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## A SEROLOGIC ASSESSMENT OF EXPOSURE TO VIRAL PATHOGENS AND *LEPTOSPIRA* IN AN URBAN RACCOON (*PROCYON LOTOR*) POPULATION INHABITING A LARGE ZOOLOGICAL PARK

Randall E. Junge, D.V.M., Dipl. A.C.Z.M., Karen Bauman, B.S., Melanie King, B.S., and Matthew E. Gompper, Ph.D.

**Abstract:** In urban environments, raccoons (*Procyon lotor*) may act as reservoirs for an array of pathogenic organisms, presenting spillover risks for human, domestic animal, and captive (zoo) animal populations. Over 5 yr, 159 raccoons from a high-density raccoon population in St. Louis, Missouri (USA), were surveyed for exposure to canine distemper virus (CDV), canine adenovirus 1 (CAV-1); feline parvovirus (FPV; =feline panleukopenia), and several serovars of *Leptospira interrogans*. Exposure to each of the viruses and two *Leptospira* serovars (*grippityphosa* and *icterohemorrhagiae*) was detected (prevalence of CDV = 54.1%; FPV = 49.7%; CAV-1 = 6.9%; *L. interrogans icterohemorrhagiae* = 8.9%; *L. interrogans grippityphosa* = 6.3%). Eighty percent of raccoons showed evidence of exposure to at least one of the five primary pathogens, and 39% were positive for multiple species. Among the viruses, there was a significant co-occurrence of CDV and CAV-1. Longitudinal data on a subset of animals revealed that among individuals who were diagnosed as seropositive on first capture, 33–100% became seronegative for the pathogen of interest when reexamined at a later date. Thus, free-ranging urban raccoons have been exposed to multiple infectious agents, some of which may pose risks to humans and to nonvaccinated domestic and captive animal populations.

**Key words:** Adenovirus, canine distemper, leptospirosis, raccoon, parvovirus, *Procyon lotor*.

### INTRODUCTION

Wildlife in urban environments has the potential to persist at high densities and to act as a reservoir for a broad array of disease-causing organisms. Because most large zoological parks also exist in urban environments, the potential exists for spillover of pathogens from free-ranging wildlife into the captive animal collection. For instance, raccoons (*Procyon lotor*) are a widespread and common North American wildlife species and can be host to several infectious pathogens transmissible to domestic animals, other native wildlife, and exotic zoo animals.<sup>7</sup> Because this species can exist at high densities in urban environments,<sup>20,25,29</sup> and because high contact rates of these populations may enhance the risk of disease transmission,<sup>33</sup> raccoons are frequently monitored or controlled in urban zoos to reduce the perceived risk of disease spread and predation on vulnerable collection animals. Insufficient data are available, however, with regard to the actual disease risk these animals may present to zoo animals or with regard to the extent of exposure to pathogens occurring among urban rac-

coons. Therefore, a survey of disease exposure among raccoons captured on the grounds of the St. Louis Zoo was undertaken as a first step toward understanding the risk posed by raccoons inhabiting the wildlife-zoo interface and toward gaining basic insights into the extent to which urban raccoon populations are exposed to pathogens representing human and animal health concerns.

Several infectious diseases have been well documented in raccoon populations in North America, and this survey was designed to assess exposure to pathogens that may represent disease risks to the captive animal collection. This survey measured antibodies to canine distemper virus (CDV), canine adenovirus 1 (CAV-1; =infectious canine hepatitis), and feline parvovirus (FPV; =feline panleukopenia or raccoon parvovirus). Each of these viruses has been previously reported in raccoons, may be an important cause of raccoon mortality, and can also infect a variety of other carnivores, including canids, procyonids, mustelids, viverrids, and felids.<sup>2,26,31,32</sup> We also surveyed for serologic evidence of exposure to *Leptospira interrogans* bacterial infection (serovars *grippityphosa*, *hardjo*, *icterohemorrhagiae*, *canicola*, and *pomona*). Raccoons are considered potentially important wildlife reservoirs for serovars *grippityphosa* and *icterohemorrhagiae*, which may infect many other species, including humans.<sup>16,24</sup>

While we have some understanding of CDV ep-

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idemiology in raccoons,<sup>11,18,26</sup> this is not the case for the other pathogens, which are poorly understood despite their potential to infect domestic and captive carnivores and, in the case of *Leptospira*, to also be zoonotic. Even for CDV, limited insights exist into how the virus fluctuates over time within any one population, and what little we know comes from populations that persist at densities far lower than are usually found in urban environments.<sup>18,26</sup> Therefore, data were examined in order to gain insight with regard to the extent of intrapopulation fluctuations in prevalence, the extent to which some of these pathogens may be enzootic in urban carnivore populations (raccoons being the dominant member of this community), and basic natural history information on the pathogen-host relationship for these diseases.

### MATERIALS AND METHODS

All captures occurred on the grounds of the St. Louis Zoo. The zoo comprises ca. 36 ha and is completely contained within St. Louis' 555-ha Forest Park, which in turn is surrounded by high-density urban development. Forest Park is one of the largest urban parks in the United States and attracts ca. 12 million visitors per year. Forest Park has large populations of raccoons (actual population size data not available) and other mid-sized mammals, and these animals are able to move freely onto the zoo grounds.

Humane box traps (Havahart, Woodstream Co., Lititz, Pennsylvania 17543, USA; or Tomahawk, Tomahawk Live Trap Co., Tomahawk, Wisconsin 54487, USA) were used to capture nuisance raccoons (suspected predators of zoo waterfowl) or as part of a broader population and landscape ecology study.<sup>10</sup> Raccoons were transferred to the zoo's veterinary care facility and anesthetized with either tiletamine/zolazepam (Telazol, Ft. Dodge Laboratories, Ft. Dodge, Iowa 30301, USA; 5 mg/kg i.m.) or ketamine hydrochloride (KetaVed, Phoenix Scientific, St. Joseph, Missouri 64403, USA; 10 mg/kg i.m.). Anesthetized animals were given a brief physical examination. Sex was determined, and age was estimated<sup>8</sup> based on morphology, dental eruption and wear patterns, reproductive status, and capture history. Reproductive status was assessed based on the presence of enlarged teats, lactation, or descended testes. Indications of general health were noted (wounds, discharges, hair and body condition), and raccoon health was classified as normal or abnormal based on clinical signs such as the presence of ocular or nasal discharge, diarrhea, ataxia, lethargy, and external wounds. Nuisance raccoons and all raccoons in abnormal health were

ethanized with pentobarbital sodium (Euthasol, Virbac Animal Health, Fort Worth, Texas 76161, USA; 1 cc/5 kg) overdose to minimize risk to the captive zoo collection. Postmortem examinations were not performed. For all animals a blood sample (ca. 10 ml) was collected via femoral or jugular venipuncture. Raccoons were ear-tagged, and a microchip transponder (Trovan Co., East Yorkshire HU13 ORD, U.K.) was inserted subcutaneously between the scapulae; a subset of adults ( $n = 17$ ) was radiocollared as part of an ongoing population monitoring project. Following recovery from anesthesia, raccoons were released at the site of capture at dusk. All work was approved by the St. Louis Zoo's Animal Care and Use Committee and was carried out under permit from the Missouri Department of Conservation.

Blood samples were placed into serum separator tubes, allowed to clot, and were then centrifuged, and the serum was transferred to cryotubes for storage at  $-70^{\circ}\text{C}$  until laboratory analysis. Serologic testing was performed at the Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University (Ithaca, New York, USA). Testing for FPV was done with a hemagglutination inhibition test, with a titer of greater than 1:10 considered positive. Testing of CDV and CAV-1 were done using serum neutralization, with titers greater than 1:8 considered positive. For the *Leptospira* serovars, microagglutination assay titers greater than 1:100 were considered positive.

Prevalence of exposure (% of examined individuals diagnosed as seropositive) to each pathogen was calculated, and statistical comparisons of prevalence by age, sex, sampling interval, and clinical health status were examined with Fisher exact or  $\chi^2$  tests. A Mantel-Haenzel (M-H)  $\chi^2$  test was used to assess patterns of prevalence due to sex when the data were stratified by age. Cross-species comparisons of prevalence were made using Fisher exact tests. Comparisons of the titers of seropositive individuals were carried out using two-tailed Mann-Whitney  $U$ -tests and by Kruskal-Wallis one-way analysis of variance, with an alpha of  $P < 0.05$  considered statistically significant. Statistical analyses were carried out using the program Systat (SPSS, Inc., Chicago, Illinois 60606, USA).

### RESULTS

Over 5 yr, 174 samples were collected from 159 individuals (79 males, 72 females, 8 in which sex was not recorded). The survey data presented are based on analyses of blood collected at initial capture for all animals and indicate exposure to CDV, FPV, CAV-1, and multiple serovars of *Leptospira*

(Table 1). Longitudinal serologic data collected for 13 animals subjected to repeat sampling indicate the potential for raccoons to seroconvert (negative to positive) and to return to seronegative status consistent with lack of persistent immunity (Table 2).

Exposure to CDV was identified in 54.1% of raccoons, with titers ranging from 1:8 to >1:1,024. There were no significant patterns in prevalence when data were contrasted by sex ( $n = 151$ ;  $\chi^2 = 2.046$ ;  $P = 0.153$ ), age class ( $n = 154$ ;  $\chi^2 = 2.124$ ;  $P = 0.346$ ), or sex stratified by age class (M-H statistic = 0.673; M-H  $\chi^2 = 1.088$ ;  $P = 0.297$ ). The subset of individuals ( $n = 15$ ) whose health status was classified as abnormal during capture also showed no difference in CDV prevalence relative to normal-health individuals (Fisher exact test;  $n = 159$ ;  $P = 0.107$ ). Among CDV-positive raccoons, the distribution of titers did not vary by sex (Mann-Whitney U test = 914.0;  $P = 0.354$ ), but did vary by age (Kruskal-Wallis = 12.024;  $P = 0.002$ ), with lower titers among subadults relative to juveniles and adults (Fig. 1).

Exposure to FPV was identified in 49.7% of the population, with titers of 1:8 to >1:10,240. There were no significant patterns in prevalence when data were contrasted by sex ( $n = 151$ ;  $\chi^2 = 0.012$ ;  $P = 0.914$ ), but there were significant age class differences, ( $n = 154$ ;  $\chi^2 = 5.90$ ;  $P = 0.052$ ), with juveniles having higher prevalence (60%) than subadults or adults (38% and 50%, respectively). However, stratifying the sex-prevalence data by age classes did not result in further insights (M-H statistic = 1.247; M-H  $\chi^2 = 0.207$ ;  $P = 0.649$ ). Abnormal-health individuals also showed no difference in FPV prevalence relative to normal-health individuals (Fisher exact test;  $n = 159$ ;  $P = 0.589$ ). Among FPV-positive raccoons, the distribution of titers did not vary by sex ( $u = 546.0$ ;  $P = 0.254$ ) or age (KW = 0.370;  $P = 0.831$ ).

Exposure to CAV-1 occurred in 6.9% of the population, with titers of 1:8 to 1:96. There were no significant patterns in prevalence when data were contrasted by sex (Fisher exact test;  $n = 151$ ;  $P = 0.538$ ), age ( $n = 154$ ;  $\chi^2 = 1.760$ ;  $P = 0.415$ ), or sex stratified by age (M-H statistic = 2.009; M-H  $\chi^2 = 0.446$ ;  $P = 0.504$ ). Abnormal-health individuals also showed no difference in CAV-1 prevalence relative to normal-health individuals (Fisher exact test;  $n = 159$ ;  $P = 1.000$ ). Titers of CAV-1-positive animals did not vary by sex ( $u = 19.5$ ;  $P = 0.185$ ) or age (KW = 0.548;  $P = 0.760$ ).

Each of the five serovars of *Leptospira* was detected (Table 1), with serovars *icterohemorrhagiae* (8.9%) and *grippotyphosa* (6.3%) demonstrating the highest seroprevalence and with both detected

**Table 1.** Serologic prevalence of three viral pathogens (canine distemper virus [CDV], feline parvovirus [FPV], and canine adenovirus 1 [CAV-1]) and two serovars of *Leptospira* spp. bacteria (*grippotyphosa* and *icterohemorrhagiae*) among urban raccoons from St. Louis, Missouri (USA). All values represent percent exposed ( $n$ ). Total seroprevalence represents the entire population, while other columns give prevalence for particular classes of individuals. For these classes, animals of undetermined or unrecorded sex or age were excluded from prevalence calculations. For age class columns, the second line indicates the number of males versus females (m/f) that were seropositive.

Pathogen	Total	Males		Females		Juveniles (m/f)	Subadults (m/f)	Adults (m/f)	Clinically		
		Total	(n)	Total	(n)				healthy	abnormal	
CDV	54.1 (159)	48.1 (79)	59.7 (72)	50.0 (40)	9/11	50.0 (56)	15/13	62.1 (58)	17/19	56.3 (144)	33.3 (15)
FPV	49.7 (159)	48.1 (79)	47.2 (72)	60 (40)	17/7	37.5 (56)	14/7	50.0 (58)	11/18	50.7 (144)	40.0 (15)
CAV-1	6.9 (159)	8.9 (79)	5.6 (72)	2.5 (40)	1/0	8.9 (56)	4/1	8.6 (58)	2/3	6.9 (144)	6.7 (15)
<i>L. interrogans icterohemorrhagiae</i>	8.9 (158)	8.9 (79)	9.9 (71)	10.0 (40)	3/1	7.3 (55)	2/2	10.3 (58)	3/3	9.8 (143)	0 (15)
<i>L. interrogans grippotyphosa</i>	6.3 (158)	3.8 (79)	7.0 (71)	2.5 (40)	1/0	9.1 (55)	1/4	3.4 (58)	2/0	7.0 (143)	0 (15)

**Table 2.** Serologic diagnoses for 13 raccoons captured on two or more occasions. Diagnoses represent results of first and last examinations.<sup>a</sup>

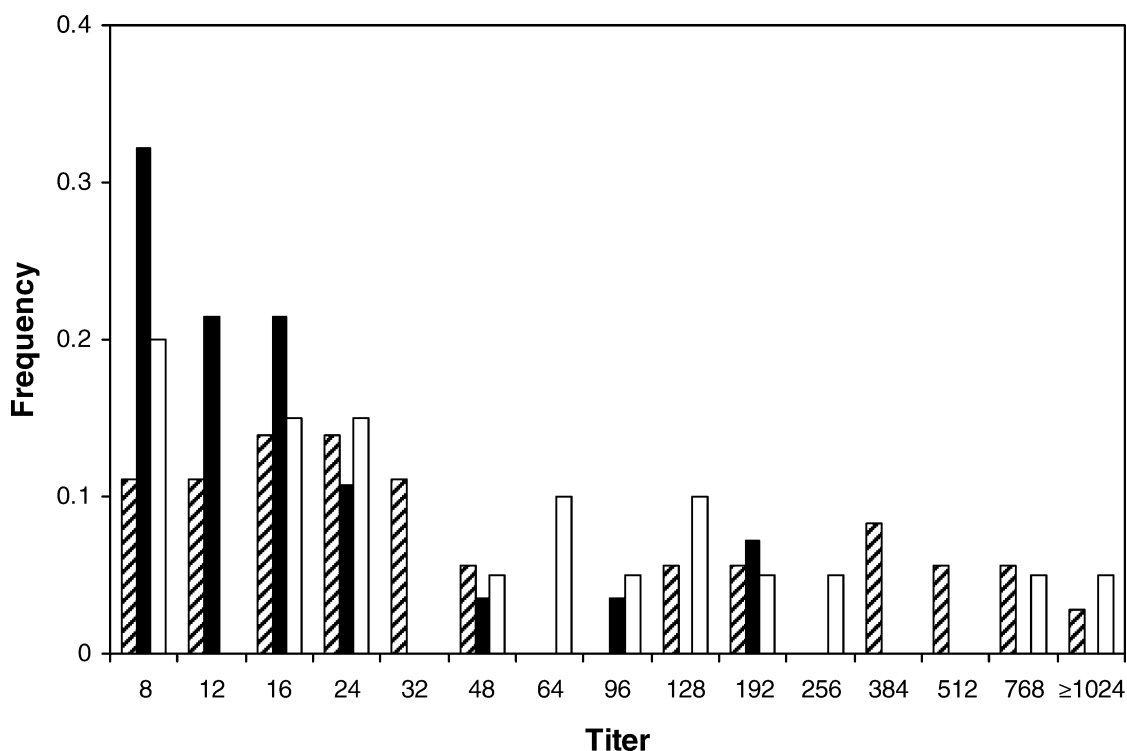
First-last result	CDV	FPV	CAV-1	<i>L. interrogans icterohemorrhagiae</i>	<i>L. interrogans grippityphosa</i>
Pos-Pos	6	4	0	1	0
Pos-Neg	3	3	3	2	0
Neg-Neg	2	6	10	9	13
Neg-Pos	2	0	0	1	0

<sup>a</sup> CDV, canine distemper virus; FPV, feline parvovirus; CAV-1, canine adenovirus 1; Pos, positive; Neg, negative.

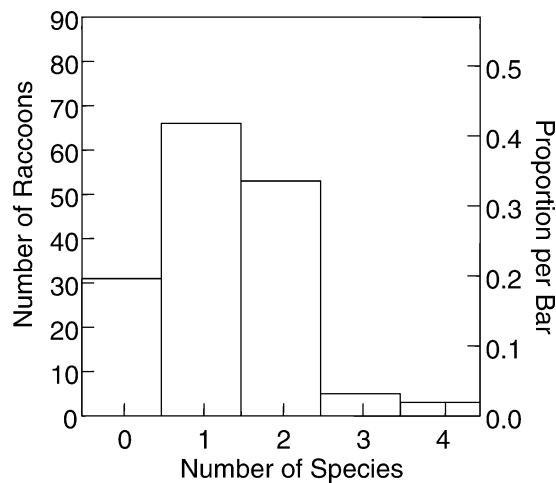
at titers of 1:100 to 1:12,800. One individual was seropositive for *pomona* (titer 1:100) and one was seropositive for *hardjo* (titer 1:400), and these individuals were both also seropositive for *icterohemorrhagiae* at a higher titer. Similarly, of the five individuals positive for *canicola*, four were also positive for *icterohemorrhagiae*, and the other was positive for *grippityphosa*, again at higher titers. There was no significant pattern of co-occurrence among serovars *icterohemorrhagiae* and *grippityphosa* (Fisher exact test;  $n = 158$ ;  $P = 1.000$ ); only one animal was positive for both serovars.

For serovars *icterohemorrhagiae* and *grippityphosa* there were no significant differences in prev-

alence by sex (Fisher exact test:  $n = 150$ ;  $P_{ict} = 1.000$ ;  $P_{gri} = 0.477$ ), age ( $n = 153$ ;  $\chi^2_{ict} = 0.368$ ;  $P_{ict} = 0.832$ ;  $\chi^2_{gri} = 2.628$ ;  $P_{gri} = 0.269$ ), or sex stratified by age (*icterohemorrhagiae*: M-H statistic = 0.892; M-H  $\chi^2 = 0.008$ ;  $P = 0.930$ ; *grippityphosa*: M-H statistic = 0.679; M-H  $\chi^2 = 0.025$ ;  $P = 0.873$ ). None of the animals identified as displaying abnormal health were seropositive for any serovar of leptospirosis. Titers of leptospirosis-positive animals did not vary by sex (*icterohemorrhagiae*:  $u = 18.0$ ;  $P = 0.373$ ; *grippityphosa*:  $u = 6.0$ ;  $P = 0.558$ ) or age (*icterohemorrhagiae*: KW = 0.724;  $P = 0.696$ ; *grippityphosa*: KW = 3.267;  $P = 0.195$ ).



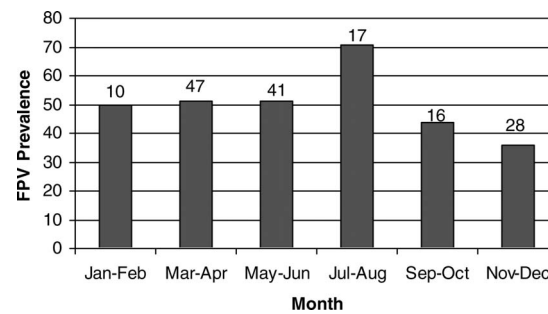
**Figure 1.** Distribution of titers for juvenile (open bars), subadult (filled bars), and adult (hatched bars) canine distemper virus (CDV)-seropositive raccoons.



**Figure 2.** Distribution of seropositivity for a St. Louis urban raccoon population ( $n = 159$ ) assayed for exposure to canine distemper virus (CDV), feline parvovirus (FPV), canine adenovirus 1 (CAV-1), and *Leptospira* spp. serovars *icterohemorrhagiae* and *grippityphosa*.

Eighty percent of raccoons showed evidence of exposure to at least one of the five primary pathogens of interest (CDV, FPV, CAV-1, *L. interrogans icterohemorrhagiae*, *L. interrogans grippityphosa*), and median number of positive titers per raccoon was 1.00 ( $n = 158$ ). Thirty-nine percent of individuals were seropositive for multiple species (Fig. 2). Among the viruses, there was significant co-occurrence of CDV and CAV-1 (Fisher exact test:  $n = 159$ ;  $P = 0.012$ ), but not of CDV and FPV ( $P = 0.267$ ) and CAV-1 ( $P = 0.534$ ). Of the 11 CAV-1-positive raccoons, 10 (90.9%) were also positive for CDV.

Raccoons were sampled over a period of 5 yr, and although sample sizes in any given year are relatively small (2000,  $n = 16$ ; 2001,  $n = 9$ ; 2002,  $n = 19$ ; 2003,  $n = 44$ ; 2004,  $n = 60$ ; 2005,  $n = 11$ ), examination of the data by year indicates fluctu-



**Figure 3.** Seroprevalence (%) of feline parvovirus (FPV) in St. Louis raccoons by 2-mo intervals. Data were collected over the course of 5 yr (2000–2005). Values above bars represent sample size ( $n$ ) per bimonthly sampling period summed over entire study.

tuations in seroprevalence for several of the pathogens (Table 3). For CDV, seroprevalence fluctuated between 38% and 46% each year with the exception of 2004, when prevalence increased to 77%. For FPV, prevalence was 25–28% during three of the five study years, but in 2001 prevalence increased, ultimately reaching 89% before declining in 2004. *Leptospira interrogans grippityphosa* and CAV-1 also showed statistically significant differences in prevalence between years, but given the relatively small number of seropositive individuals for these pathogens, these findings must be viewed as suspect until further epidemiologic studies are carried out (Table 3). For CDV and FPV, data were also analyzed by 2-mo intervals to identify intra-annual patterns. No clear pattern was observed for CDV; mean ( $\pm$  standard deviation [SD]) prevalence was 55.8% ( $\pm 7.5\%$ ) for any 2-mo period (range: March–April = 42.6%;  $n = 47$ ; November–December = 64.3%;  $n = 28$ ), with no significant variance by interval ( $\chi^2 = 4.197$ ;  $df = 5$ ;  $P = 0.521$ ). For FPV, there was an apparent increase in prevalence in late summer, followed by a decline in autumn and early winter (Fig. 3). Across all months, how-

**Table 3.** Annual serologic prevalence (%) of three viral pathogens (canine distemper virus [CDV], feline parvovirus [FPV], canine adenovirus 1 [CAV-1]) and two serovars of *Leptospira* spp. bacteria (*grippityphosa* and *icterohemorrhagiae*) among St. Louis raccoons over a 5-yr period.

Year	2000	2001	2002	2003	2004	2005	$\chi^2$ ( $P$ )
Sample size ( $n$ )	16	9	19 <sup>a</sup>	44	60	11	
CDV	37.5	44.4	42.1	38.6	76.7	45.5	0.001
FPV	25.0	66.7	52.6	88.6	28.3	27.7	<0.001
CAV-1	6.3	0	0	2.3	10	27.3	0.042
<i>L. interrogans icterohemorrhagiae</i>	0	0	22.2	9.1	8.3	9.1	0.266
<i>L. interrogans grippityphosa</i>	0	0	22.2	11.4	1.7	0	0.014

<sup>a</sup>  $n = 18$  for *L. interrogans icterohemorrhagiae* and *L. interrogans grippityphosa*.

ever, these differences were not statistically significant ( $\chi^2 = 5.458$ ;  $df = 5$ ;  $P = 0.363$ ).

Longitudinal data were collected from 13 animals surveyed two ( $n = 11$ ) or three ( $n = 2$ ) times, resulting in 15 titer values (Table 2). Median time from first to last resampling of individuals was 211 days (range = 26–428 days). Seroconversion occurred for two animals: a juvenile male became positive for CDV when resampled as a subadult (resampling interval: 178 days), and a subadult male became positive for CDV and *L. interrogans icterohemorrhagiae* when resampled as an adult (428 days). Among individuals who were diagnosed as positive for a pathogen on first capture, 33–100% became seronegative when retested at a later date (CDV: 33.3%; FPV: 42.9%; CAV-1: 100%; *L. interrogans icterohemorrhagiae*: 66.6%). Among these individuals, titers for individuals who subsequently became seronegative were 1:8–1:12 for CDV, 1:10–1:640 for FPV, 1:8–1:16 for CAV-1, and 1:100 for *L. interrogans icterohemorrhagiae*.

Fifteen (9.4%) of the 159 individuals were diagnosed as clinically abnormal based on observations of apparent illness, including depressed or obtunded attitude, nasal and/or ocular discharge, and diarrhea. These animals were predominantly adults (12 adults, 2 subadults, 1 juvenile) and included six males and nine females. For each of the five primary pathogens of interest, the prevalence of exposure in this group was lower than in the clinically healthy population when all years are combined (Table 1). CDV titers of five seropositive, clinically ill animals were not significantly higher than those of 81 seropositive, clinically normal animals ( $u = 211.0$ ;  $P = 0.874$ ). For FPV, titers of six seropositive, clinically ill animals tended to be lower than those of 73 seropositive, clinically normal animals ( $u = 120.0$ ;  $P = 0.064$ ).

## DISCUSSION

Raccoons are susceptible to a variety of infectious diseases that may be transmitted to other species. In an urban or zoological park setting, exposure of other wildlife, humans, domestic animals, and zoological collection animals to these endemic diseases could result in significant morbidity and mortality. Yet, although raccoons are one of the most abundant wild carnivore species in urbanized ecosystems, few data are available on the disease ecology of these animals in high-density populations. In addition, we are aware of no published works that report paired titers from wild raccoons or assess population-scale co-occurrence of the potentially important pathogens examined in this

study. While recognizing that serologic data can be problematic for identifying the true disease status of individuals, we nonetheless believe that this study gives significant insights into the disease ecology of urban raccoons.

Aside from raccoon rabies virus, which as of 2005 does not occur in Missouri, FPV and CDV are likely the primary pathogens of interest for researchers concerned with raccoon population dynamics, issues of carnivore conservation, and disease spillover into domestic carnivore populations and zoo animal collections.<sup>2,6,7,15,31</sup> The high level of exposure to CDV and FPV observed in this study supports this concern, as it indicates that these two viruses are enzootic in urban wildlife communities (broadly including unvaccinated feral and pet domestic dogs and cats and wild carnivores such as raccoons, skunks, coyotes, and foxes, all of which are found in and around the St. Louis zoo grounds and much of urban St. Louis).

Canine distemper virus infection in raccoons has been examined at multiple localities, and exposure levels have been found to vary from their virtual absence during particular time intervals<sup>23,27</sup> to over 80%,<sup>12</sup> and with periodicities that can result in pronounced epizootics.<sup>11,26</sup> In zoo environments, CDV transmission from wild raccoons to captive carnivores has long been a concern,<sup>1,3,15</sup> as raccoons can typically move freely from adjoining areas onto zoo grounds. Although close contact between any individual raccoon and any particular zoo animals may be infrequent, a high prevalence of CDV-exposed raccoons indicates that the raccoon population may nonetheless present a risk to zoo animals. Seroprevalence fluctuated between years, however, varying from 38–77% in this study, and it is unclear whether there is a threshold prevalence level above which interspecific contact rates represent heightened risk of spillover. Significant fluctuations were not observed in prevalence between months, however, as has been observed at large spatial scales.<sup>26</sup>

Clinical illness associated with CDV in raccoons includes lethargy, hyperkeratosis of foot pads, and ocular and nasal discharge, with significant mortality.<sup>4,31</sup> While clinically abnormal raccoons showed many of these symptoms, the post-hoc comparisons of these clinically ill animals to putatively healthy animals did not reveal higher prevalences or higher titers for those animals that were found to be seropositive. This analysis, however, is limited in scope by the small sample size of clinically ill animals. Furthermore, multiple circulating CDV strains of varying virulence were recently identified within an urban raccoon population in Chicago.<sup>15</sup> It is also likely that clinically ill raccoons have not

mounted an immune response, possibly as a result of insufficient time or immunocompromise, highlighting the potential limitations of a serology-based study.

A higher proportion of CDV seropositivity was expected in animals of older age classes, indicating continued or repeated exposure to the population, as reported elsewhere.<sup>18</sup> This was not observed; subadults had lower titers than did juveniles or adults. The cause of this difference is unclear. The higher prevalence and titers of seropositive juveniles may be due in part to early exposure or maternal antibody. The half-life of maternal antibodies to CDV in raccoons is 10.5 days, and maternal antibodies are negligible by 20 wk.<sup>19</sup> A majority of raccoons classified as juveniles were captured when their estimated age was <16 wk, and therefore these animals could have persistent maternal antibodies. On one occasion an adult female was trapped with three juvenile animals, which were assumed to be her offspring (estimated age: 3 mo). CDV serology revealed that the adult and two of her offspring were seropositive (one remained seropositive when recaptured 6 mo later), while the third offspring was seronegative. Such differences among the pups could reflect declining maternal antibodies, which may have dropped to undetectable levels in one pup, or possibly they indicate that one pup did not develop any immunity at all. Nonetheless, the fact that 56% of clinically healthy raccoons were seropositive supports the finding that many raccoons survive disease or exposure and seroconvert. This is also consistent with the finding that 50% of experimentally infected raccoons survive.<sup>19</sup> Raccoons that were euthanized with clinical signs consistent with CDV were often seronegative, possibly indicating that they died before mounting an immune response.<sup>31</sup>

Raccoon parvovirus is genetically identical to FPV, which can manifest as panleukopenia in felids.<sup>2,26</sup> It is considered one of the most important infectious diseases of raccoons and is manifested by depression, inappetence, fever, vomiting, and diarrhea.<sup>2</sup> Transmission is typically by the fecal–oral route; the virus may survive for months in the environment; and the prevalence in wildlife may be enhanced in urban systems as a result of the high numbers of unvaccinated feral and pet domestic cats.<sup>5</sup> For this reason, FPV in urban raccoon populations may be enhanced relative to the prevalence in rural (low-density) environments and may present a significant hazard to captive wildlife.

In the St. Louis raccoon population, FPV seroprevalence fluctuated between years from 25% to 89%. Juveniles tended to have higher prevalence

levels than subadults or adults, although, as for CDV, this may be due in part to high levels of circulating maternal antibodies.<sup>2</sup> Also, as seen with CDV, there is a high seroprevalence (51%) in clinically healthy raccoons, indicating an immune response to infection or exposure. We also observed an intra-annual peak in FPV seroprevalence, indicating seasonal variability in exposure. However, there have been few studies of FPV in free-ranging raccoon populations, so it is unclear whether the prevalence levels, annual fluctuations, and seasonal fluctuations observed here are typical and independent of locale and density or whether they are specific to high-density or urban populations or are possibly simply related to sample size.

Although they are not generally perceived as being as important as distemper or parvoviruses in causing morbidity and mortality, adenoviruses have the potential to be problematic in a zoo setting and for unvaccinated domestic dog populations. Adenovirus infection is manifested as hepatitis or encephalitis in a variety of carnivores, possibly including the Procyonidae,<sup>30</sup> although knowledge of CAV-1 infection in wild raccoon populations is limited. Neutralizing antibodies to CAV-1 were reported in 12% of 50 raccoons from Maryland,<sup>13</sup> but the ecological setting for the study was not given. It is therefore unclear how one should interpret the 7% seroprevalence observed in this high-density host population. The high proportion of co-occurrence of CAV-1 and CDV exposure is intriguing, however, as combined infections involving CAV-1 have been suggested to increase the persistence of infection by other viruses and to increase the morbidity and mortality for canids.<sup>9,14,21</sup>

Evidence of exposure to five serovars of *Leptospira* was detected, but given the extensive potential for cross-reactivity,<sup>16</sup> only two serovars occurred at levels sufficient to indicate valid exposure. Seroprevalence of 6–9% for the serovars *gripotyphosa*, for which raccoons are the putative maintenance hosts, and *icterohaemorrhagiae*, for which rodents are the maintenance hosts,<sup>16</sup> indicates that these serovars are enzootic in the St. Louis urban environment and that the potential exists for spillover incidents of *Leptospira* from raccoons into other wildlife, captive animal collections, or humans and their domestic animals.<sup>12,22</sup> However, the seroprevalence levels of this study are lower than those observed in other surveys of raccoons.<sup>17,18,23,28</sup> The coinfection with multiple serovars indicates either that the likelihood of infection by these serovars is not independent or that there is a need for further assessment of the specificity of microagglutination assays for serotypes *pomona*, *hardjo*, and



*canicola* in raccoons. Given that *Leptospira* bacteria may persist in renal tubules for extended periods, may be shed via urination, and may survive for days to weeks under good environmental conditions, personnel managing animal collections should be aware of the risk of transmission to animals or humans via raccoon urine.

The relationship between clinically ill raccoons and serology results was not as we expected. For clinically ill animals, prevalence of exposure was lower and viral titer values were lower or equivalent to values for the apparently healthy population. This pattern deserves further examination, as the observed pattern of titers was opposite the expected pattern. This indicates that serologic test results may not be useful diagnostically for clinically ill animals.

We are unaware of any previous studies of raccoon pathogens that have included a longitudinal component to the analyses. While our data set is constrained by small sample size, the results nonetheless emphasize two important, interrelated phenomena, particularly for researchers attempting to model the dynamics of raccoons and of these pathogens. First, raccoons may survive for extended periods following exposure to these pathogens. For example, nine individuals diagnosed as CDV-seropositive were clinically healthy when recaptured (median time to recapture = 136 days; range = 26–318 days). Second, a substantial portion of animals may become seronegative after exposure to CDV (33.3%), FPV (42.9%), and *L. interrogans icterohemorrhagiae* (66.6%). For CAV-1, all three of the resampled individuals diagnosed as seropositive at original capture were seronegative at recapture. Although titers for some individuals who subsequently became seronegative were relatively low, these results are surprising. The observation of CDV-seropositive animals becoming seronegative, for instance, is contrary to the accepted assumption that seroconversion persists for life.<sup>31</sup> For all of these recaptured animals, initial samples were collected when the animals were subadults or older; therefore, loss of maternal antibody is not a factor in the seroconversion. These findings strongly indicate a need for additional longitudinal analyses based on field-collected data.

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