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FIELD AND LABORATORY STUDIES OF SKUNKS, RACCOONS AND GROUNDHOGS AS RESERVOIRS OF *LEPTOSPIRA POMONA**

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LEPTOSPIROSIS in herds of cattle in Ontario frequently occurs under circumstances which suggest direct or indirect transmission from wildlife. Outbreaks have been most common after heavy rains in the late summer or autumn, in cattle which have had access to low-lying pastures or wooded areas. The infecting serotype is usually *Leptospira pomona.*

The results of a leptospiral survey in wild mammals in Eastern Canada were reported by McKiel *et al.* in 1961 (5). *L. icterohaemorrhagiae* was recovered from wild rats and the first isolation in Canada of *L. pomona* from a groundhog (*Marmota monax*) was reported. A skunk (*Mephitis mephitis*) collected from the same farm as the groundhog had an agglutinin titre of 1/200 against *L. pomona* antigen. More recently Abdulla *et al.* (1) used serological, cultural and histopathological tests while conducting a wildlife survey for leptospirosis in Southern Ontario. These workers reported isolations of *L. pomona* as follows: four from groundhogs, seven from raccoons (*Procyon lotor*), one from a skunk and four from deer (*Odocoileus virginianus*). Further studies of leptospirosis in deer in Ontario have been published (4).

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This report deals with the occurrence of leptospiral infections in three other species (the striped skunk, the raccoon, and the groundhog or woodchuck). These species are usually abundant in outbreak areas.

PART I. SURVEY FOR NATURAL INFECTION

MATERIALS AND METHODS

Animals were collected by shooting or trapping. Blood and kidney tissues were removed at necropsy, using aseptic procedures.

Serological Methods

Serum was obtained from the blood samples by centrifugation and was stored at 4° C. until tested. The microscopic agglutination test was used to demonstrate leptospiral antibodies. Cultures used as antigen were maintained in Korthof's medium (3) with 10% rabbit serum enrichment. Three antigens (*L. pomona, L. grippotyphosa* and *L. icterohaemorrhagiae*) were used and sera were tested in three fold serial dilutions starting with an initial dilution of 1/10. The highest serum dilution giving 50% or greater agglutination, was taken to represent the titer of the serum.

Cultural Methods

Cultures from kidneys were made using the following procedure: the kidney capsule was removed aseptically, one-half gram of tissue was homogenized in a Tenbroek tissue grinder using 4.5 ml. of

Korthof's base medium as diluent to give an approximate 10% suspension of tissue. Using this 10% suspension, ten-fold serial dilutions were made in Korthof's medium up to 10^{-4} using screw-capped tubes containing 1.8 ml. of medium. Other tissues such as liver were processed in the same way. All tubes of inoculated medium were incubated at 30° C. and examined by dark-field microscopy (150X) at weekly intervals for six weeks.

Histological Methods

Histopathological examinations of the kidneys were made on formalin-fixed, paraffin-embedded tissues, sectioned and stained with hematoxylin-eosin. In some cases Para's (6) or Levaditi's (3) techniques were used for silver staining of leptospires in formalin-fixed tissues.

Identification of Serotypes

The leptospires isolated were provisionally identified by typing with leptospiral antisera which was routinely used in the laboratory. The identities of the isolates were confirmed by the World Health Organization Typing Centre at Washington, D.C.

Nine skunks from Wellington and adjoining counties of Ontario were killed and examined. The kidneys of seven of the nine animals were found to have interstitial nephritis suggestive of leptospirosis. Six of these kidneys were obtained in suitable condition for culture and *L. pomona* was isolated from three of them. Four of the nine sera contained agglutinins for *L. pomona*; three of the four positive sera were from animals shown to be kidney carriers of *L. pomona* (Figure 1).

No evidence of leptospirosis was found in specimens from 15 raccoons collected in Wellington and adjoining counties.

Kidneys from 110 groundhogs were obtained from animals shot or trapped in Wellington and adjoining counties. A strain of *L. pomona* was obtained from the kidneys of one of these animals. This groundhog was shot on a farm where there had been an outbreak of leptospirosis in cattle one year previously. Agglutinins for *L.*

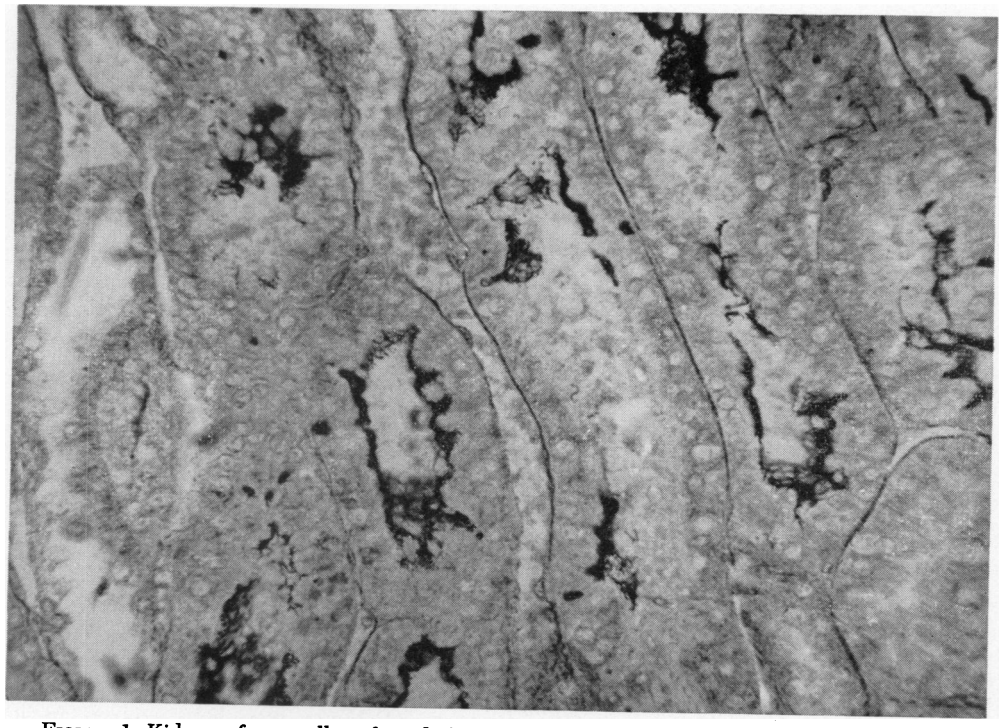

FIGURE 1. Kidney of naturally infected skunk, from which *L. pomona* was isolated. Levaditi silver stain to show masses of leptospires within tubules.

pomona were not found in the serum from this animal but a mild interstitial nephritis was found on histologic examination of the infected kidneys. One hundred and twenty-three groundhog sera were examined; of these only two had antibody to *L. pomona*. It is unfortunate that the kidneys from these two animals were not obtained in a condition suitable for culture.

Part II. Experimental Infections

A. *Skunks*

Materials and Methods

Five skunks, trapped in the wild at approximately two months of age, were used in these studies. They were surgically descented, housed in wire cages and fed a diet composed of a mixture of raw meat, fish and cereals which was prepared for mink. Skunks #1, #2 and #3 were inoculated intramuscularly with 0.3 ml. of a seven-day culture of *L. pomona* (Strain C, Table I). This strain was isolated from renal tissues of a naturally-infected skunk just prior to the experiment. It had been maintained in Korthof's medium with four serial passages in this medium. Skunks #4 and #5 were not inoculated but were housed with the inoculated skunks. The animals in this experiment were observed daily for signs of clinical leptospirosis. After the skunks were exposed to the infection specimens of blood (obtained by cardiac puncture) and urine (collected by direct bladder tap) were tested at various intervals. Occasionally pentobarbital anesthesia was used to facilitate the collection of specimens. One drop of blood from each bleeding was inoculated directly into 1.8 ml. of Korthof's medium. Ten-fold serial dilutions were made up to 10^{-4} from the inoculated tubes. The urine was examined using direct darkfield microscopy, by culture in Korthof's medium, and by inoculation of guinea pigs.

Results

Clinical signs attributable to leptospirosis were not observed in any of the skunks. All skunks inoculated with the skunk strain of *L. pomona* had leptospiremia. On day five following inoculation leptospires were demonstrated in blood cultured in liquid medium (Table II).

Agglutinins were demonstrated in all the inoculated animals. They were first detected on the 22nd day after inoculation. Titers of 1/60 to *L. pomona* were measured in serum from the inoculated skunks at this time, while agglutinins were first detected in serum obtained from the contact skunks on day 49 after exposure to their pen mates. The highest titer was 1/1620, occurring between 102 and 148 days after exposure. Contact skunk #5 died of an intercurrent bacterial infection on day 110 post-exposure. The antibody titer to *L. pomona* in serum obtained at time of necropsy of this animal was 1/540.

Leptospires were first demonstrated in urine obtained from inoculated skunks #1, #2 and #3 on post-inoculation day 22 and from contact skunks #4 and #5 on post-exposure day 49. Urinary shedding of leptospires was demonstrated in subsequent samples collected from all the experimental skunks throughout this study.

The surviving skunks in this experiment were killed on post-exposure day 148. At this time leptospires were recovered from cultures of renal tissue of all animals.

TABLE I

History of the Strains of *L. pomona* Used for Experimental Infection Studies

	Strain Isolated		
	Isolated from	Date	Maintained
Strain A	Deer fetus, Ontario	1962	Korthof's medium
Strain B	Deer, Rondeau Park, Ontario	1963	Korthof's medium
Strain C	Skunk, Ontario	1963	Korthof's medium
Strain D	Pig, Ontario	1962	Stuart's and Korthof's medium

TABLE II

RESULTS OF EXAMINATION FOR LEPTOSPIROSIS ON SKUNKS INOCULATED WITH OR EXPOSED BY CONTACT TO, *Leptospira pomona*

Skunk No.	Route of Inoculation and Amount	Serological[1] Days after Inoculation					Bacteriological Days after Inoculation					Histopathological Days after Inoculation				
							Blood	Urine[2]				Kidney				
		22	49	102	110	148	5	22	49	102	110	148	110	148	110	148
1	Intramuscular 0.3 ml.	1/60	1/180	1/1620	.	1/1620	+	+	+	+	.	+	.	+	.	+[3]
2	Intramuscular 0.3 ml.	1/60	1/540	1/540	.	1/1620	+	+	+	+	.	+	.	+	.	+
3	Intramuscular 0.3 ml.	1/60	1/180	1/540	.	1/1620	+	+	+	+	.	+	.	+	.	+
4	Contact	—	1/60	1/180	.	1/1620	—	—	+	+	.	+	.	+	.	+
5	Contact	—	1/540	1/540	1/540	.	—	—	+	+	+	.	+	.	+	.

[1]Highest dilution showing 50% or more agglutination or lysis by the microscopic agglutination test with *L. pomona* antigen.
[2]Direct darkfield examination and guinea pig inoculation.
[3]Interstitial nephritis suggestive of leptospirosis.

When examined histologically all were found to have interstitial nephritis.

B. *Raccoons*

MATERIALS AND METHODS

Six experiments were conducted with live-trapped raccoons. In Experiment 1, raccoon #3 was inoculated intraperitoneally with 1.0 ml. of a seven-day culture of *L. pomona* (Strain A, Table I). Raccoons #1, #2 and #4 served as pen contact animals. In Experiment 2, 1.0 ml. of a seven-day culture of *L. pomona* (Strain A, Table I) was added to one liter of drinking water used for experimental raccoons #5, #6, #7 and #8. This procedure was repeated daily for six days. The temperature and pH of the water was recorded daily during the exposure period. In Experiment 3, 10.0 ml. of a seven-day culture of *L. pomona* (Strain A, Table I) was added to one liter of drinking water for experimental raccoons #1, #3 and #4. This procedure was repeated daily for five days. In Experiment 4, raccoon #1 was placed in a waterproofed metal cage containing 10 liters of water with a pH of eight and a temperature of 9.5° C. Depth of the water in the cage was approximately three inches. The inoculum consisted of 10.0 ml. of a seven-day culture of *L. pomona* (Strain B, Table I), added to the water in the cage. Raccoon #1 was kept standing in the inoculated water in this cage for two hours and then returned to an isolation unit with raccoon #4. In Experiment 5, raccoon #4 was anesthetized with pentobarbital sodium given intraperitoneally. Several drops of a seven-day culture of *L. pomona* (Strain D, Table I) in Korthof's medium were instilled into the conjunctival sac of each eye. In Experiment 6, raccoon #65 was inoculated intramuscularly with 1.0 ml. of a seven-day culture of *L. pomona* (Strain C, Table I). This strain was isolated from a naturally infected skunk. It had been subcultured three times in Korthof's medium. Raccoons #66 and #67 served as pen contact animals in this experiment.

At intervals after exposure the raccoons were bled for serology. Urine was obtained for the demonstration of leptospires, using the procedures described.

RESULTS

The results of attempts to infect raccoons experimentally were generally disappointing. Clinical signs attributable to leptospirosis were not observed. In Experiment 1, only the inoculated raccoon developed an agglutinin titer of *L. pomona*. This titer had declined to less than 1/20 by the 35th day after inoculation. There was no evidence of renal shedding nor of transmission to cage mates.

Attempts to infect seven raccoons (Experiments 2 and 3) by repeatedly adding cultures of *L. pomona* to their drinking water were entirely unsuccessful, in spite of the fact that the temperature of the water averaged from two to 10° C. and the pH was approximately eight during the six days of experimental exposure.

In Experiment 4, a raccoon forced to stand in water heavily contaminated with *L. pomona* resisted infection. This was determined by lack of a subsequent immune response and failure to shed leptospires in urine.

Instillation of a culture of *L. pomona* into the conjunctival sac was successful in infecting a raccoon (Experiment 5). Agglutinins were detected in serum from this animal on the 15th day after exposure. They rose to a peak titer of 1/180. Leptospires were never found although the urine of this animal as well as its six cage contacts was examined repeatedly. Agglutinins did not appear in the sera of the pen contacts, further evidence that transmission did not take place. The raccoon exposed via the conjunctiva was sacrificed on day 80 after exposure. Leptospires were not recovered in cultures of kidney tissues although a mild interstitial nephritis was demonstrated histologically.

Agglutinins to *L. pomona* were first demonstrated in inoculated raccoon #65 on day 16 post-inoculation (Experiment 6). The peak agglutination-lysis titer of this raccoon was 1/4860. Significant levels of antibody to *L. pomona* were demonstrated in this raccoon throughout the experiment. The only time agglutinins to *L. pomona* were demonstrated in contact raccoon #66 was on post-exposure day 44. No titer to *L. pomona* was found in contact raccoon #67.

Leptospires were demonstrated in urine

obtained from inoculated raccoon #65 on days 44 and 101 post-inoculation but not in urine obtained on day 70 post-inoculation. Leptospiruria was not demonstrated in either of the contact raccoons (#66 and #67).

Raccoon #65 was killed on post-inoculation day 101 and leptospires were recovered from cultures of kidney tissue made at the time of necropsy. Histological examination revealed a mild interstitial nephritis.

C. *Groundhogs*

Materials and Methods

The groundhogs used for experimental infections were live-trapped on a local farm. They were housed in wire cages and fed good quality alfalfa hay, rolled oats and water. Greens were provided at irregular intervals. Restraint was manual, or manual plus ether anesthesia. Strain A of *L. pomona* (Table I) was used in these studies. Immediately prior to its use, two guinea pig passages were made to increase the strain's virulence. Groundhog #18 was inoculated intraperitoneally with 1.0 ml. of a seven-day culture of *L. pomona* (Experiment 1). Groundhogs #11 and #12 served as pen contact animals. In Experiment 2, groundhog #21 was inoculated intramuscularly with 1.0 ml. of a seven-day culture of *L. pomona*. Groundhogs #10, #16, and #17 served as pen contact animals. Specimens from all the experimental groundhogs were collected and processed at intervals after inoculation, using the techniques described previously.

Results

Agglutinins to *L. pomona* were first found in serum from inoculated groundhog #18 on the 21st day following inoculation (Experiment 1). Agglutinins to *L. pomona* were first detected in contact groundhog #11 on day 28 post-exposure. In these animals titers persisted for the duration of the experiment. There was no detectable antibody level to *L. pomona* in serum obtained from groundhog #12 at any time during the study period.

Leptospires were demonstrated in urine obtained from groundhog #18 on day 21 post-inoculation, also from contact groundhog #11 on days 35 and 55 post-exposure, but at no time from contact groundhog #12.

The three groundhogs in this experiment were killed on day 123 post-exposure. Bacteriological cultures of kidney tissues were negative for leptospires. An interstitial nephritis was observed in histologic kidney sections of groundhogs #18 and #11, but not in groundhog #12.

In Experiment 2, agglutinins to *L. pomona* were first demonstrated in inoculated groundhog #21 on day 23 post-inoculation. Agglutinins remained in significant levels in serum obtained from the animal throughout the study period. Antibodies to *L. pomona* were first demonstrated in contact groundhog #10 on day 30 post-exposure, and in contact groundhog #16 on day 57 post-exposure. Agglutinins to *L. pomona* were not found at any time in serum obtained from contact groundhog #17.

Leptospires were demonstrated in urine obtained from groundhog #21 on day 23 post-inoculation and from contact groundhog #10 on day 36 post-inoculation. Leptospiruria was not demonstrated in contact groundhogs #16 and #17.

On day 58 post-exposure, groundhog #17 was found dead in the cage. Extensive postmortem autolysis prevented the processing of body tissues and fluids from this animal. On post-exposure day 68 the surviving groundhogs were sacrificed under pentobarbital sodium anesthesia. Kidneys from these animals were not cultured. An interstitial nephritis was observed in histologic kidney sections from all three groundhogs.

Transmission Models

Materials and Methods

Two experimental models were constructed for the transmission of *L. pomona* from groundhogs to pigs and from groundhogs to calves.

Two groundhogs and four pigs were used in the first experiment. The groundhogs were born at the Ontario Veterinary College Research Farm. They were approximately six weeks old when used. The pigs were eight weeks old. Both

species were housed in an isolation unit, with the groundhogs confined in a wire mesh cage suspended approximately four feet above a flat metal container (3′ × 1½′ × 3″) which served as a drinking water container for the pigs. The groundhogs were inoculated intraperitoneally with 1.0 ml. of a seven-day culture of *L. pomona* (Strain C, Table I). To increase its virulence this strain was given three guinea pig passages immediately before its use. The pH and temperature of the pigs' drinking water were recorded daily during the experiment. Specimens of blood and urine were collected and examined at intervals after exposure.

In the second transmission experiment, two groundhogs and two calves were used as experimental animals. The groundhogs were live-trapped adults. Calf #1622 was approximately three months of age; calf #988 was one month of age. The groundhogs were confined in a wire mesh cage suspended approximately four feet above a metal container (2′ × 2′ × 1′) which served as a water receptacle for the calves. Drinking water supplied to both species was tap water, pH approximately eight, with no chlorine added. The groundhogs were inoculated intraperitoneally with 1.0 ml. of a seven-day culture of *L. pomona* (Strain C, Table I) and all animals were observed daily for signs of clinical leptospirosis. At intervals after exposure specimens from the animals in this experiment were collected and processed using the methods described previously.

Results

In Experiment 1, agglutinin titers to *L. pomona* were first demonstrated in both inoculated groundhogs on day 11 post-inoculation. Significant levels of antibody remained in serum obtained from these animals on days 22 and 25 post-inoculation.

Leptospires were demonstrated in urine obtained from both experimental groundhogs on days 14, 19, 22 and 25 post-inoculation. Both animals died on day 25 post-inoculation. Death resulted from hemopericardium following cardiac puncture. Leptospires were recovered from cultures of renal tissue and a severe interstitial nephritis was observed in histologic kidney sections of both animals.

During the experiment the pH of the pigs' drinking water varied from 5.7 to 8.0 and the temperature varied from 14° to 25° C.

Agglutinins to *L. pomona* were first demonstrated in contact pigs #1616 and #1618 on post-exposure day 61 and in contact pig #1611 on post-exposure day 113. Antibody to *L. pomona* was not found in serum obtained from contact pig #1612 on post-exposure days 22, 29, 61 and 113, nor were leptospires demonstrated in urine collected from this pig on days 22, 29 and 113 post-exposure. Leptospires were observed in urine collected from pigs #1611, #1616 and #1618 on day 113 post-exposure. Kidneys from these animals were not cultured or examined histologically.

In the groundhog-to-calf transmission experiment, significant levels of agglutinins to *L. pomona* were found in serum obtained from both the groundhogs (#31 and #32) on post-inoculation days 14 and 27. Leptospires were demonstrated in urine collected from these animals on the same days. Both experimental groundhogs were killed on post-inoculation day 27. Kidney cultures from both groundhogs were positive for leptospires and a severe interstitial nephritis was observed in histologic kidney sections of these animals.

During the experimental period the pH of the calves' drinking water varied from 6.2 to 8.0 while the temperature varied from 18.5° to 24.0° C.

The rectal temperatures of calves #1622 and #988 were within the normal range for this species until day 16 post-exposure when the temperature of calf #988 rose to 104.8° F. The temperature of this animal varied from 103.0° F. to 104.8° F. until it died on day 25 post-exposure. Depression, anemia and icterus were observed throughout the acute phase of the infection of this calf. Cultures of blood made on day 18 post-exposure were bacteriologically negative for leptospires. The gross findings on necropsy of this animal were extreme icterus, anemia and hemoglobinuria. Leptospires were recovered from cultures of renal tissue of this animal.

The only day the rectal temperature of calf #1622 varied from the normal range

for this species was on day 23 post-exposure when it was found to be 103.0° F. There were no clinical signs of leptospirosis observed in the calf during the experiment. Agglutinins to *L. pomona* were detected in serum obtained from this calf on day 54 post-exposure. Kidneys from this animal were not cultured or examined histologically.

Discussion and Conclusions

The persistence of antibody titers, renal lesions of focal interstitial nephritis, and leptospires in renal tubules as a result of leptospiral infection, has been used to determine the prevalence of leptospirosis in the more common wildlife species in Ontario. The results of serological and bacteriological studies indicate that the wildlife species examined were exposed only to the serotype *L. pomona*.

The frequency of leptospiral infection in skunks was found to be very high. Ten animals were captured in the wild and of these only three were without evidence of infection. Isolations of *L. pomona* were made from three of the six skunk kidneys cultured. All of these animals had significant microscopic agglutination titers to *L. pomona*. Roth (7) mentions that skunks without demonstrable agglutinins have been found to be naturally infected with *L. pomona*. The authors do not understand this apparent failure of the immune response. It was not demonstrated in skunks in this study. Two of the skunks from which isolations of *L. pomona* were made were also found to have rabies. These animals were shot on a farm within one mile of the Ontario Veterinary College.

Studies of experimental infections in skunks has confirmed that skunks are highly susceptible to infection with *L. pomona*. Following a period of leptospiremia, leptospires became localized in the kidneys where they persisted until the experiment was terminated (148 days). Immune response in contact skunks appeared as early as 27 days after antibody was first detected in their pen mates. This may be taken as evidence of natural transmission within the cage environment. The consistent demonstration of leptospires in urine collected from infected skunks indicates a highly developed host-parasite association. To further support the evidence that skunks are important maintenance hosts for leptospires, it should be mentioned that Roth (8) has demonstrated urinary shedding of *L. pomona* in naturally infected skunks for up to 220 days.

No evidence of leptospiral infection was found in raccoons collected during the wildlife leptospiral survey. Abdulla *et al.* (2) found raccoons infected with *L. pomona* during epizootics. In this study the authors found raccoons very difficult to infect experimentally although they were exposed to leptospires by supposedly natural routes. Leptospiruria was demonstrated only once in a raccoon exposed experimentally to *L. pomona*, by intramuscular inoculation. The failure in these studies to infect raccoons by "natural" exposure is not understood. Large numbers of leptospires, of strains of *L. pomona* of proven virulence for other animals, were used to expose the raccoons. The fact that leptospiral infections have occurred in Ontario, in raccoons exposed in their natural habitat (2), suggests the possibility that species adaptation is necessary. A recent isolate of *L. pomona* from a raccoon was not available for the present study. The role of raccoons in the epizootiology of leptospirosis may be merely that of amplifier hosts, in providing "fuel for the fire", but contributing little to the long-term maintenance of infection in nature.

L. pomona was recovered from the renal tissue of a mature groundhog shot on a farm where an outbreak of *L. pomona* infection in cattle had occurred the previous year. The serum of this groundhog was devoid of agglutinins to *L. pomona*. This apparent failure of the immune response is not understood. The failure of the host to produce agglutinins may possibly be characteristic of a highly developed host-parasite association, where the parasite is fed, sheltered and transported and the host remains clinically healthy and is not stimulated to produce antibody against the invading organism.

There were no clinical signs of leptospirosis in the groundhogs experimentally infected with *L. pomona*. Infection was readily established in these animals. Transmission of *L. pomona* occurred from infected to contact animals, as indicated by

the development of agglutinin titers in the contact animals. In only one animal were leptospires demonstrated in urine more than once. The duration of urinary shedding of leptospires in this animal was 55 days. Because of their habits in nature and because of the inconstant shedding of leptospires by infected animals, groundhogs appear less likely than skunks to transmit the disease to other species.

Groundhogs usually live and build burrows on high ground to avoid danger of flooding. This should limit their contamination of surface waters, considered to be the most important means of leptospiral exposure of cattle on pasture. Groundhog to groundhog transmission may be quite effective, however, if it occurs between the dam and her young, in the intimate contact of the burrow. If this vertical transmission occurs, groundhogs could then serve as reservoirs for occasional fortuitous transmission of leptospirosis to other species, possibly to grazing animals on pasture. The experiments carried out by the authors have demonstrated that *L. pomona* can be transmitted from groundhogs to calves and groundhogs to pigs, at least under the conditions of our artificial models.

Summary

A survey for leptospirosis among skunks, raccoons and groundhogs in Ontario, conducted in 1962 and 1963, revealed infection in skunks and groundhogs but not in raccoons. Strains of *Leptospira pomona* were isolated from the kidney tissues of three skunks and one groundhog. Serologic and histopathologic evidence of natural infection in these species was also found.

Experiments were conducted to assess the importance of skunks, raccoons and groundhogs as wildlife reservoirs of *L. pomona*. Raccoons were difficult to infect with the strains of *L. pomona* used, and failed to excrete leptospires in their urine in significant quantities. Skunks and groundhogs, on the other hand, proved to be highly susceptible to *L. pomona* which was given by inoculation or routes simulating natural exposure. Skunks were found to shed leptospires in their urine in large quantities and for prolonged periods. Groundhogs were less consistent renal shedders.

Résumé

Une étude sur la leptospirose chez la mouffette, le raton-laveur et la marmotte fut entreprise en Ontario en 1962 et 1963; elle révéla que la mouffette et la marmotte, mais non le raton-laveur, étaient infectées. On décela des traces de *Leptospira pomona* dans des tissus rénaux de trois mouffettes et d'une marmotte. On a aussi trouvé la preuve sérologique et histopathologique d'infection naturelle chez ces espèces.

On a effectué des expériences en vue de déterminer l'importance des mouffettes, des ratons-laveurs et des marmottes en tant que source de *L. pomona* chez les animaux sauvages. Les ratons-laveurs furent difficilement infectés avec les cultures de *L. pomona* employées, et ils n'excrétèrent pas de leptospires en quantité significative dans leur urine. D'autre part, les mouffettes et les marmottes montrèrent qu'elles étaient hautement sensibles à la *L. pomona* lorsque celle-ci était donnée soit par inoculation soit par voie simulant le contact naturel. Les mouffettes excrétaient dans leur urine de très grandes quantités de leptospires durant de longues périodes tandis que les marmottes étaient moins régulières.

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BOOK REVIEW

An Introduction to Comparative Biochemistry. Fourth Edition. Ernest Baldwin. The Macmillan Company of Canada, Toronto, Ontario. 1964. 179 pages. Price $3.

This interesting little book is concerned primarily with the biochemical approach to evolutionary problems. Material is selected from a wide variety of sources since the data of comparative biochemistry is found throughout the whole field of biological literature. It is to be regretted that no references to original material are presented in this edition.

Topics selected for consideration include genesis, the colonization of fresh water, of dry land, the regulation of osmotic pressure and the conservation of water, nitrogen metabolism including purines, the distribution of nitrogen bases and of phosphagens, respiration, respiratory and other pigments, nutrition and digestion and some general remarks about metabolism and the environment.

This book is not an introductory text on biochemistry, nor does it concern itself with the biochemistry of domestic animals or any particular species or group of animals. It provides a useful overview of a very broad subject and could be valuable to students in any of the biological sciences. *Allan G. Stewart.*

ABSTRACTS

Cowie, R. S. (1964). The use of dilute acids in the treatment of white scour in calves. Vet. Rec. 76, 1516–1518.

The use of dilute acids in the successful treatment of white scours is described and such treatment changes the pH of the faeces. Cowie's findings were supported by D. R. Lane (Vet. Rec. 77, 54 (1965)), but E. W. Fisher, J. G. Watt and R. G. Dalton (*Ibid.* p. 93) claimed that it was impossible to assess efficacy in the absence of controls, and that there was a risk of aggravating the acidosis that may occur in calves with diarrhoea.

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Batty, I., Buntain, D., and Walker, P. D. (1964). Clostridium oedematiens: a cause of sudden death in sheep, cattle, and pigs. Vet. Rec. 76, 1115–1117.

The use of fluorescent antibody staining in the detection of Cl. novyi infection in sudden death of sheep, cattle, and pigs is reported. It is suggested that Cl. novyi infection is more common in cattle in Great Britain than has previously been recognized. The majority of cases in sheep were typical of black disease. Cl. novyi infection in pigs is recorded.

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