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A Survey of the Incidence of Salmonellosis in Three Groups of Wild Animals

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A Survey of the Incidence of Salmonellosis in Three Groups of Wild Animals

(TITLE)

BY

James T. Heiberger

THESIS

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A Survey of the Incidence of Salmonellosis in Three Groups of Wild Animals

by

James T. Heiberger

a candidate for the degree of Master of Science

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Date

A Survey of the Incidence of Salmonellosis in Three Groups of Wild Animals

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James T. Heiberger

B. A. Colorado College, 1964

Submitted in partial fulfillment

of the requirements for the degree

of Master of Science in Zoology

at the Graduate School of Eastern Illinois University.

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INTRODUCTION

Salmonella are enteric pathogens of man, domestic animals, and wild animals; all known species of salmonellae are pathogenic to warm-blooded animals (Edwards and Ewing, 1967). Infection is normally squired via the oral route. Somatic and flagellar lipopolysaccharides liberated upon lysis of the bacterial cell, act as endotoxins, producing gastroenteritis, enteric fever, or septicemia in the infected host shortly after ingestion. Infected animals may die from *Salmonella* infection, or from complications initiated by the organism. Others may successfully excrete the organism in the feces, or become a reservoir-host, manifesting periodic evidence of the bacteria in the feces.

The majority of reported isolations of the genus *Salmonella* are from outbreaks in man and domestic animals. Comparitively few isolations have been reported from rodents (Khalil, 1938; Elton, 1942; Dalmat, 1944; Edwards *et al.*, 1948; Lee, 1955), and only recently have isolations been reported from reptiles (Hinshaw and McNeil, 1944; Hinshaw and McNeil, 1945; Le Minor *et al.*, 1958; Marx *et al.*, 1964), and birds (Paddoul *et al.*, 1966; Wilson and Macdonald, 1967; Cornelius, 1969).

Rodents, snakes and predatory birds are components of a food web in ecological communities of east-central Illinois, and *Salmonella* infections in these vertebrates have a potential of becoming widely disseminated in the community, particularly during rodent population explosions. This thesis is an epidemiological survey for salmonellae isolated from the intestinal fauna from a sample of these three groups of animals. The study was conducted to determine the incidence of salmonellosis and the relationship of infection to the food web of these animals.

LITERATURE REVIEW

Salmonella is a genus of the tribe Salmonellae and the family Enterobacteriaceae (Edwards and Ewing, 1967; Burrows, 1968). The first isolation of a member of the genus *Salmonella* was described in 1880 as *Bacterium typhi* (Edwards and Ewing, 1967). By 1920, a number of strains of the genus, differing in biochemical properties, had been isolated, and exact identification of members of the genus became confused. During the 1920's, the identity of the species was clarified by means of biochemical differentiation combined with antigenic analysis (Edwards and Ewing, 1967). These methods were reaffirmed and improved upon in the 1940's (Edwards and Ewing, 1967), and today there are 1800 serotypes described in the tribe Salmonellae, including the *Arizona* group (Edwards and Galton, 1967; Taylor, 1969). The *Arizona* group is biochemically and antigenically similar to the *Salmonella* group, with the exception that *Arizona* strains, in contrast to *Salmonella*, ferment lactose and fail to ferment dulcitol. Clinically, both groups produce similar diseases (Edwards and Ewing, 1967). The *Arizona* group is pathogenic for cold-blooded animals, fowl, and man (Burrows, 1968).

The genus *Salmonella* is defined in the following manner (Edwards and Ewing, 1967): "Usually motile, but non-motile forms occur. Produce acid and gas from glucose, maltose, mannitol and sorbitol (Except that in *Salmonella typhosa* and *Salmonella gallinarum* no gas is produced). Lactose, sucrose, and salicin are not attacked. Do not clot milk, form indole or liquefy gelatin. Reduce trimethylamine oxide to trimethylamine. All of

the known species are pathogenic for warm-blooded animals, including man, causing food infections and enteric fevers. A few are found in reptiles. Some or all may also live in decomposing foods." Also, "A large genus of serologically related, gram-negative and non-sporing bacilli; 0.4 to 0.6 x 1 to 3 microns in usual dimensions but occasionally forming short filaments; showing, with certain exceptions a motile peritrichous phase in which they normally occur; in fact adhering to the pattern of S. typhi in staining properties and morphology..."

Host Range

Isolations of salmonellae have been reported most frequently in human cases of salmonellosis. Next, in order of importance to public health, domestic animals, particularly swine herds and poultry flocks, have been investigated (Edwards et al., 1967). Free-living wild animals have incidentally been surveyed for the presence of salmonellae infections. Wild mammals, wild birds, and reptiles may be healthy, symptomless carriers of Salmonella, and act as reservoirs of the bacteria (Boycott et al., 1953; Buxton, 1957; Edwards and Galton, 1967; Taylor, 1969). Taylor (1969) concluded that certain Salmonella serotypes are host specific, that is, the bacterium is pathogenic for one host and relatively non-pathogenic to other species. Salmonella enteritidis var. danysz, for example, has been isolated exclusively from wild rats and mice; the organism often causes severe outbreaks in these rodents, but is not known to infe t other animals.

Order Rodentia

Salmonella have been associated with natural epizootics in rodents. During investigations of overpopulation and associated epizootics in voles (Microtus spp.) in southern England, Elton et al. (1931) conjectured that intestinal bacteria of the Salmonella type were occasionally responsible for natural infection in these populations. Salmonellae were also considered as a common infection in house mice (Mus musculus) in England (Jones and Wright, 1936; Chitty and Southern, 1954).

Several strains of salmonellae have been isolated from rodents. Loeffler, in 1892, isolated Bacterium (Salmonella) typhi-murium from white Swiss mice, and when this organism was introduced into vole (Microtus arvalis and M. agrestis) populations, an epidemic was induced. Bacillus (Salmonella) enteritidis was isolated from spleen and liver lesions in wild house mice (M. musculus) in Australia, where the organism was noted to produce natural epidemics in this rodent (Elton, 1942). In a five and ten year study of the distribution of Salmonella spp. in the United States, S. typhi-murium and S. enteritidis accounted for 92% of the salmonellae cultured from rodents (Edwards and Bruner, 1943; Edwards et al., 1948). There were 101 reported isolations of salmonellae from rodents during this ten year period. No information was given on the species of rodents from which the isolations were made, the number of rodents tested, or of the reasons for culturing them.

As a result of laboratory mortality of trapped white-footed mice (Peromyscus leucopus), Delost (1944) isolated S. typhi-murium from 19 of 70 (27%) of these mice during a subsequent trapping period. During the winter of 1959, Derrick and Pope (1960) studied a population of mice

(M. musculus) of plague proportions in Australia. Salmonella bovis-morbificans was isolated from 3 of 41 (6.8%) mice captured. Brown and Parker (1957) isolated S. stanley and S. typhi-murium from 3 of 364 (0.8%) house mice (M. musculus) trapped in the city and on the docks of Manchester, England.

Isolations have also been recorded from wild rats living in or near human habitations. A total of 842 wild rats (Rattus rattus and R. norvegicus) were tested during a four year study in Australia. An average of 3% of the rats were infected, although no Salmonella outbreaks were known to have occurred in either rats of man (Lee, 1955). Salmonella types were isolated from 55 of 750 (7.3%) and 22 of 779 (2.8%) R. rattus and R. norvegicus in two studies of rats from metropolitan areas in England (Khalil, 1938; Brown and Parker, 1957).

Experimental studies on the epidemiology of salmonellosis in rodents have provided information on stress and infection. An induced epidemic of S. enteritidis was studied on a farm near Baltimore by Davis and Jensen (1952). Twenty Norway rats (R. norvegicus) were innoculated with S. enteritidis in February and October, 1950, and released in the farm buildings. The infection spread slowly in the population on the farm for two years. An increased rate of infection, however, was noted when the rats were stressed by a depletion in food or shelter, or by a forced movement of the population. Khalil (1938) examined 750 wild R. rattus and R. norvegicus for salmonellae, and found 55 (7.3%) positive. During the winter quarter, 17.6% of the rats trapped were positive, while only 2.2% trapped during spring and summer quarters were positive. Stress

in the rats, possibly precipitated by winter weather conditions, was believed to have caused the increased rate of infection. Delmat (1944) conjectured that mortality from salmonellosis in white-footed mice (P. leucopus) in traps and in cages resulted from stress brought about by very warm fall weather, combined with the stress of trapping and caging.

Stress has been shown to cause an increase in the incidence of Salmonella infection in mice. A higher incidence of infection of S. enteritidis was noticed in white Swiss mice (M. musculus) innoculated and stressed by starvation, as compared to a control population, innoculated and experiencing normal living conditions (Miller et al., 1952). The higher incidence of infection was attributed to a lowering of host resistance. In another laboratory study, colonies of white laboratory rats and mice were infected with S. enteritidis merely by the presence of an infected rat or mouse (Welch et al., 1941), indicating that crowded conditions, increasing contact among the animals, were responsible for the decline in host resistance and therefore a higher susceptability to the disease.

Class Reptilia

The first isolation of salmonellae from snakes was from a liver culture of a gopher snake (Pituophis catenifer) caught on a farm in California (Minshaw and McNeil, 1944). Turkey flocks on the same farm were experiencing an epidemic of salmonellosis, and the snake was considered as being a possible reservoir or vector of infection. Further studies showed that 9 of 28 gopher snakes, 2 of 4 garter snakes (Thamnophis

hammondii), 0 of 4 racer snakes, and 0 of 1 rattlesnakes, caught in the wild in California yielded Salmonella types (Hinshaw and McNeil, 1945). Le Minor et al. (1964) isolated Arizona strains from 21 of 100 Sandvipers (Vipera ammodytes ammodytes) in Bavaria in 1963. An investigation of snakes, turtles, and lizards in several zoos in Germany and Switzerland resulted in isolations of 35 Salmonella strains from 191 (18.3%) reptiles tested (von Rudat et al., 1966). The incidence of infection in confined reptiles should not be compared to the incidence in wild reptiles, since crowding and stress may reduce host resistance to disease.

A high incidence of salmonellosis has been recorded in healthy turtles. Outbreaks of salmonellosis in humans in England were traced to turtles (Testudo graeca) imported from Morocco and sold as pets (Boycott et al., 1953). In the study, 17 species of Salmonella were isolated and identified from 11 turtles and two pools of larger numbers. All of the hosts were healthy carriers. Kaufmann and Morrison (1966) isolated S. [illegible] from the ovaries and bile deposits of 10 of 20 red-eared turtles (Pseudemys scripta elegans) on a turtle farm in Mississippi. Jackson et al. (1969) isolated Salmonella types from 2 of 53 (3.8%) wild turtles (Chelydra sp. and [illegible] sp.), and 11 of 123 (9.0%) captive turtles (Geochelone spp., [illegible] spp., Pseudemys spp., Macrochelys sp., and Geomyda sp.). Apparently other studies have been made on turtles since Taylor (1969) reports that in some studies, 96% of the wild tortoises (species not given) tested have carried salmonellae.

Class Aves

Salmonellosis is an important disease in poultry (Edwards et al.,

1948), but only limited investigation has been initiated on wild birds. In a one year study, 100 dead wild birds were collected in a survey of causes of death (Faddoul et al., 1966). Positive salmonellae indentifications were recorded from 8 brown headed cowbirds (Molothrus ater), 2 house sparrows (Passer domesticus), 1 white throated sparrow (Zonotrichia albicollis), and 1 herring gull (Larus argentatus). Salmonella typhi-murium was isolated from all birds except that S. derby was isolated from the herring gull. Stress, initiated by cold weather, and flocking at feeding stations was considered as a factor in the spread of the infection. Hudson and Tudor (1957) isolated S. typhi-murium from 3 starlings, 7 sparrows, 6 rusty blackbirds, and 1 cowbird (species not given). All of the birds were found dead, and all but the cowbird were collected during winter in north-central New Jersey. Flocking was believed to have been instrumental in the spread of this disease.

Wilson and Macdonald (1967) isolated salmonellae from 17 of 2,715 (0.6%) birds collected from 1939 to 1964, and concluded that birds are not an important reservoir of salmonellosis. Cornelius (1969) reported numerous sick and dead greenfinches (Chloris chloris) and house sparrows (P. domesticus) during several consecutive winters in southern England. Salmonella typhi-murium was isolated from 5 house sparrows (P. domesticus), 7 greenfinches (Chloris chloris), and 1 dunnock (Prunella modularis) in 1966. The same organism was isolated from 5 house sparrows and 8 greenfinches in winter, 1968. Outbreaks in birds were noted in all parts of southern England during the same winter. Cornelius conjectured that flocking was a factor related to the spread of infection from bird to

bird. In a study of an epizootic among seabirds in Scharnhörn, Germany, von Steiniger (1967) isolated 11 Salmonella serotypes from 62 of 108 (57%) Brandseeschwalbe, 98 of 271 Flussseeschwalbe (36%), and 17 of 68 (26%) Silbermöve. The species were not given. The author attributed the epizootic to stress precipitated by cold, wet, winter weather.

Epidemiology of Salmonellosis in Free-living Wild Animals

Salmonellosis is a zoonosis (van Kaulen, 1959). Jawetz et al. (1962) state that wild animals, cattle, rodents, and fowl may be naturally infected with the organisms, and may carry the bacteria in their tissues or feces.

The most commonly reported source of salmonellae is the consumption of food which has been contaminated with feces from an infected animal or man. Jones and Wright (1936) for example, recorded an outbreak of S. typhi-murium in five humans in England. The outbreak was directly related to the isolation of the bacteria from mouse excreta in foodstuffs.

Animals and humans may be healthy carriers of salmonellae, and periodically excrete the bacteria in the feces. Li and Davis (1952) isolated salmonellae from feces of infected wild rats for seven consecutive weeks. White Swiss mice, orally infected in the laboratory, shed an avirulent strain of S. typhi-murium for four weeks (Morello et al., 1965). The organism may also remain viable in the excreted feces. Feces of infected rats, kept at room temperature for 148 days, then cultured, showed isolates of Salmonella spp. (Welch et al., 1941).

In 5 and 10 year reports on the distribution of Salmonella spp. in the United States, Edwards et al. (1948) concluded that numerous cases of

human salmonellosis were directly attributable to fecal contamination of foodstuffs by wild rodents. *Salmonella enteritidis* was isolated mainly from rodents (species not given); therefore, human cases of *S. enteritidis* were attributed to wild rodents.

Animals which exist in an ecological community are integral parts of a food web, which can have a bearing on the spread of infectious diseases. Audy (1958) considered migratory birds of importance in the spread of various infectious diseases. Raptorial birds, feeding on rodents in Utah, have been considered as possible vectors for dissemination of rodent-borne diseases to other areas (Bushman, 1955).

The literature indicates that salmonellae are present in many species of birds, sometimes precipitating epizootics. There is some question however, as to the role of birds in the passage of the disease. Wilson and Macdonald (1967) concluded that birds were not an important reservoir of salmonellosis, while Taylor (1969) stated that it was reasonable to assume that *Salmonella* may be an important cause of death in birds, and concurred with Hudson and Tudor (1957), Faddoul *et al.* (1966), and Cornelius (1969) that flocking might cause the passage of salmonellae from bird to bird. Hudson and Tudor (1957) believed that birds may play a part in spreading *S. typhi-murium* to man and domestic animals.

Predators have aquired bacterial infections by consuming infected prey. Reilly (1966), for example, successfully infected 3 of 4 types of carnivorous predators with *Leptospira grippotyphosa* by feeding them infected laboratory mice. Although there have been no reports of salmonellae being transmitted by such a food chain, indirect evidence suggests that such relationships do exist. Elton (1942) investigated salmonellosis

epidemics during population explosions of mice (M. musculus) and voles (Microtus spp.), and observed owls and kestrels migrating to the areas of the rodent population explosions. Elton suggested that the predator-prey relationship could result in infection of predators upon feeding on the rodents.

Further indirect evidence of food chain transmission of salmonellae was reported during an outbreak of salmonellosis in poultry flocks (Hinshaw and McNeil, 1944). Cultures from wild animals captured on the farms resulted in isolation of salmonellae from 1 gopher snake, 1 garter snake, 2 domestic cats, and 4 pools of house flies. The snakes and cats may have become infected by consuming infected chickens or mice.

METHODS AND MATERIALS

The epidemiology of salmonellosis in three groups of wild animals in east-central Illinois was investigated during a 12 month period by trapping animals and culturing their intestinal contents; biochemically and serologically identifying suspected salmonellae; and by comparing the isolates to determine the relationship of salmonellae to a food web of animals. Three groups of wild animals were investigated: predatory birds, snakes, and rodents.

Animal capture

Sparrow hawks (Falco sparverius) were captured in bal-chatri traps (Berger and Mueller, 1959), and great horned owls (Bubo virginianus) were taken from nests in a 30 square mile area in Cumberland, Coles, and Shelby Counties from March to September, 1968. Cloacal swabs were taken of each bird within 24 hours of capture, using sterile cotton-tipped applicators.

All live snakes collected for the Eastern Illinois University vertebrate collection from March to December, 1968, were sampled within 30 days from the time of capture. Cloacal swabs were performed using sterile cotton-tipped applicators. Ether was used to kill three snakes, and the cloaca and lower intestine were dissected open, and a sterile applicator was used to swab the exposed lumens. The period of time between capture and swabbing varied several weeks because of the snakes ability to survive weeks without sustenance.

Rodents were trapped in nine different areas in Coles and Shelby Counties, Illinois using Sherman live-traps and snap traps. The traps were placed in a line of stations 100 yards long, four traps to a station

at intervals of ten yards. Five of the areas were grasslands adjacent to roads and railroads, while four areas were farm pastures.

Live trapped rodents were killed with ether within 24 hours of capture. The skin was removed and an abdominal incision was made antiseptically, exposing the viscera. At this point, several methods of obtaining intestinal samples were attempted:

1. A section of (1) stomach, (2) ileum, and (3) colon were removed from the rodent and dissected open. The contents of each segment were swabbed with a sterile applicator, and placed in three separate tubes of tetrathionate broth enrichment medium.
2. Two or three inches of (1) small intestine, and (2) colon were removed from the abdominal cavity, and each section was macerated and placed in a separate culture tube of tetrathionate broth medium.
3. Two or three inches of (1) colon, and (2) the entire caecum were removed from the abdominal cavity, and up to 0.5 cc of the contents were pooled into one culture tube of tetrathionate broth medium.

Isolation of Salmonella

All fecal specimens were innoculated into 10 cc of tetrathionate broth medium and incubated at 37° C for 24 to 72 hours. One loopfull from each tetrathionate culture was then streaked on one plate each of MacConkey agar, SS agar, and brilliant green agar (Difco Manual, 1968). Three loopfulls of the tetrathionate broth were also streaked on one plate of bismuth sulfite agar (Difco Manual, 1968). The MacConkey agar, SS agar, and brilliant green agar were incubated at 37° C for 24 hours. Bismuth

sulfite agar plates were incubated at 37° C for 48 hours.

After incubation, all plates were examined for colonies characteristic of Salmonella. All plates exhibiting no colonies resembling Salmonella were discarded. All plates showing positive suspect colonies were retained, and one suspect colony from each plate was restreaked on MacConkey agar for a pure culture specimen. A colony from the pure culture was then streaked on a nutrient agar slant and incubated at 37° C for 24 hours and then stored at 4° C.

Each suspect culture was then picked from the nutrient agar slant and innoculated onto a primary differential medium (triple sugar iron (TSI) agar), a secondary differential medium (urea broth), and a 1.0% tryptone broth solution. All differential media were then incubated at 37° C. The TSI agar and urea broth readings were taken at 12, 24, and 40 hours. Indole tests were performed on the tryptone broth solution after 18 hours.

Suspected salmonellae cultures were then regrown on a fresh nutrient agar slant. The cultures were suspended in sterile saline in the culture tubes, and drops of the suspension were pipetted onto a clean glass plate, and tested for agglutination reactions with polyvalent Somatic O Antisera purchased from the Difco Company (Edwards and Ewing, 1967). One drop of the suspect saline solution was mixed with one drop each of eight different polyvalent antisera, and the plate was gently swirled for five minutes. Colonies which agglutinated the polyvalent antisera were taken to the College of Veterinary Medicine, University of Illinois, where further tests were performed for action on dextrose, lysine, dulcitol, and oxidase.

A diagrammatic scheme for the procedure used to culture and identify salmonellae types from fecal specimens follows:

Fecal specimen

Tetrathionate Enrichment broth

Primary Plating media	MacConkey agar
	SS agar
	Brilliant Green agar
	Bismuth Sulfite agar
Pure culture	MacConkey agar
Storage	Nutrient agar
Differential Selective media	Triple Sugar Iron agar
	Urea broth
	Indole
	Dextrose
	Lysine
	Dulcitol
	Oxidase
Serological identification	Polyvalent, Somatic O
	Antisera

Food chain experiment

An experiment was performed to determine if salmonellae could be passed from an infected, prey animal to a non-infected predator. Five prarie king snakes (*Lampropeltis calligaster*) were stressed by fasting for 30 days. Cloacal swabs taken on day 1, 5, and 11, were cultured to demonstrate the absence of salmonellae. All snakes showing colonies resembling salmonellae were eliminated. Snakes showing no colonies resembling salmonellae on both primary plating media and differential selective media were fed suckling laboratory mice which had been

innoculated with a 24-hour Nutrient broth culture of S. typhi-murium isolated from a human by the State of Illinois Diagnostic Laboratory. Cloacal swabs were taken 12 and 24 days post-feeding, and the feces cultured. The cultures were then tested for S. typhi-murium using the prodedure described for wild animals.

RESULTS

A total of 209 birds, snakes, and small mammals collected in east-central Illinois from March, 1968 to March, 1969 were cultured for salmonellae (Table 1). The majority of the animals (86%) were collected in Coles County, although specimens were accepted from 11 other counties. Rodents and insectivores were collected predominantly during summer and fall quarters of 1968, while snakes and predatory birds were captured predominantly during the spring of 1968 (Table 2).

Fecal samples or cloacal samples from each of the animals collected were cultured in tetrathionate broth, then transferred to primary plating media (MacConkey agar; SS agar; brilliant green agar; bismuth sulfite agar). Suspect colonies were picked from the primary plating media (Fig. 1) of 176 animals (Table 3), and streaked on MacConkey agar to obtain pure cultures. Biochemical tests using triple sugar iron agar, urea broth, and tryptone (indole test)(Fig. 2), were carried out on 226 suspect cultures from the 176 animals (Table 4). A total of 52 suspect cultures were isolated. Fourteen of the 52 cultures resembled Salmonella: that is, cultures showing an acid butt and an alkaline slant, H_2S production on TSI agar, urea negative, and indole negative. An additional 38 cultures had the same biochemical characteristics except that H_2S was not produced on TSI agar.

Serological testing (Fig. 3) of the 52 suspects revealed that 12 cultures agglutinated polyvalent antisera. Additional biochemical tests carried out at the College of Veterinary Medicine, University of Illinois

(Table 5) showed 3 of the 12 specimens to be characteristic of the tribe Salmonellae. No Salmonella were isolated, however, specimens S-5 and S-170 were Arizona strains, and M-162 was a Citrobacter strain (Table 3). Culture S-170 was submitted to the National Animal Disease Laboratory, Ames, Iowa, for definitive serotyping. The culture was identified as Arizona 25: 27-28.

Specimen S-5 was a water snake (Natrix sipedon) captured near the Embarrass River, Coles County, Illinois in April, 1968. A culture specimen was taken from the snake while it was alive. Specimen S-170 was a cottonmouth snake (Agkistrodon piscivorous) captured in Johnson County, Illinois in September, 1968. A culture specimen was taken from the dissected and exposed cloaca and lower intestine of the killed snake. Specimen M-162 was a Citrobacter strain and therefore not considered a pathogen.

A control experiment, designed to show that a Salmonella sp. could be transmitted via a food chain of animals was initiated using prairie king snakes (Lampropeltis calligaster) and suckling laboratory mice. After determining two of the snakes to be free of Salmonella infection, suckling mice were innoculated with a 24-hour broth culture of S. typhi-murium and fed to the snakes. Post-innoculation fecal samples taken 12 days after feeding the snakes, were positive, i.e., polyvalent antisera Group A-I was agglutinated by a pure culture suspect from each of the 12 day fecal samples.

Table 1. Wild animals collected in east-central Illinois from March, 1968 to March, 1969 and cultured for salmonellae.

Species	Number collected by county							
	Coles	Cumberland	Douglas	Macon	Crawford	Effingham	Edgar	* other
Class Aves								
Falco sparverius	4	7						
Bubo virginianus		2				2		
Class Reptilia								
Thamnophis sirtalis	7			1			1	2
Lampropeltis calligaster	8		2		1		1	
Elaphe obsoleta	3							
Natrix sipedon	1		1					2
Thamnophis radix	2							
Pituophis melanoleucus				1				
Storeria occipitomaculata	1							
Agkistrodon piscivorous								1
Opheodrys aestivus	1							
Coluber constrictor	1				1			
Thamnophis sauritus	3							
Heterodon platyrhinos	1							
Farancia abacura	1							
Order Rodentia								
Microtus ochrogaster	22							
Mus musculus	63							
Peromyscus leucopus	11	2						
Peromyscus maniculatus	7							
Synaptomys cooperi	20							
Order Insectivora								
Blarina brevicauda	3							
Cryptotis parva	23							
Total	182	11	3	2	2	2	2	5

* Other counties: Christian, Johnson, Richland, Shelby, and McHenry

Table 2. Seasonal trapping results in a *Salmonella* survey of wild animals in east-central Illinois from March, 1968 to March, 1969.

Quarter	Number of animals cultured			
	Mammals	Snakes	Birds	Total
Spring (April to July, 1968)	8	35	10	53
Summer (July to Oct., 1968)	80	0	5	85
Fall (Oct. to Jan., 1968)	45	7	0	52
Winter (Jan. to April, 1969)	19	0	0	19
Total	152	42	15	209

Table 3. Biochemical and serological reactions of suspected *Salmonella* types isolated from wild animals captured in east-central Illinois.

Species	Number tested	Number of animals suspect as result of: primary plating	biochemical tests	serological tests	Result*
Class Aves					
Falco sparverius	11	6	2		
Bubo virginianus	4				
Class Reptilia					
Thamnophis sirtalis	11	10			
Lampropeltis calligaster	12	12	2		
Elaphe obsoleta	3	3	1		
Natrix sipedon	4	4	1	1	*Arizona* sp.
Thamnophis radix	2	2			
Pituophis melanoleucus	1	1			
Storeria occipitomaculata	1	1			
Agkistrodon piscivorous	1	1	1	1	*Arizona* sp.
Opheodrys aestivus	1	1			
Coluber constrictor	2	2			
Thamnophis sauritus	3	3			
Heterodon platyrhinos	1	1			
Farancia abacura	1	1			
Order Rodentia					
Microtus ochrogaster	22	9	4		
Mus musculus	63	63	28	2	
Peromyscus leucopus	13	13	8	2	*Citrobacter* sp.
Peromyscus maniculatus	7	7	3	2	
Synaptomys cooperi	20	10	6	4	
Order Insectivora					
Blarina brevicauda	3	1	1		
Cryptotis parva	23	21	5		
Total	209	176	62	12	

* Results of biochemical and serological testing carried out at the College of Veterinary Medicine, University of Illinois.

Table 4. Biochemical and serological reactions of 225 suspected isolates of *Salmonella* from 176 wild animals.

Primary plating media	Number of isolates	Reactions: TSI pH*	TSI H_2S	Urea	Indole	Polyvalent Somatic Antisera - Groups: A-I	A	B	C	D	E	F	G
MacConkey agar	6	+	+	-	-	-	-	-	-	-	-	-	-
	28	+	-	-	-	-	-	-	-	-	-	-	-
	10	+	+	+	-								
	32	+	-	+	-								
	9	+	-	-	+								
	1	-	+	-	-	+	-	-	-	-	-	-	-
	1	+	-	-	-	+	-	-	+	-	-	-	-
	2	+	-	-	-	+	-	-	-	-	-	-	-
SS agar	6	+	+	-	-	-	-	-	-	-	-	-	-
	25	+	-	-	-	-	-	-	-	-	-	-	-
	8	+	+	+	-								
	33	+	-	+	-								
	13	+	-	-	+								
	1	+	+	-	+								
	1	+	+	-	-	-	-	-	+	-	-	-	-
Brilliant Green agar	1	+	+	-	-	-	-	-	-	-	-	-	-
	14	+	-	-	-	-	-	-	-	-	-	-	-
	3	+	+	+	-								
	1	+	-	+	-								
	5	+	-	-	+								
	1	+	+	-	-	+	-	-	-	-	-	+	+
	4	+	-	-	-	+	-	-	-	-	-	-	-
	1	+	-	-	-	-	-	-	-	-	-	-	+
	1	+	-	-	-	+	+	+	+	+	-	-	-
Bismuth Sulfite agar	1	+	+	-	-	-	-	-	-	-	-	-	-
	13	+	-	-	-	-	-	-	-	-	-	-	-
	1	+	+	+	+								
	3	+	-	+	-								
	3	+	-	-	+								

* + = acid butt, alkaline slant

Table 5. Biochemical reactions of 12 suspected isolates of *Salmonella* from 176 wild animals.[1]

Number of isolates	Reactions											
	Kligers		Urea	Indole								
	pH	H_2S			Dextrose	Lactose	Maltose	Sucrose	Lysine	Dulcitol	Oxidase
1	+	+	-	-	G				-	-	-
1	+	+	-	-	G				-	-	
7	+	-	-	-	G				+	-	
2	+	-	-	-	G				-	-	
1	+	+	-	-	G	-	G	-	+	-	-
12											

1 Tests performed at the College of Veterinary Medicine, University of Illinois.

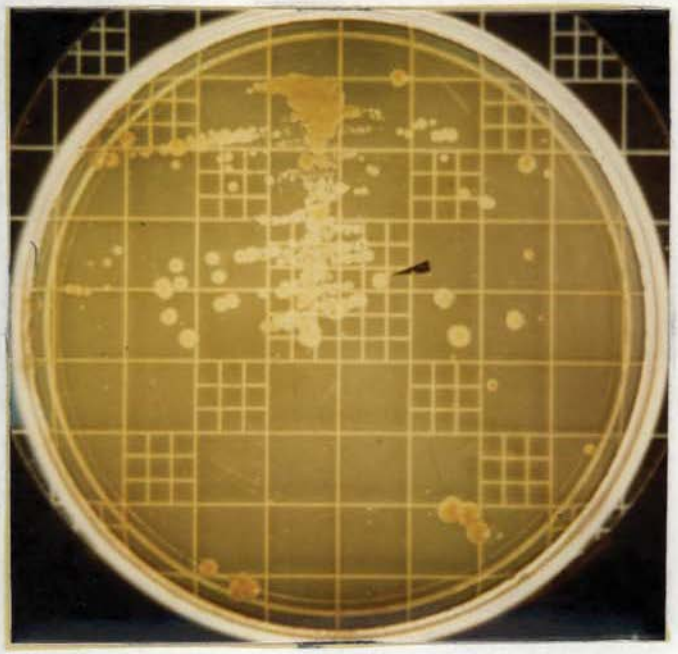
Figure 1. Typical 24-hour cultures of suspected Salmonella grown on primary plating media at 37° C.

(1) MacConkey agar: arrow points to transparent, uncolored colony suspect.

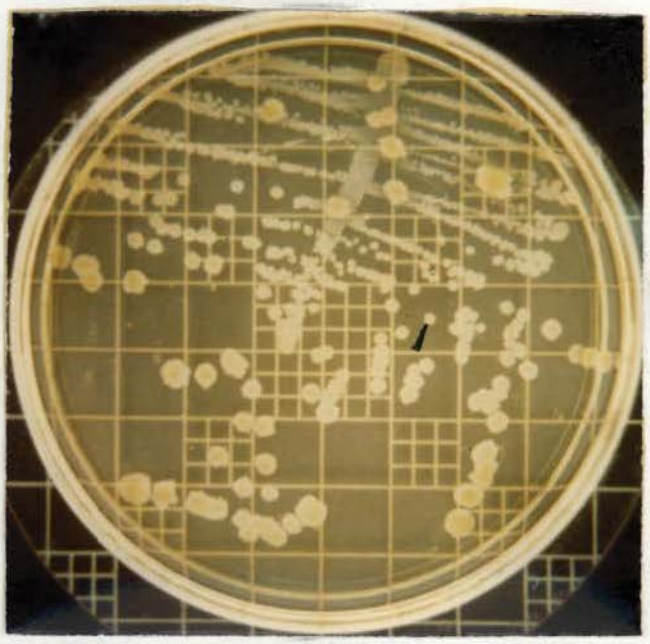
(2) SS agar: arrow points to smooth, uncolored colony suspect.

(3) Brilliant Green agar: arrow points to opaque colony suspect surrounded by brilliant red medium.

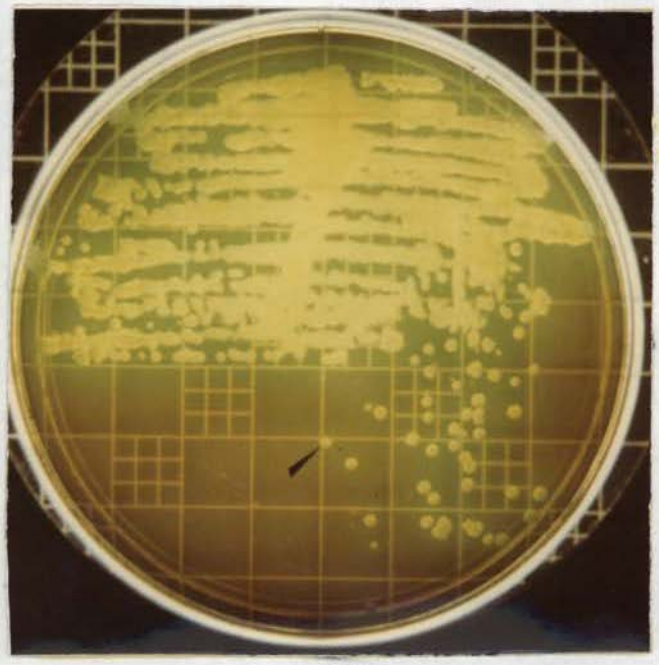
(4) Bismuth Sulfite agar: (incubated for 72 hours) arrow points to black, H_2S producing colony suspect.



(1)



(2)



(3)

(4)

Figure 2. Typical 18-hour cultures of suspected Salmonella grown on differential selective media at 37° C.

Innoculated bacteria:

Tube "A": Salmonella typhi-murium

Tube "B": Escherichia coli

Tube "C": Control (no bacteria)

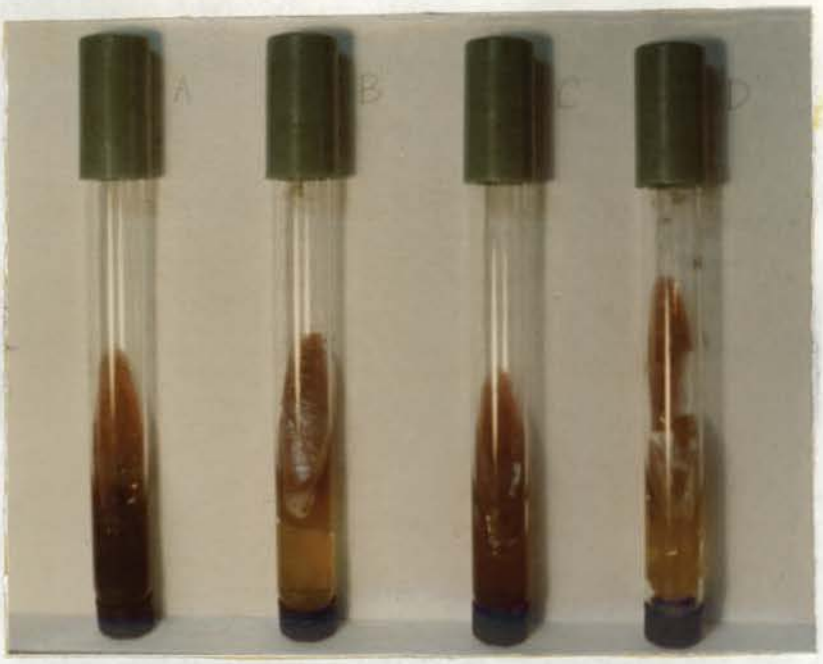
Tube "D": A pure culture suspect from a rodent

(1) Triple Sugar Iron agar: typical Salmonella culture will leave the slant unchanged, produce an acid butt (yellow), and produce H_2S (black) in the butt.

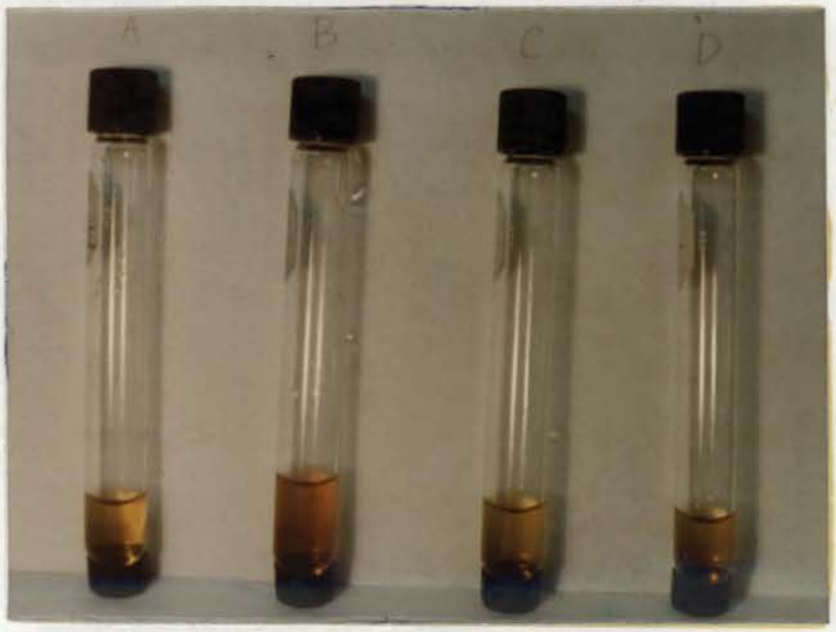
(2) Urea broth: Salmonella culture will not change urea, but contaminants will change color of broth from yellow to red.

(3) Indole production: Salmonella culture will not produce indole, but contaminants will c ange color of solution from yellow to red on the surface layer.

(1)



(2)



(3)

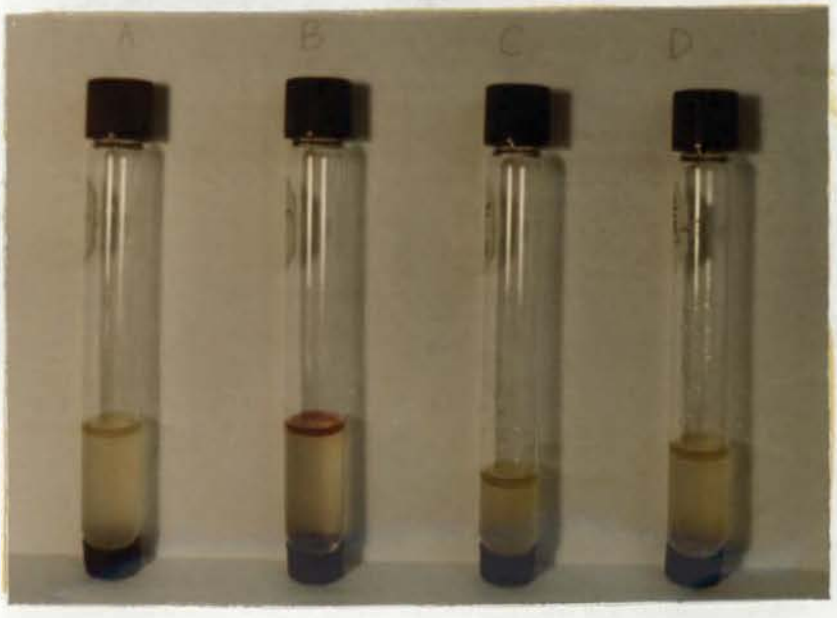


Figure 3. Agglutination tests for Salmonella using Polyvalent Somatic O Antisera - Groups: A-I, A, B, C, D, E, F, G.

Bacteria tested:

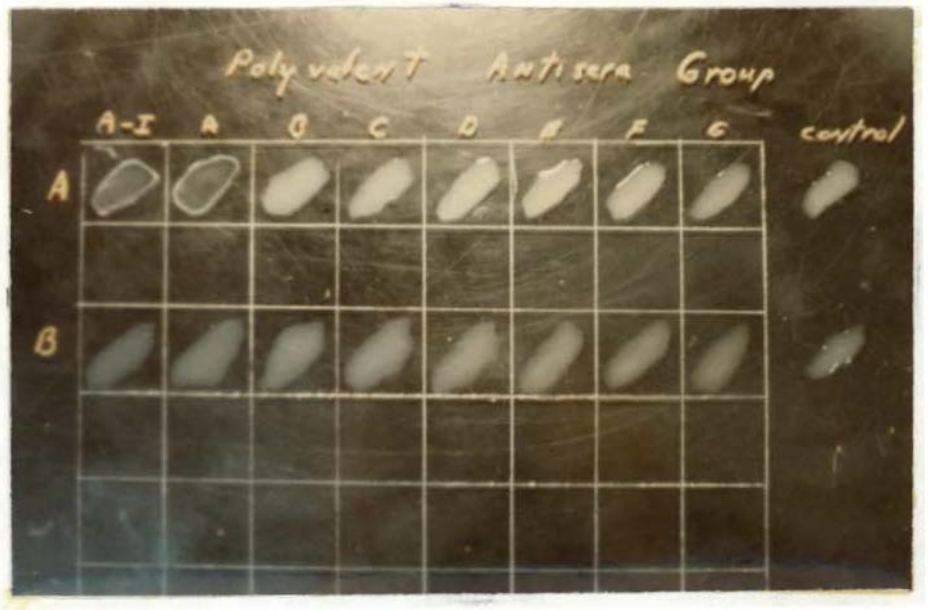
Row "A": Salmonella typhi-murium

Row "B": Escherichia coli

Row "A" (Salmonella typhi-murium) shows positive agglutination with Antisera Groups A-I, and A.

Row "B" (Escherichia coli) shows no agglutination.

Control column shows no agglutination.


Polyvalent Antisera Group
A-I
A
B
C
D
E
F
G
control
A
B

DISCUSSION

Rodents, snakes and birds are integral parts of a food web in most biotic communities. Few *Salmonella* studies (Elton, 1942; Hinshaw and McNeil, 1944) have involved all three groups of animals in this natural relationship to one another. Since it has been speculated that diseases can be transmitted via a food chain (Elton, 1942; Hinshaw and McNeil, 1944; Bushman, 1955; Audy, 1958; Reilly, 1966) an investigation of salmonellosis should include all species involved in the food web. In this study, biochemical and serological tests were performed on fecal and cloacal specimens from 152 rodents and insectivores, 42 snakes and 15 birds. Two animals, a water snake (*Natrix sipedon*) and a cottonmouth snake (*Agkistrodon piscivorous*) were confirmed to have harbored *Arizona* spp., a member of the tribe Salmonellae.

The samples from insectivore, rodent, snake and bird populations, collected mainly in Coles County, Illinois from March, 1968 to March, 1969, were apparently free of *Salmonella* infection. However, no animals were collected near sewers, garbage dumps, or human habitations, and no animals were found dead of unknown causes. Other surveys of salmonellosis in wild animals have been conducted in areas experiencing salmonellosis outbreaks. Usually animals were captured in or near human habitations; only one animal species was involved, and high rates of infection were reported. The entire animal community was not considered in these studies; therefore, comparisons between this study and others must concern isolations from rodents, snakes and birds separately.

Although no salmonellae were isolated from rodents in this survey, field mice (P. leucopus)(Dalmat, 1944), house mice (Mus musculus) (Derrick and Pope, 1950; Brown and Parker, 1957), voles (Microtus spp.) (Elton, 1942), and rats (R. rattus and R. norvegicus)(Davis and Jensen, 1952; Khalil, 1938; Lee, 1955) are commonly reported carriers of salmonellosis. However, the reported isolations always involved either overpopulation and sickness in the rodents, or the proximity of human habitations and waste dumps. Steele (1969) states that the infection rate of salmonellosis in wild rodents reflects their environment in that wild rodents commonly show lower rates of salmonellosis than rodents living in sewers and human slum districts.

No rodent population explosions or sicknesses were noted in the areas trapped in this study. Although winter temperatures were probably stressing factors, the rodent populations were apparently not effected. Salmonellae were either absent or present in very low numbers in the rodent populations.

Salmonella and Arizona groups are commonly isolated from reptiles. In tests on snakes captured on turkey farms which were experiencing outbreaks of salmonellosis, salmonellae were isolated from gopher snakes (Hinshaw and McNeil, 1944; McNeil and Hinshaw, 1944; Hinshaw and McNeil, 1945). Le Minor et al. (1956) and Dimow et al. (1964) reported isolates of Arizona spp. from 43% and 21% of various snakes captured in the wild in Bavaria, Germany and France. Edwards and Galton (1967) report that snakes are common carriers of Salmonella and Arizona groups, but that

Arizona types are present more frequently than Salmonella types.

Snakes are members of a food web in east-central Illinois, and Arizona spp. were isolated from two snakes in this survey. Of the literature reviewed, no reference was made to actual experimental proof that salmonellae could be transferred via a food chain. The control experiment conducted during this survey proved that S. typhi-murium could be transferred from rodent to snake in a food chain relationship. Reilly (1966) infected three types of carnivorous animals with Leptospira grippotyphosa by feeding them infected rodents. Therefore, it seems evident that a food chain transmission of species of Salmonella or Arizona is possible.

Predatory birds, which are components of the food web involved in this survey, could also become infected with salmonellae via the food web. Buehman (1955) and Audy (1958) reported that migratory cycles of predatory birds could involve the transfer of infectious diseases from one location to another. Hudson and Tudor (1957); von Steiniger (1967); Cornelius (1969); and Taylor (1969) reported that stress and flocking could further precipitate an epizootic of salmonellosis among birds.

None of the predatory birds trapped during this survey showed isolates of Salmonella or Arizona. Usually, cloacal specimens, cultured in tetrathionate broth and streaked on primary plating media, showed far fewer suspect colonies than rodent or snake cultures. No isolated were obtained from bird specimens either because the bacteria were present in low numbers or not at all; or isolation attempts were unsuccessful.

The incidence and etiology of salmonellosis in wild animals is influenced by stress factors such as overpopulations and adverse weather conditions. Davis and Jensen (1952) found that during changes in food or shelter, or during population movements, an increased rate of salmonellosis infection was evident in a Norway rat (R. norvegicus) population on a farm near Baltimore. Khalil (1938) noticed that the incidence of salmonellosis infection was greater in wild rats captured from January to March (17.6%) than from April to September (2.2%) in Liverpool, England. Elton (1942) recorded several instances of vole and mouse population explosions in Europe. The populations, when intentionally infected with S. typhi-murium, were severely reduced due to infection and death caused by the bacteria. Dalmat (1944) believed that an outbreak of salmonellosis in white-footed mice in central Iowa was due to very warm fall weather which stressed the population and precipitated an outbreak of the disease. White Swiss mice were observed to be more susceptable to S. enteritidis after being stressed by fasting (Miller et al., 1962). The authors believed that the fasting had caused a lowering of host resistance to infection. Morello et al. (1965) artifically infected white Swiss mice with S. typhi-murium. The bacteria were shed by 32% of the individually housed mice, while 67% of the pools of mice shed the bacteria. Hudson and Tudor (1957); Faddoul et al. (1966); Cornelius (1969); and Taylor (1969) believed that flocking and stress might be an important factor in causing salmonellosis to spread among birds.

Salmonella is the most wide-spread animal-borne disease in the world (Steele, 1969). The impact of salmonellosis on public health will become

more apparent in the future as the human use of wild animal domains increases, and the slum areas and sewage disposal areas become more numerous and further infected with rodents.

Salmonellosis is a Zoonosis, and the presence of salmonellae in rodents, snakes and birds can be of importance to public health. The common route of Salmonella infection is oral. Salmonellae frequently exist in foods eaten by domestic and wild animals (Breed et al., 1957). The close association of rodents and humans allows for fecal contamination of foods. Salmonellae may remain viable in excreted feces (Welch et al., 1941; Li and Davis, 1952; Morello et al., 1965), and there are numerous reports of salmonellosis infections aquired from contaminated foodstuffs (Jones and Wright, 1936; Welch et al., 1941; Burrows, 1968).

If Salmonella is enzootic in any species in a community, even in low numbers, it can be transmitted via a food chain, therefore effecting its spread to domestic animals or man. Stress in the form of overpopulation or climatic conditions, apparently plays an important role in inciting epizootics among wild animals (Khalil, 1938; Dalmat, 1944; Davis and Jensen, 1952; Derrick and Pope, 1959; Miller et al., 1962; von Steiniger, 1967).

SUMMARY

A total of 152 small mammals, 42 snakes, and 15 predatory birds were trapped in east-central Illinois from March, 1968 to March, 1969, and cultured for *Salmonella*. Fecal specimens, taken from each animal, were grown in an enrichment medium, streaked on primary plating media, then tested biochemically and serologically.

Of the 209 animal specimens, 176 were suspected of being *Salmonella* on primary plating media, and 52 remained suspect in the biochemical tests. Serological tests showed that 12 of the 52 suspects agglutinated polyvalent antisera.

Biochemical and serological identification carried out at the College of Veterinary Medicine, University of Illinois, revealed that a water snake (*Natrix sipedon*) and a cottonmouth snake (*Agkistrodon piscivorous*) harbored *Arizona* spp., and the remaining ten suspects were contaminants. The genus *Arizona* is a member of the tribe Salmonellae.

Since it has been speculated that diseases can be transmitted via a food chain, this study involved three groups of animals which are members of a food web. During this study, a control experiment was performed to show that *Salmonella* could successfully be transferred from a predator to a prey. *Salmonella typhi-murium* was successfully passed from laboratory mice to two prairie king snakes (*Lampropeltis calligaster*). After the snakes were proven free of *Salmonella* infection, they were fed two laboratory mice infected with *S. typhi-murium*. Post-feeding fecal samples revealed that the snakes had aquired the infection.

Although no Salmonella were isolated in this survey, it remains that the bacteria are infectious to wild animal populations, domestic animals, and therefore man.

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