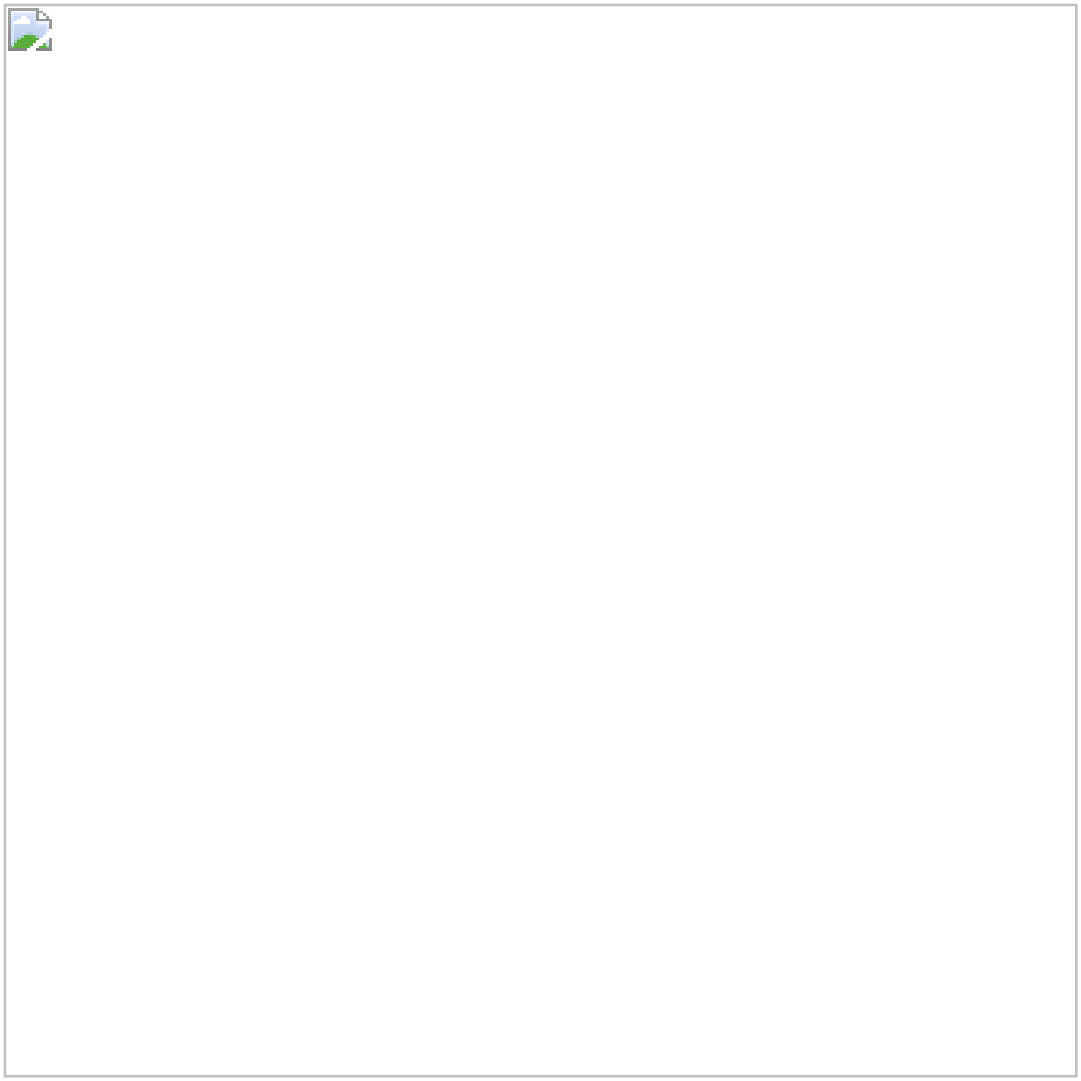

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Source: Bulletin of the Wildlife Disease Association, 3(1) : 2-10

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-3.1.2

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Parameters of a Natural Focus of *Leptospira pomona* in Skunks and Opossums

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Received for publication 10 June, 1966

ABSTRACT

The dynamics of a natural focus of *L. pomona* in skunks and opossums were investigated by live trapping for a period of 18 months, followed by removal trapping for 2 months. *Leptospira pomona* was isolated from 8 (10.6%) of 75 skunks, 3 (3.1%) of 98 opossums and 1 of 21 feral cats. Serologic reactions to *L. pomona* were found in 9 (28.1%) of 32 skunks and 3 (3.01%) of 98 opossums tested. *Leptospira pomona* was isolated from 1 to 3 times from naturally infected free-living skunks during a maximum of 77 days. Among the interesting correlations of ecologic data with infectivity was a significant relationship between both isolations and serologic titers for the colder and wetter parts of the year in skunks; no such correlation was found for opossum infections and titers. The habits of animals shedding leptospires were found to be related to specific parts of the research area. The authors conclude from the data that skunks may be able to maintain a nidus of *L. pomona*.

INTRODUCTION

Numerous isolations from skunks (*Mephitis mephitis*) of *Leptospira pomona* have been reported in the United States [6 9 10 12 17 18 22 23]. Isolations from the opossum (*Didelphis marsupialis*) have also been reported but less frequently [6 9 10 11 21]. The serotype is also found naturally in large numbers of North American cattle (*Bos taurus*) and swine (*Sus scrofa*), the latter shedding the organism for long periods[13]. The role of wildlife in the oeizpotiology of *L. pomona* is of considerable importance.

Wild animals infected with leptospirosis may be located by trapping and killing the animals, but to prove or disprove the hypothesis that the wild animals can maintain a nidus for an indefinite period, it is necessary to investigate the dynamics of a natural focus without killing the animals. The major objective of this research, therefore, was to go beyond descriptive observation of animals killed and examined and to quantitatively measure certain aspects of a leptospiral nidus. In this report the animals involved in the nidus were examined, as well as some parameters of the nidus, i.e., epidemiologic rates involving age and sex susceptibility, the length of shedding under natural conditions, seasonal distribution of reactors, population densities, intra- and interspecific relationships and habitat associations.

MATERIALS AND METHODS

The nidus of *L. pomona* that was investigated was located on the University of Illinois, Dixon Springs Agricultural Center in southern Illinois. A four-square mile research

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area encompassing what was assumed would be the most active region of the nidus was established. The central square mile of the research area was divided into 100 equal units of 6.4 acres and one box or so-called live trap was placed in each unit. These live traps were in operation for 18 months, from September, 1961 through February, 1963. Animals caught in the live traps were taken alive to the laboratory. Each animal was anesthetized with sodium pentobarbital, its sex, age, and weight recorded, a blood sample taken by cardiac puncture, a urine sample by bladder tapping, and one ear tagged. Animals trapped several times in a short period were bled only at the first capture. The day after their capture all animals were returned to the site of capture and released.

At the termination of the live-trapping, saturation trapping was employed with steel traps in an effort to remove all animals from the central square mile of the research area. In addition to the data collected from live-trapped animals, all animals taken during the removal trapping were killed and cultures were made from the kidney, liver and spleen.

The animals discussed in this report include the opossum (*Didelphis marsupialis*), skunk (*Mephitis mephitis*) and feral cat (*Felis domesticus*). Other animals collected and examined during the investigation included the woodchuck (*Marmota monax*), raccoon (*Procyon lotor*) and cottontail (*Sylvilagus floridanus*).

Populations of the species involved in the study were estimated by the ratio of marked to unmarked animals collected (Lincoln Index). As will be noted, the data available dictated the method that was used, and, when possible, multiple estimates were made to verify the results from each technique. Live-trapping data were compiled for each 3-month interval, and the ratio of marked to unmarked animals was calculated for each of two consecutive periods. The same procedure was applied to the ratio of tagged to untagged animals taken during the removal trapping.

Estimates based on the ratio of marked to unmarked were also calculated from data collected by nightlighting operations[3]. However, this technique produced sufficient data for population estimates only during the summer months. Opossum populations were also estimated from the ratio of opossums toe-clipped in the female's pouch and later recaptured as independent animals to unmarked young opossums captured as independent animals[25].

The bladder tapping technique described by Menges *et al.*[16], was used to collect urine from live trapped animals. Cultures of urine as well as cultures of material from the kidney, liver and spleen of animals killed were made as described by Ferris *et al.*[7].

Cultures were examined for leptospires each week by darkfield microscopy for a period of two or three months or more, if the cultures were not contaminated at the first inspection. Cultures contaminated with other bacteria were examined for six weeks before discarding. Contaminated cultures of the isolants were cleared by passage in guinea pigs, hamsters or gerbils. Isolants were identified by methods previously described[7], and identification was confirmed by Dr. A. D. Alexander, the World Health Organization Leptospiral Typing Center, Division of Veterinary Medicine, Walter Reed Army Medical Center, Washington, D. C. and Mrs. Mildred Galton, Veterinary Public Health Laboratory Unit, Communicable Disease Center, Atlanta, Georgia. Four tubes of the first or second uncontaminated culture in Fletcher's medium were deep frozen at -65°C or -70°C at the peak of growth. Stock cultures were maintained in Fletcher's medium by sub-culture every two to three months.

Blood samples were centrifuged at approximately 2,000 revolutions per minute, the sera pipetted off and frozen at -20°C. The microscopic agglutination test was employed to test all sera[7]. The following stock antigens were routinely used: *Leptospira pomona, grippotyphosa, sejroe, ballum, icterohaemorrhagiae, canicola,* and *hyos. Leptospira autumnalis, pyrogenes, hebdomadis, Australis A,* and *bataviae* were utilized on occasion.

RESULTS

Live traps were in operation on the central part of the research area from September 1961 through February 1963 and removal trapping was conducted during March and April of 1963. During the 20-month period 194 animals were collected and examined for a total of 630 examinations (Table 1).

Opossum population estimates based on live-trapping and removal trapping indicated an annual population fluctuation ranging from as low as 22 to as high as 77 animals per square mile (Table 2). The estimate based on recapture of young marked in the pouch was about seven times higher than these estimates. This estimate was based on a recapture of five of 77 marked young in a total capture of 26 independent young opossums (77:X :: 5:26 = 400). Production of 400 young required 60 females (the average litter was 6.7 young).

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Table 1. *Numbers of the affected mammal species collected and examined for leptospires in the focal area.*

Species	No. Examined Individuals	No. Examined Total Captures	*L. pomona* No. Isolations	*L. pomona* No. With Titers*
Opossum	98	386	3	2**
Skunk	75	210	8	9***
Cat	21	34	1	1
Totals	194	630	12	12

* Microscopic-agglutination test; 1:100 or higer.
** 64 individuals tested.
*** 32 individuals tested.

Assuming equal sex ratios of adults the total population was estimated at 120 adults and 400 young. The marking and trapping period for this estimate was from February 1962 through January 1963.

Recapture success with live traps was low for skunks, and the estimate based on those data was only for the September to November period (Table 2). The ratio of 8 tagged of 12 skunks taken during the removal-trapping was applied to the 20 skunks released on the study area during December to February, 1962-1963 for an estimate of 30 animals. Summer estimates calculated from night lighting capture -- recapture data were 67 skunks. The annual population of skunks, based on the three estimates, ranged from 30 to nearly 70 per square mile.

The limited number of cat recaptures made the live trapping data unusable as a basis for population estimates. Twenty-three cats were collected with live traps, and an additional six cats were seen on the study area, making a total of 29 cats known to have used the area over a period of 20 months. This is a conservative estimate since it was not possible to distinguish between all cats seen in the field. During the removal-trapping phase of this study one tagged and three untagged cats were trapped. Application of this ratio (1:4) to the eight cats captured and released on the area during the 6 months preceding removal-trapping indicated about 32 cats per square mile on the study area.

Leptospira pomona was isolated from 8 of 75 skunks examined (Table 1). The temporal distribution of isolations in skunks reveals a seasonal pattern (Table 3). All positive animals were collected from December through March in 1961-62 and 1962-63. Chi-square analyses of these data indicate that the temporal distribution of *L. pomona* isolants was significant at the 0.005 probability level. The spatial distribution of positive skunks on the study area (Figure 1) indicates that most positive skunks had moved in the fields and pastures adjoining the stream crossing the area.

Seven of the eight skunk isolations were from males and six were from adult animals (Table 4). The relationship between age and isolations was not signi-

Table 2. *Population estimates (number per square mile) of affected mammal species in the focal area.*

Species	Direct Count	Live-Trapping	Removal-Trapping	Marked Young	Night-Lighting
Opossum	--	26-77*	22-48*	520	--
Skunk	--	33**	30**	--	67
Cat	29***	--	32**	--	--

* Range of estimates for five 3-month periods.
** Late fall, early winter estimates.
*** Cumulative total from September 1961 through February 1963.

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ficant at the 0.01 level. The sex-isolation relationship, likewise was not significant at the 0.01 level.

Four of the seven skunks from which leptospires were isolated were captured only once. One adult male was captured twice over a 21-day period during February and March. Leptospires were isolated at only the last capture. Two of the positive skunks were captured four times. One adult male was taken 4 times over a period of 19 days during January. *L. ballum* was isolated at the second and *L. pomona* at the third and fourth captures. Another adult male was captured once in December, twice in January and once in March, over a period of 77 days; *L. pomona* was isolated from this skunk on captures 1, 3, and 4.

Serological reactions to *L. pomona* were detected in 9 skunks or 28.1 per cent of the individuals tested (Table 1). The temporal distribution of reactors was grouped even more closely than that of the isolations (Table 3). All reactors were taken during the January to March period in both 1962 and 1963. This pattern was significant at the 0.01 probability level. Eight of the serologically positive skunks were adults and six were males (Table 4); neither factor, however, was significant (0.01 level). Three of the skunks shedding *L. pomona* had no measurable antibody titer. A prozone was observed in one skunk at the 1:100,000 level.

Leptospires were isolated from 3 of 98 opossums examined (Tables 1 and 6). Two isolations were made in March and one in July (Table 5). All three isolations were from adult opossums and two were from males (Tables 5 and 6). The parameters of temporal distribution, age and sex were not significantly related (0.01 level) to the number of isolations made. The spatial distribution of the opossum isolations (Figure 1) indicates that positive opossums, like skunks, had moved on that part of the research area related to the stream and its adjacent pastures.

Serological reactions were deteccted in two opossums (Tables 1 and 6). *L. pomona* had been isolated from one opossum; its titers were 1:1000 to *L. pomona* and 1:10 to *L. ballum*. The other opossum had a prozone titer of 1:10,000 to *L. pomona*, titers of 1:10,000 to *L. ballum*, and 1:100 to *L. canicola*. *L. pomona* was not isolated from this animal in 14 captures although *L. ballum* was isolated on the 12th capture. There were no serologic reactions in two opossums from which *L. pomona* was isolated.

Table 3. *Temporal distribution of isolations and serologic titer to* L. pomona *in skunks on the Dixon Springs Research Area.*

Period	No. Examined	No. Isolations	No. with Titers*
1961			
September-December	8	0	0
1962			
January-March	20	3	2
April-June	32	0	0
July-September	52	0	0
October-December	28	1	0
1963			
January-March	70	4	7
Totals	210	8	9

* Microscopic-agglutination test; 1:100 or higher.

Table 4. *Age and sex relationships of isolations and serologic reactors for* L. pomona *in skunks.*

Class	Isolations No. Tested	Isolations No. Positive	Isolations % Postive	Serologic Reactors No. Tested	Serologic Reactors No. Positive	Serologic Reactors % Positive
Adult male	24	5	20.8	14	5	35.7
Juvenile male	20	2	10.0	3	1	33.3
Adult female	12	1	8.3	11	3	27.3
Juvenile female	19			4		
Totals	75	8	10.6	32	9	28.1

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Table 5. *Temporal distribution of isolations and serologic titer to* L. pomona *in opossums on the Dixon Springs Research Area.*

Period	No. Examined	No. Isolations	No. with Titers*
1961			
September-December	54	0	0
1962			
January-March	52	2	2
April-June	68	0	0
July-September	74	1	0
October-December	60	0	0
1963			
January-March	62	0	0
April	16	0	1
Totals	385	3	3

* Microscopic-agglutination test; 1:100 or higher.

L. pomona was isolated from one of 21 cats examined (Table 1). The cat, an adult male, was a feral animal and was not recaptured after it was released[8]. The location of this animal on the research area is shown in Figure 1.

DISCUSSION

Epidemiologic methods have been highly successful in solving problems in human disease by relating patterns of disease to biological, occupational, social, and geographic factors. The incidence, prevalence and mortality rates for some diseases are highly precise tools for use in designing experiments to determine etiology or for the control of epidemics. This investigation indicates that some of the same methods can be used to define more precisely the parameters and dynamics of a nidus of leptospirosis. If conducted as most field work has been in the past, this investigation would have involved conflicting requirements. When animals are killed, the natural focus is changed; if they are not killed, however, it is difficult or impossible to obtain proof that there is a nidus. The conflict is especially true of leptospirosis where cross-reactions and paraspecific reactions are common[23]. The methods used in this investigation preserved the nidus with a minimum of disruption while at the same time obtaining the data necessary to define some of its parameters.

The relative part of the total opossum, skunk and cat population that was examined was quite high. Opossum populations were considered to fluctuate from a low of about 22 to a high of near 80 per square mile, and a total of 98 were examined. Skunk populations were estimated to range annually from nearly 30 to over 70 per square mile for the same period, and 75 were examined. Twenty-one cats were examined while the population was estimated to have been between 24 and 32. Other investigators have utilzed large numbers of skunks[22 23] and opossums[18] but none are known to have examined as many animals from an area as small as one square mile.

Table 7 summarizes infection rates to *L. pomona* in skunks and opossums in the U. S. as reported by several teams of investigators. The reported infection rates for skunks varied from about 2% to nearly 26%. The infection rate in this nidus was the highest reported (28.1%). Of the 95 skunks collected on other parts of the Dixon Springs Agricultural Center, 11.6% were positive while only 6.7%

Table 6. *Age and sex relationships of isolations and serologic reactors for* L. pomona *in opossums.*

	Isolations			Serologic Reactors		
Class	No. Tested	No. Positive	% Postive	No. Tested	No. Positive	% Positive
Adult Male	16	2	12.5	16		
Juvenile male	45			20	1	5.0
Adult female	15	1	6.7	16	1	6.7
Juvenile female	22			13		
Totals	98	3	3.1	98	2	2.0

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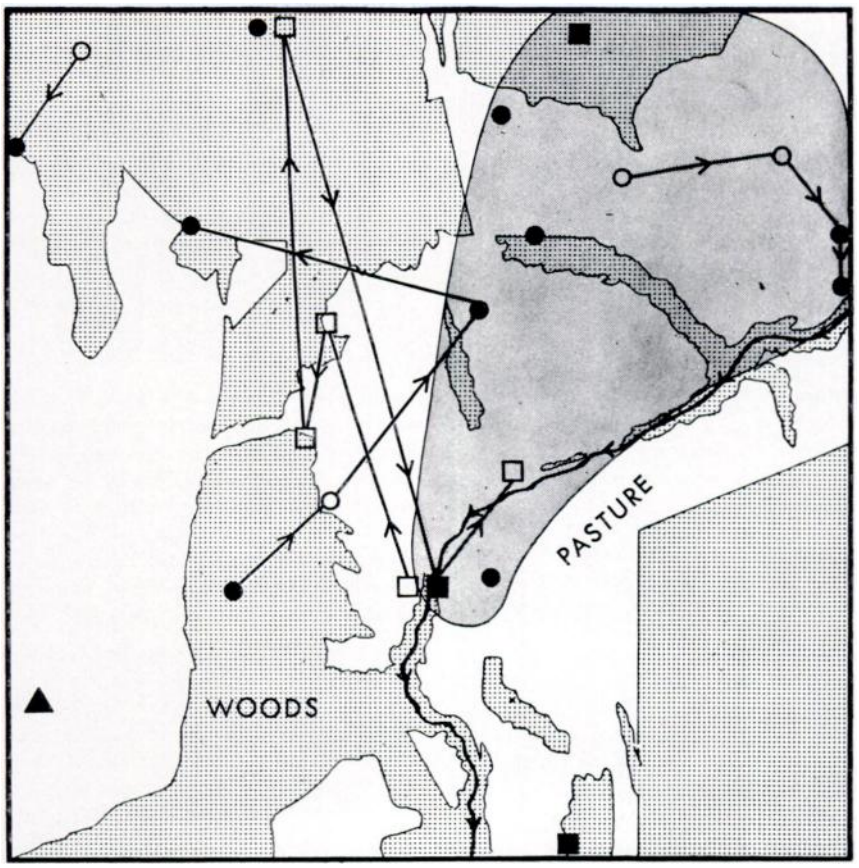

Figure 1 *Spatial distribution of* Leptospira pomona *isolations from skunks* (○), *opossums* (□) *and cats* (△) *and movement patterns of animals on the one square-mile study area. Open figures indicate captures where no isolations were made. The shaded area represents that part of the research area known to have been used by most of the positive animals. Dotted areas indicate woods while clear areas show pastures.*

of all the 239 skunks examined in southern Illinois were positive.

The concept of nidality is based upon natural concentrations of animals[5, 19, 20]. None of the animals examined during this investigation were distributed randomly over the research area. Each species used parts of the study area more intensively than others, and these tracts were deemed strategic habitat. In a previous paper[4] it was postulated that contact, direct or indirect, between the members of a species would be potentially greater on the strategic habitat since densities were greater. In addition, when the tracts of the strategic habitat for two species overlap the potential for interspecific contact was increased. When the strategic habitat of skunks, opossums, woodchucks, feral cats, and cattle was compared the area of overlap was similar, although not identical to the strategic habitat for skunks. The same area of overlap of strategic habitat also closely resembled the minimum area which most of the positive animals were known to have occcupied (Figure 1). Other positive animals may have moved

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Table 7. *Isolation and serologic reactions of skunks and opossums in the United States with* L. pomona.

Skunks		Opossums		Reference
% Isolation	% Serologic Positive	% Isolation	% Serologic Positive	
—	—	2.3 of 43	22.0 of 70	Goldstein *et al.*, 1958
7.24 of 132	—	—	—	McKeever *et al.*, 1958
—	—	0.17 of 6	—	Roth & Knieriem, 1958
8.32 of 132	—	—	—	Galton *et al.*, 1959a, 1959b
—	22.8 of 372	0 of 464	0 of 464	McKeever *et al.*, 1959
25.92 of 54	—	2.15 of 186	—	Clark *et al.*, 1961
2.25 of 312	—	—	—	Roth *et al.*, 1961
7.2 of 430	—	—	—	Gorman *et al.*, 1962
20.9 of 650	—	—	—	Roth *et al.*, 1963a
(10.65 of 75)	28.1 of 32	3.1 of 98	3.1 of 98	Ferris & Andrews, 1965

within this area, but they were not captured there. These data suggest that there is a relationship between population density, spacing and the rate of infection.

The statistical significance of the seasonal distribution of both serologic reactors and isolations is exceedingly important. The importance is magnified by the insignificance of age and sex distribution with regard to reactor and isolation rates. All isolations as well as nearly all of these reactors were found in the third of the year from December through March although nearly twice as many skunks were caught during the rest of the year (Table 3). It seems unlikely that shedding animals would have been missed during the warmer months since more than an adequate number of skunks were taken during those periods.

From these data it was postulated that the increased appearance of measurable antibody and leptospiruria in skunks from December through March is associated with climatic conditions or the animal's activities and physiologic response to stress. The observed peak period of shedding was during the colder, wetter months of the year, when the amount of surface water on the area was increased; therefore, the possibility of water-borne transmission was increased. It is also likely that sexual activity and the communal occupancy of dens by a number of animals was an effecctive means of transmission. Snow-tracking data indicated that skunks on the research area entered as many as seven separate dens in a night during the December to February period. Multiple occupancy of winter dens has been reported in which single males were found in dens with as many as 10 females[1 2].

Cattle were also a possibility as the primary source of infection in the nidus. During the 1961-63 period 23% of the cattle had titers to *L. pomona*[15]. The cattle, like the skunks, developed titers during the winter months. The serologic reactor rate in skunks was only slightly higher (28.1%) than cattle and either animal might have been responsible for contaminating the area.

The length of the carrier state is important and has been found to be quite long in skunks. Roth *et al.*[24], found that naturally infected captive skunks shed *L. hyos* for as long as 774 days; the *L. pomona* carrier state in 4 captive skunks lasted 354, 321, 303 and 229 days respectively. In this study the carrier state for 77 days in a free skunk was terminated at the trap-out by the killing of this skunk. The skunk has, therefore, been found to be readily infected and able to maintain a carrier state comparable to or longer than swine.

The complete description of a *L. pomona* nidus involving skunks and

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opossums as well as cattle will require more detailed study of many factors, including the period of leptospiruria in each host, susceptible populations, social habits, accurate life tables of the populations, a more complete knowledge of prozone reactions and the significance of carriers without immunologic reactions. A more detailed investigation of a natural focus should cover a larger amount of terrain in order to give more study to movement patterns and wild species interactions with the additional possibility of removing cattle from the focus. It is nevertheless, quite evident from this investigation that the skunk is an important host of *L. pomona* and capable of supporting a natural focus, while the opossum appears to be a less susceptible and more sporadic host.

ACKNOWLEDGMENTS

The data were collected under the terms of a grant (CC-00070, Nidality and Pathogenicity of Leptospira) from the Division of Research Grants, Communicable Disease Center, U.S. Public Health Service.

We wish to acknowledge the generous cooperation of Dr. M. E. Mansfield as well as others of the academic and non-academic staff at the Dixon Springs Agricultural Center. The collaboration of Drs. Carl Mohr, Thomas Scott and Glen Sanderson of the Natural History Survey of the State of Illinois have been indispensible. The technical assistance of Rachel Marlowe, Paul Boatman and the late Ivan Elder are also greatly appreciated.

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Quail Bronchitis[1]

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Received for publication 28 August, 1966

ABSTRACT

Quail bronchitis is an acute, contagious, respiratory disease of bobwhite quail. Tracheal rales, coughing, sneezing and mortality over 50 per cent is often observed in young infected birds. Quail bronchitis virus infects chickens and turkeys with no signs of disease. A similar agent, called chicken embryo lethal orphan virus. has been isolated from embryonating chicken eggs. Quail experimentally infected with chicken embryo lethal orphan virus have developed bronchitis. Airborne, mechanical and contact transmission of quail bronchitis virus is suspected. Diagnosis is based on signs, lesions of the respiratory system, and isolation and serological identification of the virus. No specific treatment is known. Additional research on this disease, both as it affects captive quail and the wild or released quail, is needed.

INTRODUCTION

Quail bronchitis is an acute, highly contagious, respiratory disease of bobwhite quail (*Colinus virginianus*, Linne). Mortality due to this disease can approach 100% in captive quail chicks. Quail bronchitis was first described by Olson[10] from an epornitic on a game farm in West Virginia in 1949. He isolated the causative agent, identified it as a virus and reproduced the disease in quail. Epornitics on game bird farms in Texas, one in 1956 and 4 in 1957, were reported by DuBose, Grumbles and Flowers[4] [5]. DuBose (unpublished evidence) also diagnosed an epornitic in Virginia in 1959. That prior occurrences in the U. S. were observed, possibly as

[1] Presented at the Annual Meeting of the Wildlife Disease Association, Univ. of Md., College Park, 14-19 August, 1966.

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