafson et al. (1981) evaluated the influence of avoparcin and virginiamycin in chicks challenged with relatively low levels of *S. typhimurium.* All experimental animals received feed supplemented with 10 mg/kg of either avoparcin or virginiamycin. In addition, all chickens also received 100 mg/kg of a coccidiostat monensin. The study involved three experiments. The first experiment involved inoculation of 300 chickens with 9.5×10^5 CFU of *S. typhimurium* at four days of age. Analysis compared cloacal swabs (first every three days, then weekly) of 150 chicks treated with avoparcin to those of 150 controls. On day 56, birds were sacrificed and their ceca were examined for *Salmonella*.

In the second experiment, chicks were divided into three treatment groups: 100 chicks received avoparcin, 100 chicks received virginiamycin, and 100 chicks served as controls. The *Salmonella* challenge was administered in the drinking water on days 4, 7, 11, and 14. Cloacal swabs were obtained at 5, 12, 19, 26, 33, and 40 days. In addition, cloacal and cecal samples were obtained on the day of slaughter. In the third experiment, the addition of avoparcin and *Salmonella* inoculation occurred on day 16 and day 21, respectively. Fecal sampling was performed on days 2, 7, 14, 21, and 28. All surviving birds were slaughtered at 49 days of age, 28 days after challenge with *Salmonella*.

The results of these experiments indicated the following. In the first experiment, the proportion of cultures positive for *Salmonella* was approximately 50% in both groups and declined steadily during the first three weeks, more rapidly for the controls than for the avoparcin group. In the second experiment, both the virginiamycin and avoparcin groups

demonstrated higher prevalences of positive cultures initially. However, by day 30, the percentage of positive cultures in both experimental groups fell sharply to levels that were lower than those of controls. The third experiment showed that among chickens inoculated at older age (23rd day of life) the avoparcin group had a lower prevalence of positive *Salmonella* cultures and initially demonstrated lower numbers of *Salmonella* per gram of feces. However, by day 28 of the experiment, the levels of *Salmonella* in both groups were virtually the same.

Abou Youssef et al. (1982) compared the duration and persistence of S. typhimurium infections in broilers fed virginiamycin-supplemented feed and in broilers that were fed regular feed. Fifty broiler chicks were split into four groups: A) infected control group; B) infected group fed a 10% greater amount of virginiamycin than the highest recommended feed additive level; C) non-infected control group housed with group A to determine the extent of the spread of *Salmonella* spp. between cages; and D) non-infected group housed with group B to determine the extent of *Salmonella* spp. spread. Groups A and B were inoculated with *S. typhimurium*.

A direct count of *Salmonella* from fecal samples could not be made for all samples because *Salmonella*e were undetected quantitatively and could only be detected qualitatively using an enrichment culture. Instead, the number of birds not excreting *Salmonella* was determined. Differences between test and control results were not statistically significant. Exposure to virginiamycin did not affect the response associated with induced infection by *S. typhimurium*. No *Salmonella*e were observed in the feces of groups C and D, indicating that there was no transfer of *Salmonella* spp. from cage to cage. No *Salmonella*e were detected in feces prior to inoculation. Salmonellae could not be isolated from the organs of chicks. There was no apparent effect of virginiamycin on *Salmonella* infection.

Holmberg et al. (1984) examined the impact of two medications, avoparcin (10 mg/kg of feed) and a coccidiostat monensin (90 mg/kg of feed), on the occurrence of *Salmonella infantis* in the cecum and liver of experimentally infected chickens. The study involved

two experiments. In the first experiment, all chickens received either avoparcin alone or an avoparcin-monensin combination. In the second experiment, all chickens were divided onto four treatment groups: (1) avoparcin alone; (2) monensin alone; (3) avoparcin-monensin combination; and (4) no feed additives. After 7 days of treatment some birds received a *Salmonella* inoculation and some did not (controls). In both experiments randomly selected chickens from the inoculation groups and randomly selected controls were culled and their livers and ceca examined for *Salmonella*.

The results of both experiments showed that chickens that received an avoparcin-monensin combination showed a higher frequency of *Salmonella* positive livers and ceca than chickens that received avoparcin alone. However, a comparison between chickens that received avoparcin and those that received no medications (2^{nd} experiment) showed that chickens in the avoparcin group had a statistically significant decrease in *Salmonella* isolates ($p<0.05$).

Hinton (1988) conducted a study to determine the effect of adding avilamycin at 2.5 ppm or 10 ppm on the excretion rates of young broiler chickens that were challenged with *Salmonella kedougou*. In experiment 1, five groups of 10 birds were provided with five different diets containing *S. kedougou* contaminated feed. The five diets included: 1) no antibiotic; 2) 2.5 ppm avilamycin and no monensin; 3) 2.5 ppm avilamycin and 100 ppm monensin; 4) 10 ppm avilamycin and no monensin; and 5) 10 ppm avilamycin and 100 ppm monensin. A nalidixic acid resistant strain of *S. kedougou* was used because this organism causes infections in commercially-reared chickens and food poisoning in humans in the U.K. In the second experiment, comparable groups were provided with uncontaminated diets for the first week, followed by a similar diet to experiment 1 in the second and third weeks. The dose was higher for chickens in the second experiment because young chickens are less susceptible after the first few weeks of life to infection. Chickens were fed contaminated feed for two weeks in both experiments. Fecal swabs were sampled at the end of each week, and cecal contents were sampled at the end of the second week.

No *Salmonella* species were isolated from the uncontaminated feed. In experiment 1, a higher percentage of control birds excreted *S. kedougou* compared to birds that were fed avilamycin. In experiment 2, no difference was seen in *Salmonella* carriage for birds provided both avilamycin and monensin in feed. There were no significant differences between groups either in the proportion of birds that carried *Salmonella* nor the numbers of *S. kedougou* in the ceca.

Bolder et al. (1999) evaluated the effects of flavophospholipol (FPL; Flavomycin ®, bambermycins) and salinomycin sodium (SAL; Sacox ®) in feed additives on the shedding of *Salmonella enteritidis, Campylobacter jejuni, and Clostridium perfringens.* The design of the study involved a 3x3 matrix, where 216 newly hatched broiler chicks were split into three feed groups: nonmedicated, FPL (9 mg/kg), and SAL (60 mg/kg). These concentrations are within the range of commercially recommended levels in the European Union. These three groups were further divided into 3 groups that were inoculated by gavage with one of the following: *Salmonella enteritidis, Campylobacter jejuni, or Clostridium perfringens*. Fecal samples were collected weekly to determine the presence and amount of challenge organisms. The trial was conducted for 6 weeks, after which broilers were killed.

Data from 21 broilers per group were used for statistical analyses after exclusion of birds that had died or had abnormalities. Both the number of chickens shedding a detectable level of organisms and the $\log_{10}$ concentration of organisms were measured. The feed and facilities were confirmed to be free of the challenge microorganisms prior to the study. A dose of 10^8 CFU/ml was used for inoculation on Days 2 and 3 for *Clostridium* and Days 11 and 12 for *Salmonella* and *Campylobacter*.

Mean fecal counts of FPL and SAL-supplemented chickens were lower than the control chickens at 6 weeks. Fecal counts prior to 6 weeks were not significantly different from controls. The number of *Salmonella* shedders in the FPL group was lower ($p<0.05$). There were no significant differences between medicated and control groups for chickens inoculated with *Campylobacter*. The fecal counts and the number of shedding chickens

of the FPL group were significantly lower than the control group for chickens challenged with *Clostridium perfringens*. The SAL group did not differ significantly from controls.

The authors concluded that FPL in feed significantly reduced the shedding rates of *Salmonella* and *Clostridium* in chickens compared to control groups. SAL also reduced shedding rates of *Salmonella*, although not as substantially; it did not affect the shedding rates of *Clostridium*. There were no significant effects of FPL or SAL on the shedding rates of *Campylobacter*.

The purpose of the study by Seo et al. (2000) was to examine the effect of antibiotic treatment and competitive exclusion (CE) in chickens on *Salmonella enteritidis* shedding following molting and after a 14-day feed withdrawal. *Salmonella enteritidis* is common in human foodborne illness. Eggs are a common source of this organism. Forced molting is a management practice to stimulate egg production and improved egg quality. It involves feed removal and periods of light and dark, but has been known to increase *Salmonella enteritidis* infection in hens.

Fifty-six chickens were split into two groups: molted (48 birds) and unmolted (8 birds). Feed was removed for 14 days from the molted group and on day 4, both groups were inoculated with rifampicin-resistant *Salmonella enteritidis* (approximately 10^7 cell/ml) by gavage. After feeding was resumed, the molted group was split into two sub-groups; one was administered 10 mg/kg of enrofloxacin in water for 10 days and another group acted as controls. Once the test sub-group completed its enrofloxacin treatment, normal avian gut flora (NAGF) was administered. NAGF was used as a competitive exclusion culture because it has been shown to be effective in reducing *Salmonella* infection in broilers and layers, particularly in newly hatched chicks.

Molting increased shedding of *Salmonella enteritidis* compared to unmolted chickens. Shedding was greatly reduced in the birds that were treated with enrofloxacin and CE compared to untreated birds. Only 4% of the treated birds shed the organism compared to 33% of the untreated group after four days of treatment in trial 1. After six days, no

shedding occurred in the treated group compared to 25% among the untreated group. In trial 2, similar results were observed. Birds that only received NAGF were not protected against *Salmonella* infection. This study evaluated the combined effect of antibiotic and CE treatment of *Salmonella*-infected chickens. Although NAGF alone was evaluated, antibiotic treatment alone was not addressed.

3.2 Studies not involving bacterial challenge (observational)

Smith and Green (1980) conducted an experiment to evaluate the effects of avoparcin and virginiamycin given to turkeys on incidence of naturally occurring *Salmonella* shedding. Three groups were fed the following diets: no growth-promoter, 20 ppm avoparcin, or 20 ppm virginiamycin. Turkeys were followed from one day to 84 days old. There were no significant differences between the avoparcin, virginiamycin, and control groups in respect to *Salmonella* incidence. However, the study has a substantial limitation. Due to higher than usual early mortality, all birds (except those that were sacrificed for the initial caecal examinations) were also treated with chloramphenicol and chlortetracycline. Thus, the value of this publication in determining the effect of avoparcin and virginiamycin is limited

Mamber, and Kaltz (1985) examined the feces of broiler chickens receiving antimicrobials in feed for total and anti-microbial resistant bacteria. The purpose of this experiment was to determine the levels of the most common aerobic and facultative anaerobic gram-negative enteric bacteria and antimicrobial-resistant strains in the feces of chickens. The study compared five groups of broiler chickens that received antimicrobials (50 g of bacitracin, erythromycin, penicillin, streptomycin, or oxytetracycline per ton of feed) to controls fed an unsupplemented basal ration. Microbiological analyses involved fecal cultures aimed to detect *E. coli, Proteus*, *Klebsiella*, *Enterobacter, Salmonella*, and *Pseudomonas* species. *E. coli* was the most dominant of the enteric bacilli. Antimicrobial feed did not affect levels of either antimicrobial-sensitive or antimicrobial-resistant gram-negative enteric bacilli in chicken feces. The level of resistant bacteria was directly related to the total number of bacteria.

P. mirabilis was the only organism to be significantly reduced in samples from the groups that were fed antimicrobial-supplemented feed. However, penicillin in feed appeared to cause proliferation of *K. pneumoniae* in the intestinal tracts. Based on these results, the authors concluded that the levels of antimicrobial-sensitive and resistant strains of aerobic and facultative anaerobic gram-negative bacilli in the intestinal tracts of chickens did not appear to be affected much by antimicrobial supplementation in feed. The levels appeared to be more closely linked to their presence in the environment. These organisms, whether antimicrobial-sensitive or not, are the first to colonize the intestines, becoming part of the normal flora. Therefore, the restriction of antimicrobial supplementation may not affect the levels of these bacteria in animal intestinal tracts.

Hinton et al. (1986) fed the same diet with avoparcin (10 mg/kg), nitrovin (10 mg/kg), or virginiamycin (20 mg/kg) to chickens. The fourth group of chickens received no antibiotic. *Salmonella bredeney* and *S. montevideo* were isolated from birds in all groups and the isolation rate did not differ between groups. Comparisons between each antibiotic and control group were not reported.

In a separate experiment, birds that were fed a diet containing procaine penicillin (20 mg/kg) were compared to birds fed a non-medicated diet. There was a significant increase in the shedding rate of birds fed penicillin compared to controls ($p=0.036$). *Salmonella* incidence in the crop, gizzard, and cecum contents was significantly higher among birds receiving penicillin than among control birds ($p<0.05$).

4. Discussion and Conclusions

The present review included 29 publications addressing the issue of pathogen load in relation to antibiotic use in food-producing animals. With respect to experimental design, all studies fall into two distinct categories: those that involved inoculation of animals with bacteria and those involving monitoring of bacterial shedding. All inoculation studies used *Salmonella enterica* serotypes — most commonly, *S. enterica var.* typhimurium. Only one study (Bolder et al. 1999) used other genera of pathogens in addition to *Salmonella*. The majority of studies used poultry, mostly chickens. Seven studies used pigs and two studies used calves. There were no relevant studies using other species of food-producing animals (Figure).

All challenge studies involving swine used *S. typhimurium* as the inoculum. The antibiotics under study included chlor- and oxytetracycline, apramycin, neomycin, penicillin, ceftiofur, and carbadox. None of the studies demonstrated any evidence of antibiotics increasing the pathogen load (Table 1).

The results of the challenge studies involving poultry showed substantial disagreement (Table 2). All six studies from the Houghton Laboratory (Smith and Tucker 1975a, 1975b, 1978, 1980, Barrow et al. 1984, Barrow 1989) consistently showed that chickens that received antibiotics mixed with their feed had higher levels of *Salmonella* shedding. In addition, shedding in these birds was longer than in controls. These results were particularly strong for avoparcin.

In contrast to the findings by Barrow, Smith, and Tucker, Gustafson (1981) found that avoparcin increased *Salmonella* shedding only in groups that received a single inoculation early in life. Birds that received serial inoculations and birds that were inoculated late in life showed no adverse impact of avoparcin on pathogen load. Another study (Holmberg et al., 1984) also demonstrated no increase in Salmonella shedding in chickens receiving avoparcin alone. However, the same study showed that the combined

effect of avoparcin and coccidiostat monensin was associated with increased *Salmonella* shedding. The results of other studies, which examined the effects of virginiamycin (Abou Youssef et al., 1983), nosiheptide (Benazet et al. 1980), and flavophospholipol and salinomycin (Bolder, 1999), demonstrated no increase in pathogen load.

The results of observational studies generally found little evidence to support the hypothesis that antibiotics added to animal feed substantially affect pathogen load. The only exceptions are studies that used penicillin. For example, a 1986 study by Hinton et al. (1986) which indicated that use of penicillin in chickens at 20 mg/kg of feed was associated with an increase of Salmonella shedding, and two studies by Bridges et al. (1952, 1953), which indicated that penicillin and occasionally streptomycin increased fecal shedding of total and coliform bacteria. The evidence from other observational studies showed no impact of antibiotics (Table 3).

The reviewed body of literature is limited for several reasons. First, the potential bacterial pathogens that may transfer directly from domestic animals to humans comprise a large and diverse group (Table 4). Although *Salmonella* is one of the most important bacterial pathogens, the data for *Salmonella* may or may not provide sufficient information regarding the potential impact of antibiotics on pathogen load generally. Second, challenge studies are probably not representative of real life conditions. Even studies termed here as "observational" are not truly representative because they were conducted in settings that may not correspond to conditions on farms. Third, only two types of food-producing animals — swine and chickens — underwent sufficient study; two studies included calves and two other studies used turkeys. However, no information is available on adult cattle, lamb, or other species of food-producing animals. Fourth, the diet and or genetic line of animals may affect the pathogen load and may explain some of the variability of the data, particularly for poultry. Fifth, diets are different in Europe and the United States. US formulations consist of predominantly corn and soybean meal, whereas European formulations include two or more primary energy sources, often including corn in addition to wheat, barley, rye, and other cereal grains. Those grains add different amounts of hemicelluloses, arbabinose sugars, and glucans, etc., which may

impact the characteristics of the intestinal chyme and, thereby, affect the microflora or pathogens.

In summary, the review of the available published literature leads to the following conclusions:

1. The totality of the data from poultry studies indicates that the use of antibiotics in food-processing animals is generally not associated with increased pathogen load.
2. The data for *Salmonella* and avoparcin show substantial disagreement. It appears that the age at which *Salmonella* infection is acquired is an important prognostic factor with respect to effect of avoparcin on pathogen load.
3. Data from swine studies indicate no impact of antibiotic (other than penicillin) use on pathogen load.
4. A sufficient body of literature exists only for *Salmonella* spp. and is limited to swine and poultry.
5. Although the majority of studies indicate that antibiotics do not increase pathogen load, the concern remains that the use of antibiotics may contribute to the prevalence of antimicrobial resistance.

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Table 1. Effect of antibiotics on pathogen load in swine and calves: Summary of challenge studies

Publication	Animal Species	Challenge Species Used	Antibiotic Tested	Concentration of Antibiotic in Feed	Outcome Species Analyzed	Result (excretion of test animals vs. controls)
DeGeeter et al. (1976)	Pigs	*S. typhimurium*	Lincomycin	110 mg/kg	*S. typhimurium*	~
Ebner and Mathew (2000)	Pigs	*S. typhimurium*	1) Ceftiofur sodium/oxytetracycline	1) unknown, 100 g/ton, respectively	*S. typhimurium*	1) ~
			2) Apramycin/oxytetracycline	2) 150, 100 g/ton, respectively		2) <
			3) Carbadox/oxytetracycline	3) 50, 100 g/ton, respectively		3) ~
Evangelisti et al. (1975)	1) Pigs	*S. typhimurium*	Oxytetracycline	1) 150 g/ton of each	*S. typhimurium*	1) ~
	2) Calves			2) 101.01 g/ton of each		2) < (quantity), ~ (prevalence and rate of decrease of shedding)
Girard et al. (1976)	1) Pigs	*S. typhimurium*	A combination of oxytetracycline and neomycin	1) 150 g/ton of each	*S. typhimurium*	1) <
	2) Calves			2) 94.9 g/ton of each		2) < (quantity, prevalence), ~ (rate of decrease of shedding)
Gutzmann et al. (1976)	Pigs	*S. typhimurium*	1) Chlortetracycline	1) 220.5 g/metric ton	*S. typhimurium*	1) <
			2) A combination of chlortetracycline, sulfamethazine, penicillin G	2) 110.2, 110.2, 55.1 g/metric ton, respectively		2) ~
Jacks et al. (1988)	Pigs	*S. typhimurium*	Efrotomycin	16 mg/kg	*S. typhimurium*	~
Wilcock and Olander (1978)	Pigs	*S. typhimurium*	Neomycin, oxytetracycline, nitrofurazone	110, 110–440 g/ton, 100 mg/liter nitrofurazone, respectively	*S. typhimurium*	~
Williams et al. (1978)	Pigs	*S. typhimurium* (resistant and sensitive strains)	Chlortetracycline	110 mg/kg	*S. typhimurium* (resistant and sensitive strains)	> (resistant strain), < (senstive strain)

Note: < is less than
> is greater than
~ is similar to

Table 2. Effect of antibiotics on pathogen load in poultry: Summary of challenge studies

Publication	Challenge Species Used	Antibiotic Tested	Concentration of Antibiotic in Feed	Outcome Species Analyzed	Result (excretion of test animals vs. controls)
Abou Youssef et al. (1982)	*S. typhimurium*	Virginiamycin	25 g/ton	*S. typhimurium*	~
Barrow et al. (1989)	*S. typhimurium, S. pullorum, S. cholerae-suis, S. Dublin, S. arizonae*	Avoparcin	2.5–100 mg/kg	*S.. typhimurium, S. cholerae-suis, S. dublin, S. arizonae, S. pullorum, E. coli,* streptococci and obligate aerobes, lactobacilli	> (*Salmonella* species, *E. coli* at 100 mg/kg), < (streptococci and obligate aerobes at 100 mg/kg), ~ (lactobacilli)
Barrow et al. (1984)	From bone-meal: *S. seftenberg, S. newport, S. derby, S. lexington, S. typhimurium, S. agona, S. anatum, S. schwarzengrund, S. mbandaka.* From feces of health flock: *S. montevideo*	Avoparcin	10, 100 mg/kg	Salmonella organisms from bone-meal, *S. montevideo*	>
Benazet et al. (1979)	*S. typhimurium*	Nosiheptide	20 g/ton	*S. typhimurium, E. coli*	~ (*S. typhimurium, E.coli*)
Bolder et al. (1999)	*Salmonella enteritidis, Campylobacter jejuni, Clostridium perfringens*	1) Flavophospholipol	1) 9 mg/kg	*Salmonella enteritidis, Campylobacter jejuni, Clostridium perfringens*	1) < (*Salmonella* and *Clostridium*), ~ (*Campylobacter*)
		2) Salinomycin	2) 60 mg/kg		2) <(*Salmonella*), ~(*Clostridium* and *Campylobacter*)
Evangelisti et al. (1975)	*S. typhimurium*	Oxytetracycline	200 g/ton	*S. typhimurium*	<
Girard et al. (1976)	*S. typhimurium*	A combination of oxytetracycline and neomycin	200 g/ton of each	*S. typhimurium*	<
Gustafson et al. (1981)	*S. typhimurium*	Avoparcin or virginiamycin with monensin	10 mg/kg, with 100 mg/kg monensin	*S. typhimurium*	~
Hinton (1988)	*Salmonella kedougou*	Avilamycin with and without monensin	2.5 and 10 ppm avilamycin, 100 ppm monensin	*S. kedougou*	~
Holmberg et al. (1984)	*S. infantis*	1) Avoparcin	1) 10 mg/kg	*S. infantis*	1) <
		2) Monensin	2) 90 mg/kg		2) <
		3) Combination of avoparcin, monensin	3) 10, 90 mg/kg, respectively		3) >

Table 2. (cont.)

Publication	Challenge Species Used	Antibiotic Tested	Concentration of Antibiotic in Feed	Outcome Species Analyzed	Result (excretion of test animals vs. controls)
Jarolmen et al. (1976)	*S. enteritidis, S. infantis, S. typhimurium*	Chlortetracycline	200 g/ton	*S. enteritidis, S. infantis, S. typhimurium*	<
Seo et al. (2000)	*S. enteritidis*	1) Enrofloxacin and NAGF	10 mg/kg	*S. enteritidis*	<
		2) Normal Avian Gut Flora (NAGF)			~
Smith and Tucker (1975a)	*S. typhimurium*	1) Virginiamycin, bacitracin, flavomycin, tylosin	1) 10 and 100 mg/kg	*S. typhimurium*	1) ~ or >
		2) Nitrovin	2) 10 and 100 mg/kg		2) >
		3) Sulphaqunoxaline	3) 100 and 500 mg/kg		3) <
Smith and Tucker (1975b)	*S. typhimurium*	1) Neomycin, spectinomycin, streptomycin, polymixin, ampicillin, furazolidone, chloramphenicol, oxytetracycline	1) 100 or 500 mg/kg	*S. typhimurium*	1) < or ~
		2) Trimethoprim, sulphadiazine	2) 20–100 and 100–500 mg/kg, respectively		2) <
Smith and Tucker (1978)	*S. typhimurium*	1) Lincomycin, avoparcin	1) 10 and 100 mg/kg		1) >
		2) Ampolium, monensin, dimetridazole, arsenilic acid, nitro-hydroxyphenylarsonate	2) 125, 100, 150, 250, 446 mg/kg, respectively		2) ~
Smith and Tucker (1980)	*S. typhimurium* (nalidixic-acid resistant and sensitive), *S. heidelberg, S. oranienburg, S. infantis, S. senftenberg*	1) Avoparcin	1) 10 mg/kg	*S. typhimurium* (nalidixic-acid resistant and sensitive), *S. heidelberg, S. oranienburg, S. infantis, S. senftenberg*	1) >
		2) Bacitracin	2) 10 mg/kg		2) ~
		3) Sodium arsenilate	3) 250 mg/kg		3) <

Note: < is less than
> is greater than
~ is similar to

Table 3. Effect of antibiotics on pathogen load in poultry and swine: Summary of observational studies

Publication	Animals Species Used	Antibiotic Tested	Concentration of Antibiotic in Feed	Outcome Species Analyzed	Result (excretion of test animals vs. controls)
Bridges et al. (1952)	Pigs	1) Penicillin	1) 227 mg/100 lbs.	Total bacteria, enterobacteriacae	1) >
		2) Streptomycin	2) 250 mg/ 100 lbs.		2) ~
		3) Combination of penicillin and streptomycin	3) 227 and 250 mg/100 lbs, respectively		3) ~
Bridges et al. (1953)	Pigs	1) Penicillin	1) 227 mg/100 lbs.	Coliform bacteria, *Proteus, Shigella, Staphylococcus*	1) > (coliform), ~ (*Staphylococcus, Shigella, Proteus*)
		2) Streptomycin	2) 250 mg/ 100 lbs.		2) ~ (all organisms analyzed)
		3) Combination of penicillin and streptomycin	3) 227 and 250 mg/100 lbs, respectively		3) >(coliform, *Proteus*), ~ (*Shigella, Staphylococcus*)
Fuller et al. (1960)	Pigs	1) Penicillin	1) 10 g/ton	Streptococci, lactobacilli, coliforms	1) < (Streptococci), ~ (lactobacilli, coliforms)
		2) Aurofac (3.6 g/lb chlortetracycline)	2) 3 lb/ton		2) ~
Hinton et al. (1986)	Chickens (broiler)	1) monensin sodium	1) 100 mg/kg	*Salmonella* species	1) ~
		2) avoparcin, nitrovin, virginiamycin	2) 10 mg/kg to 20 mg/kg		2) No conclusion could be made
		3) penicillin	3) 20 mg/kg		3) >
		4) furazolidone	4) 150 mg/kg		4) ~
Mamber and Kaltz (1985)	Chickens (broiler)	Bacitracin, erythromycin, penicillin, streptomycin, or oxytetracycline	50 g/ton	*E. coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas* spp.	~
Smith and Green (1980)	Turkeys (commercial hybrid)	Avoparcin, virginiamycin	20 ppm	*Salmonella* species, identified *S. hadar*	~
Woods et al. (1972)	Pigs	Chlortetracycline, oleandomycin, sulfamethazine, procaine penicillin, oxytetracycline	10–100, 2, 50, 25, and 8 mg/lb, respectively	*Bordetella bronchiseptica, Hemophilus suis, Pasteurella multicoda, Streptococcus equisimilis,* and *Mycoplasma* spp.	~ or <

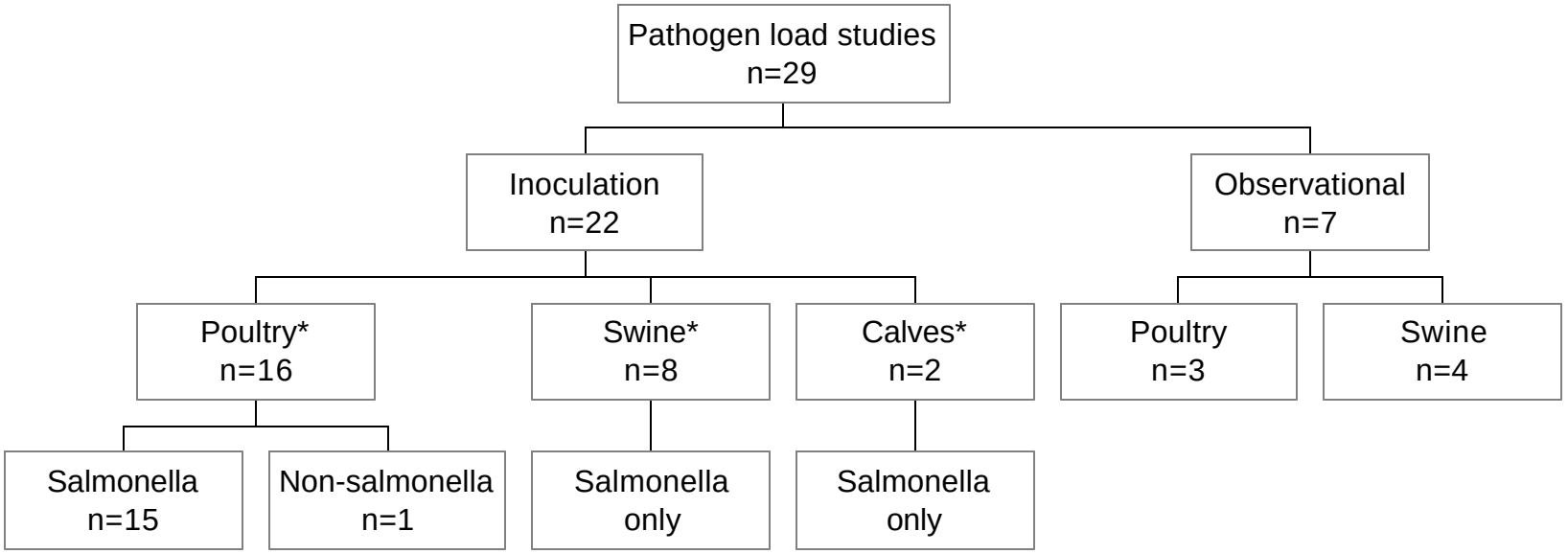
Note: < is less than
> is greater than
~ is similar to

Table 4. Bacterial pathogens in food-producing animals, and humans

Species	Pathogenicity		Transmission from Food Animals to Man
	Man	Food Animals	
Escherichia coli	+	+	+
Enteric disease-producing *E. coli*	+	+	+
Shigella sp.	+	+	+
Salmonella sp.	+	+	+
Pseudomonas sp.	+	+	
Klebsiella-Aerogenes group	+	+	
Yersinia enterocolitica	+	+	+
Yersinia psuedotuberculosis	+	+	+
Brucella sp.	+	+	+
Pasteurella multocida	+	+	+
Listeria	+	+	+
Erysipelothrix	+	+	+
Bacillus anthracis	+	+	+
Mycobacteria	+	+	+
Leptospira sp.	+	+	+
Staphylococcus aureus	+	+	+
Streptococcus agalactiae	+	+	
Bacillus anthracis	+	+	+
Clostridium perfringens	+	+	+
Chlamydia psittacii	+	+	+
Mycoplasma sp.	+	+	

Note: Adapted from Novick (1981).

Figure. Classification of studies on use of antibiotics and pathogen load in food-producing animals



***** Two studies Evangelisti (1975) and Girard (1976) conducted separate experiments on three species: chickens, swine and calves.