Canine distemper antibodies in lions of the Masai Mara


Veterinary Record (1998) 142, 662-665

Canine distemper virus (CDV) has been implicated in some recent deaths of lions, which showed clinical signs of distemper, in the the Serengeti plain. Similar clinical findings have since been reported in lions of the Masai Mara. Fifty-five per cent of serum samples obtained from wild lions of the Masai Mara have been found to contain neutralising antibody to CDV, indicating that they had been exposed to the virus. Adult orphan lions kept in captivity, were vaccinated with the live attenuated Onderstepoort strain of CDV. The results indicated that the vaccine is both safe and immunogenic, and may be potentially useful for the prophylactic vaccination of lions at high risk.

CANINE distemper virus (CDV) is a member of the Morbillivirusae and a potentially fatal disease of Canidae, although it has a wider host range under natural and experimental conditions (Appel and Gillespie 1972). In 1994, CDV infections were responsible for the deaths of a significant proportion of the wild lion (Panthero leo) population in the Serengeti plain in northern Tanzania (Spencer 1995). Seventy-one of 83 (85 per cent) of the blood samples taken from the lions were found to be CDV antibody-positive, and viral antigen was obtained from two lions (Roelke-Parker and others 1996). Barrett and others (1993) have found that the phosphoprotein (P) gene sequence varies between morbilliviruses and they have used this property to distinguish differences or show similarities between isolates. The differences in the (P) gene sequence are also useful for determining phylogenetic relationships. The (P) gene fragment of the morbillivirus isolated from the diseased lions was amplified by polymerase chain reaction (PCR) and analysed and it was concluded that the isolate was more similar to CDV than to the other morbilliviruses (Harder and others 1995). One hypothesis to account for CDV infection in lions is that the virus crossed the species barrier from the domestic dog population, of which approximately 30,000 are reported to live in close proximity to the Serengeti, and in which CDV has previously been recorded (Roelke-Parker and others 1996). The role of hyaenas, jackals, and other susceptible species in the spread of the disease is however still uncertain, although CDV was isolated from a bat-eared fox (Otocolobus megalotis) and a spotted hyaena (Crocuta crocuta) during the 1994 Serengeti outbreak (Roelke-Parker and others 1996).

There are conflicting theories about how to prevent or reduce disease conditions in wildlife. Harder and others (1995) have suggested that the best strategy would be to vaccinate the surrounding domestic dog population, the suspected reservoir of the infection, with CDV vaccines which have been shown to be safe. Their main concern was that some attenuated vaccines might induce CDV disease in susceptible wild carnivores, a concern also voiced by Morrell (1994). Conversely, Appel and others (1994), reporting on deaths due to CDV in exotic Felidae in North American zoos and wildlife parks, proposed that attenuated chick embryo-adapted vaccines could be used once their safety had been demonstrated in wildlife.

The Masai Mara in Kenya and the Serengeti plain in Tanzania form one continuous ecosystem divided only by political boundaries which are recognised by neither animals nor pathogens. There is a constant exchange of wildlife between the two areas. This paper presents the results of a small serological survey of CDV antibodies in serum samples from lions of the Masai Mara and describes the responses of the lions inoculated with a live attenuated CDV vaccine.

Materials and methods

Serum samples

Between October 1994 and February 1995, 55 wild lions (cubs less than one year old to adults over 10 years old; 27 males and 28 females) from several prides in the Masai Mara National Reserve and adjacent areas which form part of the Serengeti-Masai Mara ecosystem were bled by the Kenya Wildlife Services. Whenever possible, estimates of age based on the size of the lion and signs of tooth wear were made by the attending veterinarian. The samples were examined for antibodies to CDV because of earlier confirmed cases in Serengeti lions (Roelke-Parker and others 1996) and reports from the Masai Mara of sick lions showing the characteristic signs of distemper. For comparison, seven serum samples from six lions and one leopard (Panthera pardus) were obtained from other regions of Kenya. All the samples were heat inactivated at 56°C for 30 minutes before they were imported into the UK.

Serum neutralisation test

All the sera were diluted successively four-fold, from an initial dilution of 1/4 to 1/256 in tissue culture medium. A double strength suspension of neutralising antigen (CDV-Bussel strain), containing 200 to 600 plaque forming units/ml, was also prepared in tissue culture medium. Equal volumes of the various dilutions of sera and neutralising antigen were mixed together to give final
dilutions of 1/8 to 1/512, and the mixture was incubated at 37°C for one hour for neutralisation to occur. Vero cell monolayers in 5 cm petri dishes (three plates per dilution) were each inoculated with 200 µl aliquots of serum/antigen suspension. The plates were then incubated at 37°C for one hour to allow absorption to occur, then overlaid with a 1 per cent agar tissue culture medium and re-incubated for five days for viral plaque development. After five days, the agar was removed, the cell monolayer stained with Naphthalene Black and the plaques were counted. Serum samples with antibody titres (SN50) ≥ 1/8 were scored as CDV antibody-positive.

Statistical analysis

The proportion of seropositive lions was compared with the seroprevalence reported from the Serengeti (Roelke-Parker and others 1996) by using a χ² test. The same method was used to compare the difference in seroprevalence between the male and female lions.

To investigate the variation with age in the proportion of seropositive lions it was necessary to control for any sex difference. A multivariate analysis of variance was therefore carried out by using the statistical software package GLIM (Crawley 1993). The appropriate link function (logistic) and error structure (binomial) for the analysis of proportional data were specified. Any non-significant variables (P>0.05) were removed from the statistical model. Consecutive yearly age classes were then grouped together, provided there was no significant impact on the fit of the model, to produce a minimal adequate model. The absence of bias in the seroprevalence of the lions whose ages had been estimated compared with those for which no estimates of age were available was confirmed by using a χ² test.

Vaccine

The CDV (Onderstepoort strain) component of Nobivac vaccines (Intervet) was prepared as a monovalent vaccine (Nobivac D) containing at least 10⁵⁰ TCID₅₀ per dose and sent to Kenya Wildlife Services for inoculation into four orphan adult lions. The same dose of the vaccine was initially tested in domestic cats to determine its safety before the orphan lions were inoculated.

Vaccination

Cats. – One dose of Nobivac D vaccine was inoculated into each of eight domestic cats, approximately nine months old, which were then kept for three weeks before testing for antibody response (SN50) against CDV. The cats were bled before and three weeks after they were vaccinated.

Lions. – One dose of Nobivac D vaccine was inoculated into each of four orphan adult lions which were kept in separate compounds together with in-contact lions which were monitored to detect any potential spread of the vaccinal virus. The four vaccinated lions and in-contacts (IC) were as follows: Sheru (IC with Irene); Malaika (IC with George); Joy (IC with Singh); Pascal did not have an in-contact lion in the same compound but he was separated from Joy and Singh only by a wire fence through which contact could be made; Singh therefore doubled as the in-contact for both lions. Blood samples were taken before and three weeks after the vaccination to measure the antibody response (SN50) to CDV.

Results

Serological survey of wild lions, October 1994 to February 1995

The spatial distribution of the serological results is shown in Fig 1. A total of 30 (55 per cent) of the 55 samples contained neutralising antibodies against CDV, with the titres ranging from >1/8 to >1/1024 (Fig 2). This seroprevalence was significantly less than the 85 per cent recorded in Serengeti lions (P<0.001).

Although the sample population was relatively small, the results indicated a significant difference in seroprevalence between the sexes (P<0.001) with considerably more positive males (21 of 27) than females (nine of 28).

The age to the nearest year was estimated for 38 (69 per cent) of the 55 lions sampled. The seroprevalence in these lions was 53 per cent (20 of 38) and was not significantly different from the 59 per cent (10 of 17) recorded in the lions whose ages were not known. Seropositive lions were detected in all age groups ranging from a cub (aged between three and 12 months) to 10-year-old adult lions. Both sex (P<0.001) and age (P<0.05) were significant explanatory variables for the seroprevalence. The most parsimonious grouping of age was into two classes, less than five years of age and five years old or over, corresponding to animals born after...
This too recently of the Serological ranging below negative non-CDV models. The The between the Mara titres of were and achieved without any adverse results. The in-contact lions remained seronegative (orphaned before) and cats; the other two cats had titres below 1/2. All the cats remained clinically normal throughout the study.

Serological response of wild lions (orphaned and in captivity) to CDV vaccination

All the vaccinated lions responded within three weeks with high titres of antibody (≥1/1024) without any adverse clinical effects (Table 1). The in-contact lions remained seronegative throughout the study and showed no clinical effects, indicating that no distemper virus had been transmitted from lion to lion. The in-contact lion kept in the same compound as Malaika, died from non-CDV related causes. Singh, an in-contact lion, remained seronegative when bled two years later.

Discussion

Serological survey

The positive CDV antibody titre in a lion cub aged between three and 12 months, indicated that the disease had been transmitted recently in the Masai Mara lion population, because the cub was too old to have significant levels of maternally derived antibodies. This finding, together with other more anecdotal evidence, is consistent with the theory that the CDV epidemic reported in the lions of the Serengeti moved northwards and infected the lion population in the Masai Mara (Roelke-Parker and others 1996). The absence of any reports of CDV outbreaks in the domestic dog population close to the Masai Mara since 1990 (Alexander and Appel 1994) also provides support for this theory. The first lions in the Masai Mara to show distemper-like signs were recorded close to the border with Tanzania, and when there was active disease in the Serengeti lions. The Serengeti and Masai Mara are part of the same ecosystem with no natural barriers which could effectively block the progression of infectious disease. Lions cross between the Masai Mara and Serengeti as part of their natural home range. The timing of the first anecdotal reports of CDV infection in the Masai Mara, around August 1994, also suggests that the movement of domestic male lions, following the wildebeest migration from Tanzania to Kenya, may have been important in the spread of the epidemic.

Sero-positive lions were recorded throughout the Masai Mara and adjacent area, reaching as far as Aitong, the northern limit of the Serengeti-Masai Mara ecosystem. However, as the Aitong lions were the only prides sampled immediately outside the Masai Mara, the full range of the exposure of lions to CDV is not known. The six other lions sampled from other regions of Kenya were all CDV-negative. The range of other wildlife species in the Masai Mara which may have been infected during the CDV epidemic has not been investigated. The one seropositive lion was unlikely to have been exposed to CDV as part of the Serengeti-Masai Mara epidemic because it came from Naivasha, approximately 150 km north east of Aitong. Leopards are known to prey on domestic dogs, and around Naivasha there are domestic dog populations in which CDV may be present.

Unfortunately, unlike the Serengeti, no previous serological data are available from the Masai Mara from which the CDV status of its lion population before August 1994 can be assessed. The results suggest a 'step-like' age seroprevalence, with lions alive in 1990 having a significantly higher seroprevalence than lions born after 1990. As the antibody response to CDV lasts many years, the pattern of seroprevalence with age may be due either to previous exposure to infection, or to the variation in infection with age during a recent outbreak. More specifically, the age seroprevalence may be explained by the differential transmission of CDV with age during the 1994 epidemic, or by the differential age mortality rates due to CDV infection in 1994, or by the age-constant CDV infection during two epidemics, one starting in 1990 and the other starting in 1994.

The results from the Serengeti, however, are not consistent with the first two explanations. In the Serengeti lion population, CDV infection and increased mortality were reported among all age-classes equally (Roelke-Parker and others 1996). In the Serengeti, there have been long-term research studies of the lion population, but there have been no comparable studies with the Masai Mara. As a result, it was not possible to estimate increased disease-related mortality rates. However, there is no reason to believe that the progression of disease in the Masai Mara lions would have been different from that observed in the Serengeti.

The possibility of previous exposure to distemper infection is, however, consistent with the last reported CDV epidemic in the area which started in late 1990 in the domestic dog population surrounding the Masai Mara (Alexander and Appel 1994). An ear-
lier serological survey by Alexander and Appel (1994) suggested that during this 1990 epidemic, CDV was transmitted from the domestic dog population to other wild carnivores in the region, including wild dogs (*Lycaon pictus*) and hyenas. In Tanzania, a retrospective analysis of serum samples collected in the 1980s, indicates that lions in the Serengeti ecosystem had previously been exposed to CDV without an increase in disease-related mortality (Roelke-Parker and others 1996). It therefore seems plausible that during the 1990 CDV epidemic in the Masai Mara region, the lion population may have been exposed to CDV either directly from domestic dogs or from other wild carnivores, such as hyenas. Assuming age-constant infection and disease-related mortality rates, the observed cross-sectional seroprevalence data may therefore be explained by a CDV outbreak in 1990, followed by a more recent epidemic starting in 1994.

The significant male bias in seroprevalence in the Masai Mara is different from the Serengeti, where there were no differences in the proportions of the sexes which were seropositive. There is no clear explanation for this sex difference in seroprevalence.

The results of the serological survey and the findings from Tanzania, support the theory that the recent CDV epidemic spread through the lion populations of the Serengeti-Masai Mara ecosystems. The higher seroprevalence in animals alive at the time of the last known CDV epidemic in the Masai Mara region is consistent with the previous exposure of Masai Mara lions to CDV.

**Vaccination**

The experimental inoculation of domestic cats with canine distemper virus has produced no evidence of typical clinical signs of CDV, the only evidence of infection being found by the histopathological examination of infected tissue (Appel and others 1974). The Onderstepoort strain of CDV (Nobivac D; Intervet) passaged in tissue culture cells, has been shown to produce good antibody responses in fox, mink and ferret without spreading or being disseminated or causing adverse clinical reactions (W. S. K. Chalmers, unpublished observations). The results of this small study have shown that vaccinal virus will replicate and stimulate an antibody response in the majority of vaccinated cats. However, the vaccinal strain was more efficacious in wild lions in that the take rate was 100 per cent, it did not spread to in-contact lions, it produced much higher protective neutralising antibody titres, and no adverse clinical signs were recorded.

It has been proposed that inactivated vaccines should be used to prevent infection because it is believed that attenuated vaccines may produce distemper in susceptible carnivores. However, the neutralising antibodies produced by inactivated vaccines are often poor and short-lived compared with those stimulated by live attenuated vaccines. It is not suggested that the lion population in the Masai Mara-Serengeti should be vaccinated, first because it would be impractical, and secondly because the population is large enough to recover without intervention. Ring vaccination of the domestic dogs which are the main reservoir of infection may be feasible, because these dogs can be collected in the surrounding Masai villages for sampling, vaccination and monitoring over a defined period. The results of this study have shown that lions respond well to vaccination without adverse clinical reactions; vaccination with a safe, live, attenuated CDV vaccine strain may therefore still be an option in small isolated populations of wild or captive lions when there is need to protect individual lions against CDV disease.

**References**


**Suspected bacterial meningoencephalitis in two adult horses**

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*Veterinary Record* (1998) 142, 665-669

Bacterial infections (such as meningitis or meningoencephalitis) of the central nervous system are rare in horses. They are most prevalent in neonates as a result of septicaemia. A few cases have been reported in the adult and most have been fatal. Streptococcal species appear to be the organism most commonly identified in these cases. Thus, this disease may be a secondary complication of upper respiratory tract infections. Clinical signs are extremely variable making diagnosis difficult. In most cases, postmortem has been the definite diagnostic procedure. This paper describes the clinical course of disease, diagnosis and successful treatment of two presumptive cases of meningoencephalitis in adult horses.

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INFECTIOUS agents incriminated in equine neurological disease include viruses, bacteria, protozoa, helminths and fungi (Seiler and others 1981, Steckel and others 1982, Emmons and others 1983, Burgess and Mattison 1987). Bacterial infections of the central nervous system (CNS) of adult horses have rarely been reported and are generally fatal (Mayhew and Mackay 1982, Foreman and Santschi 1989). They can occur in all ages and breeds but are more prevalent in neonates when they are most often secondary to septicaemia (Mackay and Mayhew 1991). Beta-haemolytic streptococci are probably the most commonly isolated bacteria, and meningoencephalitis can be a complication of strep-tococci meningitis. This condition can occur in all ages and breeds but is more prevalent in neonates when they are most often secondary to septicaemia (Mackay and Mayhew 1991). Beta-haemolytic streptococci are probably the most commonly isolated bacteria, and meningoencephalitis can be a complication of streptococci meningitis. This condition can occur in all ages and breeds but is more prevalent in neonates when they are most often secondary to septicaemia (Mackay and Mayhew 1991).
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Veterinary Record 1998 142: 662-665
doi: 10.1136/vr.142.24.662