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# ANTIBODY RESPONSE OF CAPTIVE CHEETAHS TO MODIFIED-LIVE FELINE VIRUS VACCINE

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ABSTRACT: The antibody response of cheetahs (*Acinonyx jubatus*) to modified live virus vaccine against feline panleukopenia (FPLV), herpes (FHV) and calici (FCV) viruses was assessed by means of an enzyme-linked immunosorbent assay (ELISA). In the first year of study, 82 cheetahs were bled pre-vaccination. Of these, antibody levels to FPLV were found in 100% of the animals. Only 54% were found to have antibodies to FHV and 99% had antibodies to FCV. One month after booster vaccination with the same vaccine, increased antibodies to FPLV, FHV and FCV were seen in 19 (58%), 18 (55%) and 25 (76%) of these animals, respectively (*n* = 33). In the second year of study, 65 cheetahs were bled pre-vaccination. Fifty three of these animals were negative for antibodies to FPLV while 28 were positive for FHV and 64 were positive for FCV. These animals were then bled 1, 2 and 6 mo post booster vaccination. The antibody levels to the various viruses showed different trends with time.

*Key words:* *Acinonyx jubatus*, captive cheetahs, antibody response, modified live virus vaccine, ELISA, feline panleukopenia, feline herpes virus, feline calici virus, experimental study.

## INTRODUCTION

Today the cheetah (*Acinonyx jubatus*) is nearly extinct in Asia and India with perhaps only a small population of a few hundred left in Iran, U.S.S.R. and Afghanistan. The population in Africa is between 10,000 and 20,000 animals. Because of their swift and elusive character, it is difficult to obtain exact demographics. Free-ranging cheetahs presently inhabit a broad section of central, eastern and southern Africa. Although the area of the cheetahs' range is vast, population density estimates are rather low—less than one animal per 6 km² (Frame and Frame, 1981; Myers, 1975). This has led to the animals being kept in captivity and the establishment of various breeding programmes.

The early difficulties experienced in captive breeding of the cheetah prompted a rather extensive genetic physiological analysis of both captive and free-ranging cheetahs (O'Brien et al., 1985). The cheetah appears to be unique among felids and other mammals in having an extreme paucity of genetic variation as estimated by electrophoretic surveys of allozymes and cell proteins resolved by two-dimensional gels (O'Brien et al., 1983). More unusual was the observation of allogenetic skin graft acceptance among unrelated cheetahs, revealing genetic monomorphism at the major histocompatibility complex (MHC), an abundantly polymorphic locus in nearly all mammals (O'Brien et al., 1985). Based on the genetic and physiological similarities of the southern and eastern African subspecies, (O'Brien et al., 1985, 1987) concluded that the proposed bottleneck was an ancient one, perhaps dating back to the global mammalian extinctions near the end of the Pleistocene over 10,000 years ago.

Evolutionary theory predicts that a species with little genetic variation would be particularly vulnerable in a time of ecological perturbation and this has been demonstrated so for the cheetah.

The extensive documentation of an epizootic outbreak of FIP at Wildlife Safari (Oregon, USA), where 90% of the population showed clinical signs of the virus and 48% of the population died (O'Brien et al., 1985; Pfeifer et al., 1983; Evermann et al., 1986), alerted the wildlife veterinary community to the extremes of morbidity that this disease can cause in cheetahs. The morbidity of FIP in cheetahs can be ex-

plained by monomorphism of genes within the MHC sublocus.

The MHC has been the object of intensive molecular and immunological study in recent years and has been shown to consist of a group of tightly linked loci encoding at least three classes of gene products (Snell, 1981). Class I are serologically defined transplantation antigens expressed on the surface of most types of mammalian cells. Class II are cell surface proteins found on B and some T lymphocytes, which participate in the induction of antibody production. Class III are several components of the complement system.

A population that becomes monomorphic at the MHC would be particularly vulnerable to a viral strain able to circumvent the immunological defenses; therefore, any virus that adapts to one animal's immune-surveillance system would subsequently find every other system it encounters in the population to be identical to the first and easy to subvert.

Based on the above premise, it was decided to investigate the immune system of the cheetah in order to determine whether or not these animals have deficient cell mediated immune functions (in preparation), and whether or not they are able to respond adequately to modified live virus vaccines. As vaccination is the best method of preventing disease, it is important to know whether or not the vaccination regime being followed is successful or not.

Cheetahs of the National Zoological Gardens of South Africa (Pretoria, Republic of South Africa) are vaccinated annually with multivalent modified live virus (MLV) vaccines. To date there have been no outbreaks of disease in spite of the presence of feral cats. There have, however, been clinical cases of panleukopenia and suspected feline herpes induced abortions in the cheetahs. Therefore, it was decided to examine the immune status of these animals with regard to the above viruses, and also to measure the antibody response to the MLV vaccine in current use. For this purpose an enzyme-linked

immunosorbent assay (ELISA) was developed. It was hoped that the ELISA would provide a rapid, sensitive test suitable for screening large numbers of samples.

## MATERIALS AND METHODS

### Cheetahs

In 1988 82 adult cheetahs (aged between 1- and 13-yr-old) from the De Wildt Cheetah Research and Breeding Centre of the National Zoological Gardens (Pretoria, Republic of South Africa) were used. Each cheetah was vaccinated annually with Felocell CVR (SmithKline) modified live virus vaccine containing feline herpes, feline calici and feline panleukopenia viruses.

Blood samples were drawn from the medial saphenous vein at the time of vaccination and 33 animals were re-bled 1 mo later. Blood samples were allowed to clot at room temperature, centrifuged at 2,200 rpm, the serum removed and stored at −20 C until used. The animals were not sedated as they were restrained in crush cages.

In 1989 65 adult cheetahs, aged between 1- and 14-yr-old were included in this study group. Thirty nine animals were re-bled 2 mo post-vaccination. Twenty-one animals were bled 6 mo post-vaccination. Vaccine, blood collection and storage methods were the same as described above for 1988.

### Enzyme-linked Immunosorbent Assay (ELISA)

Field isolates of FPLV, FHV and FCV were grown on Feline Kidney (NLFK; Norden Laboratories, Lincoln, Nebraska 68501, USA) cells in roller bottles using equal amounts of Leibovitz and McCoy's medium (Highveld Biologicals, Kelvin, 2054, Republic of South Africa) plus 6% fetal calf serum. The viruses were purified by three cycles of freezing and thawing the cell cultures. Cell debris was deposited by centrifugation at 2,000 rpm for 15 min. The supernatants were centrifuged for 3 hr at 25,000 rpm and the virus pellet was re-suspended in 6 ml Tris, sodium chloride and EDTA (TNE, pH 7.2) buffer.

Optimal reagent dilutions were determined by means of chequerboard titrations (Bidwell et al., 1977). The optimal concentrations for each virus were 1:1,600 for FPLV, 1:1,400 for FHC and 1:1,200 for FVC.

### ELISA antibody determinations

An optimal dilution of each virus in carbonate-bicarbonate buffer (pH 9.6) was added to each well of ELISA microtitre plates (A/S Nunc, Kamstrupnef 90, Kamstrup DK-4000, Roskilde, Denmark) in 0.15 ml amounts and allowed to

TABLE 1. Summary of serologic response of captive cheetahs to vaccination with a modified-live feline panleukopenia (FPLV), herpes (FHV) and calici (FCV) virus vaccine.

| Parameter | Virus | | |
| --- | --- | --- | --- |
| | FPLV | FHV | FCV |
| Number tested | 33 | 33 | 33 |
| Increase in antibody titre | 19 | 18 | 25 |
| | (58%) | (55%) | (76%) |
| Stable antibody titre | 8 | 14 | 8 |
| | (24%) | (42%) | (21%) |
| Decrease in antibody titre | 6 | 1 | 1 |
| | (18%) | (3%) | (3%) |
| McNemar test | $P = 0.01$ | $P < 0.001$ | $P < 0.001$ |

adsorb to the wells overnight at 4 C. The excess antigen solution was removed from the wells and the plates washed three times with PBS-Tween buffer and dried. Test sera were optimally diluted 1:5 (FPLV), 1:20 (FHV) and 1:10 (FCV) in PBS-Tween and 0.1 ml was added to the wells. The plates were sealed and incubated at 37 C for 1 hr and subsequently washed three times with PBS-Tween. 0.1 ml of a 1:1,000 dilution of alkaline phosphatase conjugated anti-cat IgG (Zymed Laboratories, South San Francisco, California 49080, USA) in PBS-Tween was added to each well and incubated for an additional hour at 37 C. After three washes in PBS-Tween, 0.1 ml p-nitrophenyl phosphate (Zymed Laboratories) in 9.7% diethanolamine (pH 9.8) was added to each well and incubated at room temperature for approximately 20 min. The reaction was stopped by the addition of 50 µl 3 M NaOH. The absorbance at (405 nm) was read on a Titertek Multiskan spectrophotometer (Flow Laboratories, McLean, Virginia 22102, USA).

Test serum samples were set up in triplicate and the mean value for each sample was calculated from the three absorbance values and used as the final result of each sample.

Negative values were obtained from sera of non-immune cats and were 0.27 for FPLV, 0.33 for FHV and 0.27 for FCV.

## RESULTS

In 1988 all 82 cheetahs had ELISA absorbance values greater than 0.27 (the established negative) for FPLV antibodies at the beginning of the experiment. Of the 33 animals which were rebled 1 mo post-vaccination, 19 (58%) showed increased antibody levels, eight (24%) showed no change and six (18%) showed a decrease in antibody levels (Table 1). A McNemar test for significance of change was performed and resulted in a $P$ value of 0.01 in a one-tailed test (Siegel, 1956). This value is highly significant indicating that there is an increase in antibody levels post-vaccination.

In 1988 only 44 (54%) of the cheetahs had positive antibody levels against FHV at the first bleeding. Of the 33 rebled 18 (55%) showed an increase in antibody levels, 14 (42%) had stable antibody levels and one (3%) showed a decrease in antibody levels. Five animals had antibody levels that remained below 0.33, the established negative (Table 1).

In 1988 only one (1.2%) of the animals had negative pre-vaccinal levels for FCV (absorbance values less than 0.27). Of the 33 cheetahs rebled, 25 (76%) showed increased antibody levels, eight (21%) showed no change and one (3%) gave a decreased result (Table 1).

In 1989 53 (81%) of the 65 animals bled at the time of vaccination had absorbance levels less than 0.27 (they were negative for FPLV antibodies). Of the 39 animals bled 1 mo post vaccination, 14 (36%) showed an initial increase while five (13%) showed a decrease. The remaining 20 (51%) animals were stable. The trend thereafter was that those that increased initially, decreased slightly and remained stable to the 6 mo post-vaccination bleed. Those samples that showed an initial decrease at the first bleed post-vaccination, increased on

TABLE 2.   Result of enzyme-linked immunosorbent assay for antibodies against feline panleukopenia virus in captive cheetah following booster with modified live virus vaccine.

| Parameter | Time period | | |
|---|---|---|---|
| | 1 mo post booster | 2 mo post booster | 6 mo post booster |
| Number tested | 39 | 32 | 21 |
| Increase in antibody titre | 14 | 7 | 0 |
| | (36%) | (22%) | |
| Stable antibody titre | 20 | 19 | 18 |
| | (51%) | (59%) | (86%) |
| Decrease in antibody titre | 5 | 6 | 3 |
| | (13%) | (19%) | (14%) |

the second bleed and remained stable at the third bleeding. Those samples that showed a stable value from the pre-bleed to the first bleed post-vaccination remained stable to the 6 mo bleeding (Table 2).

In 1989 only 28 (43%) of the animals bled at the beginning of this study had pre-vaccinal antibody levels greater than the negative (0.32). Of the 39 animals rebled one month post-vaccination, 27 (69%) showed an initial increase. Three (8%) showed an initial decrease and nine (23%) remained stable. The trend thereafter was that those animals showing an initial decrease, increased and had decreased slightly by 6 mo post vaccination. The animals that showed an initial increase, decreased slightly over the next two bleeds and by 6 mo were lower than at the first bleed. Those that remained unchanged initially remained stable until 6 mo post-vaccination

when their antibody levels showed a decrease (Table 3).

In 1989 of the 65 animals bled pre-vaccination, 64 (98%) had values greater than 0.27 for FCV. Nineteen (49%) of the animals bled one month post vaccination showed an increase in antibody level. A decrease was seen in four (10%) and 16 (41%) remained stable.

Those samples showing an initial increase decreased steadily over the 2 and 6 mo rebleed. There was also a steady decrease in antibody level in the group that showed no initial change. The group that had an initial decrease declined even further (Table 4).

## DISCUSSION

The animals bled during 1988 had pre-existing antibodies to FPLV which must have been due to the 1987 vaccination programme. However, of those animals tested

TABLE 3.   Results of enzyme-linked immunosorbent assay for antibodies against feline herpes virus in captive cheetah following booster with modified live virus vaccine.

| Parameter | Time period | | |
|---|---|---|---|
| | 1 mo post booster | 2 mo post booster | 6 mo post booster |
| Number tested | 39 | 32 | 21 |
| Increase in antibody titre | 27 | 12 | 3 |
| | (69%) | (37%) | (14%) |
| Stable antibody titre | 9 | 13 | 0 |
| | (23%) | (41%) | (33%) |
| Decrease in antibody titre | 3 | 7 | 18 |
| | (8%) | (22%) | (86%) |

TABLE 4.    Result of enzyme-linked immunosorbent assay for antibodies against feline calici virus in captive cheetah following booster with modified live virus vaccine.

| Parameter | Time period | | |
|---|---|---|---|
| | 1 mo post booster | 2 mo post booster | 6 mo post booster |
| Number tested | 39 | 32 | 21 |
| Increase in antibody titre | 19 | 5 | 2 |
| | (49%) | (16%) | (10%) |
| Stable antibody titre | 16 | 10 | 7 |
| | (41%) | (31%) | (33%) |
| Decrease in antibody titre | 4 | 17 | 12 |
| | (10%) | (53%) | (57%) |

during 1989, a large proportion had no pre-existing antibody levels to FPLV. The answer to this could be due to the fact that the cheetahs bled during 1988 were older than those bled during 1989 and would therefore have had more booster vaccines. The mean age of the former group was 5 yr, whereas the mean age of the latter was 3 yr.

The antibody responses to FPLV seen in 1989 were not as dramatic as those of the previous year. Initially it was thought that the test system was at fault and in order to exclude this problem, samples from 1988 were re-tested and the same results were achieved. This indicated that the test system was not a fault. Other explanations could be that there was natural challenge during 1988 leading to increased antibody levels or else the strain of virus used in the 1989 vaccine might have been less virulent. Unfortunately, it is impossible to prove either of these theories at this stage and so they must remain purely speculative.

However, the conclusion to be drawn is that there is a good response of FPLV to vaccination with a modified-live virus vaccine. This confirms reports by Bittle et al. (1970), Gray (1971), Sedgwick (1971), and Povey and Davis (1977).

Once again more of the animals bled during 1988 had pre-existing antibodies to FHV than those bled during 1989. This could also be due to the fact that the animals from the former bleeding had had more boosters than those from the latter bleeding. However, results do tend to indicate that the response to vaccination with modified-live herpes virus is favourable. Other workers (Bush et al., 1981; Kane and Boever, 1976) also have reported success in many other species when using modified-live vaccines.

The antibody response to FCV during the 1988 bleeding was very good with only one animal having no antibodies at the start of the trial. However, all of the animals had positive antibody levels 1 mo post vaccination even though there were fluctuations in their titres. The greater number of animals being negative at the beginning of the 1989 bleeding could again be due to their mean age.

In conclusion, it can be said that the vaccination programme currently in use in the present study group is effective as the animals show anamnestic responses. This tends to further support the idea that the MHC II locus is not as severely restricted as the MHC I locus, the MHC II locus being responsible for antibody production.

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