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vide the only wintering habitat for trumpeters on the Refuge. Mixed small grains (poured into the water) are provided twice each week to supplement the natural foods available to the swans, thus bringing the birds into close contact during the feeding periods. An estimated 600 adults and cygnets wintered in the principal districts of the tri-state (Montana-Idaho-Wyoming) area in 1964-65; and, since there are movements of birds from one district to another, a major part of the trumpeter population of the United States (exclusive of Alaska) could ultimately have been exposed to infection. No other cases have been seen, however, within the intervening six months.

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Leptospiral Agglutinins In Sera from Southern Illinois Herpetofauna

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ABSTRACT

A total of 84 amphibians and reptiles were collected in southern Illinois and cultured for leptospires. All cultures were negative. Sera from 182 specimens were tested, and leptospiral agglutinins were detected in 6 of the 12 species examined. Sera from 18 (26%) of 69 seropositive turtles reacted to *Leptospira ballum* and 59 (86%) reacted to *L. hyos*. Inversely, 6 of 9 seropositive snake sera (67%) reacted to *L. ballum*, but only 1 (11%) reacted to *L. hyos*. Agglutinins were also detected for *L. canicola, L. icterohaemorrhagiae, L. pomona, L. sejroe* and *L. hardjo*. The highest percentage (89.1%) of reactors was in red-eared turtles (*Pseudemys scripta elegans*). There was no diference in the response of either sex or size classes of the red-eared turtles, although no small turtles were collected. It was postulated that high titers and high reactor rates developed in aquatic turtles in response to continue exposure to water-borne leptospires. In terrestial snakes the mode of infection was probably associated with preying on infected rodents.

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INTRODUCTION

The relationship between *Leptospira* and the herpetofauna of a community may be an essential part of the epidemiology of leptospirosis as well as the ecology of a given amphibian or reptile. Leptospiral agglutinins have been found in snakes, turtles and lizards (*Combiesco et al.* 1959, Blanc et al. 1961, van der Hoeden et al. 1961, White 1963), and in one instance leptospires have been isolated from a hognosed snake (*Heterodon platyrhinos*) (Ferris *et al.* 1961). The herpetofauna, therefore, were considered of importance in an investigation of leptospirosis in wild animals in southern Illinois. This report concerns the herpetofauna of southern Illinois that were examined for lepospires and leptospiral antibodies.

MATERIALS AND METHODS

The investigation was conducted in Pope, Johnson, and Massac Counties in southern Illinois from June 1960 through August 1964. Most of the specimens were collected on the University of Illinois, Dixon Springs Agricultural Center in western Pope County. All of the amphibians, lizards, land turtles and snakes examined were collected by hand as they were encountered in the course of field work associated with studies of leptospirosis in wild mammals or by personnel collecting specimens for the University of Illinois, Center for Zoonoses Research.

Aquatic turtles were collected with wire funnel traps from waters in several locations in the three county area. Intensive collecting was done in one pond, the Cullom pond, on the Agricultural Center in August 1964. This pond, characteristic of the artifical impoundments used as stock ponds in southern Illinois, had a surface area of about 5600 square yards and a maximum depth of 8 feet. Approximately 70% of the pond, that part with a depth of 4½ feet or less, was covered with yellow pond lilies (*Nuphar advena*). Fourteen wire funnel traps were set in the pond and operated for five days. The traps were baited with dead fish and checked morning and evening. Turtles taken in the traps were sexed, and carapase and plastron measurements were made. The turtles were killed and tissues were cultured for leptospires. Water samples collected at the surface, at 1 foot and at 5 feet in the pond were also cultured for leptospires.

Attempts to isolate leptospires from animal tissues involved aseptic removal of kidney, liver and spleen samples which were placed in leptospiral media. Stuart's (Stuart 1946) and Fletcher's semisolid (Fletcher 1928) media was used from 1960 through 1963. In 1964, water samples from Cullom pond and animal tissues were cultured in a polysorbate 80-bovine albumin media (Ellinghausen and McCullough 1965) containing 5-fluorouracil (Johnson and Rogers 1964).

Blood samples were collected by cardiac puncture. Serum was separated from the blood and tested by the microscopic agglutination-lysis test (Gochenour *et al.* 1953) using the following live antigens: *L. pomona, L. grippotyphosa, L. ballum, L. canicola, L. icterohaemorrhagiae, L. sejroe* and *L. hyos. Leptospira hardjo* was substituted for *L. sejroe* in 1964.

RESULTS

A total of 84 amphibians and reptiles were collected and cultured for leptospires; sera from 182 were tested for leptospiral antibodies (Table 1). Leptospiral agglutinins were detected in 78

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animals. Sixty-one (78%) of these animals agglutinated *L. hyos,* and 24 (31%) agglutinated *L. ballum*. Twenty-one animals agglutinated more than one serotype, and 15 of these agglutinated both *L. hyos* and *L. ballum*.

All of the cultures from amphibians were negative. No sera from these animals were tested.

Trapping at Cullom pond produced 32 red-eared turtles, 3 snapping turtles (*Chelydra serpentina*) and 1 stinkpot turtle (*Sternothaerus odoratus*). All turtles from this pond were bled and cultured. The remainder of the turtles were from other widely scattered locations. All cultures from the turtles were negative.

The highest percentage of serologic reactors was in red-eared turtles. The reactor rate in box turtles (*Terrapene carolina carolina*) and snapping turtles was only about one-third as high. Serologic reactor rates among the red-eared turtles from the Cullom pond were similar to those from other areas. Twenty nine (91%) of the 32 turtles trapped at the Cullom pond had titers while 20 (87%) of 23 of those from other areas were positive. There was not a significant difference between the reactor rates in male and female red-eared turtles from the Cullom pond or from other ponds. There was not a significant difference in plastron lengths of seropositive and negative red-eared turtles from Cullom pond, although none were caught with plastron lengths of less than 103 mm.

The tissues cultured from the four lizards were negative. No lizard sera were tested for leptospiral antibodies.

All of the cultures of snake tissues were negative. Serologic titers of snakes were, in general, lower than those of turtles. All but two of the nine seropositive snake sera had titers of 1:100. One gray rat snake (*Elaphe obsoleta spiloides*) reacted to *L. canicola* at 1:1000, and one racer (*Coluber constrictor*) reacted to *L. hyos* at the same titer. An additional 22 snakes had incomplete reactions at titers of 1:100 and were considered negative. Forty-nine (69%) of the turtles had titers of 1:1,000 or greater, and 12 (17%) had titers of 1:10,000. High titers among turtles were largely restricted to red-eared turtles.

Although *L. hyos* and *L. ballum* were primary serotypes agglutinated by both snake and turtle sera, there was a marked difference in the percentage of each serotype agglutinated. Six of the nine positive snake sera (67%) reacted to *L. ballum,* but only 1 (11%) reacted to *L. hyos*. Inversely, 18 (26%) of the 69 positive turtles reacted to *L. ballum* while 59 (86%) reacted to *L. hyos*.

DISCUSSION

The presence of *L. ballum* agglutinins in snake sera from southern Illinois, as well as other areas (White, 1963), may reflect the predatory feeding habits of snakes. One widespread prey species, the feral house mouse (*Mus musculus*), is frequently the carrier of *L. ballum* in the United States (Galton *et al.*, 1958). Such a food chain transmission of *L. ballum* has been reported between hedgehogs and infected laboratory mice (van der Hoeden, 1958), and the same theory has been postulated for infection of snakes in Malaya (Smith *et al.*, 1961). Furthermore, the only serotype isolated from a snake was *L. ballum* (Ferris et al., 1961).

The serologic reactor rate in turtles from southern Illinois parallels the re-

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Table 1. *Amphibians and reptiles collected in southern Illinois and examined for leptospires and leptospiral agglutinins.*

Species	Number Cultured	Serologic Results			
		No. Tested	Positives (1:100 and Higher) Serotype	No.	
AMPHIBIANS					
Long-tail salamander (*Eurycea longicauda*)	1	0			
Cave salamander (*Eurycea lucifuga*)	3	0			
Gray tree frog (*Hyla versicolor versicolor*)	1	0			
REPTILES					
Snapping turtle (*Chelydra serpentina*)	6	9	*L. hyos, L. hardjo*	1	
			L. ballum	1	(22.2%)
Stinkpot turtle (*Sternothaerus odoratus*)	1	1			
Eastern box turtle (*Terrapene carolina carolina*)	20	52	*L. hyos*	9	
			L. ballum	2	
			L. hyos, L. ballum	2	
			L. canicola	2	
			L. icterohaemorrhagiae	2	
			L. pomona	1	(34.6%)
Red-eared turtle (*Pseudemys scripta elegans*)	32	55	*L. hyos*	28	
			L. hyos, L. ballum	12	
			L. hyos, L. hardjo	5	
			L. hyos, L. pomona	1	
			L. hyos, L. hardjo, L. pomona	1	
			L. ballum	1	
			L. hardjo	1	(89.1%)
Fence lizard (*Sceloporus undulatus*)	2	0			
Six-lined racerunner (*Cnemidophorus sexlineatus*)	1	0			
Five-lined skink (*Eumeces fasciatus*)	1	0			
Diamond-backed water snake (*Natrix rhombifera rhombifera*)	6	2			
Midland water snake (*Natrix sipedon pleuralis*)	1	5	*L. hardjo, L. ballum*	1	(20.0%)
Northern copperbelly (*Natrix erythrogaster neglecta*)	0	3			
Eastern garter snake (*Thamnophis sirtalis sirtalis*)	0	1			
Racers (*Coluber constrictor*)	1	27	*L. hyos, L. ballum*	1	
			L. sejroe	1	
			L. canicola	1	(11.1%)
Gray rat snake (*Elaphe obsoleta spiloiders*)	0	13	*L. ballum, L. canicola*	4	
			L. sejroe	1	(38.5%)
Eastern hognose snake (*Heterodon platyrhinos*)	2	1			
Black kingsnake (*Lampropeltis getulus niger*)	4	13			
Coperhead (*Agkistrodon contortrix modeson*)	1	0			
Cottonmouth (*Agkistrodon piscivorus piscivorus*)	1	0			
Totals	84	182		78	(42.9%)

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sults of Combiesco *et al.* (1959) in Romania and van der Hoeden *et al.* (1961) in Israel. In each of these areas a high percentage of aquatic turtles had leptospiral agglutinins while land turtles reacted at lower levels. The red-eared turtles examined in this investigation and most of the aquatic turtles reported in the literature had much higher titers than those found in snakes. Turtles inoculated with cultures of *L. pomona* or *L. grippotyphosa,* however, produced titers no higher than 1:100 (van der Hoeden, 1961; Abdulla and Karstad, 1962). Development of high titers in aquatic turtles under natural conditions may be the result of continued long-term exposure to leptospires in the water rather than an occassional exposure as with snakes and infected mice. The difference in serologic response observed in red-eared turtles and snapping turtles, however, suggests that other factors may also be involved. The serologic difference may have been due to species variations, although it is more likely they were due to the feeding habits or habitat niches of each species.

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