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Infectious Diseases in Wild Animals in Utah

VI. Experimental Infection of Birds with *Rickettsia rickettsii*

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ABSTRACT

LUNDGREN, D. L. (University of Utah, Salt Lake City), B. D. THORPE, AND C. D. HASKELL. Infectious diseases in wild animals in Utah. VI. Experimental infection of birds with *Rickettsia rickettsii*. J. Bacteriol. **91**:963–966. 1966.—Chickens, pigeons, pheasants, sparrow hawks, red-tailed hawks, ravens, magpies, and a marsh hawk were inoculated with *Rickettsia rickettsii*, the etiological agent of Rocky Mountain spotted fever. The development and persistence of complement-fixing (CF) antibodies and rickettsemias were tested for in these birds. Rickettsiae were recovered from the blood of a number of birds up to the 16th day after inoculation, whereas only the pigeon was found to develop high CF antibody titers. It was concluded that certain species of birds have the potential of contributing to the dissemination of *R. rickettsii* in nature, and that the CF test is generally unsuitable for serological diagnosis of this organism in birds.

It has been well established that lagomorphs, rodents, and some larger mammals are important hosts of *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (RMsf; 5, 6). In addition to these animals, it has been suggested (12) that ground-frequenting birds could contribute to the dissemination of the organism by transporting infected ticks, *Haemaphysalis leporis-palustris* (Bishopp), from one locality to another. In North America, many species of birds have been reported to be hosts of this tick (1, 4) and others as *Dermacentor andersoni* (3) and *Amblyomma americanum* (1), which are known vectors of *R. rickettsii*. The occurrence of these ticks on birds would indeed intimate that birds may be of potential importance in the dissemination of *R. rickettssii* in nature. Serological evidence of birds naturally infected with *R. rickettsii* has been reported by others (7) as well as ourselves (16, 17). The role of birds in the ecology of tick-borne diseases in the Eastern Hemisphere has been discussed by Hoogstrall et al. (9).

Although the evidence suggests that birds may be involved in the cycle of *R. rickettsii* in nature, the responses of birds to experimental infection have not previously been investigated. It is the purpose of this paper to report observations on the susceptibility of certain species of birds to experimental infection with *R. rickettsii* and to report on the complement-fixing (CF) antibody responses resulting from the inoculation of this organism.

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MATERIALS AND METHODS

Animals. Sixteen sparrow hawks, *Falco sparverius* Linnaeus; three red-tailed hawks, *Buteo jamaicensis* (Gmelin); one marsh hawk, *Circus cyaneus* (Linnaeus); three common ravens, *Corvus corax* Linnaeus; and six black-billed magpies, *Pica pica* (Linnaeus), were obtained as nestlings in the field. These birds were maintained on a diet of ground beef by-products and laboratory mice. The 130 domestic pigeons, *Columba livia*; 19 ring-necked pheasants, *Phasianus colchicus* Linnaeus; and 5 white leghorn (Aimes Incross breed) chickens used were obtained from local commercial sources. These latter birds were maintained on a diet of cracked corn and pigeon game mix. Male albino guinea pigs (350 g) of the Hartley strain were used as indicator animals.

No differentiation of birds was made on the basis of sex. The ages of the birds at the time of testing are given in Table 1.

Rickettsiae. The SFR isolate (from H. G. Stoenner, Rocky Mountain Laboratory, Hamilton, Mont.) of the R strain of *R. rickettsii* used was originally isolated from *D. andersoni* ticks collected in western Montana, and was characterized as being virulent for guinea pigs. Suspensions of rickettsiae for animal inoculation were prepared and titrated in embryonated hen's eggs as described previously (10).

Inoculation of birds. Subcutaneous (sc) inoculations of the desired concentration of rickettsiae were made in the dorsum between the wings. Spleens from in-

fected guinea pigs were fed to some sparrow hawks to determine *per os* susceptibility.

Testing for rickettsiae. At various intervals, 0.5 to 1.0 ml of blood was collected from each bird by cardiac puncture or from a wing vein and was tested for rickettsiae. Tissues were examined for organisms by preparing a 20% homogenate in a sucrose-phosphate-glutamate (SPG) solution (2). Each blood or tissue sample was inoculated intraperitoneally into two indicator guinea pigs. The rectal temperatures of these guinea pigs were taken at the same time each day for 12 days. Spleen smears of those dying were stained by the method of Gimenez (8) and were examined for rickettsiae. Four weeks after inoculation, the surviving guinea pigs were bled by cardiac puncture to obtain serum for RMsf CF antibody determinations. These survivors were then challenged with 100 guinea pig 50% infective doses (ID_{50}) of the infecting strain of rickettsia. The criteria used to determine whether inoculated guinea pigs were infected with RMsf rickettsiae were: (i) a sustained rectal temperature of 40 C or higher followed by death and demonstration of the organisms in spleen smears, or (ii) development of CF antibody titers and immunity to challenge with *R. rickettsii*.

CF antibody responses. At desired intervals (Table 2), blood samples were collected from the birds to determine CF antibody titers. All birds were prebled and found to be negative for *R. rickettsii* antibodies prior to inoculation of rickettsiae. Cross-reactions against psittacosis and *Coxiella burnetti* antigens were also checked.

CF test procedures have been described previously in detail (16, 17). Although the choice of this test may be questioned by some, its use was dictated by the availability of commercial antigens and the desire to determine the value of the CF test in experimental infections.

RESULTS

Quantitative determination of susceptibility of pigeons. Pigeons were the only species available in sufficient numbers to determine susceptibility to infection on a quantitative basis. Tenfold serial dilutions of the stock culture of *R. rickettsii* were prepared in SPG and inoculated into six groups of five or more pigeons. These doses ranged from 1.5 to 1.5×10^6 chick embryo LD_{50} (ELD_{50}) of rickettsiae. These birds were observed for 4 weeks, then bled by cardiac puncture to obtain sera to test for CF antibody. Three of the five inoculated with 1.5×10^6 and 6 of the 14 inoculated with 1.5×10^5 ELD_{50} developed CF antibody titers ranging from 1:16 to 1:256. All pigeons inoculated with lower dosages failed to develop detectable antibody at titers of 1:16 or greater. The ID_{50} for the pigeons, as calculated by the Reed and Muench method (14), was 2.9×10^5 ELD_{50} of rickettsiae.

Persistence of rickettsiae in pigeon tissue. Four pigeons were inoculated with 10^6 ELD_{50} doses of the organism. Ten birds were then sacrificed on the 1st, 2nd, 4th, and 6th weeks after inoculation. Heart, lung, liver, spleen, kidney, and brain tissues were removed aseptically, pooled according to the type of tissue, and tested for viable organisms. Rickettsiae were recovered from all tissues on the 1st week, and from all except the heart and lung on the 2nd week. All samples collected on the 4th and 6th weeks were negative.

Rickettsemia in various birds. Five adult chickens were inoculated with 10^6 ELD_{50} doses of *R. rickettsii* and were checked for rickettsemia at 48-hr intervals for 14 days. The organisms were recovered from the blood of one chicken on the 6th, 8th, and 10th days after inoculation; from one on the 6th day; from another on the 8th day; and from a fourth on the 10th day (Table 1). No positive samples were collected from the fifth chicken.

The inoculation of 10^4 or more ELD_{50} doses of rickettsiae resulted in the development of a rickettsemia in the 6-month-old pheasants (Table 1). Organisms were recovered from several pheasants on the 6th and 10th days after inoculation, but not from the same birds on the 8th day. In the adult pheasants, as in the younger birds, the inoculation of both doses of rickettsiae resulted in a rickettsemia which persisted for several days (Table 1).

Three different studies of a possible rickettsemia in pigeons were conducted. In the first study, each of 45 birds was inoculated with 10^7 ELD_{50} doses of the SFR isolate; five birds were killed and tested for rickettsemia at 2-day intervals (Table 1). Rickettsiae were found in one to three of the blood samples at each interval tested between the 2nd and 14th postinoculation day, except for the 8th day, when all samples were negative. Similar results were noted in a second experiment with 40 pigeons injected with 10^6 ELD_{50} doses, and the third experiment where 10 birds were infected with 10^5 ELD_{50} doses of the organism (Table 1).

Four adult sparrow hawks were inoculated with 10^7, three adults with 10^5, and seven 1 to 3-week-old hawks with 10^5 ELD_{50} doses of rickettsiae. Only one sample collected from an adult inoculated with 10^7 ELD_{50} doses and one from a young hawk were positive on the 6th day. All other samples collected were negative (Table 1).

Two adult hawks were each fed 7 g of spleen tissue from infected guinea pigs. Rickettsiae were not evident in blood samples collected from these birds on the 2nd, 4th, 6th, and 8th days.

One 3-month-old marsh hawk, six juvenile magpies, and three juvenile ravens were each inoculated with 10^5 doses, whereas three adult magpies were given 10^3 ELD_{50} doses of the organism. Rickettsiae were not recovered from any of the blood samples collected from these birds at the intervals sampled (Table 1).

CF antibody responses. None of the chickens, pheasants, sparrow hawks, magpies, or ravens developed detectable CF antibodies at titers of 1:16 or greater. In contrast, most pigeons readily formed CF antibody after the inoculation of an infective dose of rickettsiae (Table 2). Antibody titers of 1:32 to 1:128 usually developed in these birds by the 2nd week after inoculation. Maximal titers to 1:256 were found between the 3rd and the 5th week. These titers decreased rapidly and were not detectable by the 12th week; in some instances, as early as the 6th week. However, one pigeon (no. 931) was unusual in that it remained sero-positive through the 25th week.

One red-tailed hawk, inoculated with 10^7 ELD_{50}, and the one marsh hawk, receiving 10^5 ELD_{50}, were

TABLE 1. *Rickettsemia in birds inoculated subcutaneously with Rickettsia rickettsii as determined by inoculation of blood into guinea pigs*[a]

Species	Age	Dose	Days after inoculation[b]								
			2	4	6	8	10	12	14	16	18
		ELD_{50}									
Chicken	5 months	10^6	0/5	0/5	2/5	2/5	2/5	0/5	0/5		
Pheasant	6 months	10^6	0/3	0/3	2/3	0/3	2/3	0/3	0/3	0/3	0/3
		10^4	0/4	0/4	3 4	0/4	3,4	0/4	0/4	0/4	0/4
Pheasant	30 months	10^6	0/5	1/5	2/5	1/5	2/5	1/5	0/5	0/5	0/5
		10^4	0/6	2/6	3/6	0/6	1/6	1/6	1/6	1/6	0/6
Pigeon	Adult	10^7[c]	1/5	2/5	2/5	0/5	2/5	3/5	2/5	0/5	0/5
		10^6	0/10	5/10	3/10	3,5			0/5		
		10^5[d]	−	+	+	+	+	+	+		
Raven	Adult	10^5	0/3	0/3	0/3	0/3	0/3		0/3		
Magpie	Adult	10^5	0/6	0/6	0/6	0/6	0/6		0/6		
Sparrow hawk	1 to 3 weeks	10^5	0/7	0/7	1/7	0/7	0/7		0/7		
Sparrow hawk	Adult	10^7	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
		10^5	0/3	0/3	0/3	0/3	0/3		0/3		
Red-tailed hawk	3 months	10^6	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	
		10^5	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	
Marsh hawk	3 months	10^5	0/1	0/1	0/1	0/1	0/1		0/1		

[a] Unless otherwise indicated, the same birds were bled repeatedly at the various intervals.
[b] Blood was collected from the same five pigeons and pooled at each interval.
[c] Five pigeons were killed at each interval.
[d] Positive birds per total tested.

TABLE 2. *Rocky Mountain spotted fever complement-fixing antibody response in birds inoculated subcutaneously with Rickettsia rickettsii**

Species	Bird no.	Dose	Weeks after inoculation†										
			2	3	4	5	6	8	10	12	14	16	25
		ELD_{50}											
Pigeon	920	10^6	64		128	64	—	—	16	—	—	—	—
	908	10^6	32		16	32	16	—	—	—	—	—	—
	617	10^6	—		32	32	64	32	16	16	—	—	—
	931	10^6	—		64	128	128	128	64	64	64	64	64
	919	10^6	32		64	128	128	64	32	16	—	—	—
	698	10^6	—		16	64	64	32	16	16	—	—	—
	224	10^7	64	64	64	32	16	—					
	225	10^7	128	256	128	128	64	32					
Marsh hawk	1	10^5	—	32	32	—	—	—					
Red-tailed hawk	3	10^7	16	16	16	16	—	—					

* Titers of 1:16 or greater reported only.
† Titers expressed as the reciprocal of the highest serum dilution giving 100% lysis of sensitized sheep red blood cells. Titers at 0 and 1 week were less than 1:16.

found to have CF antibody titers of 1:16 to 1:32 during the 2nd and the 3rd week. The antibodies were not detectable by the 5th week.

Symptoms of infection. Throughout the study, none of the birds under study developed any overt symptoms of infection.

DISCUSSION

It is evident, from the limited experimental data presented here, that at least five of the eight species of birds tested developed asymptomatic infections, as indicated by the appearance of rickettsiae in the blood of the apparently healthy birds at various time intervals after the inoculation of *R. rickettsii*. The persistence of the organisms in the blood of infected pigeons, chickens, and pheasants was similar to that observed in rodents and lagomorphs known to be involved in the cycle of *R. rickettsii* in nature (12, 13). Rickettsiae were not observed to persist for more than 1 day in the blood of the sparrow hawks and the red-tailed hawks tested, and were not recovered from any of the samples collected from ravens, magpies, and the marsh hawk.

It is concluded that certain species of birds might become infected in nature and have the potential of providing infective blood meals for feeding ticks, thus establishing a tick-bird-tick or tick-bird-tick-mammal cycle of *R. rickettsii*, in addition to the transporting of infected ticks, as suspected by Parker et al. (12).

Several pigeons, one marsh hawk, and one red-tailed hawk were the only birds observed to develop spotted fever CF antibody. Chickens, pheasants, magpies, ravens, and sparrow hawks failed to develop antibody that would fix complement in the presence of soluble *R. rickettsii* antigen, even though many of these same birds were observed to have developed a rickettsemia. Similar observations on the inability of certain species of birds to produce detectable CF antibody have been reported by others (11, 15). These observations of our studies confirm these reports and indicate other serological tests should be used in testing bird sera. Our studies on this problem will soon be published.

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