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# FREQUENCY AND DURATION OF PARATYPHOID ORGANISM SHEDDING BY EXPERIMENTALLY INFECTED BOBWHITE QUAIL (Colinus virginianus)

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Abstract: Four-week old bobwhite quail (Colinus virginianus) were experimentally infected with Salmonella urbana, S. infantis, S. newport, S. gaminara, S. braenderup, and S. litchfield. Rates of mortality varied from 0 to 50%. The rate of shedding of para-typhoid organisms varied from 14 to 100% for 18 or more days after infection. The maximum duration of shedding was 53 days by 12% of the quail infected with S. braenderup and the minimum duration was 18 days by 14% of the quail infected with S. litchfield.

#### **INTRODUCTION**

Salmonellosis remains an important problem in wildlife populations,<sup>10,13</sup> food animal production,  $^{2,8,12,14}$  and the pet industry,  $^{1,11,13}$  The mechanisms of propagation and dissemination of paratyphoid orgamisms into the environment are not fully understood and need further definition.

The isolation of salmonellae from birds suggests that free-flying populations should be considered as possible reservoirs.<sup>10,13,15</sup> Domestic birds have been shown to survive infections by different paratyphoid serotypes and subsequently shed the organisms for months.<sup>2,3,5,9</sup> Outbreaks of paratyphoid infections among young quail in confinement have occurred,<sup>4,6</sup> but the ability of bobwhite quail (*Colinus virginianus*) in confined or free-flying populations to serve as a reservoir host for salmonellae has not been reported.

This study was initiated to evaluate the potential role of bobwhite as a reservoir and a disseminator of paratyphoid organisms, using the three following criteria: rate of survival, frequency of sheddings of the paratyphoid organism, and duration of shedding by survivors.

#### MATERIALS AND METHODS

Pen-raised bobwhite from a parent flock known to be free of salmonellae for several generations were used in the study. Each experimental group consisted of either six or nine quail, housed when 3-weeks old in cages with wire floors in plastic film isolation chambers. I A commercial game bird ration, I sterilized by irradiation with 3,000,000 rads from a Co<sup>60</sup> source, was used. At 39 days post-infection, each group was moved to a modified Horsfall-Bauer isolation unit to facilitate handling. The study was terminated at 116 days post-infection, and all survivors were killed and examined at necropsy.

Paratyphoid serotypes, and the dosage used for infection are shown in Table 1. Paratyphoid agents selected for the study were isolated from turtles or turtle pond water. All serotypes (typed by the National Animal Disease Center, Ames, Iowa) used had been reported previously

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<sup>&</sup>lt;sup>II</sup> Standard Safety Equipment Co., Palatine, Illinois.

<sup>&</sup>lt;sup>2</sup> Ralston Purina Co., St. Louis, Illinois.

TABLE 1. Number of deaths in groups of bobwhite quail infected with different Salmonella serotypes.

Serotype	Infective dose*	No. of deaths		
S. urbana	$1  imes 10^7$	2/6**		
S. infantis	$1  imes 10^7$	3/6		
S. newport	$1  imes 10^7$	2/6		
S. gaminara	$1  imes 10^7$	0/6		
S. braenderup	$5 imes 10^6$	1/9		
S. litchfield	$5 imes 10^6$	2/9		

\*Number of bacteria administered.

\*\*No of deaths/total No. in experimental group.

as infective for avian species.<sup>14</sup> When concurrent experimental groups were evaluated, serotypes of different somatic antigenic groups were used to facilitate detection of possible cross infections. The bacterial agents were administered *per os* to quail at four weeks of age in 0.2 ml of an 18 h. broth culture.

Cloacal swabs were taken from each bird on days 3, 6, 11, 18, 25, 32, 39, 46, 53, 67, 81, and 95 post-infection. One or more birds from each group was killed on days 81, 95, and 116 post-infection to determine the possibility of a latent infection. Samples of tissue and contents of the small intestine, large intestine, cecum, spleen, and liver were taken at necropsy of birds that succumbed to infection or that were killed during the study.

Equal-sized pieces of each tissue (approx. 150 mg, depending upon organ size) and cloacal swabs were placed into selenite cystine broth and tetrathionate broth containing 0.001% brilliant green for primary enrichment. After incubation for 24 h. at 37C, the enrichment cultures were streaked on brilliant green, SS, and bismuth sulfite agar selective plating media. Suspect colonies were assayed on triple sugar iron agar, lysine iron agar, and urea agar slants, and in modified malonate broth. After incubation for a week at room temperature, a one ml aliquot of the tetrathionatebrilliant green broth was transferred into a second tetrathionate-brilliant green broth. The subsequent procedures used for this secondary enrichment culture were the same as those used for primary cultures. Isolates having characteristics of salmonellae in these biochemical tests were confirmed with commercial groupspecific antiserum.

## RESULTS

The rate of mortality in quail from infections by different paratyphoid serotypes varied from 0% by *Salmonella gaminara* to 50% by *S. infantis.* Mortality attributed to the different serotypes is shown in Table 1.

A cloacal swab from quail was positive for *S. braenderup* on the 53rd day postinfection, much later than from quail infected with other serotypes. The number of positive cloacal swabs obtained from each group infected with respective serotypes is shown in Table 2.

The percentage of tissue samples positive for salmonellae taken from birds that died were as follows: large intestine, 100%; small intestine, 84.2%; cecum, 94.7%; liver, 84.2%; and spleen, 68.4%. There were no differences in localization of the organisms of six serotypes within the various tissues studied.

Cultures of tissue samples taken from quail examined at necropsy on days 81, 95, and 116 days post-infection were negative for salmonellae.

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TABLE 2. Number of cloacal swabs from bobwhite quail positive for *Salmonella* at various intervals post-infection.

Serotypes	Days post-infection							
	3	6	11	18	25	32	39	53
S. urbana	1/5*	1/4	1/4	0/4	0/4	4/4	0/4	0/4
S. infantis	3/5	4/5	2/3	1/3	0/3	0/3	1/3	0/3
S. newport	5/5	4/5	4/4	1/4	0/4	1/4	0/4	0/4
S. gaminara	3/6	3/6	4/6	2/6	0/6	0/6	0/6	0/6
S. braenderup	9/9	7/9	1/8	2/8	0/8	2/8	0/8	1/8
S. litchfield	2/7	3/7	0/7	1/7	0/7	0/7	0/7	0/7

\*Number of birds with cloacal samples positive for *Salmonella*/total No. of birds sampled.

# DISCUSSION

Numerous factors not evaluated in this study may influence bacterial dissemination by quail. Mortality in young quail in the two reported natural outbreaks was higher than in the four-week-old quail used in this study. The difference may be attributed to age, method of housing, infective dose, method of infecting, or unknown stress factors. The survival rate of birds infected with salmonellae usually increases correspondingly with the age of the host at the time of infection.<sup>9</sup> Experimentally, size of the infecting dose has been shown to influence survival of young chickens but not the duration of shedding.<sup>9</sup> The reduction in number of bacteria administered to quail infected with S. braenderup and S. litchfield was based on the observed mortality of quail previously infected with the other four serotypes and on the report that size of the dose does not influence duration of shedding.

Chickens reared on litter become reinfected from the environment and shed longer than birds kept on wire floors.<sup>9</sup> If food contamination or coprophagy occur in confined or freeliving quail populations, dissemination of paratyphoid organisms may continue longer than was observed in this study, although some vent picking of moribund birds was noted. Stress factors that may arise in freeliving populations have been shown to increase shedding of paratyphoid organisms by confined chickens. Deprivation of water did not significantly influence rate of infection, but duration of shedding was greater when water was withheld 3 days either before or after inoculation.<sup>3</sup> Heat stress caused chickens with latent infections to become active shedders.<sup>3</sup>

Although the shedding rate of quail in this study was low after the 18th day post-infection with paratyphoid organisms, the duration of shedding was long enough to allow extensive dissemination of many serotypes. The shedding rate was similar to that reported in chickens, which dropped markedly between 12 and 18 days after oral infection.9 Shedding was not detected after the 53rd day in quail in this study, but reportedly persisted at the 0.5% level in chickens until the 94th day post-infection. The number of experimental quail in this study was too small to accurately detect a low rate of shedding for a prolonged period. It is highly probable that the duration of shedding from some birds in a large population of quail also would be longer.

Latent infections and intermittent shedding may not have been detected with the method and frequency of sampling used. Rectal swabbing of humans with salmonellae infections detected only 50% of the infections when fecal samples contained 100 or less organisms per gram of feces.<sup>7</sup> Possibly the carrier state was more common at times in quail in this study than was revealed by the experimental procedures used.

Necropsies and bacterial culturing of tissues from quail at 81 to 116 days postinfection did not detect latent infections. In contrast, necropsies with bacterial culturing from 77 to 115 days postinfection revealed intestinal infection in 7 of 191 (about 4%) chickens that had stopped shedding 22 to 79 days previously.<sup>3</sup> Again, the experimental numbers used in the present study were too small to accurately detect a 4% rate in intestinal infection.

No difference could be detected between serotypes in the localization of the organism in tissues at necropsies. This finding corresponds with those reported from studies in the chicken.<sup>5</sup> The large intestine always yielded isolates on necropsy of quail dead from active infection. No other organ was so uniformly a source of isolates. Localization of the organisms in surviving quail was not found; all survivors were negative for paratyphoid organisms at necropsy. Necropsies of chickens that survived infections have revealed, however, that more recoveries of the infective agent could be obtained from cecal contents.<sup>5</sup>

Although salmonellosis in wild quail populations has not been reported, it has been observed on game-bird farms.<sup>1,6</sup> The susceptibility of quail to infection with paratyphoid organisms in this study, the ubiquity of paratyphoid organisms in the environment, and the common practice of releasing quail grown in confinement on game-bird farms strongly suggest, however, that *Salmonella* infections do occur in freeliving quail populations.

Fecal contamination of pasture land by carnivores has been shown to be a source of salmonellae for livestock." If shedding patterns occur in wild quail populations as observed in this study, infected quail may serve as a primary source of infection for herbivorous as well as for carnivorous animals.

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