Efficacy of Neomycin Sulfate Water Medication on the Control of Mortality Associated with Colibacillosis in Growing Turkeys

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ABSTRACT The objective of this investigation was to evaluate the efficacy, safety, and toxicity of neomycin sulfate (Neomix® 325) water medication to control mortality associated with colibacillosis (*Escherichia coli*) in growing turkeys. One efficacy trial was conducted at five locations; each location included 2,880 sexed 21-d-old turkey poults that were naturally challenged with litter from turkey flocks that had colibacillosis. Between 5 and 7 d after challenge, and when mortality had reached 0.5%, poults were randomized within sex into three treatment groups of 0, 11, or 22 mg neomycin sulfate/kg body weight. In each location, each treatment was replicated 12 times with 40 poults per sex per replicate. All treatments were administered in the drinking water for 5 d. The pivotal decision criterion was mortality. Mortality was defined as 1) supported mortality (SM): positive microbial culture for *E. coli* and gross lesions, 2) diagnosed mortality (DM): diagnosed as associated with *E. coli* but not supported by lesions or positive microbiological cultures, 3) overall mortality (OM): mortality associated with *E. coli* or other microorganisms and miscellaneous reasons such as accidents (trampling or suffocations). Performance data (growth and feed utilization) also were measured and are reported without statistical analysis. Results from this efficacy study clearly demonstrated the effectiveness of neomycin sulfate against *E. coli* as measured by a reduction in mortality. In the target animal safety and toxicity study (done in conjunction with the efficacy study), neomycin sulfate in the drinking water at 66, 110, or 220 mg/kg per d for 15 d had no observable adverse effects on poult performance, as measured by feed or water consumption, body weight, gross pathology, or mortality.

(*Key words*: colibacillosis, *Escherichia coli*, turkeys, neomycin, clinical trial).)

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INTRODUCTION

Neomycin is the general term for a mixture of antibiotics obtained from cultures of *Streptomyces fradiae* (Waksman and Lechevalier, 1949). Two active components, which are isomeric, of the mixture have been identified as neomycin B and neomycin C. The neomycins belong to the aminoglycoside class of antibiotics, form salts with organic or inorganic acids, and are sold, usually as a sulfate salt, as neomycin sulfate. Neomycin is water soluble and is stable in animal feeds. Neomycin is also classed as broad spectrum as it is effective against Gram-positive and Gram-negative bacterial species (Waksman *et al.*, 1950).

Neomycin has been widely used in the animal industry (Kitchen and Waksman, 1955) since its introduction in 1949. Neomycin use continues in cattle, swine, and goats. Neomycin was removed from use in turkeys and chickens after a National Academy of Science/National Research Council Drug Efficacy Study Implementation Program review in 1971 reported a lack of controlled studies to support efficacy and safety in these species. The objectives of the studies reported herein were to evaluate the efficacy, safety, and tolerance of neomycin sulfate water medication to control mortality associated with colibacillosis in turkeys.

MATERIALS AND METHODS

Test Article

The drug evaluated was the commercial grade antibacterial neomycin sulfate NEOMIX® 325 (Lot No. OL.7818).[4] Neomycin was provided in 100-g packages in the form of a soluble powder.

Animals and Housing

In each trial location, sexed, day-old turkey poults were obtained from a commercial hatchery and were placed

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Abbreviation Key: DM = diagnosed mortality; OM = overall mortality; SM = supported mortality.

TABLE 1. Overall mortality[1]

Treatment	SM[2]		DM[3]		OM[4]	
	Male	Female	Male	Female	Male	Female
	(%)					
Control	3.88	2.48	4.64	3.73	6.60	6.03
11 mg/kg	1.45	1.29	2.04	2.49	3.68	3.88
22 mg/kg	1.16	1.20	1.81	1.96	3.28	3.95

[1]Total mortality = 627 poults.

[2]Supported mortality = mortality caused by colibacillosis (*Escherichia coli*) as supported by gross lesions and positive microbiological cultures.

[3]Diagnosed mortality = mortality diagnosed as associated with *E. coli* but not supported by either lesions or positive microbiological cultures.

[4]Overall mortality = overall mortality includes mortality associated with *E. coli* as well as other microorganisms and other reasons, such as trampling, overcrowding, and suffocation.

in sex-separated pens in a confinement house, on clean wood shavings, until 21 d of age. All had *ad libitum* access to feed and water during this period.

Experimental Design

Efficacy Study. At 21 d of age, the poults were naturally challenged by the addition of litter to the pens, which was obtained from turkey flocks known to have clinical colibacillosis confirmed by necropsy or cultures. Between 5 and 7 d after the litter challenge, and when the mortality rate had reached 0.5%, the poults were randomized within sex into 40 poult groups. Each group was weighed by sex and was assigned to one of three treatment groups of 0, 11, or 22 mg neomycin sulfate/kg body weight. In each trial, each treatment group was replicated 12 times with 40 poults per sex per replicate. All treatments were administered *via* the drinking water for 5 d, followed by 10 d posttrial observations. A total of 14,400 sexed 21-d-old turkey poults were utilized in the trial over the five different locations. These trial locations were contract poultry research facilities located in Virginia, Maryland, Georgia, and California.

Target Animal Safety. The purpose of this study was to determine the safety and toxicity of neomycin sulfate (formulated commercially as Neomix® 325 Soluble Powder) administered orally in the drinking water of 28-d-old turkey poults for 15 d at one of three concentrations to deliver doses of 66, 110, or 220 mg/kg per d (3×, 5×, and 10× the therapeutic dose of 22 mg/kg per d). Six hundred forty were wing-banded at 1 d old and were housed by separate sexes, equally divided into 20 pens for 14 d. On Day 14, each of the 20 pens were subdivided into blocks of four smaller pens (total of 80 pens). Within each block, six poults were assigned to each of the four pens using a random sequence of wing-band numbers generated using PROC PLAN of SAS® (SAS Institute, 1989). All poults that were considered unsuitable for the

TABLE 2. Summary of three calculated rates of mortality (%)

Trial	Treatment	SM[1]		DM[2]		OM[3]	
		Male	Female	Male	Female	Male	Female
A	Control	1.47	1.63	3.42	3.83	8.86	11.18
	11 mg/kg	1.04	1.81	1.67	4.55	7.14	8.99
	22 mg/kg	0.51	2.22	1.57	2.92	5.99	9.10
B	Control	7.52	3.96	7.50	3.96	7.71	3.96
	11 mg/kg	4.59	2.71	4.58	2.71	4.79	2.71
	22 mg/kg	3.36	2.29	3.33	2.29	3.75	2.29
C	Control	5.42	3.14	5.42	3.33	5.63	3.54
	11 mg/kg	1.46	1.68	1.46	1.67	1.46	2.08
	22 mg/kg	1.05	1.05	1.04	1.25	1.88	2.29
D	Control	5.03	3.14	5.00	3.13	5.42	3.75
	11 mg/kg	0.21	0.00	0.21	0.00	0.42	0.00
	22 mg/kg	0.00	0.00	0.00	0.21	0.21	0.21
E	Control	0.00	0.54	1.88	4.38	5.42	7.71
	11 mg/kg	0.00	0.24	2.29	3.54	4.58	5.63
	22 mg/kg	0.92	0.45	3.13	3.13	4.58	5.83

[1]Supported mortality = mortality caused by colibacillosis (*Escherichia coli*) and supported by gross lesions and positive microbiological cultures.

[2]Diagnosed mortality = mortality diagnosed as associated with *E. coli* but not supported by lesions or positive microbiological cultures.

[3]Overall mortality = overall mortality includes mortality associated with *E. coli* as well as mortality caused by other reasons, such as trampling, overcrowding, suffocation, and other microorganisms.

TABLE 3. Probability values for pairwise comparisons and least squares means for the DM[1] (%) group

	LSMean[2]	11 mg/kg	22 mg/kg
Control	4.18	0.006	0.002
11 mg/kg	2.26		0.560
22 mg/kg	1.90		

[1]Diagnosed mortality (%) = mortality diagnosed as associated with *Escherichia coli* but not supported by lesions and positive culture results.

[2]The means square (trial × treatment) was used as the error term.

study were eliminated from consideration prior to this randomization. The extra poults from each pen were placed in one of four holding pens and were used to replace any poults that died or were judged unsuitable prior to Day 1 of the start of the study. The poults were exposed continuously to treated water for 15 consecutive d, which was 3× the anticipated 5-d treatment therapy for colibacillosis in turkeys. The dose levels of 66, 110, and 220 mg/kg per d were 3×, 5×, and 10× the therapeutic dose of 22 mg/kg per d as required by the US Food and Drug Administration/Center for Veterinary Medicine (US FDA/CVM) guidelines for target animal safety-toxicity and tolerance studies. Thus, this experimental design met the guidelines (US FDA/CVM) for safety-toxicity studies.

During the study the poults were observed twice daily to determine mortality or any evidence of toxicity. Body weights were determined on Day 1 and every 5 d during dosing (Days 6 and 11) throughout the study and on Day 16 when the study was terminated. Feed consumption was also measured at Days 1 and 16.

Water consumption was measured daily during the week preceding dosing titration (Day 1) and during the 15 d of dosing. The water was weighed in and out for each day of the dosing period.

Mortality. Mortality was the decision criterion for the efficacy trials. All poults that died during the experimental period were necropsied, examined for lesions of colibacillosis in the pericardial and perihepatic regions, and cultured for bacteria identification.

Mortality Defined. Supported mortality (SM) was a positive microbial culture for *E. coli* and gross pathologic lesions and positive microbiological culture.

$$\frac{\text{(number died from } E.\ coli\text{)}}{\text{number in pens}}.$$

Diagnosed mortality (DM) was diagnosed as associated with *E. coli* infection but not necessarily supported by lesions or positive cultures.

$$\frac{\text{(number died assumed to be } E.\ coli\text{)}}{\text{number in pens}}.$$

Overall mortality (OM) was mortality associated with *E. coli* or other microorganisms and miscellaneous reasons

TABLE 4. Estimated average (n = 12 pens) gain and feed efficiency during the 15-d trial period

Trial	Sex	Dose	Gain in kg (% over control)	Feed efficiency (% over control)
A	M	0.0 mg/kg	20.03	1.90
		11.0 mg/kg	20.94 (4.54)	1.88 (1.05)
		22.0 mg/kg	20.28 (1.25)	1.92 (–1.05)
	F	0.0 mg/kg	16.79	2.06
		11.0 mg/kg	17.92 (6.73)	2.02 (1.94)
		22.0 mg/kg	18.02 (7.23)	1.97 (4.36)
B	M	0.0 mg/kg	25.12	1.96
		11.0 mg/kg	27.28 (8.60)	1.88 (4.08)
		22.0 mg/kg	28.06 (11.70)	1.85 (5.61)
	F	0.0 mg/kg	28.03	1.70
		11.0 mg/kg	28.49 (1.64)	1.65 (2.94)
		22.0 mg/kg	28.80 (2.75)	1.65 (2.94)
C	M	0.0 mg/kg	27.03	2.23
		11.0 mg/kg	28.58 (5.73)	2.13 (4.48)
		22.0 mg/kg	29.03 (7.40)	2.09 (6.28)
	F	0.0 mg/kg	25.17	2.33
		11.0 mg/kg	27.15 (7.87)	2.20 (5.58)
		22.0 mg/kg	26.39 (4.85)	2.24 (3.86)
D	M	0.0 mg/kg	31.31	1.63
		11.0 mg/kg	33.70 (7.63)	1.52 (6.75)
		22.0 mg/kg	34.54 (10.32)	1.49 (8.59)
	F	0.0 mg/kg	28.74	1.59
		11.0 mg/kg	30.25 (5.25)	1.53 (3.77)
		22.0 mg/kg	30.46 (5.98)	1.52 (4.40)
E	M	0.0 mg/kg	24.92	2.10
		11.0 mg/kg	25.47 (2.21)	2.08 (0.95)
		22.0 mg/kg	24.68 (–0.96)	2.13 (–1.43)
	F	0.0 mg/kg	24.77	1.99
		11.0 mg/kg	24.57 (–0.81)	2.03 (–2.01)
		22.0 mg/kg	24.94 (0.69)	2.00 (–0.50)

TABLE 5. Microorganisms cultured from the pericardium or liver of dead poults[1]

Microorganism	A	B	C	D	F	Total	Poults[2] (%)
Bacillus spp.	1	0	0	0	0	1	0.2
Escherichia coli	102	117	68	40	16	343	54.7
Providencia spp.	0	0	0	1	0	1	0.2
Proteus spp.	0	0	0	3	1	4	0.64
Salmonella spp.	0	0	0	0	14	14	2.23
Klebsiella spp.	0	0	0	0	1	1	0.2
Acinetobacter spp.	0	0	0	0	1	1	0.02
Serratia spp.	0	0	0	0	2	2	0.32

[1]Total number of poults that died = 627.

[2]More than one microorganism may have been cultured from a single poult.

such as accidents resulting from overcrowding (suffocation and trampling).

$$\frac{\text{(number died)}}{\text{number in pens}}.$$

Performance Data. Treatment group body weights by pen were measured at the start and conclusion of the trial. Feed consumption was determined by weighing the feed at the start and end of the trial.

Statistical Analysis

Analysis of Variance (ANOVA) was performed using the general linear model procedure of SAS® (SAS Institute, 1989) after testing homogeneity of variance by the Bartlett's (1937) test. All statements of statistical significance were based on $P \leq 0.05$.

The interaction between sex and treatment were tested first for significance. The least significant differences method was used for pairwise comparisons to determine whether the mortality of either group was significantly lower than that of the control.

RESULTS AND DISCUSSION

Poults in this study were naturally challenged with litter from commercial or research turkeys that had recently experienced colibacillosis. Poults raised on this type of litter will inhale large numbers of virulent *E. coli* daily (Harry, 1964). Thus, the natural route of infection (though not fully understood) is believed to be from the respiratory tract into the systemic circulation (Arp *et al.*, 1976). In the trial reported herein, the onset of colibacillosis (identified by gross observations) varied from a 3-d minimum to a maximum of 7 d after the poults were challenged at 21 d of age, a response similar to that reported by Newberry *et al.* (1993) and Leitner *et al.* (1992). The decision criterion (pivotal) was SM. Means for SM are reported in Table 1. The ANOVA showed a significant ($P \geq 0.05$) sex by treatment interaction; thus, the analysis of treatment effects was determined for males and females separately. There were significant treatment effects for males ($P = 0.0001$) and females ($P = 0.0001$). The percentage mortality for males was 3.88 (control) and with neomycin sulfate at 11 mg/kg and 22 mg/kg was 1.45 and 1.16%, respectively. These results represent a reduction in mortality of approximately 63 and 70% for the drug-medicated groups. The percentage mortality for females was 2.48 (control) and with neomycin sulfate at 11 and 22 mg/kg was 1.29 and 1.20%, respectively. The reduction in mortality for females was approximately 48 and 51%. No significant ($P > 0.05$) differences in percentage mortality were detected between the 11 and 22 mg/kg treatment groups for either males or females.

TABLE 6. Other microorganisms (other than *Escherichia coli*) diagnosed as responsible for poult mortality[1]

Microorganism	Control	Neomycin sulfate (11 mg/kg)	Neomycin sulfate (22 mg/kg)
Bacillus spp.	1 (0.02)[2]	0 (0.0)	0 (0.0)
Morgonella morganii	1 (0.02)	0 (0.0)	0 (0.0)
Salmonella spp.	6 (0.12)	6 (0.12)	2 (0.04)
Acinebacter calcoaceticus	0 (0.0)	1 (0.02)	0 (0.0)
Serratia spp.	0 (0.0)	1 (0.02)	0 (0.0)
Serratia marcescens	0 (0.0)	0 (0.0)	1 (0.02)

[1]Total number of poults were control, 4,775; neomycin sulfate at 11 mg/kg, 4,775; neomycin sulfate at 22 mg/kg, 4,775.

[2]Percentage of total in parentheses.

Analysis of Mortality Associated with E. coli *as Diagnosed*

Means for DM are reported in Table 1. The sex and treatment interaction was not significant ($P > 0.05$); thus, the treatment effects were tested across both sexes by the trial and treatment error term. The percentage mortality was reduced by 46 to 55%, a reduction similar to that obtained with the SM analysis.

Means for OM are reported in Table 1. Although there were no statistically significant ($P > 0.05$) differences detected, the mortality pattern was similar to that for SM and DM. A mortality reduction was found for the 11 and 22 mg/kg treatment groups of 44 and 50% for males and 36 and 34% for females (Table 1). Inspection of individual location data (Table 2) identified similar mortality rates three locations (B, C, D) but varied response at two locations (A and F). Both locations had the lowest number of confirmed deaths attributable to colibacillosis in SM and DM. It is possible that the *E. coli* in the litter at these sites were not as virulent as expected or desired for this trial. In contrast, the OM for all trial locations, and especially

TABLE 7. Mean water consumption per pen in grams for study Days 1, 6, 11, and 15

Group[1]		1	6	11	15
Males					
1	Mean	1,864.5	2,461.8	2,396.5	2,863.0
	SD	258.8	272.8	415.6	125.0
2	Mean	1,823.9	2,354.4	2,434.8	2,828.9
	SD	250.8	319.2	361.1	204.0
3	Mean	1,864.4	2,325.6	2,296.2	2,857.5
	SD	276.1	210.8	338.7	156.2
4	Mean	1,702.6	2,131.7	2,153.9	2,788.8
	SD	145.1	211.2	168.1	178.8
Females					
1	Mean	1,614.4	2,009.4	2,152.8	2,460.4
	SD	217.3	390.8	188.0	294.3
2	Mean	1,584.2	1,967.4	2,137.7	2,603.2
	SD	150.3	278.2	141.6	332.4
3	Mean	1,697.1	1,986.2	2,061.2	2,616.9
	SD	346.2	332.6	333.4	314.1
4	Mean	1,531.4	1,840.0	1,829.6	2,293.3
	SD	115.8	130.7	204.4	296.6

[1]Dosage groups: 1 = 0 mg/kg per day; 2 = 66 mg/kg per day; 3 = 110 mg/kg per day; and 4 = 220 mg/kg per day.

the high OM seen among those two locations, would seem to indicate that other pathogenic organisms were present that were detrimental to the poults. The results by sex and treatments for males were control, 6.60 for neomycin sulfate at 11 and 22 mg/kg body weight, 3.68 and 3.28%, respectively. For the females, control was 6.03 for neomycin sulfate at 11 and 22 mg/kg body weight, 3.88 and 3.95%, respectively (Table 3).

Performance

Performance data included body weight and feed efficiency (feed:gain) but were not used as decision criteria in this trial; thus, the data were not analyzed statistically. The data on estimated weights (weight gain) and feed efficiency are summarized in Table 4. In four of five trial locations poults receiving neomycin showed appreciable gains in weight and improved feed efficiency over the controls (Table 4).

Microorganisms (Other Than E. coli) That Were Diagnosed as Causing Poult Mortality

Microorganisms other than *E. coli* were identified by the diagnostician as the cause of mortality among the poults (Table 5). The number (Table 6), however, was extremely small, and no conclusion could be made regarding the efficacy of neomycin sulfate against these organisms.

Target Animal Safety Study

The target animal safety study done in conjunction with the efficacy study was conducted to evaluate the safety of neomycin sulfate at 3×, 5×, and 10× the therapeutic dose of 22 mg/kg per d when administered in the drinking water to 4-wk-old turkey poults. Although daily water consumption per pen was measured, the mean water consumption is shown only for Days 1, 6, 11, and 15, the days when the poults were weighed (Table 7). The data

TABLE 8. Mean daily doses[1] of neomycin consumed per pen at three different intervals

		Males			Females		
		Study day actual dose (mg/kg/day)			Study day actual dose (mg/kg/day)		
Dosage group (mg/kg/day)		1	6	11	1	6	11
66	Mean	80.21	85.52	70.94	82.48	85.66	74.98
	SD	8.50	9.55	8.98	7.77	13.11	5.03
110	Mean	136.66	143.34	113.12	147.75	146.46	121.93
	SD	18.59	11.19	16.58	33.31	22.71	17.30
220	Mean	248.38	271.88	219.08	252.86	270.17	215.95
	SD	21.24	24.16	15.27	21.57	20.14	23.09

[1]Actual dose was calculated from theoretical concentrations and actual pen body weight data.

TABLE 9. Mean body weights on study Days 1, 6, and 11

Dosage group (mg/kg/day)		Males			Females		
		Body weight (kg) study days			Body weight (kg) study days		
		1	6	11	1	6	11
0	Mean	2.40	5.08	8.57	1.95	4.13	6.76
	SD	0.114	0.2004	0.257	0.089	0.132	0.302
66	Mean	2.47	5.21	8.70	2.00	4.24	6.89
	SD	0.125	0.319	0.367	0.078	0.176	0.2553
110	Mean	2.48	5.22	8.81	1.99	4.22	6.91
	SD	0.148	0.295	0.380	0.174	0.180	0.218
220	Mean	2.39	5.08	8.74	1.99	4.23	6.88
	SD	0.180	0.338	0.505	0.116	0.221	0.328

suggest a decrease in water consumption in both sexes at the 10× dose. The mean of the actual daily doses of neomycin per pen by treatment groups, consumed per kilogram of pen body weights, for dosage Days 1, 6, and 11 are summarized in Table 8. All neomycin doses consumed, except the Day 11 dose for females at the 220 mg/kg per d dose group, exceeded the targeted dose. The doses consumed ranged from 70.94 to 85.52 and 74.98 to 85.66 for the males and females in the dose group of 66 mg/kg per d. At 110 mg/kg per d, the ranges were 113.12 to 143.34 for the males and 121.93 to 147.75 for the females, and at 220 mg/kg per d ranges were 219.08 to 271.88 and 215.95 to 270.17 for males and females, respectively. As evidence that the consumption of high doses of neomycin sulfate had no deleterious effect on the poults, the body weights for the dose groups collected at Days 1, 6, and 11 and summarized in Table 9 show equal to or a better growth response when compared with their controls. Although, the amounts of neomycin sulfate were 3×, 5×, and 10× that used in the efficacy study, the trends in growth were very similar to those observed in the efficacy trials (Table 4). This response in growth is further evidence of the safety of neomycin sulfate when administered in water to turkey poults. There was no mortality in this part of the trial. Poults selected (1 pen – 10/sex per treatment group) for a complete necropsy at the end of the study exhibited no treatment-related lesions nor were there any treatment-related changes in the organ weights from these identified poults.

The results of this trial establish evidence of the effectiveness of neomycin sulfate in controlling mortality associated with colibacillosis (*E. coli*) in young male and female turkeys. Neomycin sulfate was effective at 11 or 22 mg/kg body weight, when administered in the drinking water for 5 consecutive d. Neomycin was further shown to be safe and well tolerated at doses up to and including the expected use level.

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REFERENCES

Arp, L. H., C.L.G. Graham, and N. F. Cheville, 1976. Comparison of clearance rates of virulent *Escherichia coli* in turkeys after aerosol exposure. Avian Dis. 23:386–391.

Bartlett, M. S., 1937. Some examples of statistical methods of research in agriculture and applied biology. J. R. Stat. Soc. (Suppl.)4:137–183.

Harry, E. G., 1964. The survival of Escherichia coli in the dust of poultry houses. Vet. Rec. 76:466–470.

Kitchen, H. B., and S. A. Waksman, 1955. Streptomycin and neomycin in veterinary medicine. JAVMA 127:261–274.

Leitner, G., and E. D. Heller, 1992. Colonization of *Escherichia coli* in young turkeys and chickens. Avian Dis. 36:211–220.

Newberry, L. A., J. K. Skeeles, D. L. Kreider, J. N. Beasley, J. D. Story, R. W. McNew, and B. R. Berridge, 1993. Use of virulent hemorrhagic enteritis virus for the induction of Colibacillosis in turkeys. Avian Dis. 37:1–5.

SAS Institute Inc., 1989. SAS/STAT® Users Guide, Version 6, Fourth Edition. Volume 2. SAS Institute, Inc., Cary, NC.

Waksman, S. A., E. Katz, and H. A. Lechevalier, 1950. Antimicrobial properties of Neomycin. J. Lab. Clin. Med. 36:93–99.

Waksman, S. A., and H. A. Lechevalier, 1949. Neomycin a new antibiotic active against Streptomycin resistant bacteria—Including tuberculosis organism. Science (Washington, DC) 109:305–307.