

# Leptospirosis in raccoons in Quebec: 2 case reports and seroprevalence in a recreational area

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**Abstract** — Raccoons may represent a source of leptospires for humans and domestic animals. We describe a case of severe interstitial nephritis associated with the serovar *bratislava* of *Leptospira interrogans* (1st report in wildlife), and the seroprevalence to 4 leptospire serovars in a recreational area in Quebec.

**Résumé** — La leptospirose chez les ratons laveurs : Rapport de 2 cas et séroprévalence dans un lieu récréatif du Québec. Les ratons laveurs peuvent représenter une source de leptospires pour les humains et les animaux domestiques. Nous décrivons un cas de néphrite interstitielle grave associée au sérovar bratislava du *Leptospira interrogans* (1<sup>er</sup> rapport chez les animaux de la faune) et la séroprévalence à 4 sérovares de leptospires dans un lieu récréatif du Québec.

(Traduit par docteur André Blouin)

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Leptospirosis is a zoonotic disease of mammals caused by serovars belonging to the spirochete *Leptospira interrogans*. Most serovars have 1 or several maintenance host species in which they generally cause chronic diseases characterized by reproductive failures or interstitial nephritis. Acute disease primarily occurs in young maintenance host species or in incidental host species. Important populations of raccoons inhabit urban and recreational areas of North America where they may represent a source of leptospires for humans (1). Since the 1970s, the knowledge and understanding of leptospirosis in other species has grown. New serovars have been identified and new leptospiral relationships have been documented among humans, domestic animals, and wildlife (2). However, there have been no recent reports relating to leptospirosis in raccoons (*Procyon lotor*), despite the close association of raccoons with human habitation. This paper reports the detection of leptospires in 2 diseased raccoons, as well as the results of a serological survey carried out on 25 presumably healthy raccoons captured in a recreational area.

Forty-two raccoons were received at the Faculté de Médecine Vétérinaire de l'Université de Montréal from 1993 to 1996 for postmortem examination. Kidney

sections from 27 of the 42 raccoons were available for microscopic study. Kidneys with any evidence of inflammation were stained by the Warthin-Starry technique. When filamentous bacteria were detected, immunostaining techniques for the identification of the leptospiral serovar were performed.

The choice of the immunostaining technique was guided by the availability of appropriate tissues. Direct immunofluorescence was done on frozen renal tissues, using monoclonal antibodies against serovars *pomona*, *icterohaemorrhagiae*, *hardjo*, and *bratislava* (supplied by Dr. John F. Prescott, Ontario Veterinary College, Guelph, Ontario).

The streptavidine-biotin immunoperoxidase technique was done on paraffin-embedded sections using polyclonal antibodies against serovars *pomona*, *icterohaemorrhagiae*, *hardjo*, *grippityphosa*, and *bratislava* (rabbit polyclonal antibodies, University of California Davis, California, USA).

Microscopic examination of kidneys revealed interstitial lymphoplasmocytic nephritis in 8 of 27 raccoons (30%). Filamentous bacteria were evidenced by the Warthin-Starry staining technique in the renal convoluted tubules of 2 of the 8.

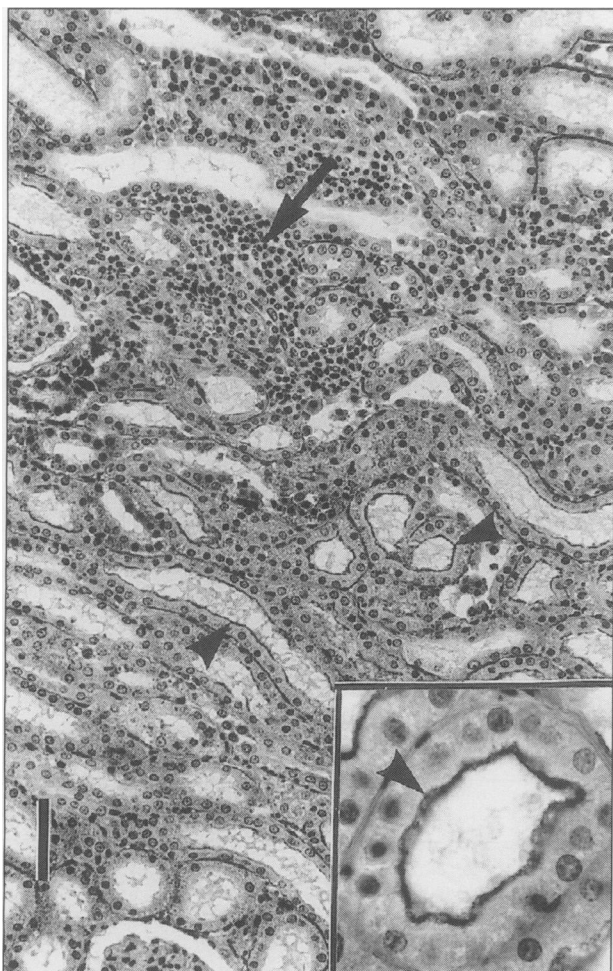
Case 1 was a 1-year-old, thin, pregnant female, which had been found dead in an urban park of the City of Montreal. The cortex and the medulla of the right kidney were severely and homogeneously enlarged and pale. The cortex of the left kidney showed a spherical, 1.5 cm in diameter, ill-defined, homogeneously pale area. Microscopically, the cortical and medullary structures of the grossly affected areas were almost totally replaced by myriads of lymphocytes and plasmacytes, admixed with a few neutrophils and macrophages. At the periphery of the inflammatory infiltrate, a few Warthin-Starry-positive filamentous organisms formed small clusters in the lumen of a few convoluted renal tubules. These organisms reacted with monoclonal antibodies strongly to the serovar *bratislava* and weakly to the serovar *pomona* using the immunofluorescence technique. The spleen

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**Figure 1.** Kidney from a raccoon: moderate interstitial lymphoplasmocytic nephritis (→) with abundant argyrophilic material along the luminal surface of the distal convoluted tubule cells (▶). Warthin-Starry. Bar = 140 µm. Insert: higher magnification; streptavidine-biotin peroxidase stain with antibodies for *Leptospira interrogans* serovar *pomona* antigen.

showed marked lymphoid depletion and mild, multifocal foci of coagulative necrosis affecting the corpuscles. The liver showed mild, irregular, periportal and perilobular lymphoplasmocytic infiltration and fibrosis. *Leptospira* isolation was not attempted due to the poor preservation of the specimens.

Case 2 was an adult female raccoon found with neurological signs in a barn in a farming area of southwestern Quebec. The animal seemed unaware of its surroundings and showed no fear of humans. Gross examination was unremarkable. Microscopically, the kidneys showed multifocal to coalescing interstitial infiltration by a moderate number of lymphocytes and plasmocytes and by a few macrophages (Figure 1). Myriads of Warthin-Starry-positive filamentous bacteria were present in the proximal convoluted tubules. These organisms reacted strongly with polyclonal antibodies to *L. interrogans* serovar *pomona* using the immunoperoxidase technique (Figure 1, insert). *Pomona* antigens were also detected in the cytoplasm of tubular cells and in the cytoplasm of macrophages in areas of interstitial nephritis. The death of this animal was attributed to cerebral larva migrans.

**Table 1. Microscopic agglutination test (MAT) titers for 25 raccoons from a recreational area in Quebec**

Serovar	Number reactive, per dilution				Total positive <sup>a</sup>	%
	50	100	400	1600		
<i>pomona</i>	2	2	1	...	3	12
<i>hardjo</i>	...	1	...	...	1	4
<i>bratislava</i>	2	2	2	1	7	28
<i>grippityphosa</i>	...	1	...	...	1	4

<sup>a</sup>MAT was considered positive for a final serum dilution of  $\geq 1:50$  for *hardjo* and *bratislava* serovars and  $\geq 1:100$  for *pomona* and *grippityphosa* serovars

Twenty-five raccoons (24 adults, 1 juvenile) were trapped and tagged from June to August 1995 in the Mont Orford recreational area (72°15'E, 42°18'N) in Quebec, and tested by the microagglutination test (MAT). The population level of raccoons in the study area was approximately 100 per km<sup>2</sup> (Lefebvre F, unpublished observation). The most commonly diagnosed serovars in domestic animals in Quebec are serovars *pomona*, *hardjo*, *bratislava*, and *grippityphosa* (Major M, unpublished observation). Our objective was to determine whether these serovars also infected raccoons in Quebec. Titers  $\geq 1:100$  for serovars *pomona* and *grippityphosa* and  $\geq 1:50$  for serovars *hardjo* and *bratislava* were considered positive for the purpose of this survey. A higher cutoff dilution was selected for serovars *pomona* and *grippityphosa*, because these serovars are considered to be more immunogenic compared with serovars *hardjo* and *bratislava*.

Evidence of exposure to serovar *bratislava* was found in 7/25 (28%) raccoons, with 1 animal having a titer of 1:1600 (Table 1). Five of the 7 animals also showed low titers to other serovars: 3 showed a reaction to *pomona*, 1 to *hardjo*, and 1 to *grippityphosa*. These results confirm that leptospirosis is present in raccoons in the Mont Orford recreational area, Quebec.

In animals, the extent of renal lesions caused by leptospires is dependent on the serovar-host relationship. An association between interstitial nephritis and leptospiral infection has been proposed for a variety of serovars in domestic (3) and wild animals (4). Because renal lesions were histologically compatible with leptospirosis in the raccoon infected by serovar *bratislava* (case 1), it is likely that this serovar was the etiologic agent of the severe interstitial nephritis.

In recent infections, leptospires are localized in the tubular lumen. At this stage, they are numerous, intact, and easy to visualize by both the immunochemical and silver-staining methods; the numerous organisms found in the convoluted tubules of the raccoon infected by serovar *pomona* (case 2) and the moderate nephritis suggest that this animal had been recently infected.

In the past, several wildlife species have been identified as maintenance hosts for serovars causing disease in domestic animals and man; concern was raised about the possible role of raccoons (1), skunks (4) and red foxes (5). Studies have demonstrated that serovar *pomona* is maintained in pigs (6) and cattle (7), not in skunks (7) and red foxes (5). Serovar *bratislava* is maintained in pigs and horses and has been incriminated in acute leptospirosis in man (8) and dogs (9). Seroprevalence of this serovar

has been reported from a variety of wild animals and among trappers in Quebec (2). Data presented herein suggest that serovar *bratislava* might be endemic in raccoons from the Mont Orford area, a species in which it has not been detected previously. Further studies are needed to confirm by nucleotide sequencing the classification of leptospiral strains in raccoons and to assess the role of raccoons in the epidemiology of leptospirosis. CVJ

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