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Epidemiology of clinical feline herpesvirus infection in zoo-housed cheetahs (*Acinonyx jubatus)*

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Abstract

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[f.] R, version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria. Available at: www.R-project.org/. Accessed Dec 1, 2015.

OBJECTIVE—To determine the incidence of and risk factors for clinical feline herpesvirus (FHV) infection in zoo-housed cheetahs and determine whether dam infection was associated with offspring infection.

DESIGN—Retrospective cohort study.

ANIMALS—144 cheetah cubs born in 6 zoos from 1988 through 2007.

PROCEDURES—Data were extracted from the health records of cheetahs and their dams to identify incident cases of clinical FHV infection and estimate incidence from birth to 18 months of age. Univariate and multivariable Cox proportional hazards models, controlling for correlations among cheetahs with the same dam, were used to identify risk factors for incident FHV infection.

RESULTS—Cumulative incidence of FHV infection in cheetah cubs was 35% (50/144). No significant association between dam and offspring infection was identified in any model. Factors identified as significant through multivariable analysis varied by age group. For cheetahs up to 3 months of age, the most important predictor of FHV infection was having a dam that had received a preparturition FHV vaccine regimen that included a modified-live virus vaccine versus a dam that had received no preparturition vaccine. Other risk factors included being from a small litter, being born to a primiparous dam, and male sex.

CONCLUSIONS AND CLINICAL RELEVANCE—This study provided the first population-level characterization of the incidence of and risk factors for FHV infection in cheetahs, and findings confirmed the importance of this disease. Recognition that clinical FHV infection in the dam was not a significant predictor of disease in cubs and identification of other significant factors have implications for disease management.

Feline herpesvirus infection is endemic in cheetahs (*Acinonyx jubatus*) housed in zoos. Whereas some affected cheetahs develop a self-limiting, mild disease similar to that of domestic cats,[1,2] others acquire a chronic, severe upper respiratory tract disease and debilitating cutaneous and ocular ulcers, with life-long problems due to viral latency and reactivation.[1,3–5] Severe cases of FHV infection require considerable and repeated intervention by caretakers and veterinarians (eg, surgery, prolonged administration of medication, and immunosuppressive treatment), for which the success rate is variable.[3–5] Chronic, severe infection can also affect quality of life and has led to the euthanasia of several cheetahs,[4,5] which further impedes breeding efforts and affects population sustainability of this endangered species. Free-ranging cheetahs have evidence of exposure to FHV,[6–8] but disease has not been reported[7–9] and, therefore, the population-level importance of FHV infection in free-ranging cheetahs is unknown.

Research involving domestic cats has shown that FHV is horizontally transmitted via close contact with bodily fluids from infected animals, such as nasal, ocular, and oral secretions.[10] Infection is common in animal shelters and has been attributed to poor hygiene and close housing conditions that promote stress and facilitate disease transmission.[11–14] Kittens have the highest risk, compared with all other age groups, owing to the waning of maternally derived antibody and viral shedding by the lactating dam.[10] Infections in cheetahs appear to follow similar patterns, with cubs developing clinical signs in their first few months after birth[2–4]; however, to the authors' knowledge, no studies have been performed to

quantitatively evaluate the relationship between FHV infection in offspring and their dams and species-specific risk factors have yet to be identified.

Management practices developed to minimize FHV transmission and outbreaks in cheetahs are largely based on disease prevention and management in domestic cats.[15,16] The Association of Zoos and Aquariums Cheetah Species Survival Plan[2] recommends physical separation of pregnant cheetahs from other cheetahs and minimization of the number of naïve cubs at a given time. If cubs develop FHV lesions, removal of the cubs from the dam should be considered to eliminate continual exposure to the virus and facilitate treatment. Sometimes cubs are preemptively removed and hand reared when a dam has a history of passing FHV to her offspring.

Killed-virus vaccines are currently recommended to be administered at 6, 9, 12, and 16 weeks of age as maternally derived antibody wanes. Regular booster vaccination for breeding and pregnant females is also recommended. However, vaccination against FHV infection does not completely prevent infection or disease, nor does it prevent viral shedding by infected individuals, although it may reduce infection severity or viral load.[17–19] Hygienic practices of animal caretakers during outbreaks are encouraged to minimize the potential for FHV transmission through fomites.[2] Although these recommendations have merit, they are based on educated guesses and anecdotal experiences along with what is known about FHV transmission in domestic cats. A need remains for the development of strategies on the basis of the unique biological characteristics of cheetahs and their management. Given the continued transmission of FHV among cheetahs, the purpose of the study reported here was to evaluate the relationship between dam and offspring FHV status and to identify factors associated with development of clinical FHV infection in zoo-housed cheetahs.

Materials and Methods

Animals

The cohort of cheetahs used in this study was a subset of a previously described20 group of 322 cheetahs housed between 1988 and 2007 in 6 North American zoological institutions where cheetahs have been historically housed and bred (Columbus Zoo and Aquarium, Fossil Rim Wildlife Center, Smithsonian National Zoo, San Diego Zoo Safari Park, St Louis Zoo, and White Oak Conservation Center). The source cohort was identified by means of institution inventory and global record databases.[a]

For the present study, cheetahs were only included if they were born at the 6 institutions mentioned during a period when there was potential FHV exposure. For most of the institutions mentioned, this included cheetahs born throughout most of the study period, but 1 institution did not have any known cases of FHV infection until 2001. Cheetahs were excluded if they died or were shipped to another zoological institution prior to 7 days of age. Each cheetah was monitored in the medical records from birth to up to 18 months (78 weeks) of age, which represented the time during which cheetahs were generally housed in

[a]. International Species Information System, Bloomington, Minn.

family groups with their mother and siblings. These criteria resulted in a study population for which complete medical histories were available and incident episodes of clinical FHV could be identified and further evaluated.

FHV infection

Feline herpesvirus infection status was determined for included cheetahs and their dams on the basis of previously described criteria.[20] Briefly, medical histories of all cheetahs were reviewed by use of electronic data[b] and paper medical records to identify cheetahs with diagnostic evidence or clinical signs consistent with FHV infection. Cheetahs were considered to have FHV infection when they had documentation of at least 1 of the following 3 criteria: corneal ulcers or keratitis without a history of trauma to the eye or evidence of neoplasia; a pattern of clinical signs persisting ≥ 7 days that included conjunctivitis, epiphora, blepharospasm, ocular discharge, corneal lesions, sneezing, congestion, nasal discharge, and focal to multifocal skin ulcers, dermatitis, or skin lesions (limited to areas of ocular and salivary secretion and where cheetahs commonly lick themselves [eg, forelimbs]) described as crusted or scabbed; or diagnostic confirmation of FHV infection by PCR assay, immunofluorescent antibody techniques, viral culture, or presence of characteristic viral inclusion bodies on histologic examination. Exclusion criteria outlined in the previous report[20] were applied to all case definitions.

Outcome

The study outcome was time at risk until a cheetah had a clinical episode that met the criteria for FHV infection. Cheetahs were monitored in the records for up to 18 months from birth to diagnosis of FHV infection. Cheetahs with no diagnosis of FHV infection continued to be followed in the records and accumulate time at risk until 18 months of age, unless any of the following happened first, at which point they were censored: shipment to other zoological institutions, death from a cause other than FHV infection, or conclusion of the study (December 31, 2007). Although the time unit for all analyses was weeks, some data presented herein are summarized as months to facilitate interpretation.

Risk factors

The FHV infection status of the dam was the main factor of interest. This was evaluated as a dichotomous variable (yes or no) on the basis of the medical history of the dam before the cub was born. Information on missing observation periods for the dam was also summarized. Severity of infection in the dam was explored as an alternative indicator of dam infection status by use of previously reported criteria.[20] Briefly, a history of a dam ever having aggressive or invasive treatments for FHV disease prior to a cub's birth was classified as indicating severe disease in the dam. This included surgical procedures (keratectomy, tarsorrhaphy, cryosurgery, or debridement of the cornea), use of immunosuppressive interferon treatment, treatment with antiviral drugs, or treatments administered subconjunctivally. A history of treatment only for minor signs of FHV infection prior to a cub's birth (ie, ophthalmic drops) or no treatment was classified as indicating mild disease.

[b] MedARKS, International Species Information System, Bloomington, Minn.

Additional evaluated factors included sex (male or female), birth year (evaluated as a continuous variable with actual year of birth; range, 1988 to 2007), litter size (range, 1 to 6 cubs), size of facility cheetah population when each cub was born (range, 6 to 36 cheetahs), whether the cub had been hand reared (pertained to cubs that had been removed from the dam during the first week after birth; yes or no), dam parity (primiparous or multiparous), whether the dam had at least 1 cub from a previous litter infected with FHV (only evaluated in known multiparous dams; yes or no), dam age at cub birth (natural logarithmic transformation; range, 3.5 to 10 years), and FHV vaccination status of the dam prior to parturition. For dam FHV vaccination status, 3 groups were created: MLV booster vaccination (dam initially vaccinated with a KV vaccine,[c] then revaccinated 3 weeks later with an MLV vaccine[d] prior to breeding, followed by a KV vaccine booster while pregnant), vaccinated only with a KV vaccine while pregnant, and no booster vaccination while pregnant. Institutional effects by themselves were not evaluated because of sparse data and low statistical power for estimating effects across the 6 institutions mentioned. Age by itself could not be evaluated because it was used as the time scale but was incorporated into the baseline hazard function of the survival analysis.

Statistical analysis

Risk factors for clinical FHV infection in cubs were evaluated for the entire cohort and then for the subset of cubs with an FHV-infected dam by calculation of HRs and corresponding 95% CIs. Univariate Cox proportional hazards regression was first used to calculate HRs and to test proportional hazards assumptions by entering an interaction term for each variable with the natural logarithm of time to FHV infection. Purposeful multivariable model selection[21] was then performed by creation of a multivariable Cox proportional hazards model containing the main variable of interest (dam infection status), birth year (to account for management and diagnostic changes over time), and other variables identified as having a liberal P value (< 0.25) in univariate models for the association with FHV or for changes in the HR over time.

In addition, variables for which the proportional hazards assumption was violated indicating potential effect modification were further evaluated. Because evidence was found that hazards were not always proportional, models were stratified by time period (ie, 0 to 3 months of age [when cubs were likely nursing] and > 3 to 18 months of age [when cubs were generally housed in family groups]). Variables were then removed 1 at a time on the basis of Wald P values, with the variable that had the highest P value removed first and the process continued until included variables were either significant ($P < 0.05$) or purposely retained. Dam infection status and birth year were retained in all models; some variables were retained in the model constructed for 1 time period despite a lack of significance because they were significant in the model for the other time period. Presence of effect modification was also evaluated by inserting an interaction between 2 predictor variables into the model, and this interaction term was retained if significant.

[c]Fel-O-VAX and FELOVAX-PCT, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
[d]PUREVAX Feline 3, Merial Inc, Duluth, Ga.

Each variable previously removed was reentered into the model to determine whether it represented an important confounder of the relationship between cub FHV infection status and dam infection status as indicated by a ≥ 10% change in the HR for that variable. The final, most parsimonious models were chosen on the basis of the inclusion of significant predictors or those important in other models, identified confounders and effect modifiers, biological plausibility, and robustness of models.

In all analyses, a robust sandwich covariance matrix was used to adjust the variance to account for correlation between cheetahs with the same dam.[22,23] Tied events, or cheetahs with FHV infections that occurred at the same survival time, were handled by use of the exact conditional probability methods.[24]

The potential effect of misclassification of FHV status in cubs was evaluated by excluding 30 cubs with signs of FHV infection but no laboratory confirmation, while controlling for birth year because the availability of tests increased over time. The potential effect of missing information for dams that joined the population as adults and were not observed for FHV infection from birth was assessed by excluding the 90 cubs that had dams with no clinical signs but without complete early observation, while controlling for birth year. All tests were 2-sided, and values of $P < 0.05$ were considered significant. All analyses were performed with the aid of statistical software.[e–g]

Results

One hundred forty-four cheetahs were born into the source cohort during the study period and were included in the study, representing 46 litters from 26 dams. Of these, 50 cheetahs developed signs of an FHV infection during the 18-month follow-up period (Table 1) for a cumulative incidence of 35% (95% CI, 27% to 43%) and an incidence rate of 4 cases/10 cheetah-years at risk (50 cases/6,483 cheetah-weeks at risk). One cheetah was euthanized because of severe FHV infection, yielding a case fatality rate of 2.0% (1/50). Cheetahs excluded from analysis of particular putative risk factors included 3 cubs cross-fostered to other dams (analyses involving hand rearing), 19 cubs for which there productive history of the dam was unknown (analyses involving dam parity), and 77 cubs that lacked a known multiparous dam (analyses involving previous litter infected with FHV).

Development of a clinical FHV infection was first noted to occur from 8 days through 16 months of age (Figure 1). Twelve (8%) cheetahs had first clinical signs of FHV infection as early as 2 weeks of age, suggesting that a large proportion of total infections (12/50 [24%]) were acquired within a few days after birth, assuming the 2- to 6-day incubation period for domestic cats applies to cheetahs.[25] Sixty (30/50) percent of affected cheetahs first developed clinical signs during the nursing period (0 to 3 months), for an incidence rate of 11.3 cases/10 cheetah-years at risk (30 cases/1,375 cheetah-weeks at risk). Most FHV infections in this group (19/30 [63%]) were first noticed during the first month after birth. The incidence rate during the postweaning, juvenile period when cheetahs were generally

[e] PROC PHREG, SAS/STAT, version 9.3, SAS Institute, Cary, NC.
[g] Therneau T. A package for survival analysis in R, version 2.38. Available at: CRAN.R-project.org/package=survival. Accessed Dec 1, 2015.

still housed with their mother and siblings (> 3 to 18 months of age) was 2 cases/10 cheetah-years at risk (20 cases/5,108 cheetah-weeks at risk).

Overall, 28% (41/144) of cheetahs were born to dams that had a history of clinical FHV infection (ie, FHV-positive dams; Table 2), with an incidence rate of 15 cases/10 cheetah-years at risk (41 cases/1,443 cheetah-weeks at risk). A higher proportion of cubs with an FHV-positive dam developed an FHV infection (18/41 [44%]) than did those with FHV-negative dams (32/103 [31%]); however, this difference was not significant ($P = 0.14$). Outcomes for FHV infection in cubs differed by dam infection status. For cheetahs from FHV-positive dams and multicub (> 1 cub) litters, all offspring in 2 such litters and no offspring in 3 such litters developed an FHV infection; for offspring in the remaining 6 litters, outcomes were mixed. Regarding cheetahs from FHV-negative dams and multicub litters, all offspring in 4 such litters and no offspring in 15 such litters developed an FHV infection; for offspring in the remaining 8 litters, outcomes were mixed.

Risk factors for development of FHV infection

Dam FHV status (infection or no infection prior to cub birth) was not a significant predictor of subsequent FHV infection in cubs in the univariate analysis (Table 1; Figure 2), nor was it a significant predictor in multivariable analyses that considered potential confounders, effect modifiers, and follow-up period (Table 3). Limiting cases of FHV infection to only cheetah cubs with laboratory-confirmed infection (n = 20; excluding 30 nonconfirmed but clinically compatible cases), while controlling for birth year, did not change this relationship (HR, 2.4; 95% CI, 0.6 to 9.2; $P = 0.21$). Exclusion of 90 cheetahs with dams that had never been recorded as having an FHV infection and had not been completely observed since birth, while controlling for birth year, yielded an HR of 7.2 (95% CI, 0.9 to 56.5), but this association was not significant ($P = 0.06$). Severity of the dam's prior FHV infection (severe, mild, or no FHV infection) was also not a significant predictor of subsequent FHV infection in the cub.

Other than the dam's prior infection status, which despite a lack of significance was believed a priori to have a biologically plausible association with the outcome of interest, factors that met the selection criteria for evaluation in the multivariable analyses involving all cheetahs included dam parity, dam vaccination status prior to parturition, and cheetah litter size (Table 1). Population size at the time of each cub's birth was also examined in multivariable models because of evidence of effect modification. The time-adjusted final models revealed a substantial risk of developing FHV infection during the first 3 months after birth among cheetahs with a dam that had received a preparturition FHV vaccine regimen involving an MLV vaccine or only a KV vaccine while pregnant, compared with the risk for cheetahs with dams that had received no preparturition vaccine (Table 3). Effects of the MLV vaccine regimen could not be evaluated for the later period (> 3 to 18 months of age) because all cubs with a dam that had received that regimen either had already developed an FHV infection (all infections occurred by 6 weeks of age) or were censored (n = 3; 2 cubs were transferred to another facility, and 1 cub died of causes unrelated to FHV). In neither age group were significant differences identified in FHV infection risk between cheetahs with

dams that received a KV vaccine during pregnancy and those with dams that received no preparturition vaccine (Figure 3).

Time-stratified model estimates of the HR indicated that cheetahs from larger litters were less likely to develop FHV infection during the first 3 months after birth than cheetahs from smaller litters, but this association was not sustained in the later age period (Table 3). The hazard did not differ by dam parity during the first 3 months after birth; however, from > 3 to 18 months of age, cheetahs with a primiparous dam more often developed FHV infection than those with a multiparous dam.

When analysis was limited to 41 cheetahs that had a dam with a history of clinical FHV infection, no significant difference in hazard was identified with respect to a dam's severity of infection (Table 2). All other factors met the selection criteria for evaluation in the multivariable analysis. This final model for the first 3 months after birth included cheetah birth year (HR, 0.90; 95% CI, 0.80 to 1.10; $P = 0.28$), cheetah sex (male vs female; HR, 4.60; 95% CI, 1.50 to 14.30; $P = 0.008$), and dam preparturition vaccine status. By this model, an FHV infection was more likely to develop in cheetahs with dams that had received the MLV vaccine regimen before parturition than in cheetahs with dams that had received no preparturition vaccine (HR, 8.70; 95% CI, 1.80 to 43.10; $P = 0.008$). However, cheetahs with dams that received only the KV vaccine before parturition had no different hazard of developing an FHV infection than cheetahs from unvaccinated dams (HR, 2.80; 95% CI, 0.70 to 11.90; $P = 0.15$). Because only 23 cheetahs were available for the 12-week to 18-month period, a multivariable model was not developed for this period.

Notably, hand rearing was not a significant predictor of cheetahs developing an FHV infection in any of the univariate or multivariable models.

Discussion

The study reported here represents the first population-level characterization of disease incidence and evaluation of risk factors for clinical FHV infection in zoo-housed cheetahs. Our findings confirmed the importance of this disease in young cheetahs, with 35% (50/144) developing clinical signs during the first 1.5 years after birth. The high incidence of clinical disease during this period represents an important component of the high prevalence previously reported[20] and supports the consensus that FHV is widespread and infection is endemic in captive cheetahs.[2] Further, a large proportion of these infections occurred during the first month after birth. This was consistent with FHV case series reports[3,4] involving cheetah cubs and findings reported for domestic cats, in which kittens are believed to be important in maintaining the infection cycle.[10] However, incident infections should not be considered exclusive to cubs. Cheetahs in the present study continued to develop incident clinical FHV infections throughout (and also beyond) the 18-month follow-up period. Although individual cheetahs were not monitored through adulthood for the purposes of this study, it is noteworthy that at least 8 cheetahs considered negative for clinical FHV infection at 18 months of age had documented clinical (and sometimes severe) disease later in life (age range of incident clinical disease, 1.6 to 8.6 years; data not shown).

The primary focus of the present study was on the risk of cheetahs developing a clinical FHV infection during the early months after birth, particularly as related to potential transmission from the dam. We found that a history of clinical FHV infection in the dam was not a predictor of development of clinical FHV infection in cubs, although the *P* value for this association was close to the cutoff for significance ($P = 0.07$) for cheetahs during the > 3- to 18-month period. This lack of association was unexpected given that FHV transmission has commonly been attributed to recrudescence and viral shedding by latently infected cheetah dams. Although this finding may indicate that FHV-related clinical illness is not a reliable predictor of true FHV status, it may also reflect the inconsistency of viral shedding by infected dams. In domestic cats, the prevalence of shedding by dams is variable, even following experimental infection.[10] Transmission of FHV from queens to their kittens following experimental infection has been demonstrated; however, not all infected queens in that study[10] shed FHV, only 4 of 12 kittens from queens with detectable shedding developed clinical disease, and some kittens never developed signs yet became carriers with measurable immunity against the virus.[10]

The shedding and transmission pattern of FHV may be similarly variable among cheetahs, given that FHV-positive dams in the present study had a combination of affected and unaffected cubs in 55% of litters and had no clinically affected cubs in 27% of litters. We also found that 31% of cheetah cubs with dams with no history of an FHV infection developed clinical disease. Alternatively, the potential exists for exposure from close contact with other infected felids.[5,10] Although the virus does not persist well in the environment, indirect transmission through fomites is considered to play a role in animal shelters and catteries[16] and was considered a possible mode of transmission for an outbreak observed in a group of semicaptive cheetahs.[1] Therefore, consideration of other potential sources of FHV, isolation, sanitation, and disinfection remain critical in cheetah facilities.

Whereas findings of the present study suggested that sources of FHV exposure beyond the dam may be important for cheetahs in zoos, they also raised a question regarding the role of undetectable, subclinical carriers in the transmission cycle. A true association between FHV infection in offspring and dams may have been masked if the presence or absence of clinical signs was not a good indicator of the true FHV disease status or shedding in dams.[26]

In domestic cats, studies have involved identification of FHV cases on the basis of viral shedding, which was not measured in the historical cohort of cheetahs in the present study and which would be difficult to evaluate, even prospectively, in this endangered species. In clinical zoo practice, diagnosis of FHV infection is often based on clinical signs alone owing to logistic challenges and risks associated with anesthesia and sample collection from a large carnivore. Specific patterns of clinical signs, coupled with results of laboratory diagnostic tests, in health records of cheetah cubs and their dams were used to identify cases of FHV infection.[20] Although other causes of upper respiratory tract disease exist (eg, feline calicivirus, *Chlamydophila felis*, *Bordetella* sp, and *Mycoplasma* spp), disease due to FHV appears to be the most widespread given the numerous reports in the literature and expert knowledge.[2,27]

Case definitions in the present study excluded other causes of respiratory disease when identified,[20] but this did not eliminate the possibility that clinical signs observed in the 30 cheetahs without laboratory confirmation were related to other pathogens or concomitant infections. To evaluate potential effects of misdiagnosis of FHV infection in cubs, a smaller subset of 20 laboratory-confirmed cases and 94 cubs with no clinical signs was used. This analysis also revealed no association between dam FHV status and development of clinical FHV infection in cubs.

Another potential source of misclassification bias for dams in the present study was the incomplete nature of some health records. If observation was not continuous since birth and if a dam had clinical FHV infection as a cub, then clinical FHV infection may not have been noted in the reviewed medical data. This could have led to classification of a latently infected dam as FHV negative. Therefore, a subset analysis was performed to include only cheetahs with dams that had complete follow-up data available since birth (n = 62). Although these dams disproportionally represented dams born in the earlier years of the study and the reference group of dams without clinical signs was small, the HR estimate was larger for this comparison and the *P* value was close to the cut-off for significance (HR, 7.2; 95% CI, 0.9 to 56.5; $P = 0.06$). This finding could be perceived as supporting a role of the dam in the epidemiology of FHV infection in cheetahs, but also highlights the difficulty in identifying dams that may shed FHV.

Like domestic cats,[10] some cheetahs may be subclinical carriers that never develop clinical signs. Thus, clinical signs alone may not be a sensitive measure of infection status. With respect to clinical management in zoo settings, the present study showed that the observed clinical status of a dam was not a robust predictor of whether her offspring would develop a clinical FHV infection. Disease management decisions, such as removing cubs from dams, should not be based solely on the clinical history of the dam, but should take into account subclinical carrier status and other important drivers of FHV transmission and disease susceptibility.

The most important predictor of clinical FHV infection in the cheetahs of the present study was vaccination of the dam with a regimen that included a KV vaccine followed by an MLV vaccine as a pre-breeding booster, then another KV booster while pregnant. Careful interpretation of this finding is warranted because the data were limited by small numbers of dams at 1 institution receiving the MLV FHV vaccine regimen late in the study period. However, the strong association between FHV infection and a dam having received the preparturition MLV vaccine regimen persisted even when data analysis was limited to that institution (data not shown). Bias could have been introduced by administration of the MLV vaccine to dams that had a history of passing FHV to their cubs, although these dams also had a history of having cubs with no clinical signs. Residual confounding by time or other institution-specific factors that were not accounted for in the present study may also have existed. Despite these limitations, the dam vaccination predictor was highly significant and the effect was large when considering all cheetahs and also just those with FHV-positive dams.

Modified-live virus vaccines against FHV are more protective than KV vaccines,[28] and response to MLV vaccination in cheetahs is favorable[29]; however, MLV vaccines can cause disease.[30,31] Such vaccines may have been associated with severe, chronic FHV infection in a cheetah,[5] and vaccine-associated disease has been identified in other nondomestic feline species.[32] If MLV infection promotes undetected viral shedding in cheetah dams, then transmission to offspring may occur. Pending efficacy studies, it has been suggested that MLV vaccines could be used as boosters for cheetah dams before breeding to further enhance the immune system and may help decrease the severity of clinical lesions in offspring.[2] The cheetah cubs that developed clinical FHV infection in the present study after their dam received the MLV vaccine all had mild clinical signs and did not develop severe disease after the study ended. Our findings, therefore, raise an important question as to whether the preparturition MLV vaccine regimen for cheetah dams could both promote mild disease expression (as we observed) and also be protective against severe manifestations of disease in offspring. Additional studies are needed to investigate this possibility.

Until the risks are well understood and vaccine efficacy has been established in cheetahs, we recommend caution in use of the MLV FHV vaccine near the time of parturition. Existing recommendations for disease prevention include vaccination of the dam with a KV vaccine during pregnancy to increase the amount of maternal antibody transferred to the cubs.[2,33] In the cheetahs of the study reported here, preparturition vaccination of the dam with a KV vaccine was not effective in significantly reducing the risk of infection in cubs, but there also was no greater risk of cubs subsequently developing an FHV infection than that for cubs with unvaccinated dams, regardless of the cohort examined. Therefore, these vaccines may not be warranted as a population-level method of preventing FHV infection but should not increase the risk of FHV when a polyvalent vaccine that includes protection against FHV is administered to manage other diseases. Our findings suggest the need for well-controlled, randomized trials to determine FHV vaccine effectiveness in cheetah populations.

Clinical FHV infection was more likely in cheetah cubs in the present study that had primiparous versus multiparous dams, but only from > 3 to 18 months of age. First parity cheetah dams, as opposed to multiparous dams, have less mothering ability and their cubs grow slower,[34,35] which may promote disease transmission or expression. In livestock, primiparous dams reportedly have lower amounts of colostrum and colostral antibody.[36,37] Longer intervals from birth to nursing may also be characteristic of first-time dams and can decrease colostral antibody concentrations as well as the ability of the neonate to absorb antibodies.[37,38] Adequate colostral antibody transfer is important for cheetahs given that the feline endotheliochorial placenta allows only limited transplacental passage of antibody. The negative effect of a primiparous dam may manifest in the period after 3 months of age as circulating concentrations of maternally derived antibody decline below a protective level. In domestic kittens, colostral antibody interfered with the immune response to FHV vaccination from 2 to 10 weeks of age in some studies to > 16 weeks of age in others, with the duration of interference related to initial maternally derived titers.[39]

Cubs from larger litters, which would allow more susceptible cubs to be in contact with and potential amplification of viral exposure from sick siblings, were no more likely to develop an FHV infection than cubs from smaller litters in the present study. For many of the

affected litters, clinical disease spread to only a portion of the cubs and the risk of FHV infection decreased during the first 3 months after birth as litter size increased. For other alphaherpesviruses with adverse reproductive effects, smaller litter size could indicate infected dams; however, pregnancy losses in domestic cats are uncommon with FHV and have been attributed to disease severity rather than the direct effect of the virus.[40] Litter size can affect all-cause mortality rates in domestic cats[41] and captive tigers,[42] with the highest rate in singleton litters, although the reasons are unknown. Findings of the present study suggested that it is important to consider risk of clinical FHV infection at the cub level rather than litter level and that infection in 1 cub in a litter does not predict the outcome for its siblings.

Males are reportedly at higher risk of and have a poorer prognosis for infectious diseases than females across species. This is attributed to sex-related differences in both immune function and behavior.[43] In humans, respiratory disease is more common in males than females at all ages, including infancy.[44] For FHV infection in the present study, the increased risk for males was significant only for cubs with FHV-positive dams. Although this finding suggests the need for further studies of sex-related health differences, it offers limited opportunity for management intervention.

Removal of the cub from the dam for hand rearing within the first week was not significantly associated with a reduction in the incidence of FHV infection in the present study. It is recommended that young cubs with severe clinical signs be removed from the dam and hand reared to help improve the clinical course of disease.[2] Because we focused exclusively on incident cases of clinical infection, we did not evaluate whether removal of the offspring after signs were observed helped improve the clinical course of disease. Additional research is needed to determine whether this practice is beneficial.

The retrospective cohort design of the present study allowed the capture of clinically relevant incident FHV infections as they naturally occurred and allowed calculation of the instantaneous hazard of developing an infection at any point during the study period. Censoring the population according to the 18-month timeline aided in ascertaining an appropriate group for ascribing risk profiles related to the dam and minimized the effects of potential biases resulting from prolonged follow-up periods.[45] Analyses took into account the expected correlation between individuals with the same dam, so findings are applicable to individual cheetahs rather than exclusive to a litter. To obtain a large enough sample size with variability in the distribution of disease and exposures, data were pooled across institutions. Sparse data precluded the ability to examine effect modification or control for institution-level effects in the analyses. Although some inherent differences among institutions were captured in the evaluated variables (eg, population size, hand rearing, and dam vaccinations), institutional effects may have been unaccounted for, thereby limiting the generalizability of our findings.

In the study reported here, variables were analyzed for which a biologically plausible hypothesis existed for explaining differences in disease risk between cheetahs and for which high-quality information was available for most members of the population. Factors not well captured in the medical data that may have further shed light on the epidemiology of FHV

infection in cheetahs included the precise time at which clinical signs began, duration of apparent infection, results of all FHV diagnostic tests (often missing in clinical records), concomitant illnesses, maternal care received, days of bottle feeding, and birth weight. Acute and chronic stress in captive populations may be associated with disease expression.[9,46] We could not specifically evaluate stressors, such as medical treatments, corticosteroid administration, animal movements, enclosure characteristics, nutrition, and human contacts. Measurement of maternal viral shedding, amount of maternally derived antibody received by cubs, and maternal response to vaccination may also be important to understand disease patterns, given that these can vary across individuals.[29,47] Direct contact with or proximity to other animals (infected conspecifics or predators) could not be assessed for most of the population because of a lack of detailed housing records. Efforts to more specifically capture episodes of FHV infection and their predictors in the medical and management records are needed to provide additional insight into the epidemiology of FHV infection in cheetahs.

Findings of the present study can help guide efforts to improve management of FHV infection in cheetahs housed in zoos and may have implications for wild cheetah populations in which exposure to FHV has been documented.[6–8] Any manifestation of incident FHV-related clinical disease was evaluated in young cheetahs, including mild, self-eliminating infections. The results can provide a framework for further investigations of the identified risk factors and determination of drivers of chronic and severe manifestations of disease that are the most clinically challenging. Several findings are important to cheetah management, including the high incidence (35%) identified in cheetahs during the first 18 months after birth. Dam history of FHV infection was not a good predictor of whether a cub also developed an infection; therefore, management decisions for cubs should not be based solely on the clinical history of the dam. Preparturition FHV vaccine regimens that include MLV vaccines should be further evaluated prior to wide adoption as preventive measures because they may increase the risk of offspring developing an FHV infection. Finally, offspring of primiparous cheetah dams, young males, and cubs from small litters should be closely monitored for development of clinical signs for early intervention if FHV infection occurs.

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ABBREVIATIONS

CI	Confidence interval
FHV	Feline herpesvirus
HR	Hazards ratio
KV	Killed virus

MLV	Modified-live virus

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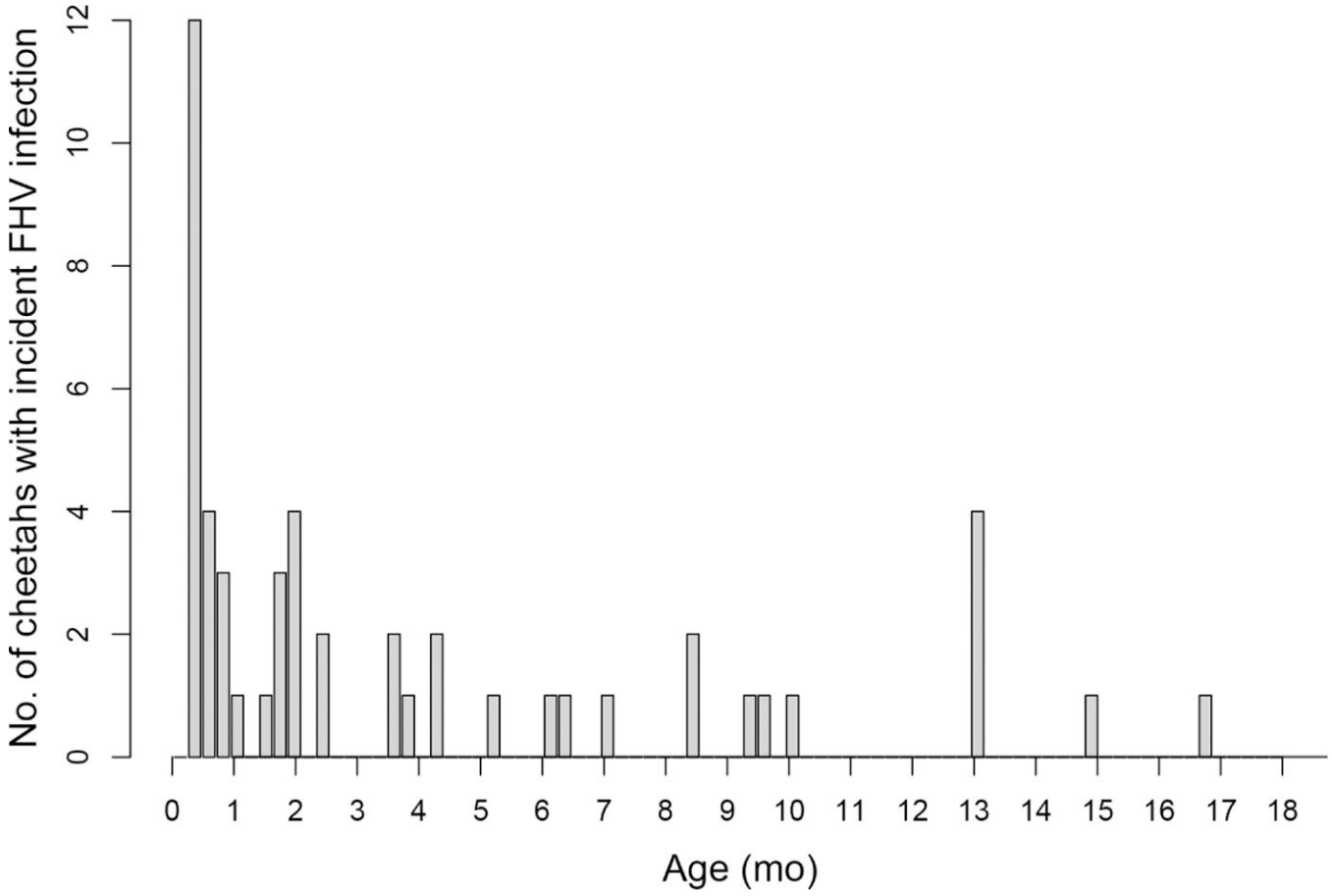

Figure 1—.

Age distribution of incident cases of clinical FHV infection from birth to 18 months in 50 young cheetahs (*Acinonyx jubatus*) housed at 6 zoos.

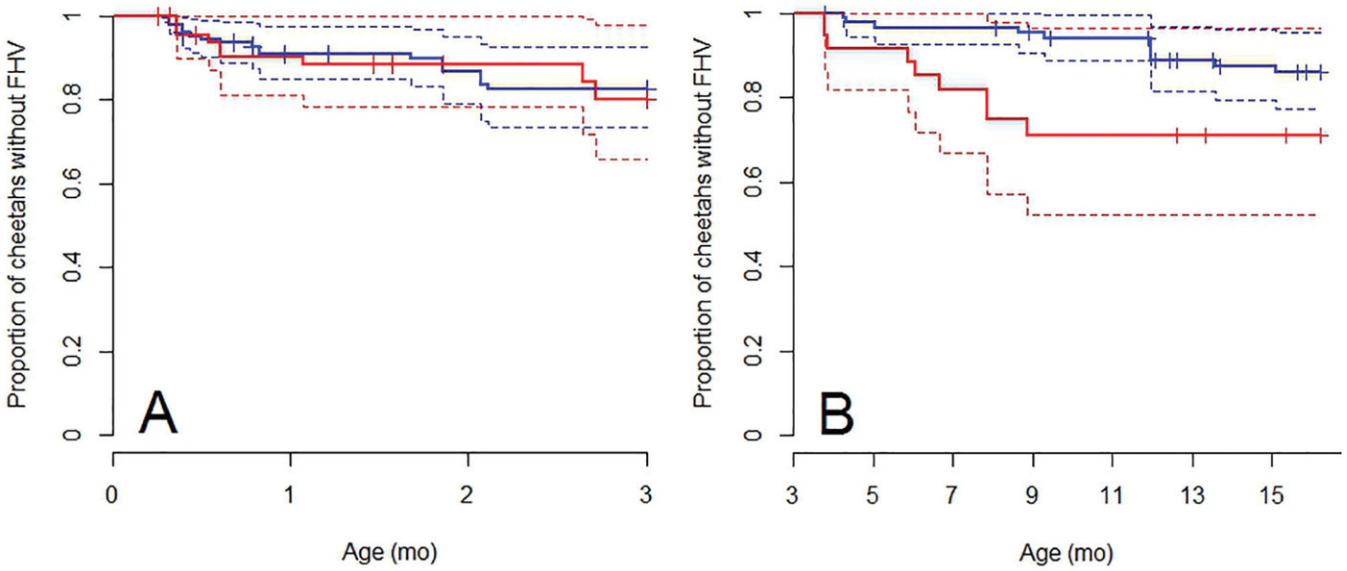

Figure 2—.

Kaplan-Meier curves showing the probability of young zoo-housed cheetahs (n = 144) developing a clinical FHV infection as a function of age from birth to 3 months (A) and > 3 to 18 months (B), stratified by whether the dam did (red line) or did not (blue line) have a clinical history of FHV infection at any time in the past. Dashed lines represent 95% CIs.

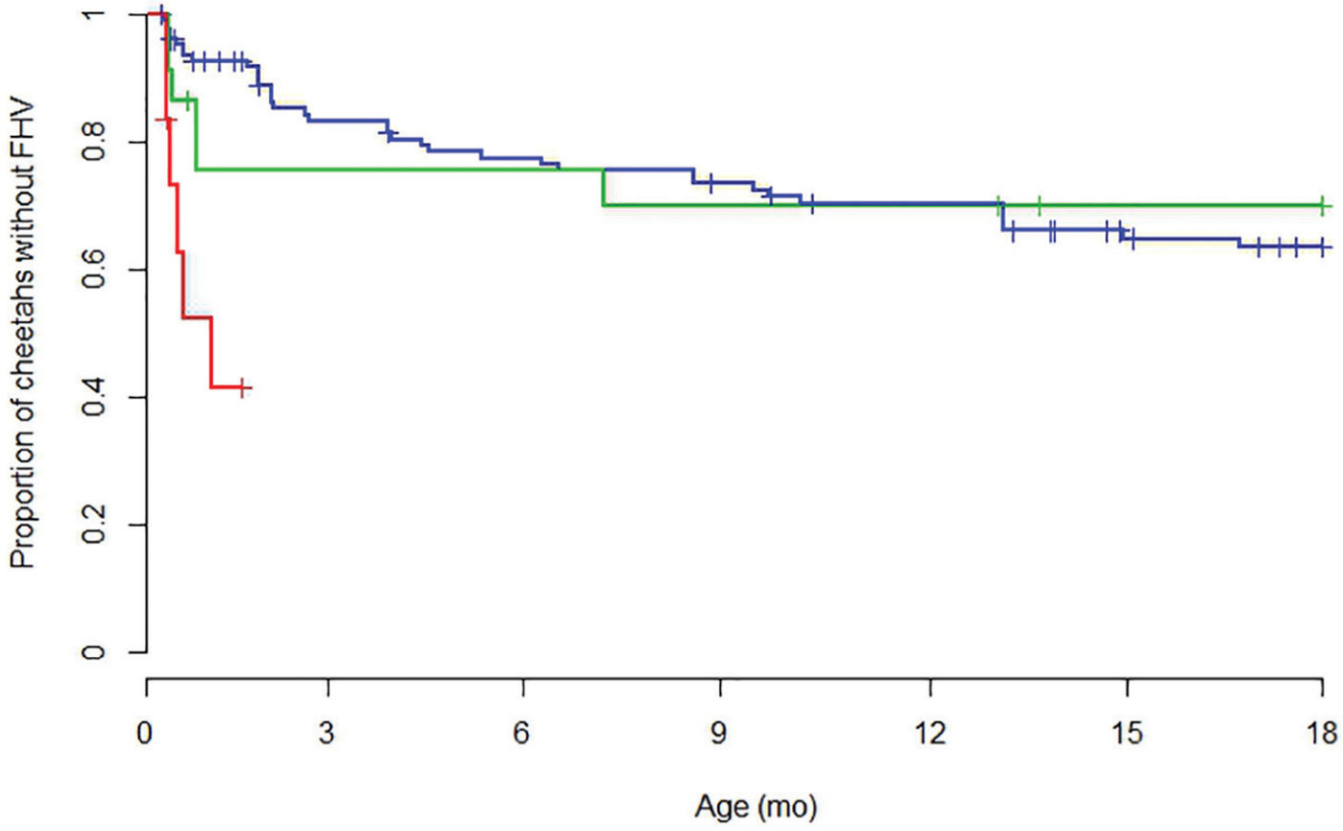

Figure 3—.

Kaplan-Meier curves showing the probability of young zoo-housed cheetahs developing a clinical FHV infection as a function of age, stratified by whether the dam received a preparturition vaccine regimen that included an MLV FHV vaccine[b] (red line), only a KV booster vaccine while pregnant (green line), or no preparturition vaccine (blue line). The MLV vaccine regimen included a KV vaccine, followed 3 weeks later with an MLV vaccine prior to breeding, followed by a KV booster while pregnant.

Table 1—

Characteristics* of 144 young zoo-housed cheetahs (*Acinonyx jubatus*) and HRs for univariate associations of these characteristics with development of FHV infection from birth to 18 months of age.

Characteristic	FHV positive (n = 50)	FHV negative (n = 94)	HR†	95% CI†	Wald *P* value
Follow-up period (wk)	9 (3–28)	78 (45–78)	—	—	—
Dam clinical history of FHV infection					
Positive	18 (36)	23 (24)	1.76	0.67–4.63	0.25
Negative	32 (64)	71 (76)	Referent	—	—
Dam FHV infection severity (before cub was born)					
Ever had severe infection	4 (8)	10 (11)	0.98	0.24–3.96	0.28
Only mild signs of infection	14 (28)	13 (14)	2.28	0.79–6.61	—
No infection	32 (64)	71 (76)	Referent	—	—
Dam age at offspring birth (y)‡	5.8 (1.7)	6.1 (1.5)	0.54	0.15–1.92	0.34
Dam parity‡					
Primiparous	25 (60)	33 (42)	1.72	0.78–3.80	0.18
Multiparous	17 (40)	50 (60)	Referent	—	—
Dam previously had an FHV-positive litter‡					
Yes	4 (24)	11 (22)	1.15	0.42–3.16	0.78
No	13 (76)	39 (78)	Referent	—	—
Dam received a preparturition FHV vaccine					
MLV vaccine during breeding, followed by KV booster while pregnant	6 (12)	3 (3)	13.38	4.48–39.97	< 0.001
Only KV booster while pregnant	6 (12)	10 (11)	1.34	0.26–6.90	—
No preparturition vaccination recorded	38 (76)	81 (86)	Referent	—	—
Cheetah sex					
Male	25 (50)	43 (46)	1.22	0.72–2.06	0.45
Female	25 (50)	51 (54)	Referent	—	—
Cheetah birth year	2003 (1992–2005)	2002 (1997–2005)	1.02	0.94–1.11	0.60
Cheetah litter size	3.5 (1.3)	3.9 (1.2)	0.72	0.51–1.01	0.06
Cheetah population size at facility when cheetah was born	20.7 (6.6)	20.1 (8.7)	1.01	0.98–1.05	0.62
Cheetah hand reared within the first week after birth‡					

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Characteristic	FHV positive (n = 50)	FHV negative (n = 94)	HR[†]	95% CI[†]	Wald *P* value
Yes	4 (8)	19 (20)	0.50	0.08–3.04	0.45
No	44 (92)	74 (80)	Referent	—	—

[*] Values of continuous data are reported as mean (SD) if normally distributed (cheetah litter size, dam age, and cheetah population size) and median (interquartile range) if nonnormally distributed (follow-up period and cheetah birth year). Categorical data (all other variables) are reported as number (%).

[†] Robust sandwich variance estimates were used to account for correlations among cheetahs with the same dam. Cox proportional hazards analysis was used.

[≠] Analyzed for cheetahs with complete data only (dam age, n = 130; dam parity, 125; dam previously had an FHV-positive litter, 67 [primiparous dams removed]; cheetah hand reared, 141).

— = Not applicable.

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Table 2—

Characteristics* of a subset of 41 young zoo-housed cheetahs with dams that had a clinical history of FHV infection and HRs for univariate associations of these characteristics with development of FHV infection from birth to 18 months of age.

Characteristic	FHV positive (n = 18)	FHV negative (n = 23)	HR†	95% CI†	Wald *P* value
Follow-up period (wk)	14 (3–28)	74 (7–78)	—	—	—
Dam infection severity (before cub was born)					
Severe	4 (22)	10 (43)	0.45	0.10–2.04	0.30
Mild	14 (78)	13 (57)	Referent	—	—
Dam age at offspring birth (y)	6.1 (2.0)	7.0 (1.6)	0.88	0.61–1.25	0.47
Dam parity					
Primiparous	14 (78)	9 (39)	2.92	0.66–12.85	0.16
Multiparous	4 (4)	14 (61)	Referent	—	—
Dam previously had an FHV-infected litter‡					
Yes	2 (50)	10 (71)	0.80	0.07–7.71	0.80
No	2 (50)	4 (29)	Referent	—	—
Dam received a preparturition FHV					
MLV vaccine during breeding, followed by KV booster while pregnant	2 (11)	3 (13)	4.90	1.48–16.26	0.02
Only KV booster while pregnant	3 (17)	4 (17)	0.86	0.09–8.15	—
No preparturition vaccine recorded	13 (72)	16 (70)	Referent	—	—
Cheetah sex					
Male	8 (44)	9 (39)	1.81	0.74–4.44	0.19
Female	10 (56)	14 (61)	Referent	—	—
Cheetah birth year	2005 (2002–2006)	2005 (1999–2005)	0.97	0.82–1.14	0.68
Cheetah litter size	3.6 (1.3)	3.8 (1.3)	0.80	0.65–0.98	0.03
Cheetah population size at birth	18.6 (7.0)	14.5 (5.8)	1.09	1.02–1.18	0.02
Cheetah hand reared within the first week after birth‡					
Yes	1 (6)	9 (39)	0.20	0.01–2.81	0.23
No	16 (94)	14 (61)	Referent	—	—

‡Analyzed for cheetahs with complete data only (dam previously had FHV-positive litter, n = 18 [primiparous dams removed]; cheetah hand reared, 40).

See Table 1 for remainder of key.

Table 3—

Results of multivariable Cox proportional hazards models* of factors associated with the development of FHV infection in young zoo-housed cheetahs from birth to 3 months of age and from > 3 to 18 months of age.

	Birth to 3 months of age					> 3 to 18 months of age†				
Factor	**β**	**SE**	**HR**	**95% CI**	***P* value**	**β**	**SE**	**HR**	**95% CI**	***P* value**
Dam had (vs did not have) history of clinical FHV infection	0.16	0.78	1.17	0.26–5.36	0.84	1.024	0.56	2.78	0.93–8.31	0.07
Cheetah birth year	−0.06	0.05	0.94	0.85–1.04	0.24	−0.071	0.05	0.93	0.85–1.02	0.13
Dam preparturition FHV vaccine status										
MLV vaccine followed by KV vaccine (vs no FHV vaccine)	0.43	0.87	13.32	2.10–84.59	0.006	—	—	—	—	—
KV vaccine only (vs no FHV vaccine)	2.59	0.94	1.54	0.28–8.48	0.62	−0.016	0.90	0.98	0.17–5.75	0.99
Cheetah litter size	−0.48	0.16	0.62	0.45–0.85	0.003	0.300	0.30	1.35	0.76–2.41	0.31
Primiparous (vs multiparous) dam	0.27	0.62	1.31	0.39–4.39	0.66	1.880	0.81	6.55	1.34–31.93	0.02

*Robust sandwich variance estimates were used to account for correlations among cheetahs with the same dam; 144 cheetahs were evaluated in the model building process. The final model for 0 to 3 months included only 125 cheetahs because of missing dam parity values; however, similar HRs and significance were observed when all cheetahs were included and dam parity was excluded from the model. The final model for > 3 to 18 months included 89 cheetahs owing to censoring and missing data.

†The period of > 3 to 18 months included only cheetahs that had not developed an FHV infection by this point and that remained in the cohort after 3 months of age.

See Table 1 for remainder of key.