SALMONELLOSIS IN WATERFOWL IN OKLAHOMA

By

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PREFACE

This study was concerned with the possible influence that salmonellosis might have on migrating and wintering migratory waterfowl populations in the state of Oklahoma. The primary objectives of this study are to ascertain the prevalence of Salmonella organisms in these populations in the field and, through the use of laboratory reared ducks, to determine any deleterious effect that might be caused by Salmonella infection. Data from the laboratory experiments serves as the reference upon which interpretation of the field results is based.

The author wishes to express his thanks and appreciation to his major adviser, Dr. A. Alan Kocan, for his guidance, support and patience throughout this study. Thanks and appreciation are also extended to the other committee members, Dr. Patrick M. Morgan, Dr. John A. Bissonette, and Dr. Paul B. Barto for their assistance in the preparation of the final manuscript.

The author wishes to thank Colonel Howard R. Jarrell and the Environmental Institute of Oklahoma State University for their support in funding and for their permission to utilize Ham's Lake as a research site. Thanks are also expressed to the Max MacGraw Wildlife Foundation for providing ducklings which were raised and used in this study. In addition, appreciation is expressed to Dr. J. E. Williams of the Southeastern Cooperative Poultry Disease Unit for supplying the group-specific Salmonella antigens used in the serologic studies of this project and to

the management of Washita National Wildlife Refuge for allowing a portion of this study to be done on their facility. Finally, thanks are extended to Dr. William Warde for his assistance with the statistics portion of this study.

A special note of thanks is expressed to my wife, Darcy, for her support and sacrifice and her efforts in preparing the initial drafts of this manuscript, and to my parents, Mr. and Mrs. Arlen Waldrup, and my in-laws, Mr. and Mrs. Delbert H. Tammen for their support and understanding.

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CHAPTER I

INTRODUCTION

The importance of Salmonella bacteria in domestic ducks is well documented in the disease literature (Clarenburg, 1939; Lucas, 1956; Rettger and Scoville, 1920; Truscott, 1956). Little information is available, however, on the possible role of Salmonellosis in free-ranging migratory duck populations. It is assumed by some that salmonellosis is a common disease problem in migratory waterfowl (Fowler, 1978), but no supportive evidence is given to uphold this assumption. Although articles have been published which describe the isolation of a Salmonella species from wild ducks (Keymer, 1958), to date, only two surveys (one in the United States and one in England) are available which address this subject in any detail (Bradshaw and Trainer, 1966; Mitchell and Ridgwell, 1971).

Since little information on salmonellosis in wild waterfowl is available, it is not surprising that there is virtually nothing recorded from Oklahoma or the Central Flyway. Biological data from migratory waterfowl population studies indicates that nearly 10,000,000 ducks migrate through the Central Flyway of which Oklahoma is a part (Belrose, 1976). This large number of ducks in a single area represents a disease potential both to the ducks themselves and to other animals including man through dissemination of Salmonella bacteria into water sources and food stuffs.

Salmonellosis or the infection of a host of bacteria of the genus Salmonella is known to be pathogenic to ducks under specific conditions. The usual route of infection is by ingestion of the bacterium which results in an enteritis of varying degrees due to intestinal invasion and dysfunction. A fatal septicemia often develops as a complication of infection. The largest numbers of mortalities are usually observed in younger animals. Even if the infection is not fatal, the Salmonella organisms may colonize the intestine of the recovered individual and may be shed in the feces for the life of the recovered individual. Both vertical (older ducks to younger ducks) and horizontal (ducks to other animals and other animals to ducks) transmission has been recorded (Clarenburg and Vink, 1948; Levine and Graham, 1942; Neilsen, 1960; Stenius, 1932).

Because of the disease potential and the lack of information on the subject, a study designed to show the prevalence of this disease in free-ranging ducks and experimental determination of Salmonella detection was undertaken.

The objectives of this study were of a twofold nature. The first set of objectives were accomplished through laboratory experimentation. They were: 1) to determine the virulence of Salmonella typhimurium var. copenhagen in laboratory reared non-juvenile mallards, 2) to determine the relationship between isolation by cloacal swab and serologic changes over time in non-juvenile mallards inoculated orally with S. typhimurium var. copenhagen, and 3) to compare the efficiency of two different enrichment systems for the isolation of Salmonella from non-juvenile ducks using cloacal swabs. The second part of this study involved field investigation, the objective of which was to determine the prevalence

of Salmonella species in wild waterfowl migrating through and wintering in Oklahoma by the use of cloacal swabs and the measurement of serologic reactivity.

CHAPTER II

LITERATURE REVIEW

Most of the available literature concerning Salmonellosis of ducks deals with domestic birds and those rearing operations associated with them. In a report of an outbreak on a commercial duck farm, Rettger and Scoville (1920) reported the etiologic agent of "keel" disease as Salmonella anatum. This disease affected ducklings up to three weeks old with a resulting high mortality. The foci of infection in the reported outbreak were adult breeder ducks having S. anatum-infected gonads (vertical transmission). Many other similar reports exist. Levine and Graham (1942) reported the death of 400 wood ducklings out of a flock of 500 due to Salmonella typhimurium infection. Again, the source of the bacteria was an adult duck which intermingled with the young ducklings. Truscott (1956) reported Salmonella moscow as the etiologic agent of an outbreak involving ducklings on a duck farm in Ontario, Canada.

Adult ducks are also susceptible to virulent Salmonella infections. Keymer (1958) reported the isolation of S. typhimurium from a distressed wild tufted duck. The existence of a non-virulent shedding state in wild ducks was demonstrated in a survey by Mitchell and Ridgwell (1964) who reported an incidence rate of 4.2% for Salmonella organisms shed in the feces of wild ducks. Bradshaw and Trainer (1966) surveyed 55 ducks

for serologic reactivity to S. typhimurium and S. pullorum with negative results. Nielsen (1972) reported the transmission of S. typhimurium from wild mallards to domestic mallards at a game farm in Norway.

In addition to the vertical transmission mentioned afore, instances of horizontal transmission have also been recorded. Clarenburg and Vink (1948) described the transmission of S. anatum from domestic ducks to a domestic cow. Reports of cross-transmission of S. pullorum between young chicks and domestic ducklings have been published by Stenius (1932) and Hinshaw and Hoffman (1937).

The pathogenesis of Salmonellosis in avians has primarily been researched in chickens. Fanelli et al. (1972) found that in four week old chickens Salmonella organisms were most consistently isolated from the cecal contents and cecal tonsils. Sadler et al. (1969) correlated age and inoculum level of S. typhimurium in chickens and found that no mortality occurred in chickens inoculated after two days of age. In the same study, it was observed that the shedding of Salmonella organisms dropped sharply after 12 to 18 days regardless of age or inoculum. The serologic reactivity, however, increased to this point and decreased after 24 days.

CHAPTER III

METHODS AND MATERIALS

Laboratory Experiment I: Virulence of *Salmonella typhimurium* var. *copenhagen* in MacGraw Mallard Ducks

The Max MacGraw Wildlife Foundation of Dundee, Illinois provided an inbred strain of mallard ducklings which are known as MacGraw mallards. The birds were shipped from the MacGraw Foundation as day-old ducklings. Upon arrival at Oklahoma State University they were pinioned and hand-reared under *Salmonella*-free conditions. To determine the virulence of *S. typhimurium* var. *copenhagen* in MacGraw strain 10-week old mallard ducks, 27 hand-reared birds were divided in five experimental groups each containing five birds and a two bird control group. Each group of experimental ducks received an oral inoculation of one milliliter per bird of one of the following concentrations: 10^8 cells per milliliter, 10^6 cells per ml, 10^4 cells per ml, 10^2 cells per ml and 10^1 cells per ml. The control group received an oral inoculation of sterile saline. The *S. typhimurium* cells were harvested from 18 hour growth on blood agar Petri plates by washing with sterile saline. The washed cells were diluted to the concentration of a MacFarland #1 standard and then further diluted to the appropriate concentration in sterile saline.

After inoculation, the birds were housed in enclosed, screen-

floored cages. The temperature was maintained at 25°C and the food and water was changed daily. The food was tested to be Salmonella-free by inoculating random samples into selenite-cystine enrichment broth followed by inoculation onto Brilliant Green Agar. The birds were sampled every two days until shedding as detected by cloacal swab had stopped. Sampling was accomplished by taking two cloacal swabs, one of which was inoculated in strontium chloride B broth, an enrichment medium designed by Iveson (1971) for the enrichment of Salmonella and other enteric bacteria from snakes, and the other in selenite-cystine broth, a medium originally designed by Leifson (1936) for the enrichment of typhoid and paratyphoid bacteria. After inoculation, the enrichment tubes were incubated at 42°C for 48 hours. The use of an elevated incubation temperature (42°C as opposed to 37°C) has no adverse effect on the performance of the strontium-chloride B broth (Iveson, 1971) and has been reported to enhance the performance of the selenite medium (Greenfield and Bankier, 1962) with respect to the enrichment of Salmonella bacteria.

After incubation, material from the strontium chloride B broth was inoculated and streaked on Salmonella-Shigella (SS. Difco) agar. Material from the selenite-cystine broth was inoculated and streaked on Brilliant Green (BG) agar (Difco). The inoculated agar media were incubated at 37°C for 18 hours. Suspect colonies were inoculated onto Heart Infusion (HI, Difco) slants. After sufficient growth was present, the suspect organisms were tested with polyvalent "O" anti-Salmonella serum. Triple Sugar Iron (TSI, Difco) slants and Christensen's urea slants (BB). Suspect organisms were considered positive as Salmonella species if they were lactose and urease negative, glucose positive (fermentation) and either hydrogen sulfide positive or agglutinated by

the polyvalent antisera. The comparison of the two enrichment systems was continued through this experiment.

After the shedding had stopped, the surviving birds were killed by injecting 2 ml of pentabarbital directly into the saphenous vein of the leg and necropsied. At necropsy, specific organs were cultured for the presence of Salmonella bacteria. The gizzard, the small intestine, the colon, the cecae, the spleen, the heart, and the gonads were cultured by aseptically removing the entire organ, placing it in 200 ml of enrichment broth, and then mascerating it thoroughly with sterile scissors. The gall bladder was also cultured in a similar manner after it was removed from the liver. Portions of the liver, kidney and lung were cut from the tissue and inoculated into enrichment media. The inoculated media was then incubated at 42° C for 48 hours and streaked on solid differential media. After additional incubation, the solid media was checked for the presence of Salmonella colonies.

Laboratory Experiment II: Relationship of Cloacal Detectability and Antibody Titer Changes Over Time in Ten-week Old MacGraw Mallards

Twelve experimental birds were inoculated orally with one milliliter inoculum of Salmonella typhimurium var. copenhagen having a concentration of 10^4 cells per ml. The Salmonella cells were harvested by washing 18 hour growth from blood agar Petri plates using sterile saline. The concentration of the suspended cells was adjusted to the

concentration of a MacFarland #1 standard and then further diluted to the desired level. The two uninfected controls received an oral inoculation of one ml of sterile saline. After infection the experimental birds were housed together in outside, covered runs with gravel floors. The runs were enclosed with one inch mesh chicken wire. Commercial feed used in this experiment was tested as before to prevent any extraneous Salmonella contamination. Food and water containers were raised off the floor to prevent fecal contamination. The control birds were housed in the same facility but in a different pen. At three day intervals post-infection, all birds were sampled with two cloacal swabs (one for each enrichment system), and one ml of blood was removed by syringe from the brachial vein. By allowing the blood to stand at room temperature for 24 hours, the serum was separated from the other blood components by coagulation. The anti-Salmonella titer was measured using a microagglutination technique designed by Williams and Whittemore (1971). This technique utilized a group-specific, tetrazolium-stained antigen, and the specific antigens for Salmonella groups B, C, and D were used. Since S. typhimurium is a group B organism, the titer observed against group B would indicate the response directed by the ducks against S. typhimurium. Titers against groups C and D would indicate cross-reactions. When the Salmonella organisms were no longer detectable by cloacal swab and the antibody response was diminished, the surviving birds were killed by decapitation and necropsied. The organs were cultured as in the previous experiment.

Sampling of Wild Adult Ducks

The capture and sampling of wild wintering and migrating waterfowl

began in January, 1976, and continued through December, 1978. All sampling was accomplished at two sites in the state of Oklahoma. The first site was Washita National Wildlife Refuge located in Custer County on Foss Lake. This site was sampled only once, that occurring in January 1976. The other site was Ham's Lake located eight kilometers west of Stillwater in Payne County. Ham's Lake is a private impoundment with a surface area of approximately 40 hectares that is leased by Oklahoma State University for research purposes, and no waterfowl hunting is allowed.

The adult ducks were captured either by rocket or cannon net and by a baited swim-in trap. At Washita NWR, a cannon net only was used while both a rocket net and swim-in trap were utilized at Ham's Lake. After capture, each bird was identified to species and sex, sampled, banded with U.S. Fish and Wildlife Service aluminum leg bands and released.

The sampling procedure involved taking two cloacal swabs using cotton-tipped applicator sticks and obtaining 1-3 milliliters of blood from which the serum was later extracted. One of the cloacal swabs was placed in a screw-cap, five-inch test tube containing approximately eight milliliters of selenite-cystine broth. The other swab was placed in a similar tube containing approximately eight milliliters of Strontium-chloride B broth. The blood was collected from the brachial vein with a three cubic centimeter syringe equipped with a 23 gauge needle. The blood was allowed to clot by letting it stand at room temperature for 18-24 hours. The serum was poured off, centrifuged to remove any erythrocytes still present, and frozen at -20^{o}C. These serum samples

were later tested using a microagglutination technique designed by Williams and Whittemore (1971).

Upon returning from the field, the inoculated enrichment tubes were incubated at 42°C for 48 hours. After incubation, material from the Strontium-chloride B broth was inoculated and streaked on Salmonella-Shigella (SS, Difco) agar. Material from the selenite-cystine broth was plated on Brilliant Green agar (Difco). The inoculated agar media were incubated at 37°C for 18 hours. Suspect colonies were treated exactly as described for the laboratory experiment.

CHAPTER IV

RESULTS

Laboratory Experiment I

The results of Experiment I, which was designed to demonstrate pathogenicity of Salmonella typhimurium var. copenhagen in ducks and the short-term shedding pattern of infected ducks is illustrated in Figures 1-5. All ducks infected with Salmonella typhimurium var. copenhagen survived. Group I received the greatest inoculum (10^8 cells). The detectable shedding pattern of the group was double peaked. The percentage of the population detectably shedding Salmonella organisms reached its first peak at two days postinfection (PI). Eighty percent (4/5) of the ducks were detected as positive. This percentage dropped to zero by six days PI but peaked again at ten days PI. At that time, 40% (2/5) were detected. Detectable shedding had again ceased by 14 days PI (see Figure 1). All five ducks were killed and necropsied at 18 days PI. At that time, 60% (3/5) of Group I still had Salmonella typhimurium infecting some internal tissue (see Table I).

Group II received 10^6 cells, and the detectable shedding pattern was different than any of the other experimental groups. The level of detectable shedding remained at 60% (3/5) from two to ten days PI. At 14 days PI, only 20% (1/5) was detected (see Figure 2). At necropsy at 18 days PI, 40% (2/5) of the ducks were still infected (see Table I).

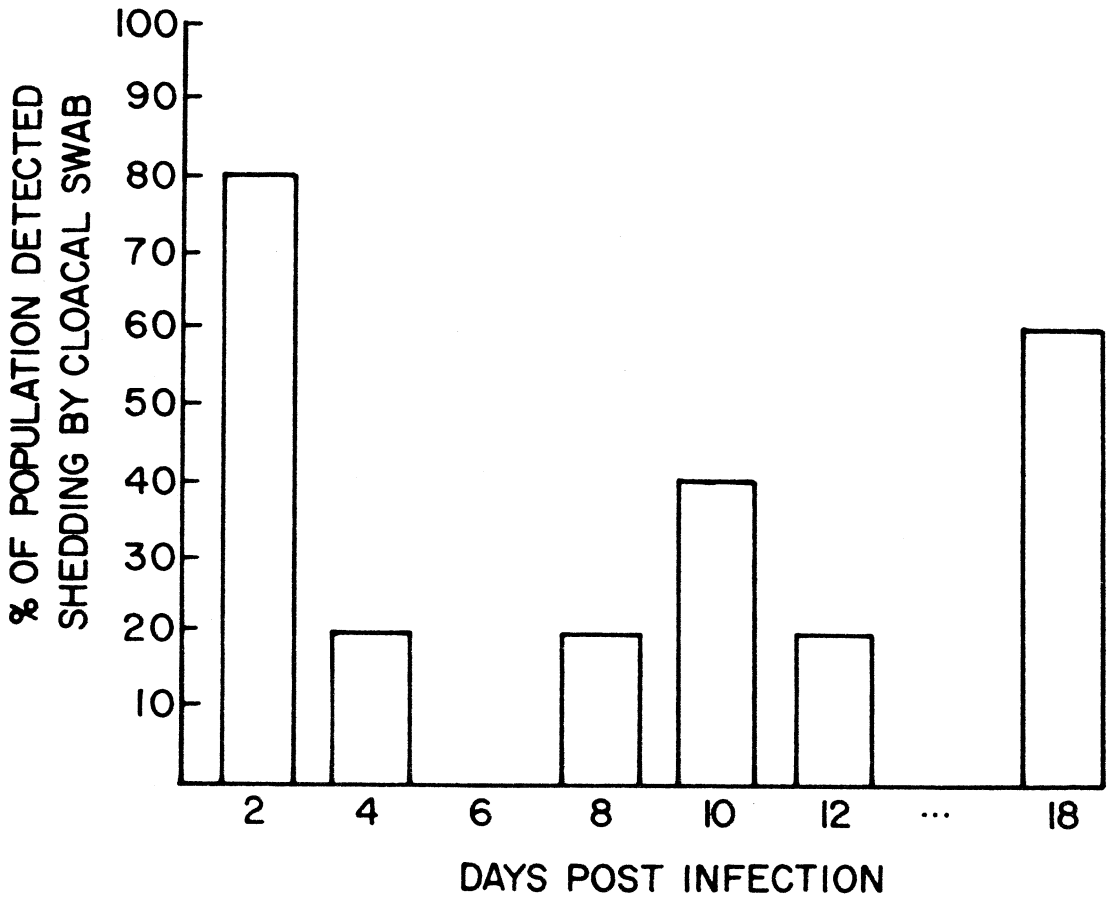

Figure 1. Shedding Pattern of MacGraw Mallards Inoculated Orally with 10^8 Cells of Salmonella typhimurium var. copenhagen through 14 Days Postinfection and at Necropsy on Day 18 Postinfection.

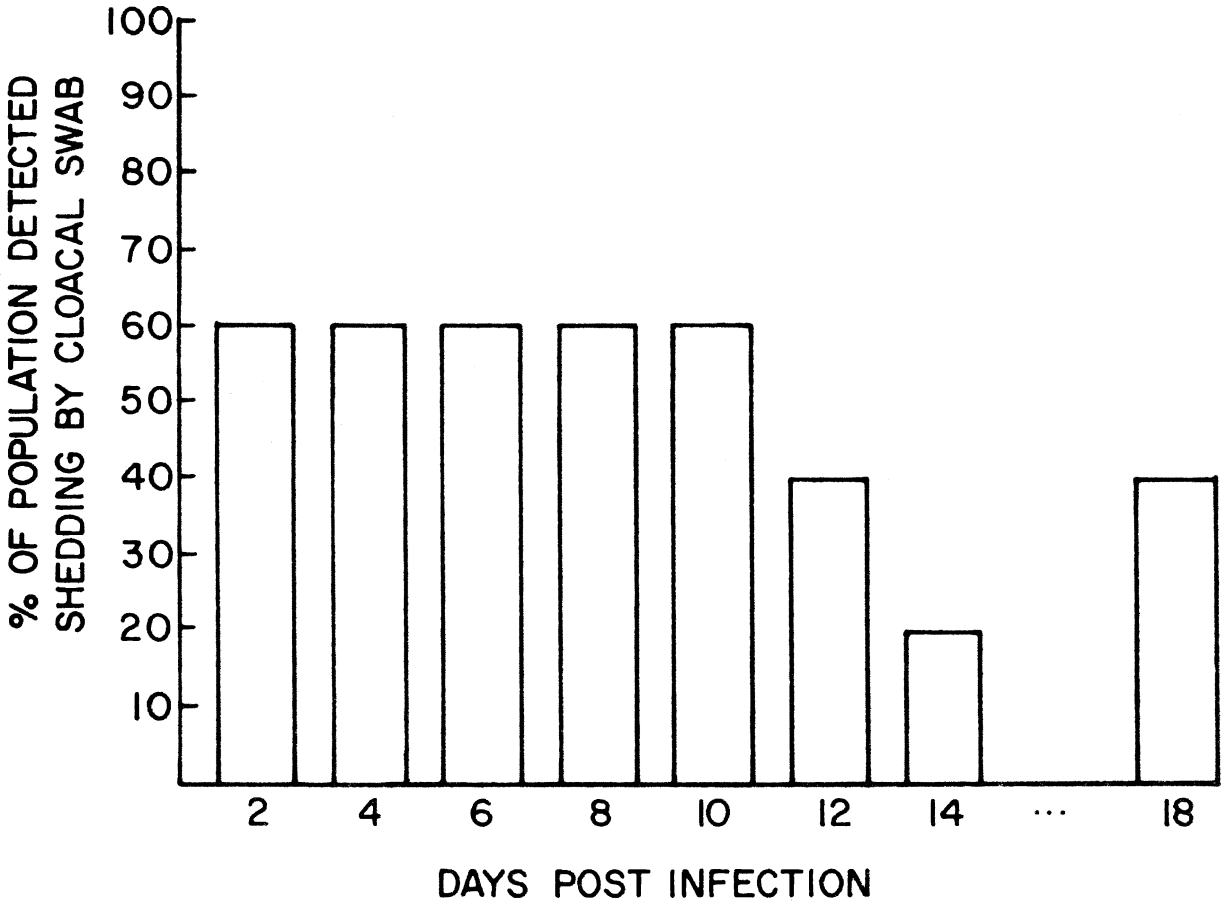

Figure 2. Shedding Pattern of MacGraw Mallards Orally Inoculated with 10^6 Cells of Salmonella typhimurium var. copenhagen through 14 Days Postinfection and at Necropsy on Day 18 Postinfection.

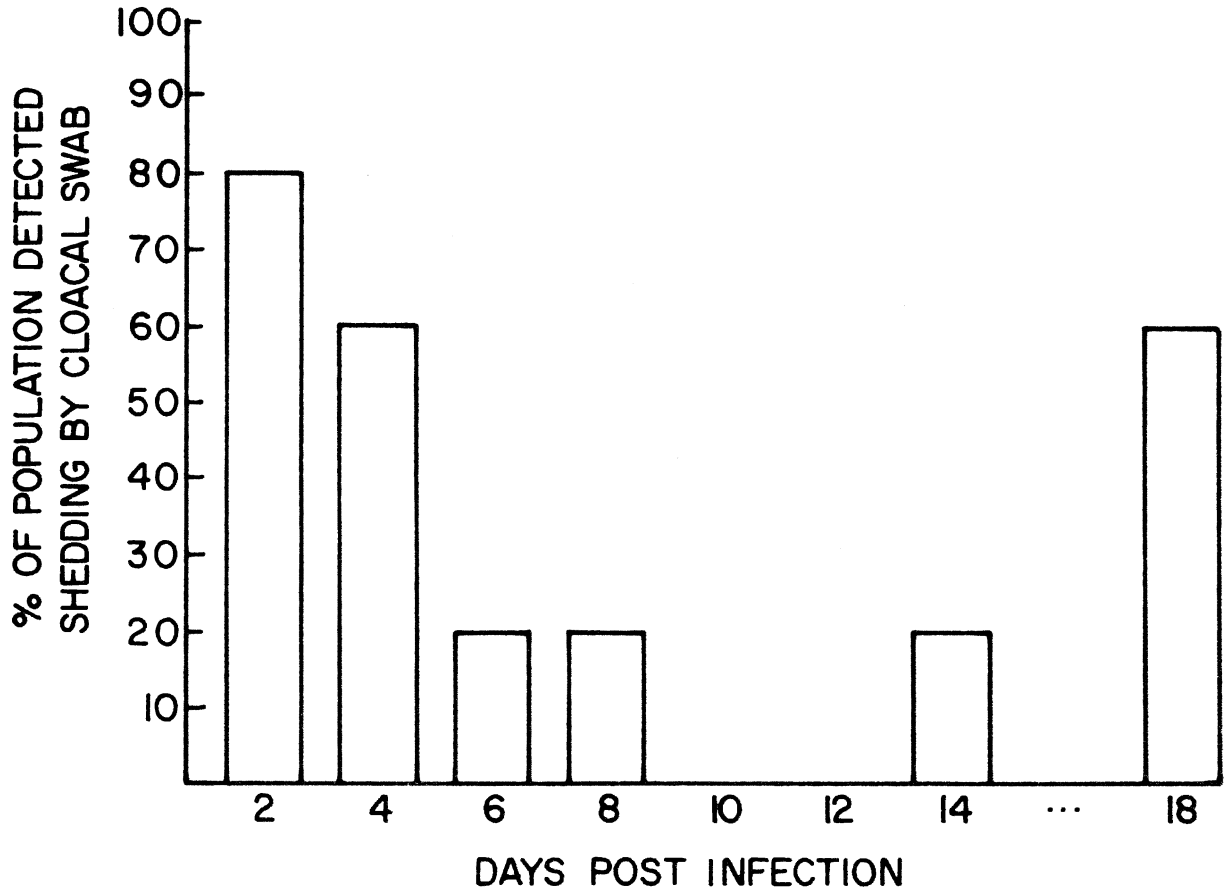

Figure 3. Shedding Pattern of MacGraw Mallards Orally Inoculated with 10^4 Cells of Salmonella typhimurium var. copenhagen through 14 Days Postinfection and at Necropsy on Day 18 Postinfection.

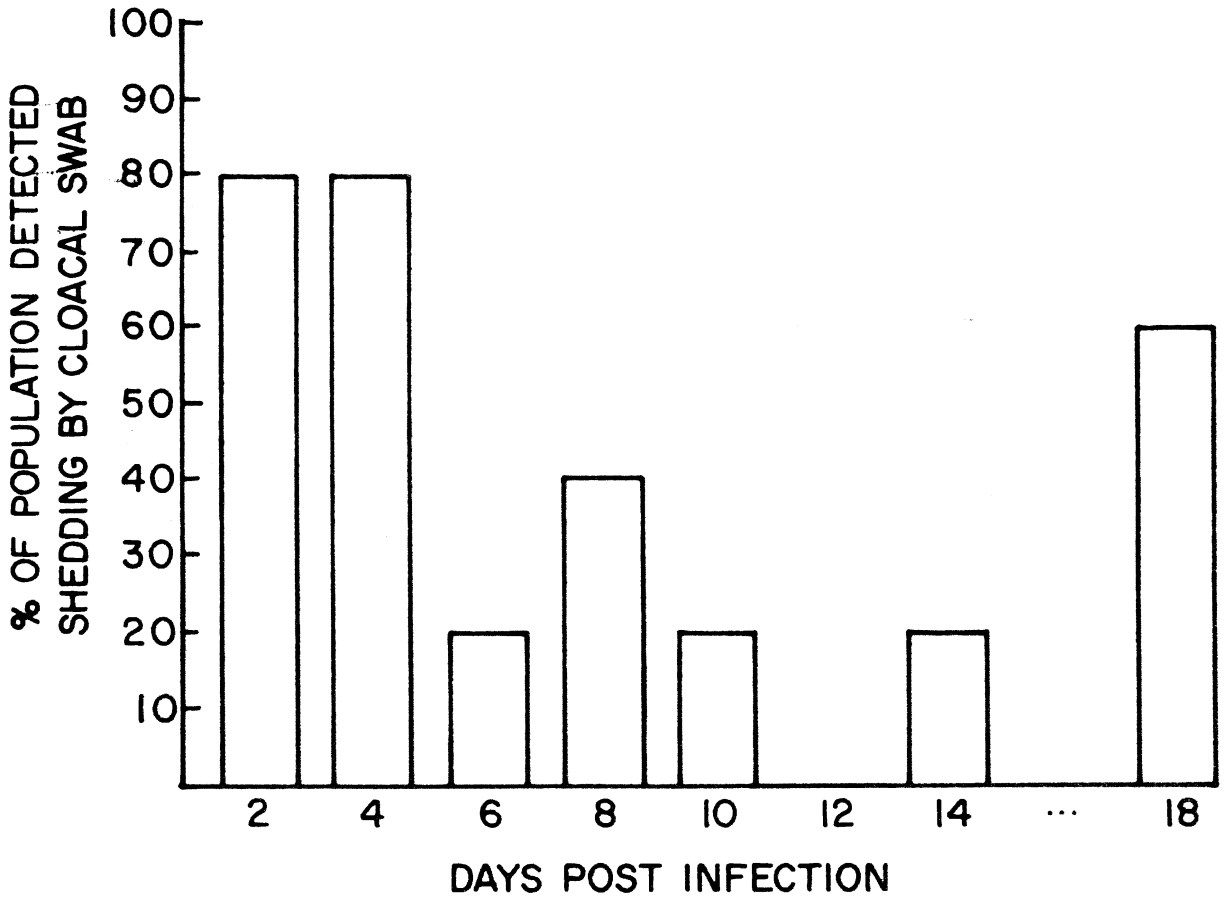

Figure 4. Shedding Pattern of MacGraw Mallards Orally Inoculated with 10^2 Cells of Salmonella typhimurium var. copenhagen through 14 Days Postinfection and at Necropsy on Day 18 Postinfection.

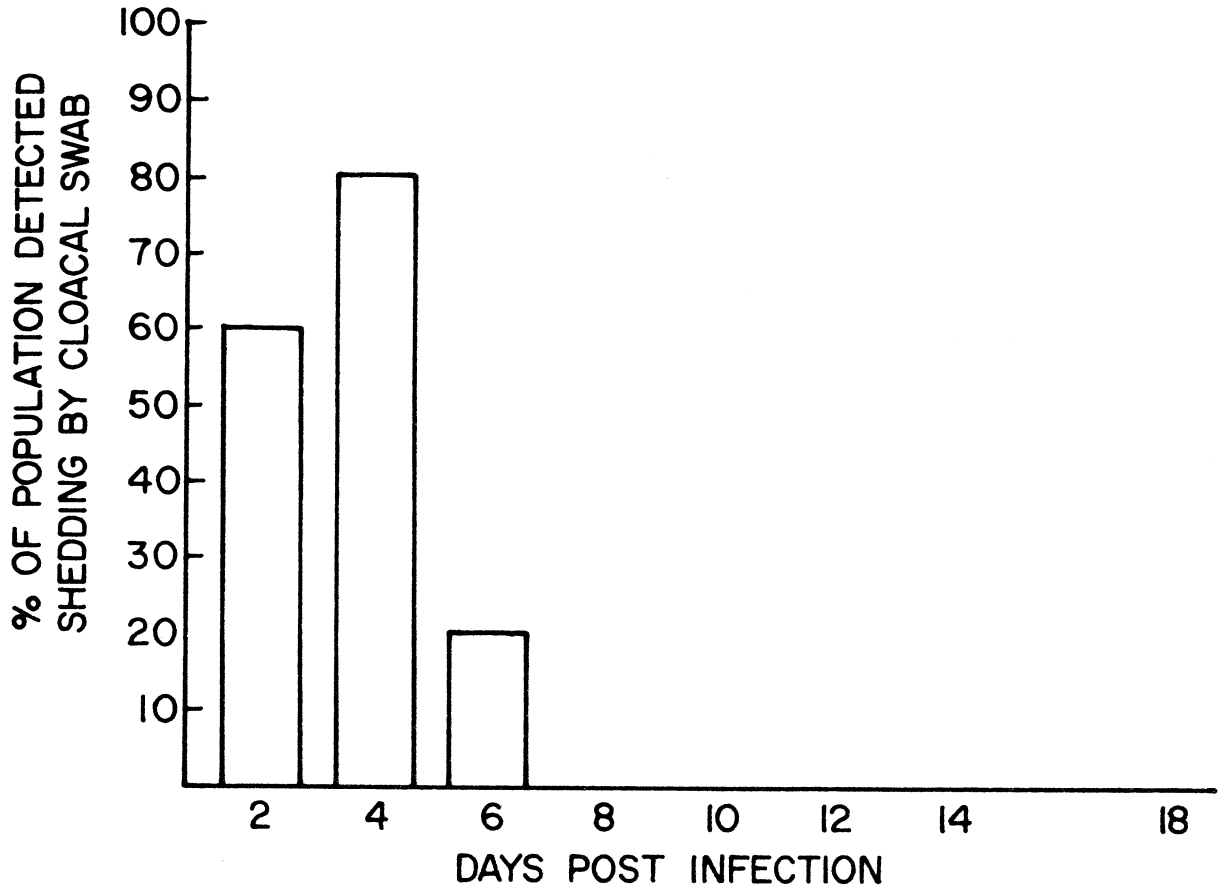

Figure 5. Shedding Pattern of MacGraw Mallards Orally Inoculated with 10 Cells of Salmonella typhimurium var. copenhagen through 14 Days Postinfection and at Necropsy on Day 18 Postinfection.

TABLE I

ISOLATION OF SALMONELLA TYPHIMURIUM VAR. COPENHAGEN FROM THE ORGANS OF ORALLY INOCULATED MACGRAW MALLARDS AT NECROPSY AT 18 DAYS POSTINFECTION

	Bird #	Gizzard	Sm Intest.	Colon	Ceca	Gall bl.	Liver	Kidney	Spleen	Lungs	Heart	Genitalia Ovaries	Genitalia Testes
Group I	226	-	-	-	-	-	-	-	-	-	-		-
	227	-	-	-	-	-	-	-	-	-	-		-
	228	-	+	-	-	+	-	-	-	-	-	-	
	229	-	-	+	-	+	-	-	-	-	-		+
Group II	230	-	-	-	-	-	+	-	-	-	-	-	
	231	-	+	-	-	+	-	-	-	-	-	-	
	232	-	-	-	-	-	-	-	-	-	-		-
	233	-	-	-	-	-	-	-	-	-	-		-
Group III	234	-	-	-	-	-	-	-	-	+	-		+
	235	-	-	-	-	-	-	-	-	-	-		-
	236	-	-	-	-	-	-	-	-	-	-	-	
	237	+	-	-	-	-	-	-	-	-	-	-	
Group IV	238	-	-	-	-	-	-	-	-	+	+	-	
	239	-	+	-	-	-	-	-	-	-	-		-
	240	-	-	-	-	-	-	-	-	-	-		-
	241	+	-	-	-	-	-	-	-	+	-	-	
	242	-	+	-	-	-	-	-	-	-	-		-
Group V	243	-	-	+	-	-	-	-	-	-	-		-
	244	-	-	-	-	-	-	-	-	-	-		-
	245	-	-	-	-	-	-	-	-	-	-		-
	246	-	-	-	-	-	-	-	-	-	-	-	
	247	-	-	-	-	-	-	-	-	-	-		-
	248	-	-	-	-	-	-	-	-	-	-		-
	249	-	-	-	-	-	-	-	-	-	-	-	
	250	-	-	-	-	-	-	-	-	-	-	-	
	251	-	-	-	-	-	-	-	-	-	-	-	
	252	-	-	-	-	-	-	-	-	-	-	-	

Group III received 10^4 cells. Eighty percent (4/5) of these ducks were detected shedding at two days PI. This percentage decreased until ten days PI, when no birds were detected. At 14 days PI, one bird was again detected (20%) (see Figure 3). At necropsy at 18 days PI, 60% (3/5) of this group still harbored the Salmonella organisms (see Table I).

Groups IV and V received 10^2 cells and 10^1 cells respectively. In Group IV, the percentage of the population initially detected shedding was 80% (4/5). This percentage had dropped to zero at 12 days PI, but one bird (20%) was positive at 14 days PI (see Figure 4). At necropsy, 60% (3/5) of Group IV were still infected internally (see Table I). Detectable shedding of S. typhimurium had completely stopped by eight days PI in Group V (see Figure 5). At necropsy, no ducks were found to be infected (see Table I). The two control ducks did not shed Salmonella bacteria at any time and were totally negative at necropsy.

Of the 25 experimentally infected ducks necropsied, a total of 11 were still infected though the prevalence of detectable shedding was low. Table II illustrates the recovery of S. typhimurium from the organs cultured from the animals necropsied in Experiment I. Of the 11 persistent infections, eight were located in the gastrointestinal tract. Two of these eight also had Salmonella organisms present at sites outside the gastrointestinal tract (testes and lungs). Three other ducks had infected organs not in the gastrointestinal tract. In one case the affected organ was the liver, in another case it was the lungs and testes, and in the third case, it was the lungs and heart.

Figure 6 illustrates the combined shedding pattern of all birds in the five experimental groups. About two-thirds of the infected birds shed Salmonella organisms through four days PI. At six days PI, less

TABLE II

SEROLOGIC REACTIVITY OF WILD DUCKS TO SALMONELLA GROUP B ANTIGENS, 1977-1978

Species	Sex	No. of Samples	Titer (inverse) 32	64	128
Mallard	M	117	2		
	F	93	1		
American Widgeon	M	11			
	F	3			
Pintail	M	6			
	F	3			
GW Teal	M	26			
	F	11			
BW Teal	M	20			
	F	7			
Ringneck	M	8		1	
	F	4			
Lesser Scaup	M	8	1		
	F	6			
Redhead	M	5			
	F	3			
Total		331	4	1	0

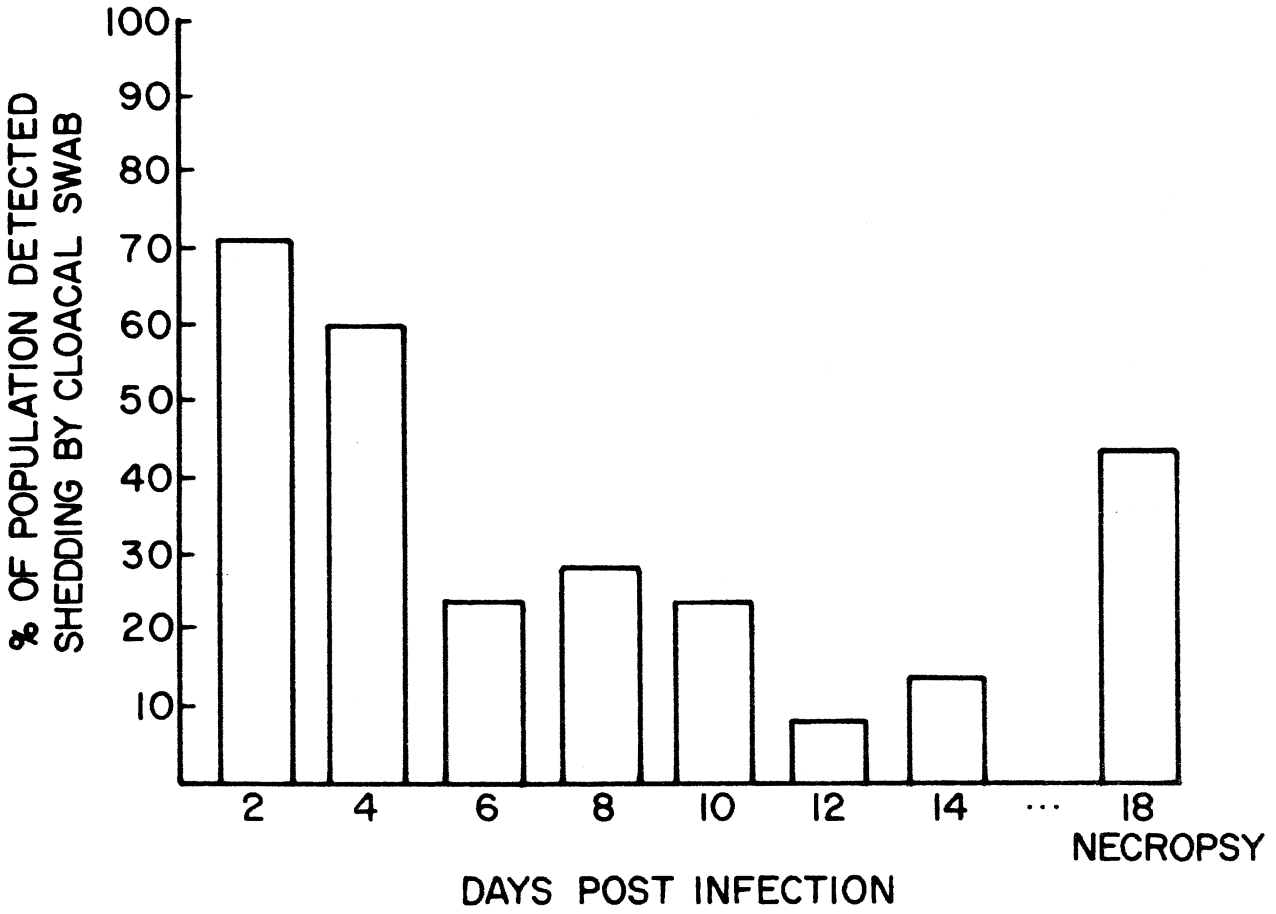

Figure 6. Composite Shedding Pattern of 25 MacGraw Mallards which Received Various Oral Inoculations of Salmonella typhimurium var. copenhagen through 14 Days Postinoculation and at Necropsy on Day 18 Post-inoculation.

than one-third of the infected birds were detected shedding. Detectable shedding in all groups was less than 10% at 14 days PI, but at necropsy on day 18 PI, over 40% of the infected birds still maintained an infection with Salmonella bacteria.

Laboratory Experiment II

Twelve MacGraw mallards were inoculated orally with 10^4 cells of Salmonella typhimurium var. copenhagen in the same manner as in Experiment I. The two control mallards were inoculated with sterile saline. The shedding data collected from this study is displayed chronologically in Figure 7. With only one exception, all detectable shedding ceased after 15 days PI (the single exception occurred at 23 days PI). Three of the twelve infected ducks (25%) demonstrated appreciably sustained increases in antibody titer (equal to or greater than 1:32 or 5.0 on a log base$_2$) against group B antigens. Three other birds had low level increases (equal to or less than 1:8 or 3.0 on a log base$_2$), and the other six experimental birds showed no change in anti-group B titer. At 90 days PI, the seven remaining birds were killed by decapitation and examined. Five experimental birds and the two control birds were killed and eaten by an unknown predator prior to the termination of the experiment and were unavailable for examination. Of the seven birds necropsied, only one harbored S. typhimurium, that infection occurring in the small intestine. Table III illustrates the organs examined and the sites of isolation for birds necropsied in Experiment II.

Figure 8 demonstrates the titer changes exhibited by the individual experimental ducks. The titers are recorded as base$_2$ logarithms, five (or 1:32) being the minimum titer considered reactive. Figure 9

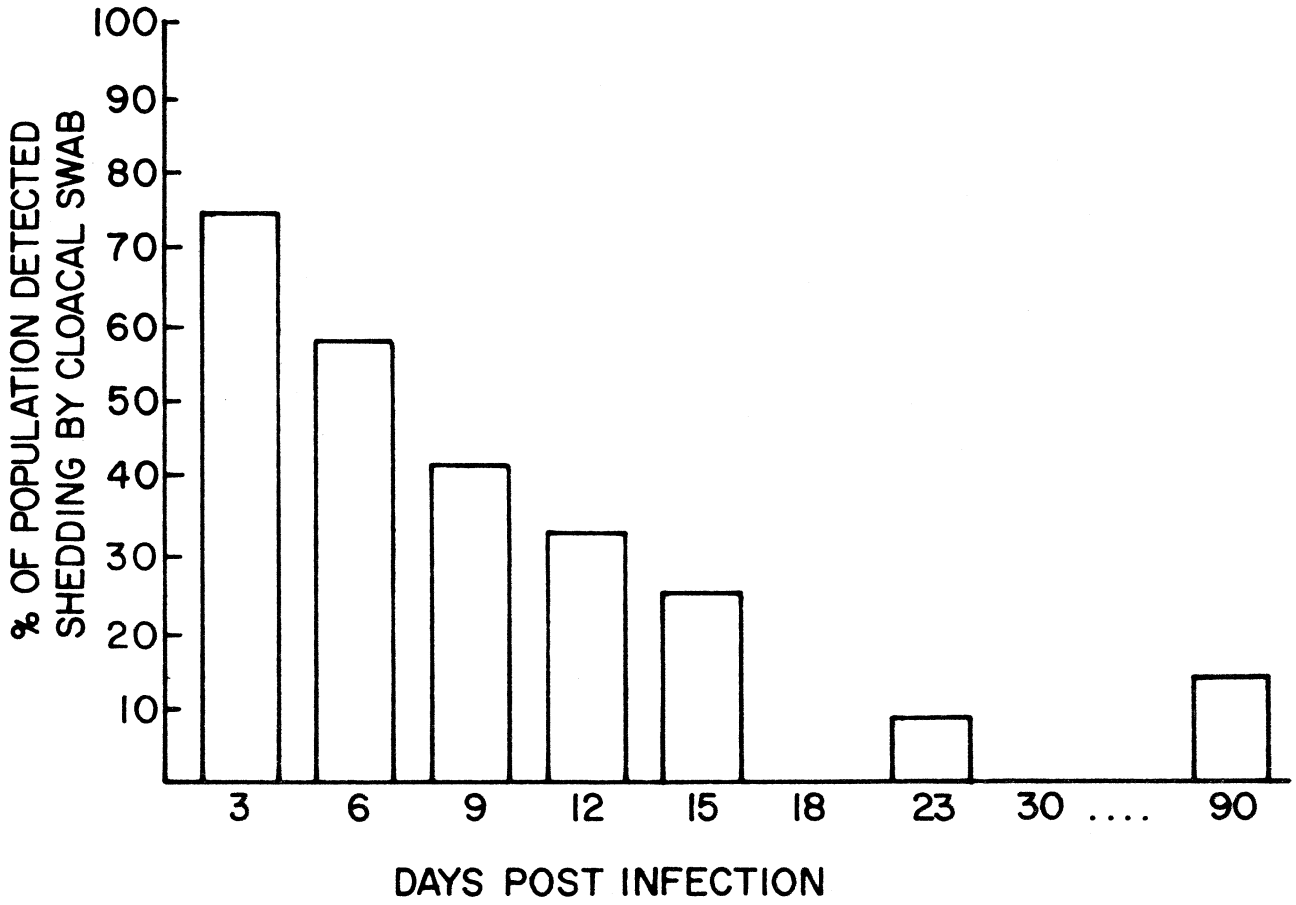

Figure 7. Shedding Pattern of MacGraw Mallards Inoculated Orally with 10^4 Cells of Salmonella typhimurium var. copenhagen through 30 Days Postinfection and at Necropsy on Day 90 Postinfection.

TABLE III

EXPERIMENT II NECROPSY RESULTS

Bird No.	Gizzard	Small Intestine	Colon	Cecae	Liver	Kidney	Spleen	Lung	Gall Bladder	Heart	Testes	Ovary
275	-	+	-	-	-	-	-	-	-	-	-	
278	-	-	-	-	-	-	-	-	-	-	-	
279	-	-	-	-	-	-	-	-	-	-		-
283	-	-	-	-	-	-	-	-	-	-	-	
284	-	-	-	-	-	-	-	-	-	-	-	
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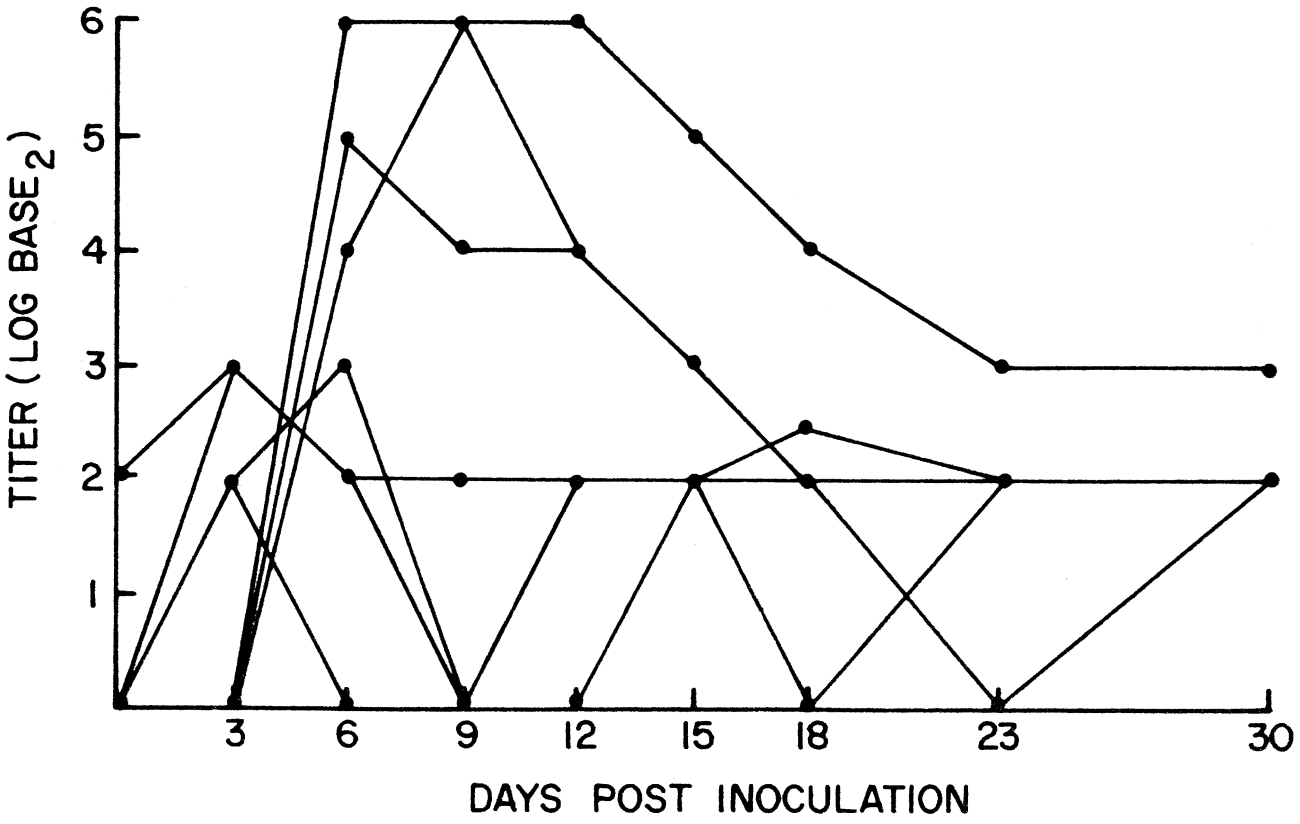

Figure 8. Changes in Antibody Titer Expressed as Base, Logarithms, to Salmonella Group B Antigens in MacGraw Mallards Orally Inoculated with Salmonella typhimurium var. copenhagen through 30 Days Postinfection.

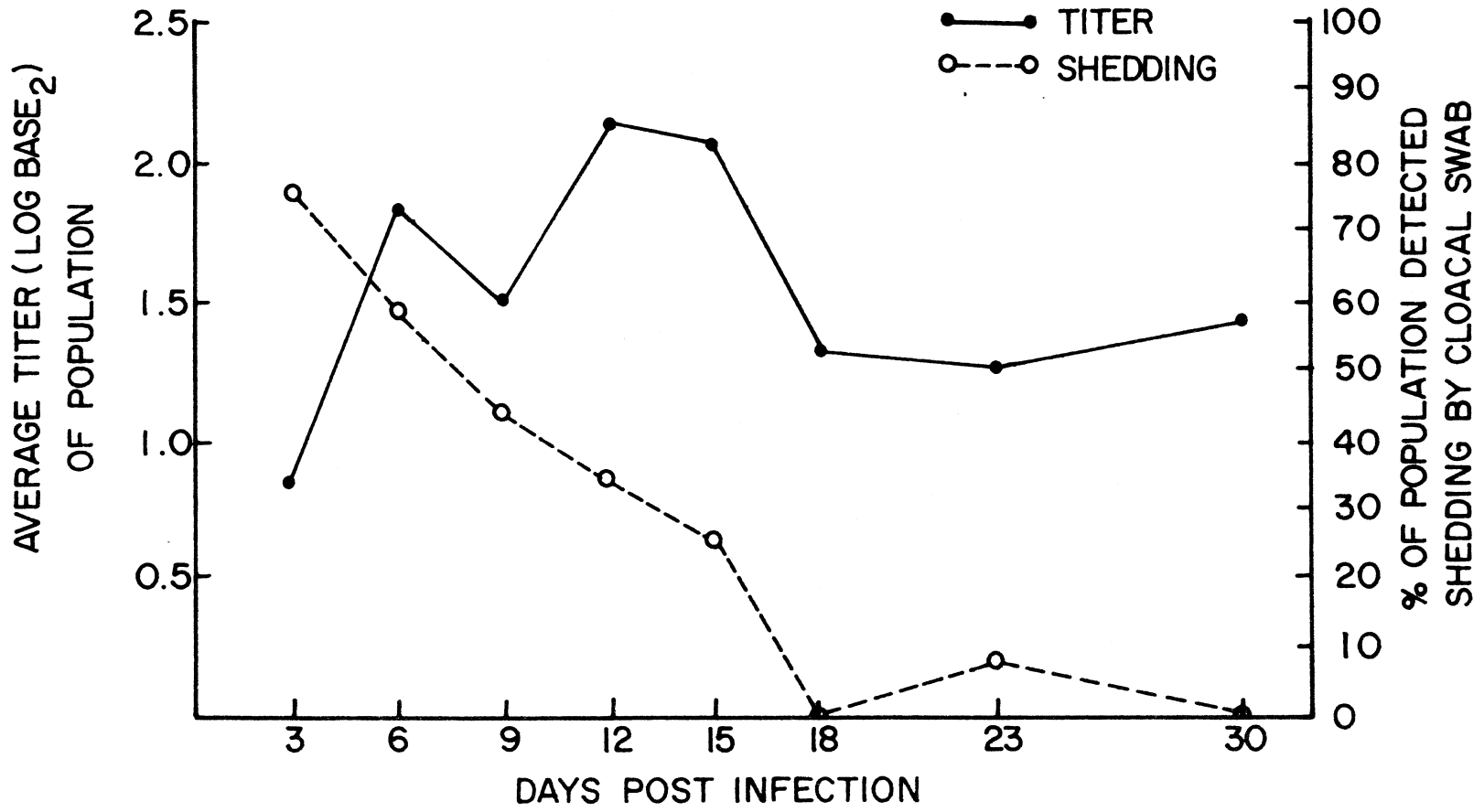

Figure 9. Comparison of the Changes in Average Population Titer (Log Base$_2$) and Shedding Pattern in MacGraw Mallards Orally Inoculated with Salmonella typhimurium var. copenhagen through 30 Days Postinfection.

illustrates the relationship between the time frame and the shedding and the antibody patterns. The highest prevalence of detectable shedding of Salmonella typhimurium occurred three days PI while the highest population titer (demonstrated as the average of the $base_2$ logarithms of the titers of the individual birds) occurred at nine days PI. Both parameters decreased steadily after their respective peaks, and at 18 days PI, both shedding and titer levels were effectively zero.

Field Experiment

The data compiled from the field experiment is presented in Tables II, IV, and V. As presented in Table IV, there were no Salmonellae isolated from a total of 413 cloacal samples. Table II presents the species of ducks examined and sex of the ducks that were serologically active against group B antigens. Five of a total of 331 birds tested showed serologic activity at a titer of 1:32 or higher. There were no reactors to group C antigens at a titer of 1:32 or higher. Table V illustrates the species of ducks and sex of the ducks that were serologically active against group D antigens. Ten of a total of 331 birds tested showed serologic activity at a titer of 1:32 or higher. The total of 15 reactors of a total of 331 gave a prevalence of 4.5%. Drakes of all species combined had a prevalence of 5.5% reactivity at a titer of 1:32 or higher when hens of all species combined had a prevalence of 3.1%. There was, however, no significant difference at the 5% ($P>0.05$) level ($P=1.023$).

TABLE IV

CLOACAL *SALMONELLA* ISOLATION FROM WILD DUCKS, 1977-1978

Species	Sex	No. of Samples	No. of Isolations
Mallard	M	124	0
	F	118	0
American Widgeon	M	17	0
	F	3	0
Pintail	M	7	0
	F	3	0
GW Teal	M	51	0
	F	19	0
BW Teal	M	25	0
	F	8	0
Ringneck	M	8	0
	F	4	0
Lesser Scaup	M	9	0
	F	6	0
Redhead	M	8	0
	F	3	0
Total		413	0

TABLE V

SEROLOGIC REACTIVITY OF WILD DUCKS TO *SALMONELLA* GROUP D ANTIGENS, 1977-1978

Species	Sex	No. of Samples	Titer (inverse) 32	64	128
Mallard	M	117	2	1	1
	F	93	1	1	
American Widgeon	M	11			
	F	3			
Pintail	M	6			
	F	3			
GW Teal	M	26			
	F	11	1		
BW Teal	M	20	1	1	
	F	7			
Ringneck	M	8			
	F	4			
Lesser Scaup	M	8		1	
	F	6			
Redhead	M	5			
	F	3			
Total		331	5	4	1

Discussion

Interpretation of Laboratory Results

In Experiment I, three basic facts were observed. First, *Salmonella typhimurium* var. *copenhagen* infection in adult ducks did not cause any mortality at any of the concentrations used. All 25 orally-infected birds survived, and no outward signs of infection were noted. Secondly, following *per os* infection, the resultant persistent shedding state is short-lived (at 14 days PI, only 9% of the birds in all groups combined were still shedding). Thirdly, even though the actual shedding of *Salmonella* bacteria may be at a low prevalence after 14 days, 44% of the inoculated ducks remained infected internally when cultured at necropsy 18 days PI. While the majority of these remaining infections were in the gastrointestinal tract, infection of the gonads and other organs also occurred.

In Experiment II, three additional facts were observed. First, as a population, the initial shedding period is short-lived (15 days). An intermittant shedding state may continue in some individuals for some indeterminant time after the initial persistent shedding has stopped. These individuals may serve as infectious foci for horizontal transmission to other ducks or other animals, but this is probably rare. Only one bird out of twelve shed *Salmonella* organisms after 15 days PI. Secondly, almost half of the Experiment I birds remained internally infected (though not detectably shedding organisms). At 90 days PI, only one of seven birds maintained an intestinal infection. This reinforces the idea that salmonellosis in adult ducks is a transitory infection not prone to chronic tendencies. Thirdly, testing of antisera for

agglutinins to Salmonella antigens can be of value in identifying Salmonella-infected individuals. The individual antibody response, however, varies greatly and may not show a change at all. Three of twelve infected birds showed titers of 1:32 or above. This corresponds well to the guidelines established by the National Poultry Disease Guidelines (USDA) which list titers between 1:25 and 1:50 as positive reactors. However, serologic testing is not an absolute parameter as 9 of the 12 infected ducks in Experiment II did not have a significant antibody response. Therefore, the presence of a significant titer would be indicative of a positive reactor while the absence of a significant titer does not necessarily rule out a Salmonella infection.

In summation of the experimental data, the following parameters should be remembered. Adult ducks infected with Salmonella do not appear to suffer mortality, and a shedding state begins almost immediately after inoculation and usually lasts 14 to 15 days. Eventhough shedding may have ceased or may only occur intermittantly, Salmonella bacteria may be present in the internal organs of almost one-half of those birds infected. By 90 days postinfection, most of these cases are negative. Serologic reactivity may or may not increase to significant levels. Titers of 1:32 or higher should be considered a positive reaction. Reactions of 1:16 may be considered suspicious, but no evidence exists in this study to include this as a positive titer.

Comparison of Strontium Chloride B Broth and Selenite-Cystine Broth for the Enrichment of Salmonellae

By analyzing the results of Experiment I in test day blocks using a two-tailed t test, a comparison of the two media was accomplished.

Table VI illustrates the t statistic associated with each test day. The null hypothesis of the analysis was that there was no difference between the ability of each medium to recover Salmonella bacteria. Significance of difference at the .05 level and the medium to which the significance was favorable was noted in parenthesis. Each medium was significantly favorable on one of seven test days. The difference between the two media was not significant on five test days. With this analysis, there is no evidence that Selenite-cystine broth is different in its ability to recover Salmonella bacteria from a cloacal swab than Strontium chloride B broth.

Interpretation of Field Results

The low prevalence of Salmonella isolates and serologic reactivity from the field data indicates that salmonellosis may not be as common as is generally assumed. Since there were no Salmonella isolated out of 413 birds examined, it might be speculated that no disease potential exists in Oklahoma. However, the presence of even low levels of positive serologic reactors coupled with the fact that the data from Experiment II indicates that the serologic reactors may only reflect 25% of those birds actually infected and indicates that up to 18% of the population may actually be infected. It is difficult to pinpoint the time of infection since this study dealt with only non-juvenile birds. If one assumes, however, that the positive reactors were adult when exposed to Salmonella, then the birds were presumably infected before entering Oklahoma.

The fact that adult ducks can be infected with Salmonella bacteria and can shed these organisms in feces for up to 15 days after inoculation

TABLE VI

CALCULATED TWO-TAILED t STATISTICS COMPARING SELENITE-CYSTINE BROTH AND STRONTIUM CHLORIDE B BROTH FOR THE RECOVERY OF SALMONELLA FOR SEVEN TEST DAYS IN EXPERIMENT I

Day PI	t Statistic
2	1.414 (not significant)
4	2.585 (significant in favor of Strontium Cl B)
6	2.750 (significant in favor of Selenite-Cystine)
8	0.500 (not significant)
10	0.500 (not significant)
12	0.569 (not significant)
14	0.569 (not significant)

indicates that a dissemination potential does exist. This potential is of even greater importance when one considers the concentrations of waterfowl on wintering grounds. Fall migration flights could bring the bacteria to the wintering areas where it could be disseminated to other birds thereby propagating and magnifying the infection. Infected, shedding birds leaving the wintering areas pose a transmission threat to other animals, other waterfowl, and, upon their return to their breeding grounds, to that year's hatch of ducklings. Since the high mortality sometimes attributed to salmonellosis is usually associated with very young birds, this threat would indeed be serious if the infection were widespread. Studies of salmonellosis on the breeding grounds might be warranted to see if this disease is being brought in and from where the returning adults are becoming infected.

Since Salmonella typhimurium is a group B organism and there were twice as many reactors to group D antigens than group B antigens, S. typhimurium did not appear to be the major serotype encountered by those birds who were serologically reactive. A possible explanation for the higher number of group D reactors could be the continued presence of S. gallinarum and S. pullorum, both group D organisms, in domestic poultry in those states which comprise the Central Flyway (Avian Diseases, 1978). There are, however, many other group D serotypes with which free-ranging waterfowl could come in contact.

Summary

Laboratory studies showed that infection of non-juvenile ducks by Salmonella typhimurium resulted in no mortality and no recognizable morbidity. The shedding state as detected by cloacal swab lasted 14-15

days but intermittant shedding did occur. Even after the initial shedding state had ceased, some of the organs of the inoculated birds remained infected. Infection in these internal organs was usually lost by 90 days postinfection. Organs most commonly infected were in the gastrointestinal tract. The intestines also were found to retain the infection longer than other non-alimentary organs. Serologic changes did not occur in all cases of infection, and only titers of 1:32 or greater actually reflected infection.

In the field study, no Salmonella species were isolated out of 413 samples. Of 331 serologic samples, five were reactive to Salmonella group B antigens, none were reactive to group C antigens, and ten were reactive to group D antigens. The total of 15 reactors of 331 gave a prevalence of 4.5%. Because of the lack of a serologic response in percentage of experimentally infected cases, the true prevalence of infection in the wild population could be higher. There was no statistical difference between hens and drakes concerning serologic prevalence rates.

There was no statistical difference between Strontium Chloride B enrichment broth and Selenite-cystine enrichment broth for the recovery of Salmonella organisms from a cloacal swab.

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