INVESTIGATION OF A FELINE PANLEUKOPAENIA VIRUS INFECTION IN VACCINATED ARABIAN SAND CATS (*FELIS MARGARITA HARRISONI*)

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Summary

Post-mortem findings following the sudden death of 2 Arabian sand cat kittens at the Al Wabra Wildlife Preservation indicated a feline panleukopaenia (FPLV) infection as the probable cause of death. FPLV was confirmed, albeit indirectly, by PCR in the intestinal swabs of the kittens. Since the kittens had been vaccinated a little over 2 weeks before death, with a modified live vaccine (MLV) against FPLV, the question arose of whether the disease was vaccine-induced or a result of vaccination failure. Further investigations carried out on the surviving littermate and mother of the kittens, as well as a sero-survey in a sample of the AWWP sand cat population, contributed to the understanding of the persistence of virus in sand cats and the efficacy of the vaccination protocol at AWWP. Viral DNA was detected in rectal swabs of 2 apparently healthy sand cats up to 6 weeks after vaccination, which is significantly longer than the duration of faecal virus shedding reported in the literature. The sero-survey demonstrated that the antibody titres to vaccination with a MLV in sand cats are similar to those in domestic animals with haemagglutination inhibition titres of 1:80 to 1:160 recorded in all sand cats studied. The findings of this study helped re-evaluate the vaccination protocol at AWWP to minimize the possibility of vaccination failures and adequately protect animals from potentially fatal infection.

Introduction

Feline panleukopaenia, feline infectious enteritis and feline distemper are synonyms for the disease manifestation in cats caused by the feline panleukopaenia virus (FPLV). FPLV belongs to the Feline Parvovirus (FPV) subgroup, which consists of viruses that are antigenically and phylogenetically closely related, including the canine parvovirus (CPV-2), mink enteritis virus (MEV), blue fox parvovirus (BFPV) and raccoon parvovirus (RPV), among others (TRUYEN et al., 1995; NAKAMURA et al., 2001; SHACKELTON et al., 2005). Since the 1980's, however, CPV-2 has been completely replaced by antigenic variants, CPV-2a and CPV-2b, and acquired a broader host range including felids (STEINEL et al., 2000; NAKAMURA et al., 2001; IKEDA et al., 2002).

FPLV displays a distinct tropism for actively dividing cells, and therefore, age of the animal plays an important role in epidemiology of the disease since neonatal tissue is a rich source of mitotically dividing cells (TRUYEN and PARRISH, 1992; SHACKELTON et al., 2005). In adults, the virus infects the epithelium of the gut and the lymphatic tissue (TRUYEN, 2000; STEINEL et al., 2001). Mortality is high, especially when foetuses and young kittens are infected, with peracute cases often dying within 24 hours of showing symptoms. Often, a feline ataxia syndrome is observed caused by cerebellar hypoplasia (ADDIE et al., 1998; AIELLO, 1998a; STEINEL et al., 2000). In older kittens, illness is

characterised by loss of appetite, pyrexia, diarrhoea, vomiting and a panleukopaenia. In adult cats, infections, subclinical or with mild symptoms is common, and infection induces a life-long immunity and complete elimination of the virus (STEINEL et al., 2001; IKEDA et al., 2002). Virus is commonly shed 3 - 9 days post infection in the faeces, with peak titres occurring at the time or prior to clinical signs (SCHUNCK et al., 1995; CHALMERS et al., 1999; TRUYEN, 2000). Since the virus has a high resistance to heat and disinfectants and can survive in the environment for up to a year, the predominant route of infection is the oro-faecal route (TRUYEN and PARRISH, 1992; AIELLO, 1998a).

Highly specialised nocturnal predators, Arabian sand cats (*Felis margarita harrisoni*) occur in potentially threatened numbers in desert regions in the Arabian Peninsula (Near Threatened, IUCN 2008). The Al Wabra Wildlife Preservation (AWWP) is home to 27 Arabian sand cats. The sand cats are housed in enclosures of varying sizes with 2 - 3 individuals sharing one enclosure. In terms of veterinary management, the sand cat population at AWWP is routinely dewormed at 2 weeks, 4 weeks and 8 weeks of age with Pyrantel embonate (BANMINTH®Katze, PFIZER, Karlsruhe, Germany), and vaccinated with a live attenuated feline enteritis (panleukopaenia) virus, 'snow leopard' strain, feline rhinotracheitis virus strain FVRm, and feline calicivirus strain F9 (FELOCELL®CVR, PFIZER, Karlsruhe, Germany) as well as inactivated rabies vaccine (RABDOMUN®, ESSEX Pharma GmbH, Munich, Germany) at 4 weeks and 8 weeks of age followed by an annual booster. This study reports the outcome and inferences drawn from an investigation carried out following the sudden death of 2 Arabian sand cat kittens from possible FPLV infection shortly after vaccination.

Case report

Case history

On the 20th of April, 2006, a male Arabian sand cat kitten (AWWP stock list # 6151), from a litter of 2 males and 1 female, was found dead with no clinical signs noted ante-mortem. The following day, the other male of the same litter was also found dead (AWWP stock list # 6152) under similar circumstances. The former died at 46 days and the latter at 47 days of age. Incidentally, the surviving female littermate had undergone veterinary treatment for signs of lacrimation from its left eye accompanied by mild diarrhoea just 2 days before the first kitten died. At the time of death, however, 6151 weighed 339 g and 6152 weighed 274 g, having lost 23 g and 93 g, respectively, in a little over 2 weeks.

Gross pathological examination was carried out at an estimated interval of 24 h and 10 h post mortem for the cats, with the carcass being stored at 0 - 4 °C during this time. Histopathological examination of organs fixed immediately at post mortem in 10 % formalin was carried out at a collaborating laboratory using Haematoxylin-Eosin staining of organ sections embedded in paraffin. In addition, histo-pathological examination of the brain was performed in both cases at a specialised institute for neuropathology.

To confirm infection with parvovirus at a molecular biological level, swabs of the thawed intestinal contents from the small intestine of both sand cats were submitted to Vet Med Labor GmbH (Germany) for detection of feline parvovirus by a PCR technique adapted from PEREIRA et al., (2000).

Further investigations

The death of the 2 kittens from a possible parvovirus infection prompted a decision to investigate the efficacy of the FPLV vaccination protocol followed at AWWP. To this end, a sample of 5 Arabian sand cats was selected, including the surviving littermate (AWWP stock list # 6153) and the mother (AWWP

stock list # 5066) of the kittens from the same enclosure, and 3 other randomly selected individuals (AWWP stock list # 5064, 5065 and 4512).

Serum samples were collected from all the cats and paired serum samples, pre- and post annual revaccination, were obtained from 3 out of the 5 sand cats. Blood collection by femoral venipuncture was carried out under isoflurane (ISOFLURANE®, BAXTER DEUTSCHLAND GmbH, Unterschleisheim, Germany) at 5 % level for induction and 3 % for maintenance with oxygen supplied at 1 l per minute. Each time a blood sample was collected, it was stored in Li-heparin tubes, and a complete blood count (CBC) was performed, for evidence of panleukopaenia or other abnormalities. Both domestic cat ranges (AIELLO, 1998b) and ranges reported for sand cats (*Felis margarita*) in the International Species Information System (ISIS) were used as reference for interpretation. Further, in all cases, plasma was separated within 2 hours of collection and stored at -20 °C until dispatch to the laboratory for analysis. The Haemagglutination Inhibition (HI) assay for antibodies against feline parvovirus was based on the method described by APPEL et al. (1979). Due to the unavailability of a test for detecting antibodies specifically for FPLV, this test, developed for detecting CPV-2 antibodies in dogs can be used for detecting antibodies to FPLV since the 2 viruses cannot be differentiated serologically (APPEL et al., 1979).

In addition to serum samples, rectal swabs were collected on 3 occasions, 1 week, 6 weeks, and 15 weeks after primary vaccination and revaccination, respectively for the littermate (6153) and mother (5066) of the dead kittens with the modified live vaccine for FPLV and stored at -20 °C until submission to the laboratory. The PCR detection of FPV in rectal swabs was adapted from PEREIRA et al. (2000) with slight modifications.

Results

Case history

During the gross pathological examination of sand cat 6151, foul-smelling material was observed around the perineal area and on the posterior surface of hind legs, suggestive of diarrhoea. This was accompanied by accumulation of dirt around the mouth and bilateral nasal discharge. The lungs were congested with petechial haemorrhages scattered on the surface, and the edge of the right lung showed a greenish discolouration, possibly due to necrosis. In the stomach, only watery contents were found, with the intestinal mucosa appearing haemorrhagic. Finally, the skull showed longitudinal subdural haemorrhagic streaks. Bacterial swabs collected from the intestine and kidney revealed *Escherichia coli* on culture, whereas the lung, heart, spleen and liver showed no microbial growth.

Sand cat 6152 was found to be very emaciated at the gross post mortem examination. The cervical muscles appeared damaged with evidence of a sharp object penetration at the base of the skull. The location and nature of the lesion leads to the presumption that it was a bite wound inflicted by the mother probably in an effort to stimulate the sick or dying animal. In addition, scattered areas of both lungs were congested. Hepatomegaly, petechiation and jaundice of the liver were noted and diffuse petechial haemorrhages were also observed in the parenchyma of the spleen. The digestive system was empty but for a small amount of watery faecal content in the colonic part of the intestine. The rest of the organs showed no significant abnormalities. Microbiology again revealed *Escherichia coli* only in the intestine of this kitten with other organ swabs showing no growth.

Examination of the faecal samples of both sand cats, using sedimentation and floatation techniques, did not reveal any endoparasites.

Histopathological examination of the organs from both the dead sand cats resulted in nearly identical findings. The significant findings were atrophic enteritis with marked dilatation and necrosis of crypts

(figure 1), interstitial pneumonia with bacterial colonies observed in some alveoli, and a mild depletion and haemosiderosis of the spleen.
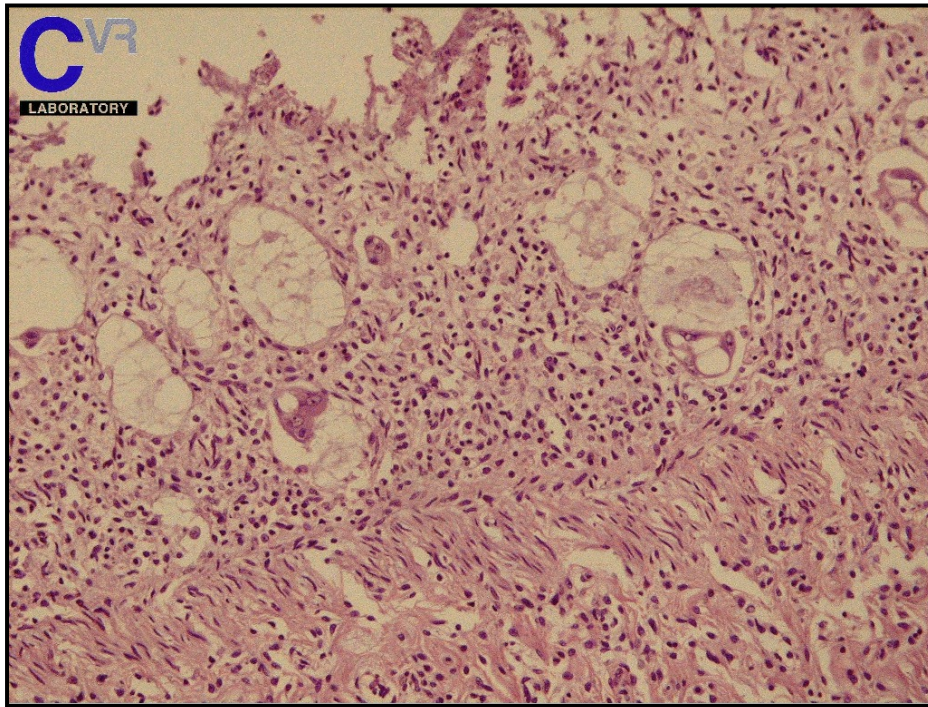

Figure 1: Histological picture of the intestines of an Arabian sand cat with lesions typical of FPLV infection.

No histomorphological abnormalities were observed in the brain of either animal. The histopathological finding in both cases was strongly suggestive of a feline panleukopaenia infection accompanied by a possible secondary bacterial pneumonia.

In addition, feline parvovirus DNA was confirmed in intestinal content swabs of both kittens using the PCR test.

Further investigations

The results of the complete blood count in all instances of blood sampling showed white blood cell counts well within reference ranges described for cats and with ISIS reference values for this species with no evidence of leukopaenia or lymphopaenia (table 1).

For the ISIS reference ranges, maximum and minimum values reported for each parameter were used as the limits for the range.

The results of the PCR tests for detection of FPLV DNA in rectal swab of the littermate and mother of the dead kittens indicated the presence of viral DNA shedding at 1 week and 6 weeks but both had stopped shedding before 15 weeks post vaccination with the modified live vaccine (MLV) for FPLV (table 2).

In addition, the HI titre of the cats post vaccination fell below pre-vaccination titres in 2 out of 3 cats, from   which paired serum samples were obtained (table 3).

Table 1: Haematological findings in 5 Arabian sand cats at AWWP.

AWWP stock list	Date of sampling	PCV %	RBC x 10⁶/ µl	WBC x 10³/ µl	Differential WBC Count							
					L %	L ab	N %	N ab	M %	M ab	E ab	B ab
6153	04.05.2006	20	4.3	14.6	29	4.2	69	10	2	0.3	0	0
5066	04.05.2006	40	7.6	11.2	35	3.9	63	7	2	0.2	0	0
5065	10.05.2006	40	7.6	12.4	39	4.8	55	6.8	6	0.7	0	0
5064	04.05.2006	46	8.6	16.6	34	5.6	64	10.6	2	0.3	0	0
4512	04.05.2006	50	9.3	10.8	40	4.3	57	6.2	3	0.3	0	0
ISIS Reference Range (*Felis margarita*)		26 - 46	5.6 - 13.5	3.6 - 20.2	8 - 27	0.3 - 5.5	15.7 - 48	0.6 - 9.7	1 - 5	0.03 - 0.9	0.03 - 1.2	0.05 - 0.1
Domestic Cat (Aiello, 1998b)		30 - 45	5 - 10	5.5 - 19.5	20 - 55	-	35 - 75	-	1 - 4	-	2 - 12	rare

KEY: PCV, RBC and WBC denote Packed Cell Volume, Red Blood Cell count and White Blood Cell count, respectively. In the differential count L stands for lymphocyte, N- neutrophil, M- monocyte, E- eosinophil and B- basophil counts, with the units % , indicating the percentage of each cell type in the total WBC count, and ab, denoting absolute counts (x 10³/µl).

Table 2: Results of antigen (PCR) testing for Feline Panleukopaenia virus in 2 Arabian sand cats.

AWWP stock list #	Sex	Relationship to dead kittens	Age	Date of sampling	FPV PCR	Vaccination status in 2006	Duration from last vaccination
6153	f	littermate	9 wks	12.05.2006	++	vaccinated	1 week
			14 wks	15.06.2006	++	vaccinated	6 weeks
			23 wks	27.08.2006	-ve	vaccinated	15 weeks
5065	f	mother	> 1 yr	11.05.2006	++	vaccinated	1 week
			>1 yr	15.06.2006	++	vaccinated	6 weeks
			>1 yr	27.08.2006	-ve	vaccinated	15 weeks

Table 3: Results of serological testing for FPLV in Arabian sand cats at AWWP in 2006.

AWWP stock list #	Sex	Age	HI titre for FPLV	Post-vaccination interval (days)	Vaccination status for 2006
5066	f	> 1 year	1:160	0	unvaccinated
			1:80	42	vaccinated
5065	f	> 1 year	1: 160	0	unvaccinated
			1:80	36	vaccinated
5064	m	> 1 year	1:40	0	unvaccinated
			1:80	42	vaccinated
6153	f	14 weeks	1:160	42	vaccinated
4512	m	> 2 year	1:320	109	vaccinated

Nevertheless, titres recorded in all sand cats after re-vaccination were above the limit of 1:80 to 1:160 considered protective in domestic cats and dogs (TRUYEN, 2000; MOUZIN et al., 2004; MARTELLA et al., 2005). In addition, individual variation in the strength of the HI titres induced in the cats ranged from 1:80 to 1:320. In fact, in all the sand cats where pre-vaccination titres were measured, with the exception of 1 animal (5064), antibodies against FPLV from the previous year's vaccination were still at levels considered adequate to confer protection against infection in domestic cats.

Discussion

Most of the viruses infecting domestic cats are known to co-exist in non-domestic felid populations (ARTOIS and REMOND, 1994). A number of sero-surveys of free-ranging felids revealed a high sero-prevalence of FPLV (PAUL-MURPHY et al, 1994; HOFMANN-LEHMAN et al., 1996). In case of captive felids, outbreaks accompanied by clinical disease due to FPLV have been described in tigers, leopards, cheetahs, wild cats, servals, tiger cats and African wild cats (STEINEL et al., 2000). Moreover, investigations into viral infections affecting European wild cats (*Felis sylvestris sylvestris*) have established without doubt the susceptibility of wild cats to FPLV (MCORIST et al., 1991; ARTOIS and REMOND, 1994; DANIELS et al., 1999; LEUTENEGGER et al., 1999). Conversely, little is known about its occurrence and prevalence in the Middle East (OSTROWSKI et al., 2003) but, in general, evidence points towards a greater susceptibility of small cats to FPLV virus as opposed to the CPV-2a and 2b generally encountered in larger non-domestic cats (STEINEL et al., 2000; IKEDA et al., 2002).

The acuteness of the disease, the gross lesions and the histological findings are consistent with reports in domestic cats suffering from feline panleukopaenia infection (GREENE and SCOTT, 1990; AIELLO, 1998a; ADDIE et al., 1998; STEINEL et al., 2000; TRUYEN, 2000). BARKER and PARRISH (2001) reported that the clinical expression of parvovirus infection in wild animals often involves profound depression and lassitude which are likely to cause the affected animal to hide rather than become more conspicuous, and this provides a likely explanation for no clinical symptoms having been observed prior to the death of the sand cat kittens at AWWP. In fact, gross post mortem lesions, especially in cats and raccoons with enteric parvovirus infections, are often subtle, with scant fluid filled faeces in the colon being the only observable signs attributable to a parvovirus infection (BARKER and PARRISH, 2001). Moreover, β-haemolytic and non-haemolytic *E.coli* has been reported previously as a secondary finding, along with *Clostridium spp.*, in case of feline panleukopaenia infections in cats (ADDIE et al., 1998).

Histologically, crypt-necrotising enteritis is considered to be a lesion typical for FPLV infections in felids (GREENE and SCOTT, 1990; AIELLO, 1998a; BARKER and PARRISH, 2001), as the virus spreads from the lymphoid tissue of the Peyer's patches to the rapidly dividing crypts of Lieberkühn, after the initial viraemic phase. In fact, the authors described fatal FPLV infections in 3 captive cheetahs wherein the main histological finding was sub-acute to chronic crypt-necrotising enteritis, with ante-mortem symptoms ranging from a complete absence of any signs to chronic diarrhoea (STEINEL et al., 2001). The pneumonic lesions in the lungs of the sand cats, however, are not consistent with feline parvovirus infections reported in literature and could possibly be attributed to a secondary bacterial infection. However, the organism could not be isolated on culture. The positive PCR results provided evidence of FPLV virus DNA in the intestinal swabs of the kittens. In addition, the PCR confirmed that it was indeed FPLV by exclusion, as the test is able to differentiate FPLV from the other viruses of the FPV subgroup that are currently known to infect felids, i.e. canine parvovirus (CPV) strains 2a and 2b (PERREIRA et al., 2000).

The possibility of the death of the 2 Arabian sand cats being the result of a parvovirus infection is complicated by the vaccination of both individuals with a MLV for FPLV 2.5 weeks before death. Vaccination failure - or failure of the vaccine to induce active immunity in the host - followed by exposure to the pathogen, resulting in natural infection, is one possible reason for the death of the kittens. Residual maternal derived antibodies (MDA) at a level, that interferes with primary immunisation but is insufficient to protect against natural infection with a virulent virus, is considered to be the most common cause of "vaccination failure" in domestic and captive wild carnivores (JANSSEN et al., 1982; AIELLO, 1998a; BARKER and PARRISH, 2001; WACK, 2003). This period of several days or weeks, when maternal antibodies are at a level when the animal is susceptible to infectious virus but interferes with active immunisation, is commonly termed the "window of vulnerability" (TRUYEN, 2000).

The first vaccination in Arabian sand cats at AWWP at 4 weeks of age can be considered premature in comparison to recommended schedules in literature, where the minimum recommended age is 8 weeks - in order to compensate for the effect of maternal antibodies (WACK, 2003; JOOST, 2006). In fact, fatal FPLV infections in pedigree kittens following vaccination at 6 weeks of age with a MLV vaccine against the virus has been reported (ADDIE et al., 1998). The feline parvovirus is known to be extremely resistant - surviving in the environment for up to 1 year (PAUL-MURPHY et al., 1994; AIELLO, 1998a; WACK, 2003). Moreover, the abundance of stray cats inhabiting the AWWP premises act as potential source of infection contaminating the environment with the virus which can challenge the inadequately protected individuals (STEINEL et al., 2000). In this regard, serum from the dead kittens would have been invaluable for determining presence and titres of antibodies to the virus.

The history of vaccination using the MLV 2.5 weeks prior to the consecutive deaths of the 2 kittens inevitably conjures up the possibility of a vaccine-induced infection. As the debate on the potency versus safety of MLV over killed vaccines (KV) continues (THEOBALD, 1978; STEINEL et al., 2000; WACK, 2003), the possibility remains that in the absence of controlled studies, the MLV vaccines reportedly safe for use in domestic species may be insufficiently attenuated for use in non-domestic species and may, in fact, cause "vaccine-induced" disease (CARPENTER et al., 1976; AIELLO, 1998a; STEINEL et al., 2001). VISEE (pers.comm.1974 and 1975) reported clinical panleukopaenia in 3 Siberian tigers and 1 leopard that had been vaccinated, of which 2 were fatal (JOOST, 2006). Although it is possible to differentiate FPLV vaccine strains from "wild" strains using Restriction Enzyme Analysis (ADDIE et al., 1998), this resource was not available at the laboratories used, precluding a definite conclusion in this regard. It is noteworthy that the same vaccination protocol was implemented in 2005 in sand cats, including kittens at 4 weeks of age, with no adverse effect.

Finally, the possibility of the FPLV DNA detected being remnant DNA material from the vaccination itself remains. However, both published literature and the pathological findings do not support this hypothesis. The leaflet accompanying the vaccine categorically stated that there is no viral shedding in domestic cats after vaccination. The outer limit previously demonstrated for detection of vaccine virus in faeces has been 2 weeks (CHALMERS et al., 1999; NEUERER et al., 2008). On the other hand, most recent reports of natural infections also concur that the virus is shed in the faeces for a period of 2 - 10 days post infection and stops completely once virus neutralising antibodies are induced (BARKER and PARRISH, 2001; NAKAMURA et al., 2001; STEINEL et al., 2001), although a duration of faecal shedding following natural infection of up to 6 weeks post infection has been reported (AIELLO, 1998a).

The detection of the FPLV DNA by PCR amplification in the intestinal contents of the dead kittens prompted a similar investigation in the mother and surviving female of the same litter, since they had shared the same enclosure at AWWP. However, since both cats had received a booster vaccination of

the MLV for FPLV only 8 days prior to the first sampling, there was a high likelihood that the viral DNA detected was, in fact, that of the vaccine virus. Thereafter the viral DNA was detected again at six weeks post vaccination but not at 15 weeks. If indeed it was the vaccine virus that the PCR detected in the first 2 sampling instances in the sand cats, a hypothesis corroborated by the absence of a significant (4 fold) rise in serum titres reported in domestic cats with natural infections, as well as a complete absence of clinical signs; our findings happen to be contradictory to recent published literature (CHALMERS et al., 1999; NEUERER et al., 2008). However, the PCR is 10 - 100 fold more sensitive than the conservative electron microscopy or haemoagglutination used in the past for detecting virus levels in the faeces (SCHUNCK et al., 1995; DECARO et al., 2005) and this might be the reason viral DNA was detected for a longer period in this study. Although the possibility of natural infection during the testing interval cannot be ruled out, a controlled study demonstrated no virus shedding using rectal swabs and no significant increases in serum titres post challenge in cats vaccinated with an MLV and then challenged with virulent FPLV virus (CHALMERS et al., 1999).

The titres recorded in all our Arabian sand cats are considered protective in vaccinated domestic dogs and cats (TRUYEN, 2000; MOUZIN et al., 2004; MARTELLA et al., 2005). Titres in case of acute natural infections with FPLV tend to be much higher, as in the case of titres more than 1:10240 in free-ranging lions (HOFMANN-LEHMANN et al., 1996). The titres following re-vaccination in this study are quite similar to those reported after vaccination in dogs using the same commercial product (APPEL et al., 1979) indicating that the vaccination was effective. The drop in serum titres following boosters by 1 dilution step in 2 of the sand cat (table 2) was considered insignificant enough to be attributed to individual variation and possibly reflected the ineffectiveness of vaccination in the presence of existing protective titres. Studies in domestic felids have established that titres to FPLV using MLV vaccines persist beyond 48 months (MOUZIN et al., 2004), and thereby deeming re-vaccination necessary only once in 3 years.

The findings allow us to conclude that the cause of death of the 2 captive Arabian sand cats at AWWP was probably an acute, fatal infection with feline panleukopaenia virus (FPLV) - evidence point towards a likely case of vaccination failure, although a vaccine-induced infection might be responsible for the pathological changes observed. However, follow-up testing in the rest of the population indicated that an outbreak of the infection in the population was unlikely and that the vaccination had conferred adequate protection. In addition, this study has cast a shadow of uncertainty over the persistence of faecal shedding of the FPLV viral DNA following vaccination. The serological responses to primary vaccination, and re-vaccination, with a commercially available modified live vaccine (MLV) have been reported in Arabian sand cats. As with European wild cats (*Felis sylvestris sylvestris*), spill-over infections like FPLV from domestic felid populations can pose a considerable threat to the survival of threatened populations and hamper Species Survival Programs (SSP) (ARTOIS and REMOND, 1994). In conclusion, the death of the 2 kittens and the ensuing investigations, and findings thereof, has provided the impetus for a review and restructuring of the vaccination protocol followed at AWWP to start vaccinations at 9 weeks of age, with revaccination at 12 weeks followed by an annual booster to minimize the possibility of vaccination failures in kittens.

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