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ANTIBODIES TO CANINE AND FELINE VIRUSES IN SPOTTED HYENAS (*CROCUTA CROCUTA*) IN THE MASAI MARA NATIONAL RESERVE

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ABSTRACT: Spotted hyenas (*Crocuta crocuta*) are abundant predators in the Serengeti ecosystem and interact with other species of wild carnivores and domestic animals in ways that could encourage disease transmission. Hyenas also have a unique hierarchical social system that might affect the flow of pathogens. Antibodies to canine distemper virus (CDV), feline immunodeficiency virus (FIV), feline panleukopenia virus/canine parvovirus (FPLV/CPV), feline coronavirus/feline infectious peritonitis virus (FECV/FIPV), feline calicivirus (FCV), and feline herpesvirus 1 (FHV1) have been detected in other Serengeti predators, indicating that these viruses are present in the ecosystem. The purpose of this study was to determine whether spotted hyenas also had been infected with these viruses and to assess risk factors for infection. Serum samples were collected between 1993 and 2001 from 119 animals in a single clan for which behavioral data on social structure were available and from 121 hyenas in several other clans. All animals resided in the Masai Mara National Reserve. Antibodies to CDV, FIV, FPLV/CPV, FECV/FIPV, FCV, and FHV1 were present in 47%, 35%, 81%, 36%, 72%, and 0.5% of study hyenas, respectively. Antibody prevalence was greater in adults for FIV and FECV/FIPV, and being a female of high social rank was a risk factor for FIV. Hyenas near human habitation appeared to be at lower risk to have CDV, FIV, and FECV/FIPV antibodies, whereas being near human habitation increased the risk for FPLV/CPV antibodies. Canine distemper virus and FECV/FIPV antibody prevalence varied considerably over time, whereas FIV, FPLV/CPV, and FCV had a stable, apparently endemic temporal pattern. These results indicate that hyenas might play a role in the ecology of these viruses in the Serengeti ecosystem. The effect of these viruses on hyena health should be further investigated. The lower prevalence of CDV antibody–positive hyenas near human habitation suggests that reservoirs for CDV other than domestic dogs are present in the Serengeti ecosystem.

Key words: Canine distemper virus, *Crocuta crocuta*, feline immunodeficiency virus, hyena, Kenya, serology, viruses.

INTRODUCTION

Spotted hyenas (*Crocuta crocuta*) are the most abundant large carnivores in the Serengeti ecosystem, and they interact regularly with sympatric predators and domestic animals in ways that might encourage disease transmission. Hyenas have large home ranges that overlap habitats of lions (*Panthera leo*), jackals (*Canis* sp.), endangered African wild dogs (*Lycaon pictus*), and endangered cheetahs (*Acinonyx jubatus*) (Kruuk, 1972). However, little is known about which viruses infect spotted hyenas and how hyenas influence pathogen transmission in the Serengeti ecosystem.

Hyena groups, or clans, have unique social hierarchies that might influence their risk of acquiring and transmitting viral diseases. In clans, females rank above adult male hyenas (Kruuk, 1972). High-ranking female hyenas are more likely to defend the clan's home range by fighting with other hyena clans along territorial borders, to attack females intruding into their home range, and to challenge lions at kill sites (Kruuk, 1972; Holekamp et al., 1997; Boydston et al., 2001). Juvenile hyenas

achieve the social rank of their mother (Holekamp and Smale, 1993; Engh et al., 2000), and female hyenas retain these "maternal ranks" in their natal clan. Female hyenas are philopatric and tend to stay in their natal clans for their entire lives. In contrast, male spotted hyenas disperse from their natal clan around 24–62 mo of age and enter a new clan at the bottom of the social hierarchy (Smale et al., 1993; East and Hoffer, 2001; Engh et al., 2002). This behavior should theoretically make males more likely than females to contact other hyenas in neighboring home ranges before they join new clans as adults, to attack other males that invade the home range, and perhaps also to encounter domestic animals in suboptimal hunting areas during dispersal (Smale et al., 1993; Boydston et al., 2001). If spotted hyenas are susceptible to feline and canine viruses, these hierarchical differences in space utilization and social interactions could influence how infectious diseases are transmitted within the ecosystem. Susceptibility to these viruses is likely because hyenas have a close phylogenetic relationship to felids and canids (Schreiber et al., 1998).

Antibody surveys have been performed on lions (Hofmann-Lehmann et al., 1996; Packer et al., 1999), cheetahs (Heeney et al., 1990), and wild dogs (Alexander and Appel, 1994) in the Serengeti ecosystem; however, no comprehensive serosurvey has been performed in hyenas. Lions in the Serengeti have high prevalences of antibodies to feline viruses, including feline immunodeficiency virus (FIV), feline panleukopenia virus (FPLV), feline coronavirus/feline infectious peritonitis virus (FECV/FIPV), feline calicivirus (FCV), and feline herpesvirus 1 (FHV1) (Hofmann-Lehmann et al., 1996; Packer et al., 1999). It appears that FIV and FHV1 occur at a constant low level in these lions, whereas FPLV, FECV/FIPV, and FCV occur as epidemics (Hofmann-Lehmann et al., 1996; Packer et al., 1999). Serologic evidence of periodic infection of cheetahs with FECV/FIPV has also been noted in the Serengeti ecosystem (Heeney et al., 1990).

One of the most important causes of morbidity and mortality in Serengeti predators is canine distemper virus (CDV). Canine distemper virus has been implicated in a 35% decline in the lion population and in deaths of hyenas that occurred during 1994–95, resulting in a major loss of both important predators in this ecosystem (Haas et al., 1996; Roelke-Parker et al., 1996). Although the source of CDV in wildlife has not been identified, domestic dogs have been implicated as a possible reservoir in the Serengeti National Park and the Masai Mara National Reserve (MMNR; Kenya) (Cleaveland et al., 2000) because increased canine distemper in domestic dogs in the region has been associated with observation of wildlife exposure (Alexander et al., 1995; Roelke-Parker et al., 1996). Because hyenas range among human habitations that have domestic dogs and can be infected with CDV, hyenas could be involved in CDV ecology as was hypothesized during the devastating 1994–95 Serengeti CDV epidemic (Roelke-Parker et al., 1996). If hyenas are susceptible to disease caused by CDV, they could possibly succumb after infection, transport the virus among hyenas, or serve as a reservoir.

The purpose of this study was to evaluate the prevalence of antibodies to CDV, FIV, FPLV, FECV/FIPV, FCV, and FHV1 in hyenas and to examine the associations of antibody prevalence with age, sex, social rank, proximity to human habitation, and year of sampling. All study hyenas resided in the MMNR, which is in the northern portion of the Serengeti ecosystem. A long-term behavioral study of a clan of hyenas (Talek clan; Holekamp et al., 1996) provided the information on social rank needed for these investigations.

MATERIALS AND METHODS

Sampling

Blood samples were collected from spotted hyenas in the Talek clan (n=119) between 1993

and 2001. Talek clan hyenas were sampled within a home range that occupied approximately 65 km^2 south of the Talek River in the MMNR (35°50′E, 1°40′S) near the Talek village and Maasai homesteads (manyattas). A subset of 17 hyenas were sampled twice between 1993 and 2001, and two hyenas were sampled three times between 1995 and 2000 to assess seroconversion over time. Hyena social rank was known for individuals in this clan through ongoing behavioral studies. Social rankings of hyenas were based on outcomes of dyadic agonistic interactions (Smale et al., 1993). Blood samples were also collected from spotted hyenas (n=121) from approximately nine other clans between 1993 and 2001 in conjunction with behavior and genetic studies in the Serengeti ecosystem (Libants et al., 2000; Engh et al., 2002). Age was known for Talek clan individuals and was estimated for non-Talek hyenas by dental measurements (Van Horn et al., 2003), but social ranks of individuals in non-Talek clans were not known. Only animals older than 4 mo were included in the study so that residual maternal antibodies were not likely to be measured. The oldest hyena included in the study was 16.5 yr old.

Animals were anesthetized with tiletamine hydrochloride and zolazepam hydrochloride (Telazol®, 2.5 mg/kg intramuscularly; A. H. Robbins, Richmond, Virginia, USA). Blood samples were collected from the jugular vein and separated into serum and cell components in the field. Serum samples were stored in liquid nitrogen in the field, shipped in dry ice, and subsequently stored at −80 C in the laboratory until analyses.

Serologic assays

All serologic assays were completed at the New York State Animal Health Diagnostic Laboratory (Ithaca, New York, USA). Serum-neutralizing antibodies against CDV were measured by the Onderstepoort strain (Appel et al., 1994). Titers greater than 1:10 were considered positive for CDV. For FECV/FIPV, FCV, and FHV1, sera were also tested with a microplate serum neutralization technique in a format similar to the CDV serum neutralization test with modifications: the number of cells in the assays detected by 50% tissue culture infective dosage ($TCID_{50}$) were 100–300, and the test cells were Crandell Reese feline kidney (CRFK). The viruses used in the assays were FECV 1683 (positive titer > 1:10), FCV ATCC VR 653 (positive titer > 1:10), and FHV1 ATCC VR 636 (positive titer > 1:10). Antibodies against FPLV were detected by a hemagglutination inhibition assay (positive titer > 1:20). The FPLV assay used in this study also detected antibodies against canine parvovirus 2 (CPV), so results are reported as FPLV/CPV.

Feline immunodeficiency virus antibodies were detected by IDEXX FIV PetCHEK plate enzyme-linked immunosorbent assay (ELISA; IDEXX, Portland, Maine, USA). For this study, the FIV test was considered positive if the kinetic ELISA (KELA) slope was greater than 8 and a sample-to-positive (S/P) ratio was greater than 100. A test was considered negative if the KELA slope was less than 8 and the S/P ratio was less than 150. These criteria were based on western blot results in domestic cats, a lion, a leopard (*Panthera pardus*), and a Florida panther (*Felis concolor coryi*) (Barr et al., 1989, 1991; Roelke et al., 1993; Osofsky et al., 1996). A single hyena sample that was FIV ELISA–positive was also tested for specificity on a western blot with Pallas cat and domestic cat antigens that have been used to confirm ELISA results in wild carnivores (Roelke et al., 1993; Barr et al., 1997). This strongly positive serum on ELISA was weakly positive for the FIV core protein on western blot.

Analyses

Hyenas were grouped by age class (juvenile, 5–24 mo old, n=84; adult, 2–16.5 yr old [mean age, 6.1±3.1 yr; median age, 5.4 yr] n=153; unknown age, n=3), sex (female, n=130; male, n=110), and social rank (high-ranking Talek clan females and their juvenile cubs, n=38; medium- and low-ranking Talek clan females and their juvenile cubs, n=54; adult low-ranking Talek clan males, n=24; unknown ranking of Talek clan members, n=3; rank was not known and therefore not considered for non-Talek clan individuals, n=121). Animals of unknown classification (n=3 for age, n=3 for rank) were used for overall prevalences but not for logistic regression calculations.

For the purpose of statistical analyses, hyenas in the Talek clan (n=119), which is located on the border of the MMNR near the Maasai manyattas, were considered to be near human habitation. Hyenas in the other clans sampled had home ranges in the interior of MMNR and were considered to be distant from human habitation (n=121).

Associations between antibody prevalence of each virus and age, sex, social rank, proximity to human habitation, and year of sampling were evaluated by multiple logistic regression. Only Talek clan animals were used in analyses for social rank because ranks were not available for the other hyenas. Multivariate logistic regression models were selected by backward stepwise selection (P=0.10 to remove). The logistic

TABLE 1. Prevalence of seropositivity in spotted hyenas (*Crocuta crocuta*) to canine distemper virus (CDV), feline immunodeficiency virus (FIV), feline panleukopenia parvovirus/canine parvovirus (FPLV/CPV), feline coronavirus/feline infectious peritonitis virus (FECV/FIPV), feline calicivirus (FCV), and feline herpesvirus 1 (FHV1) in the Masai Mara National Reserve, Kenya, between 1993 and 2001. The numbers in parentheses represent the total number of animals in each category.

	Seroprevalence (%)										
Pathogen	Overall prevalence		Juvenile	Adult		Overall males	Overall females		High-rank Talek females	Medium/low-rank Talek females	Low-rank Talek males
CDV	47	(217)	9 (77)	69	(137)	42 (98)	52	(119)	21 (33)	18 (50)	61 (18)
FIV	35	(239)	17 (84)	44	(152)	28 (110)	41	(129)	41 (37)	9 (54)	29 (24)
FPLV/CPV	81	(235)	89 (82)	76	(150)	85 (107)	77	(128)	95 (37)	100 (52)	96 (24)
FECV/FIPV	36	(236)	18 (83)	47	(150)	33 (108)	48	(128)	19 (37)	25 (53)	33 (24)
FCV	72	(236)	60 (83)	78	(150)	69 (108)	74	(128)	65 (37)	66 (53)	83 (24)
FHV1	0.5	(217)	0 (71)	0.7	(145)	0 (99)	0.8	(118)	0 (34)	0 (43)	0 (23)

regression model fit was determined by the Hosmer-Lemeshow test statistic (Hosmer and Lemeshow, 1989). Strengths of associations were estimated by multiple logistic regression odds ratios with 95% confidence limits (SPSS for Windows, standard version 8.0.0, 1997, Chicago, Illinois, USA). Hyena sample locations were mapped with ArcView GIS® software (ESRI, Ver. 3.1.1, ©1992–1999, Redlands, California, USA). Spatial statistics were by SaTScan software (Ver. 2.1, Biometry Research Group, Division of Cancer Prevention, National Cancer Institute, Frederick, Maryland, USA). The probability model used was purely spatial and Bernoulli based (Kulldorff and Nagarwalla, 1995). The data were analyzed for geographic clusters of antibody prevalence that were either lower or higher than expected. A cluster with $P<0.05$ was considered significant. Because FIV infections in lions do not usually result in seroconversion before 6 mo of age (Spencer et al., 1992), additional spatial analyses for FIV were conducted that included only animals older than 6 mo old. Annual variations in antibody prevalence from 1993 to 2001 were evaluated for the population as a whole, as well as in 19 hyenas from which multiple serum samples were available. Because the majority of non-Talek clan animals were sampled in 2000 (93/121), chi-square analyses for seropositivity were also used to evaluate annual variation for the more evenly distributed Talek clan samples (EpiInfo statistical software, Ver. 1.1.2, November 2001, Centers for Disease Control and Prevention, Atlanta, Georgia, USA).

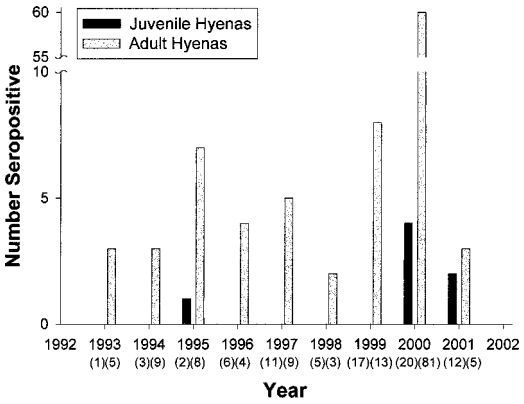

FIGURE 1. Juvenile and adult spotted hyenas (*Crocuta crocuta*) seropositive to canine distemper virus (CDV) by year in the Masai Mara National Reserve, Kenya, between 1993 and 2001. The numbers in parentheses represent sample sizes for each age class.

RESULTS

Hyenas in the study population had antibodies against all viruses tested (Table 1). Although overall CDV antibody prevalence was 47%, there was considerable temporal variation (Fig. 1). Four sequentially sampled study animals that were born before 1995 seroconverted to CDV in 1995, and three of six animals born after 1995 seroconverted in 2000. The number of seropositive juveniles increased in 1995 and 2000–2001 (Fig. 1). Canine distemper antibody prevalence increased with age ($P<0.0001$), adults being 23.2 times more likely to test positive than juveniles (95% confidence interval [95% CI]=9.8–54.7). The youngest animal with a positive CDV antibody titer was 8 mo old. Spatially, a low-risk CDV cluster (15% [10/65] sero-

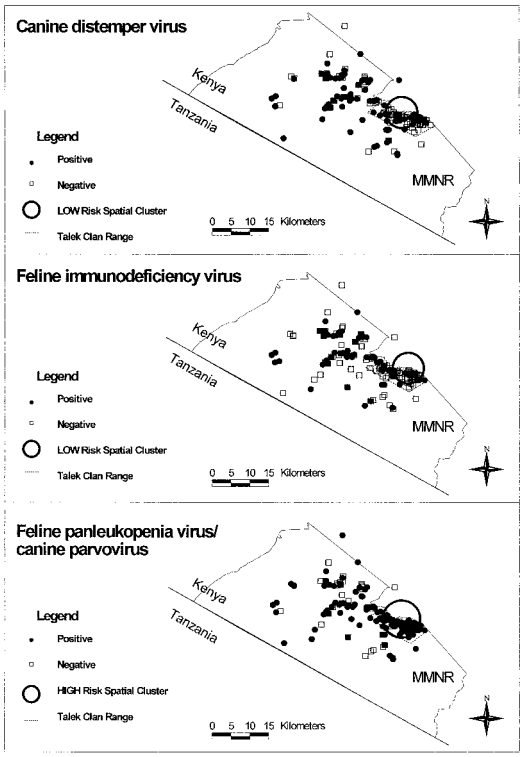

FIGURE 2. Distribution of spotted hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve (MMNR), Kenya, seropositive to canine distemper virus (CDV), feline immunodeficiency virus (FIV), and feline panleukopenia virus/canine parvovirus (FPLV/CPV) between 1993 and 2001. The risk of seropositivity is shown relative to human habitations that exist along the northeast border of the MMNR. Note that hyenas have a lower risk for CDV and FIV seropositivity and an higher risk for FPLV/CPV seropositivity near human habitation. The risk for feline enteric coronavirus/feline infectious peritonitis virus (FECV/FIPV) seropositivity was similar in distribution to CDV and FIV.

positivity observed where 47% seropositivity was expected; overall relative risk, 0.33; log likelihood ratio (λ)=20.2; P=0.001) was found within a 6.7 km-diameter area near human habitation (Fig. 2). Canine distemper virus antibody prevalence by year was significantly different for the study population as a whole (χ^2=31.4, P<0.0001) as well as for the subpopulation of Talek clan hyenas (χ^2=15.2, P=0.055). Antibody prevalence to CDV did not differ by sex or social rank.

Of the 239 animals tested for FIV by ELISA, 84 hyenas had positive results (35%). Social rank was associated with antibody prevalence (P=0.0016), with high-ranking Talek females being 7.2 times more likely to be seropositive than Talek females of medium and low social rank (95% CI=2.1–24.7). High-ranking Talek females were not significantly more likely than low-ranked adult Talek males to be seropositive for FIV (P=0.53). Age was associated with FIV antibody prevalence (P<0.0001), with adults being 4.1 times more likely to be seropositive for FIV (95% CI=2.0–8.2). The youngest animal to test positive was almost 8 mo old. Of those hyenas that were sequentially sampled, seroconversion occurred in four of 15 FIV-negative hyenas. However, two of four FIV-positive hyenas with repeat samples became negative again on subsequent samples. A low-risk spatial cluster 7.3 km in diameter was found near human habitation when all ages were considered, as well as when the calculation was adjusted for age (10% [7/67] seropositivity observed where 34% seropositivity was expected; overall relative risk, 0.31; λ=13.2; P=0.001; Fig. 2). Feline immunodeficiency virus antibody prevalence varied during the study period, ranging from 11% to 67%, with antibody prevalence 40% or higher in six of the nine years sampled. Antibody prevalence among juveniles ranged from 0% to 40% over the 9 yr sampled. Feline immunodeficiency virus antibody prevalence did not differ between sexes.

Antibody prevalence for FPLV/CPV remained high throughout the study period, ranging from 65% to 100% for juveniles and from 56% to 100% for adults, except in 1993, when the only juvenile tested was negative. A high-risk cluster of FPLV/CPV antibody prevalence 7.9 km in diameter was found near human habitation (98% [91/93] seropositivity observed where 81% seropositivity was expected; overall relative risk, 1.2; λ=18.3; P=0.001; Fig. 2). The youngest animal to test positive for FPLV/CPV was almost 5 mo old and was the

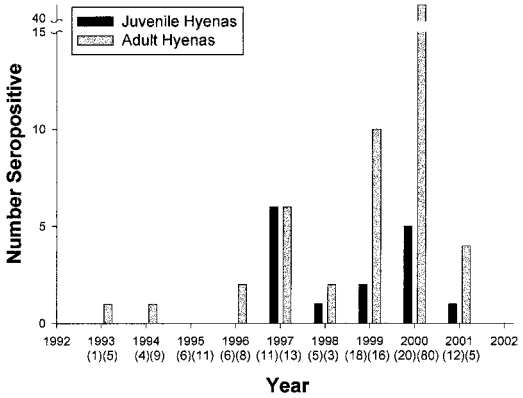

FIGURE 3. Juvenile and adult spotted hyenas (*Crocuta crocuta*) seropositive to feline enteric corona virus/feline infectious peritonitis virus (FECV/FIPV) by year in the Masai Mara National Reserve, Kenya, between 1993 and 2001. The numbers in parentheses represent sample sizes for each age class.

youngest animal included in this study. There was no significant association between antibody prevalence for FPLV/CPV and age, social rank, or sex of the hyena.

Antibody prevalence for FECV/FIPV during the study period ranged from 0% to 80% by year (Fig. 3). Of the 50 animals tested between 1993 and 1996, only four adults (8%) had antibodies. Antibody prevalence of FECV/FIPV increased in both juveniles and adults in 1997 to 55% and 46%, respectively. After 1997, antibody prevalence ranged from 46% to 80% in adults and from 8% to 25% in juveniles. Adult hyenas were 4.1 times more likely than juveniles to test positive (95% CI=2.2–7.9; $P<0.0001$). Similar to those found for CDV and FIV, a low-risk spatial cluster of antibody prevalence to FECV/FIPV 7.4 km in diameter was found near human habitation (19% [17/90] seropositivity observed where 37% was expected; overall relative risk, 0.51; $\lambda=10.6$; $P=0.009$). The youngest animal to test positive for FECV/FIPV was almost 8 mo old. There was no significant association between FECV/FIPV antibody prevalence and sex or social rank.

Feline calicivirus antibody prevalence remained high throughout the study period, ranging from 33% to 80% in juveniles and from 38% to 89% for adults, except in 1993, when the only juvenile tested was negative. The youngest animal to test positive for FCV was 7 mo old. There was no significant statistical association between age, sex, social rank, or location and FCV antibody prevalence.

A single animal had antibodies against FHV1. This hyena was an adult female located far from human habitation.

DISCUSSION

This study demonstrated that free-ranging hyenas in the MMNR are exposed to and become infected with feline and canine viruses. The increased CDV antibody prevalence in study hyenas in 1994–95 and 2000–01 suggests that epidemics of CDV occurred in hyenas during these periods. This observation is further supported by the seroconversion of juvenile animals and sequentially sampled adults in 1995 and in 2000–01. Seroconversion of adult hyenas mirrored the seroconversion of adult lions during the 1994–95 CDV epidemic in the Serengeti ecosystem (Roelke-Parker et al., 1996; Kock et al., 1998; Packer et al., 1999). The increase in CDV antibody prevalence detected in 2000–01 might have been the result of an epidemic that was not recorded. Although more non-Talek animals were sampled during that period, the peak in antibody prevalence was not the result of a higher proportion of seropositive animals in this group; when evaluating the Talek clan alone, the difference in annual variation persisted. Because antibody prevalence in the study hyenas varied by year, hyenas might not be a reservoir species for this virus; rather, they could encounter the virus periodically from another source.

Canine distemper virus antibody prevalence was greater in adults. This age effect might be an outcome of exposure during previous epidemics or because it is more likely they will encounter the virus over their lifetime (Packer et al., 1999). Also, juvenile hyenas might have higher mortality rates than adults when infected. The

response in MMNR hyenas was similar to the highly susceptible lion population, with a low antibody prevalence detected prior to the 1994–95 epidemic and a more elevated postepidemic antibody prevalence (Roelke-Parker et al., 1996; Packer et al., 1999).

Domestic dogs have been implicated as the source of CDV for wildlife in numerous studies (Alexander et al., 1993; Roelke-Parker et al., 1996; Cleaveland et al., 2000; Frolich et al., 2000). If dogs were the primary source, the Talek clan, with a home range that encompasses some local Maasai manyattas, would be expected to have a higher level of exposure to domestic dog viruses than hyenas from the interior of the park. The Talek clan is especially at risk because these hyenas have sometimes denned near the manyattas and have had the opportunity to contact domestic dogs and cats in these areas on a daily basis (Alexander et al., 1994, 1995). Interestingly, there were significantly fewer than expected CDV antibody–positive hyenas in close proximity to human habitation and more antibody-positive hyenas within the interior of the park, further from contact with domestic animals. This spatial distribution suggests that there are sources of CDV in this ecosystem in addition to domestic dogs; alternatively, the domestic dogs in this region might not have had CDV during the study period. Hyenas can be exposed from infected hyenas or other carnivores living in or immigrating into the MMNR from Tanzania, as suggested during the 1994–95 epidemic (Kock et al., 1998).

This study marks the first time that FIV antibodies have been reported in spotted hyenas. Because the ELISA test used in this study will detect antibodies to closely related viruses, it is not known whether the hyena antibodies were against FIV from lions or an endogenous hyena lentivirus. Feline immunodeficiency virus is endemic in Serengeti lions (Hofmann-Lehmann et al., 1996; Packer et al., 1999), so the fairly constant prevalence of FIV antibodies in MMNR hyenas suggests that hyenas might share the virus with lions. The strong positive ELISA test results and the positive western blot supports the premise that the hyenas are infected with FIV. However, the hyena antibodies could be against a closely related immunodeficiency virus and not FIV, because the western blot FIV core protein reaction was only weakly positive. Seroconversion of four of 15 FIV-negative hyenas could mean that antibody levels were below detection level on initial testing or that exposure had occurred during the sampling period. The decrease in FIV antibodies in some hyenas might indicate that the hyenas are not persistently infected and do not mount a long-lasting immunologic response. A more specific test that detects hyena antibodies needs to be developed to validate these findings. Also, isolation and characterization of the virus in hyenas should be attempted to determine whether they carry a unique immunodeficiency virus.

Social rank played an important role in FIV, but not in the other viruses tested. Bite wounds, the primary means of transmission for FIV among domestic animals (Ueland and Nesse, 1992), are most commonly noted in low-ranking female and low-ranking adult male hyenas. However, rates of interspecific aggressive interaction might be highest among high-ranking adult females and low-ranking adult males, and this opportunity for exposure might account for the relatively high levels of FIV antibody prevalence in these two groups. Adult hyenas are also more likely to be involved in aggressive encounters than juveniles, which could lead to increased viral exposure. The trend of increasing antibody prevalence with increasing age and social status follows a pattern similar to rabies virus exposure in Serengeti hyenas (East et al., 2001). Furthermore, the spatial distribution of low FIV antibody prevalence near human habitation suggests that the primary source of hyena exposure to FIV is not likely to be domestic cats.

Unlike CDV and FIV, a significant high-risk cluster of FPLV/CPV was associated with human habitation, which increases suspicion that the primary source of hyena exposure is domestic animals for this virus. However, the spatial distribution of low FECV/FIPV antibody prevalence near human habitation was similar to CDV and FIV, which suggests that hyenas might not be solely exposed to FECV/FIPV from domestic animals. Adult hyenas were more likely to have been exposed to FECV/FIPV than juveniles, as was true for CDV and FIV. This tendency for more adults to be seropositive for FECV/FIPV is likely because of increased viral exposure with age.

The spatially significant clusters for CDV, FIV, FPLV/CPV, and FECV/FIPV occurred in the same general area and were approximately the same diameter. These clusters consisted of areas where there were more seronegative or seropositive animals in a location than would be expected if the amount of seropositive or seronegative animals were evenly distributed throughout the study area. Because the Talek clan occupies the area identified by the spatial analyses, Talek clan individuals were more frequently included in the spatial clusters, although spatial statistics were calculated independent of clan categorization. The spatial distribution of seronegative or seropositive animals was not evenly distributed throughout the study area for CDV, FIV, FPLV/CPV, and FECV/FIPV, suggesting regional exposure to viruses. Clusters of more seropositive or seronegative animals than expected by chance occurred in the same general area in proximity to human habitation and were approximately the same diameter. The low-risk cluster for CDV and FIV near human habitation might indicate that domestic pets are not the main reservoir for these viruses. Interestingly, there were no spatially significant clusters located far from human habitation, although sampling effort was slightly lower in these areas.

Antibodies to FHV1 were detected in only one hyena. Because exposure appears to be very low, it is unlikely that herpesvirus is endemic in MMNR hyenas. A higher antibody prevalence would be expected in this population if an endogenous herpesvirus was present.

Antibodies against viruses such as CDV and FECV/FIPV occurred at varying levels in the spotted hyena population over time, possibly indicating that these viruses occur as epidemics in MMNR. The temporal pattern of FECV/FIPV antibody prevalence suggests that there was little exposure before 1997, yet the virus appears to have subsequently persisted in the ecosystem. It is possible that further sampling might reveal an epidemic cycle. The relatively constant FIV, FPLV/CPV, and FCV antibody levels suggest that these viruses are endemic in the spotted hyena population or their environment. The persistent seropositivities in hyenas are similar to the FIV pattern seen in Serengeti lions, in which FIV is also endemic, but different in that FPLV/CPV and FCV appear to be endemic in hyenas but not in lions (Packer et al., 1999).

The absence of overt clinical disease in Talek clan spotted hyenas does not mean that viral diseases are not a concern. Seropositive animals are the survivors of infection, and individual spotted hyenas might have experienced clinical signs of disease that went unnoticed because of their common nocturnal behavior and the large sizes of hyena home ranges. Additionally, although humans and lions are known to be responsible for most deaths among adult hyenas (Kruuk, 1972), many deaths occur without identifying causes that could include disease, particularly among juveniles. During the 1994–95 CDV epidemic in Tanzania, spotted hyenas had neurologic signs and died from CDV (Roelke-Parker et al., 1996; Haas et al., 1996); however, only a few cubs in the Talek clan were observed to show clinical signs of CDV during the 1994–95 epidemics, despite the death of many sympatric lions in the MMNR (Kock et al., 1998; K.

E. Holekamp, unpubl. data). To determine the extent of mortality due to viral infections, efforts should be made to collect postmortem samples and confirm infection through pathology.

These results demonstrate that spotted hyenas in the MMNR have been infected with and mounted immune responses to feline and canine viruses. This evidence of infection indicates that spotted hyena likely play a role in the ecology of these viruses within the Serengeti ecosystem. Whether hyenas are a reservoir for these viruses or are exposed by another wildlife reservoir species is not known. The results of this study suggest that domestic animals associated with Maasai manyattas are not the sole source for CDV, FIV, and FECV/FIPV. The presence of potentially pathogenic viruses in hyenas, other carnivores, and the ecosystem should be considered before translocating or reintroducing highly susceptible species.

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