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Author: Kingscote, B. F.

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LEPTOSPIROSIS IN RED FOXES IN ONTARIO

B. F. Kingscote[1]
Agriculture Canada, Animal Diseases Research Institute,
P.O. Box 640, Lethbridge, Alberta T1J 3Z4, Canada

ABSTRACT: The role of the red fox (*Vulpes vulpes*) in the epizootiology of leptospirosis in southwestern Ontario was investigated in 1973–1974. *Leptospira interrogans* serovar *pomona* (*kennewicki* by DNA analysis) was isolated from the kidneys of three of eight foxes tested. Severe hemorrhagic nephritis and interstitial nephritis were common to these foxes and to five others out of nine foxes examined. *Autumnalis* antibodies were detected at titers 10^{-2} to 10^{-5} in 12 of 100 fox sera. *Pomona* antibodies occurred in 6% of the sera, always accompanied by *autumnalis* antibodies, and at titers never exceeding the *autumnalis* titers. Cultural, serological, and pathological findings together indicated that the red fox could have been acting as an amplifier host, but not as a maintenance host, for *pomona*.

INTRODUCTION

Leptospirosis caused by *Leptospira interrogans* serovar *pomona* (*pomona*) has been recognized in Ontario for at least 30 yr. Barnum and Grinyer (1957) confirmed its pathogenicity by isolating it from a dead calf. Karstad and co-workers (Abdulla et al., 1962; McGowan and Karstad, 1965; Tabel and Karstad, 1967) studied the roles of wild mammals in the epizootiology of *pomona* infection in domestic animals in southern Ontario. Their studies showed that white-tailed deer (*Odocoileus virginianus*) and moose (*Alces alces*) were more likely to be accidental than to be maintenance hosts, that raccoons (*Procyon lotor*) could act as amplifiers, and that striped skunks (*Mephitis mephitis*) and groundhogs (*Marmota monax*) could serve as maintenance hosts. *Pomona* infection in cattle and other livestock was shown by Kingscote (1970) to be widespread in southern Ontario, on the basis of test results on 27,000 serum samples. Market sows representative of the population in southwestern Ontario in 1973–1974 had antibodies to *pomona* and to *autumnalis* at prevalences of 4.2% and 3.3%, respectively (n = 334), while only *pomona* was identified in cultures from clinical cases in swine and cattle (Kingscote, unpubl. data). Clark et al. (1960) isolated *pomona* from a red fox on a farm in Pennsylvania.

The innovation of DNA analysis by restriction endonuclease as a tool for typing *Leptospira* within the past few years (Thiermann et al., 1985) has facilitated the accurate comparison of *pomona* strains isolated from cattle, swine, skunks, and foxes in Ontario. The potential of the red fox to function as a reservoir for *pomona* in southwestern Ontario is the subject of this paper.

MATERIALS AND METHODS

Red foxes were live-trapped throughout southwestern Ontario, in a rabies research program in 1973–1974. The foxes were killed with nembutal, bled and necropsied at the Ontario Veterinary College. Blood and kidneys were refrigerated and delivered to the leptospirosis laboratory within 12 hr of collection. A single exception was a fox which was shot on a farm, chilled but not frozen, and delivered for necropsy 2 days later. In total, 100 sera and nine kidneys were examined. Serum was tested in 10-fold dilution for the presence of agglutinins against leptospiral serovars *pomona, autumnalis, canicola, grippotyphosa, icterohaemorrhagiae, ballum,* and *hardjo,* by the microscopic agglutination (MA) test. The titer of a serum was the highest dilution which agglutinated 50% of the antigen.

Kidney tissue including cortex and medulla was homogenized aseptically in phosphate buff-

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[1] Present address: P.O. Box 1468, Innisfail, Alberta TOM 1A0, Canada.

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ered saline, pH 7.2, in a Ten Broeck grinder, to produce a 10% suspension. This suspension was diluted 10-fold to 10^{-3}, and inoculated in 1-ml aliquots to semi-solid albumin polysorbate-80 medium (EMss) (Ellinghausen and McCullough, 1965). Cultures were incubated at 29 C for a maximum of 8 wk. One weanling guinea pig was inoculated intraperitoneally with 1.0 ml of each kidney homogenate, 10^{-2} dilution. Three wk later, guinea pigs were bled for serological testing and their kidneys were cultured as described above. Positive cultures were transferred to fresh EMss and adapted to liquid EM. Antiserum to each isolate was produced in rabbits by intravenous inoculation of live antigen. Cultures were serotyped by standard World Health Organization method and DNA-typed by restriction endonuclease analysis (Thiermann et al., 1985).

The possibility of a mixed infection by *pomona* and *autumnalis*, resulting in the presence of a minority population of *autumnalis* in the kidney cultures, was addressed in the following way. Antiserum (titer 10^{-4}) prepared in rabbits against *pomona* reference strain was added in 0.5-ml volume to 9 ml of liquid culture medium. Isolates were inoculated to this medium, incubated for 8 wk, and examined for growth.

In addition to culture, kidneys were examined for lesions grossly, and also histologically by standard hematoxylin-eosin staining of formalin-fixed, paraffin-embedded sections.

RESULTS

Autumnalis antibodies were found in 12 of 100 sera tested, accompanied by *pomona* antibodies in six sera. Titers ranged from 10^{-2} to 10^{-5}, with *autumnalis* titers equal to or exceeding *pomona* titers (Table 1). Kidneys of three foxes yielded isolates which were serotyped as *pomona* and DNA-typed as *kennewicki,* in the Pomona serogroup. Leptospires were isolated from a kidney removed from a fox carcass necropsied 2 days after death.

Two guinea pigs inoculated with fox kidney developed *pomona* infection, with no evidence of a dual *autumnalis* infection. Rabbits in which antiserum was produced developed only *pomona* agglutinins. No growth was obtained in culture medium containing *pomona* antiserum.

The gross and microscopic lesions seen in eight of nine fox kidneys were spectacular. Intense hemorrhagic nephritis affected the whole kidney, with moderate to severe interstitial nephritis developing. No lesions were seen in the ninth kidney.

DISCUSSION

The identification of *pomona* only, from red foxes whose serology strongly suggested the presence of *autumnalis* infection, illustrates the need to verify serology by culture. No evidence was obtained in this study to support a hypothesis of a dual infection. Therefore the infecting serovar, *pomona*, was presumed to be a strain which elicited in foxes a serological response with a high degree of cross-reaction against *autumnalis* antigen. The observation of Clark et al. (1960) supports this conclusion. *Autumnalis–pomona* cross-reactions are not uncommon in diagnostic serology, especially early in the course of *pomona* infection in man. Considering the extent of renal lesions in the foxes in this study, the serological findings do not suggest the classic paradoxical reaction of early leptospirosis.

Despite the conclusions stated above, the possibility of isolating *autumnalis* in Canada should not be overlooked. This serovar occurs in North America in man (Kaufmann, 1976) and wild mammals (Gorman et al., 1962; Alexander et al., 1972). Kingscote detected *autumnalis* antibodies, alone or at higher titers than coincident *pomona* antibodies, in striped skunks, groundhogs, a coyote (*Canis latrans*), fisher (*Martes pennanti*), mink (*Mustela vison*), swine and cattle around a site where a red fox with high *autumnalis* titer in serum was obtained (unpubl. data).

The isolation of *pomona* (*kennewicki*) from a red fox is a new host record for Canada. *Kennewicki* is the serovar in the Pomona serogroup which occurs in swine, cattle, and striped skunks in Ontario and Alberta (Thiermann et al., 1985). Possible sources of infection to red foxes include

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TABLE 1. Results of laboratory tests for leptospires, leptospiral antibodies, and lesions in red foxes in Ontario, 1973–1974.

	Serum antibodies (titer)[a]		Kidney	
Fox no.	*autumnalis*	*pomona*	Culture	Histology
1	neg[b]	neg	neg[c]	ND[d]
2	10^{-2}	neg	ND	ND
3	10^{-2}	10^{-2}	ND	ND
4	10^{-2}	neg	ND	ND
5	10^{-3}	neg	pos[e]	pos[f]
6	10^{-5}	10^{-3}	ND	ND
7	10^{-3}	neg	neg	pos
8	neg	neg	neg	pos
9	10^{-3}	neg	neg	pos
10	10^{-2}	neg	neg	pos
11	10^{-3}	10^{-2}	ND	pos
12	10^{-5}	10^{-5}	pos	pos
13	10^{-4}	10^{-3}	pos	pos
14	neg	neg	ND	neg[g]
15	10^{-3}	10^{-2}	ND	ND
(85)[h]	(neg)	(neg)	(ND)	(ND)
	12/100 = 12%	6/100 = 6%	3/8 = 38%	8/9 = 89%

[a] Measured by Microscopic Agglutination Test for serovars *pomona, autumnalis, canicola, grippotyphosa, icterohaemorrhagiae, ballum,* and *hardjo*. Titer is the highest serum dilution agglutinating 50% of the antigen.

[b] Absence of agglutination of 50% of antigen at serum dilution 10^{-2}.

[c] No leptospires detected in culture after 8 wk incubation.

[d] ND = not done.

[e] *Leptospira interrogans* serovar *pomona* isolated.

[f] Histological lesions seen, including interstitial nephritis and hemorrhagic nephritis.

[g] No lesions seen.

[h] Eighty-five sera with which no tissues were submitted.

water contaminated by domestic or wild mammals, carrion, or infected prey. The possibility of oral infection occurring in nature was demonstrated by Reilly et al. (1970) in carnivores, including foxes which were fed mice infected with leptospires of the serovar *grippotyphosa*. Reilly reasoned that the leptospires were protected from gastric acidity because the gulped bolus passed partially unmacerated into the alkaline duodenal environment.

The striped skunk is recognized as a maintenance host for *pomona* (*kennewicki*) in North America. This role is characterized by an absent or weak antibody response in many carrier animals (Roth et al., 1963; Tabel and Karstad, 1967). The high antibody titers in the foxes in this study and the severe nephropathy indicated a poorly balanced host–parasite relationship, such as the relationship reported by Shotts et al. (1971) to exist between rabbits and serovar *grippotyphosa*. Thus it is unlikely that the red fox was acting as a maintenance host for *pomona* at the time of the study. It was certainly capable of functioning as an amplifier host, and it could have served well as an indicator host. The present status of red foxes as carriers of *pomona* in Ontario is unknown, since changes in host–parasite relationship occur over time. The reader is referred to Torten's discussion of the epizootiology of leptospirosis as ". . . a constantly changing dynamic process . . ." (Torten, 1979).

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