Case Report—
Effective Control of a Gentamicin-Resistant
Salmonella arizonae Infection in Turkey Poult

H. E. Ekperigin, S. Jang, and R. H. McCapes
Veterinary Medical Teaching Hospital
University of California
Davis, California 95616

Received 7 October 1982

SUMMARY

A gentamicin-resistant Salmonella arizonae isolate was identified as the cause of an unusually high early mortality rate in several flocks of poult produced by a primary turkey breeder. The company routinely dipped its hatching eggs in 500 ppm gentamicin before incubation and injected each poult at 1 day of age with 1 mg gentamicin. Mortality was reduced to normal, but S. arizonae was not eliminated by injecting the day-old poult with higher doses of gentamicin. S. arizonae was not isolated from sample normal-sized poult in treated groups when tetracyclines were used for antibiotic inoculation of day-old poult. Tetracyclines seemed to be completely effective only when a 5-mg subcutaneous injection per day-old poult was combined with an approximately equal dose in drinking water daily for 4 days, and therapy was accompanied by the culling of runts and other debilitated poult.

INTRODUCTION

Current techniques for egg-dipping do not ensure that all eggs will absorb adequate amounts of antibiotic (1). This is most probably why the technique has been found to reduce, but not eliminate, egg transmission of certain pathogens (1). Ghazikhanian and Yamamoto (3) isolated tylosin-resistant mycoplasma from eggs and vaginas of fifth-generation turkeys hatched from eggs dipped in tylosin, but they isolated tylosin-susceptible mycoplasma from turkeys hatched from undipped eggs. This shows that repeated exposure of the microbes in dipped eggs to ineffective levels of antibiotic can result in the development of strains of organisms resistant to the antibiotic.

The present report describes how an unusually high mortality rate in several flocks of poult hatched from eggs dipped in gentamicin
was shown to be caused by a strain of *Salmonella arizonae* that was resistant to gentamicin. Also described are experiments conducted to determine the appropriate routes and regimen of therapy for some of the antibiotics to which the organism was susceptible *in vitro*.

**MATERIALS AND METHODS**

**Case history.** On 16 April 1981, the Veterinary Medical Teaching Hospital of the University of California at Davis was asked to investigate a case of unusually high mortality occurring during the first week of life in several flocks of broad-breasted white pouls. The client was a primary turkey breeder, and the problem was widespread in the pedigree stock raised on its ranches and in the multiplier and meat-bird flocks raised by customers. The company practiced an egg-sanitization program in which eggs were collected from nests every hour and washed immediately after on the ranch in an automatic egg-washing machine with a hot (40–45°C), aqueous solution containing equal parts of formalin and a quaternary ammonium compound (QAC). Before incubation at the hatchery, the eggs were placed in a closed room with adequate air exhaust and fumigated with a mixture of potassium permanganate and 37% formaldehyde (0.6 g KMnO₄ and 1.2 ml formalin per cubic foot) while being heated to about 38°C. The warm eggs were then dipped in a cold (4°C) solution containing 500 ppm gentamicin and 250 ppm QAC. Pouls hatching out of the eggs were injected subcutaneously in the dorsal aspect of the neck at 1 day of age with a dextrose-vitamin-antibiotic solution containing gentamicin. Each poult received 1 mg of gentamicin. The antibiotic component of the solution used for poult injection was alternated after every two or three hatches between gentamicin and spectinomycin (10 mg). It was assumed that this would reduce the possibility of organisms developing that were resistant to either antibiotic.

**Clinical signs.** Symptoms observed or reported in all affected flocks included lethargy, huddling, ruffled feathers, anorexia, diarrhea and pasted vents, blindness, ataxia, and some leg paralysis. Mortality ranged from 5 to 15%.

**Gross pathology.** Affected pouls from several flocks were examined post-mortem at various times over a 1-month period.

**Microbiology.** Samples of livers, ceca, duodeni, and yolk sacs from each group of pouls necropsied were carefully excised for microbiological examination. Other samples examined for microorganisms were cardiac blood samples, brains from two pouls exhibiting ataxia or leg paralysis, organs of pips, and samples of solutions from the dip tank and egg-washing machine.

**Parasitology.** The contents and mucosal scrapings of the ceca and duodeni were examined for coccidia by the direct smear and flotation methods.

**Virology.** Antibiotics were added to suspensions of livers, yolk sacs, and ceca to inhibit bacteria. These were then inoculated into and passaged through chicken embryos for virus isolation.

**Antibiotic trials.** Four trials were conducted in the field and in experimental facilities of the hospital to determine the most appropriate route and regimen of therapy for some of the antibiotics to which *S. arizonae* had been shown to be sensitive *in vitro*. Antibiotics tested were tetracyclines (Oxyject: Diamond
Table 1. Effect of antibiotics on mortality and isolation of *S. arizonae* from livers and ceca of naturally infected poults.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Mortality (no. dead/total)</th>
<th><em>S. arizonae</em> isolations (no. positive/no. examined)</th>
<th>Liver</th>
<th>Ceca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>3/24</td>
<td>19/20</td>
<td>14/20</td>
<td></td>
</tr>
<tr>
<td>Gentamicin 2 (mg/poul)</td>
<td>1/24</td>
<td>16/20</td>
<td>15/20</td>
<td></td>
</tr>
<tr>
<td>Amikacin 1.5 (mg/poul)</td>
<td>0/24</td>
<td>0/20</td>
<td>11/20</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline 5 (mg/poul)</td>
<td>1/24</td>
<td>4/20</td>
<td>20/20</td>
<td></td>
</tr>
<tr>
<td>Trial 2B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>5/10</td>
<td>6/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin 2 (mg/poul)</td>
<td>-</td>
<td>0/10</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>Amikacin 5 (mg/poul)</td>
<td>-</td>
<td>1/10</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline 5 (mg/poul)</td>
<td>-</td>
<td>2/10</td>
<td>3/10</td>
<td></td>
</tr>
</tbody>
</table>

A The antibiotics were injected into each poult once daily for 4 days starting at 1 day of age. Poults were killed and examined for *S. arizonae* at 1 week of age.

B The poults were injected with antibiotics at 1 week of age and given neomycin in drinking water for 4 days. They were killed and examined for *S. arizonae* at 2 weeks of age.

C Not determined.

Shamrock, Cleveland, Ohio; Terramycin soluble powder: Pfizer Inc., New York, New York, amikacin sulfate (Amikin: Bristol Laboratories, Syracuse, New York), gentamicin sulfate (Gentocin: American Scientific Laboratories, Syracuse, New York), and neomycin sulfate (Neomix 326: Upjohn Co., Kalamazoo, Michigan). The antibiotics were administered either orally or parenterally for 4 days to day-old or week-old broad-breasted white poults. Appropriate dosages of antibiotics to be administered parenterally were contained in 0.2 ml of a dextrose-vitamin-antibiotic solution used routinely at the client's hatchery for day-old poults injection, and injected subcutaneously in the dorsal aspect of the neck.

The poults used were naturally infected with *S. arizonae* 7A,7B:1,7,8. They were supplied by the client and divided randomly into treatment groups as shown in Tables 1 and 2. Poults in trials 1 and 2 were raised in wire-floored, electrically heated brooders in rooms in which temperature (24 C) and lighting (14 hr) were kept constant. Poults in trials 3 and 4 were raised in the field on deep litter using gas-heated brooders. Poults were fed and watered *ad libitum*.

The four groups of poults in trial 1 were injected once daily for 4 days, starting at 1 day of age, with gentamicin (2 mg/poul), amikacin (1.5 mg/poul),...
oxytetracycline (5 mg/poult), or nothing (controls). The poult in trial 2 were injected with the same antibiotics once at 1 week of age and then given neomycin in drinking water (as recommended by manufacturer: 35.7 g/128 gallons) for 4 days. The groups of poult in trials 3 and 4 were injected once at 1 day of age with either gentamicin or oxytetracycline and given either plain drinking water or water containing tetracycline for 4 days. The tetracycline solution contained 4 g tetracycline per gallon of drinking water. This equates to a daily consumption of 4 mg tetracycline per poult, if a daily water-consumption value of 10 gallons/1,000 large-type turkeys during the first week of life is used as a guide (Merck Chemical Co., Rahway, New Jersey).

One week after the initiation of each trial, poult were killed by cervical dislocation, and their livers and ceca were excised and examined individually for *S. arizonae*.

**RESULTS**

**Gross pathology.** Gross lesions consistently observed in poult examined postmortem included retained and markedly distended yolk sacs, enlarged, yellowish, mottled or inflamed livers, and enlarged ceca with cecal cores. However, the organs of some of the affected poult appeared normal.

**Microbiology.** *Salmonella arizonae* was isolated and identified by the usual laboratory procedures (5) from the livers, ceca, and yolk sacs of pips and poult and the blood samples. All isolates were resistant to gentamicin, spectinomycin, penicillin, streptomycin, erythromycin, oxacillin, ampicillin, trimethoprim-sulfamethoxazole, and kanamycin but susceptible to tetracycline, amikacin, neomycin, chloramphenicol, and cephalothin when tested by the *in vitro* disc or microdilution (MIC 2000 System, Dynatech Laboratories, Inc., Alexandria, Virginia, 1976) methods. In all cases, the serotype of the organism was *S. arizonae* 7A.7B:1,7,8 (confirmed by the Enteric Bacteriology Unit, Diagnostic Bacteriology Section, VSL, APHIS, USDA, Ames, Iowa).

The organism was isolated neither from the two brains examined nor from the samples of solutions used in the dip tank or egg-washing machine.

**Parasitology.** Coccidia were found only occasionally, and the number of oocysts present in such cases were too few to be considered significant.

**Virology.** No virus was isolated in three embryo passages.

**Antibiotic trials.** Rates of mortality and isolation of *S. arizonae* from livers were much lower in poult given multiple injections of each of the antibiotics than in untreated poult (Table 1, trial 1). However, antibiotic therapy had no appreciable effect on the rates of cecal isolation.
The rates of isolation of *S. arizonae* from livers was also much lower in pouls given oral neomycin and a single injection of one of the three antibiotics than in untreated pouls. In addition, a lower rate of cecal isolations was observed in pouls treated with parenteral tetracycline and oral neomycin (Table 1, trial 2).

In trial 3 (Table 2), *S. arizonae* was isolated from organs of sample pouls picked randomly from groups injected with tetracycline or gentamicin alone or treated with a combination of parenteral gentamicin and oral tetracycline. On the other hand, no *S. arizonae* was isolated from the livers or ceca of sample pouls picked from the group injected with 5 mg tetracycline and given about the same dose of oral tetracycline daily for 4 days.

Except for the isolation of *S. arizonae* from one cecum, the effects of parenteral-oral tetracycline therapy in trial 4 (Table 2) were the same as in trial 3. The one cecal isolation observed in trial 4 was from a runt. The parenteral-oral tetracycline regimen was prescribed for use on several subsequent customer hatches with the advice that it be accompanied by culling runts and other debilitated pouls. Reports indicated that the tetracycline therapy resulted in superior poult performance, absence of *S. arizonae* from sample pouls, and a reduction in mortality to normal or levels below normal.

**DISCUSSION**

The clinical signs and gross lesions observed in pouls naturally infected with *S. arizonae* in the present study are compatible with those described earlier (5). Isolation of the organism from the ceca as well as from livers and blood samples of affected pouls confirmed its enteric and systemic nature. The fact that *S. arizonae* was not isolated from the solution used in the egg-washing machine or dip tank indicates that these solutions were most probably not the source of the *S. arizonae* isolated from the organs of the pouls examined. This, and the isolation of the organism from yolk sacs of pouls and organs of pips, support evidence presented by others (5) as to the egg-transmissibility of *S. arizonae*.

The effects of multiple injections of antibiotics on rates of mortality and isolation of *S. arizonae* from pouls in trial 1 are similar to those observed by Zolli and Polewaczyk (6) and Williams (5), who used a single dose of various antibiotics to which the organism was susceptible *in vitro*. The rate of liver isolations was much lower, whereas the rate of isolation of *S. arizonae* from ceca was virtually the same in untreated pouls as in pouls properly injected with full
Table 2. Effect of parenteral-oral antibiotic therapy on isolation of *S. arizonae* from livers, ceca, and yolk sacs of naturally infected pouls. The pouls were killed and examined for the organism at one week of age.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. of pouls</th>
<th>S. arizonae isolations (no. positive/no. examined)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin + water</td>
<td>800</td>
<td>Liver 4/7 Ceca 3/7 Yolk -</td>
</tr>
<tr>
<td>Gentamicin + tetracycline</td>
<td>800</td>
<td>6/7 3/7 -</td>
</tr>
<tr>
<td>Oxytetracycline + water</td>
<td>800</td>
<td>4/6 0/6 -</td>
</tr>
<tr>
<td>Oxytetracycline + tetracycline</td>
<td>800</td>
<td>0/6 0/6 -</td>
</tr>
</tbody>
</table>

Trial 4

| Oxytetracycline + tetracycline | 770 tons (house 1) | 0/2 0/2 - |
| Oxytetracycline + tetracycline | 1140 hens (house 2) | 0/8 1/8 0/5 |

A Pips and newly hatched but unthrifty pouls were cultured for *S. arizonae*. The isolation rate from livers, ceca, and distended yolk sacs varied from 50 to 100%. The serotype of all isolates was *S. arizonae* 74, 78/1, 7, 8.

B The pouls were injected at 1 day of age with gentamicin (2 mg/poul) or oxytetracycline (5 mg/poul) and given either plain drinking water or water containing tetracycline (4 mg per poult daily) for 4 days.

doses of antibiotics to which the organism was susceptible. This indicated the need for a combined parenteral-oral antibiotic therapy if the organism is to be eliminated from infected pouls. However, Greenfield et al. (4) did not succeed in eliminating *S. arizonae* from the intestines of artificially infected pouls treated with the parenteral-oral combinations of gentamicin-neomycin, spectinomycin-neomycin, or spectinomycin-chloramphenicol. In the present study, oral neomycin seemed to have some effect on cecal isolation only when it was used in conjunction with parenteral tetracycline. The failure of the parenteral-oral combinations to eliminate *S. arizonae* in these studies could have been due to antibiotic incompatibilities. However, since neomycin is largely unabsorbed from the gut and only one injection of the other antibiotic in the combination was administered, failure of the parenteral-oral combination could also be attributed to the absence of a high enough level of any of the two antibiotics in the systemic circulation.
The results of trials 3 and 4 give credence to these hypotheses. In trial 3, the high rate of isolation of *S. arizonae* from organs of poults injected with gentamicin and given plain drinking water was not decreased by supplementing the injection with oral tetracycline. On the other hand, no *S. arizonae* was isolated from the livers or ceca of sample poults picked randomly from the group injected with 5 mg tetracycline and given about the same dose of oral tetracycline for 4 days. The parenteral-oral tetracycline therapy most probably failed to eliminate *S. arizonae* from the ceca of the runt in trial 4 because the physical condition of that poult prevented it from gaining ready access to, and consuming enough of, the medicated drinking water. Although *S. arizonae* was not isolated from normal-sized infected poults treated with parenteral-oral tetracycline, it would have been necessary to examine many more of them before it could be concluded that the organism had been eliminated. It was not possible to do that in the present study, because the poults used in the field trials constituted either a prime genetic pool or valuable commercial products, and market forces at that time made examination of more poults economically imprudent.

Egg-dipping and day-old poult injection, as now practiced, are good examples of improper use of antibiotics. In each case, the organisms against which treatment is aimed are continually exposed to inadequate quantities of the antibiotic. This increases the probability of early development of resistant strains and is most likely the cause of the tylosin resistance reported earlier (3) and the gentamicin and spectinomycin resistance observed in the present study. Therefore, although both practices have helped greatly in minimizing the adverse economic effects of egg-transmitted diseases, they also appear to have resulted in the potential loss of the effective use of valuable antibiotics. The procedures need to be improved upon or discarded.

Egg-dipping techniques could be improved, and Ekperigin and McCapes (1,2) have described techniques that could ensure that each dipped egg absorbs an effective dose of the antibiotic in the dip solution. Those techniques are being studied further.

The major problem with day-old poult injection as practiced is that antibiotics designed to be effective after administration for 3 or more consecutive days are injected only once. In the present study, a 4-day parenteral-oral tetracycline therapy was utilized to overcome that problem and to effectively control the high mortality and poor
poults performance caused by a gentamicin-resistant *S. arizonae* infection.

Williams (5) stated that therapeutic methods have no place in an overall program to eliminate arizona infection from poultry flocks. The present report indicates that the possibilities for therapeutic methods have not been exhausted.

REFERENCES


ACKNOWLEDGMENTS

The manuscript was typed by Yolanda G. Ferguson of the School of Veterinary Medicine, University of California, Davis. Her excellent technical aid is warmly acknowledged.