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Immunogenicity of an Inactivated Oil-Emulsion Canine Distemper Vaccine in African Wild Dogs

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ABSTRACT: The immunogenicity of an inactivated oil-emulsion vaccine against canine distemper virus was evaluated in nine captive African wild dogs (*Lycaon pictus*). Antibody levels were determined by neutralization test in Vero cells. No significant local or systemic adverse reactions were observed in the animals. Virus neutralizing antibody levels >1:20 were detected, especially in animals that were vaccinated twice. The use of oil adjuvants is suggested as a good way to enhance the immune response to inactivated canine distemper vaccine.

Key words: African wild dogs, canine distemper virus, immunization, *Lycaon pictus*.

Canine distemper (CD) is a highly infectious, frequently lethal viral disease of many species in the order Carnivora. Clinical signs may include respiratory, gastrointestinal, and neurologic signs and cutaneous lesions.

Canine distemper virus (CDV) is a member of the *Morbillivirus* genus within the family *Paramyxoviridae* (Lamb et al., 2000). These viruses have enveloped virions that contain a nonsegmented, negative RNA genome that is packaged by the nucleocapsid protein into a helical symmetry. The envelope proteins of CDV include a membrane protein and two glycoproteins: hemagglutinin, the attachment protein, and fusion protein (Appel, 1987).

African wild dogs are extremely susceptible to infection with CDV (Montali et al., 1983; Zhang et al., 1983) and to modified live virus (MLV) vaccines, which may induce severe, lethal disease (McCormick, 1983; Van Heerden et al., 1989; Durchfeld et al., 1990).

The ecology of CDV infection in African wild dogs is not well understood. An outbreak of CDV infection resulting in the death of all the pups and four of six adults

living in a park in Botswana was well documented in the mid-1990s (Alexander et al., 1996). More recently, a CD outbreak was diagnosed in Tanzania; 49 of 52 dogs died within 2 mo (van de Bildt et al., 2002). Moreover, several episodes of mortality of wild dogs in past years have been related to CD on the basis of clinical signs. For instance, a CD-like disease was described in wild dogs in the Serengeti (Tanzania) in 1972 (Schaller, 1972), and the near extinction of wild dogs in the Serengeti/Masai Mara area in 1990–91 was attributed to an epidemic of CD (Alexander and Appel, 1994).

Domestic dogs may act as a reservoir of CDV for African wild dogs; in areas where there is high seroprevalence for CDV in dogs, CD-like disease has been described in wild dogs (Alexander and Appel, 1994; Roelke-Parker et al., 1996; Laurenson et al., 1997). Other wild canids might be acting as a reservoir of infection for African wild dogs (Woodroffe and Ginsberg, 1997).

Several experiments of the immunization of African wild dogs against CDV have been reported with either MLV or inactivated vaccines. Modified live virus vaccines, in particular the vaccinal strains attenuated by serial passages on canine cells, may retain residual virulence for a variety of wild carnivores (Appel and Harris, 1988; Durchfeld et al., 1990). Inactivated CDV vaccines have been used, but they have shown little or no serologic evidence of protection (Visee, 2001; van de Bildt et al., 2002; Van Heerden et al., 2002).

The present study was carried out on nine adult African wild dogs, housed in the

Biopark of Rome (Italy; 41°53'43"N, 12°28'57"E) and identified with letters A–D (males) and E–I (females); animals D and F were born in December 1996, and all the others were born in November 1998. The animals were not inoculated with CDV vaccines before the trial. Fifteen days before vaccination, they were treated with Ivermectin (0.2 mg/kg; Ivo-mec, Merck and Co. Inc., Rahway, New Jersey, USA).

Our vaccine was prepared using the attenuated Onderstepoort CDV strain, propagated in African green monkey kidney cells (Vero cells) that were grown in Dulbecco's minimal essential medium (Sigma-Aldrich, St. Louis, Missouri, USA) that contained 10% bovine fetal serum. Infected monolayers were incubated at 37 C. In the presence of extensive cytopathic effect, the cells were frozen and thawed three times. After centrifugation at $5,000 \times G$ for 10 min to discard cellular debris, the supernatant was inactivated with 0.4% formaldehyde for 24 hr at 27 C. Before inactivation, aliquots of the supernatant were stored for virus titration ($10^{5.3}$ 50% tissue culture infectious doses [TCID₅₀]/ml). The inactivated viral suspension was inoculated onto Vero cells, and the cells were tested by immunofluorescence 72 hr after infection, using monoclonal antibody to CDV (Merial, Lyon, France), which provided the evidence for complete inactivation of the virus. The inactivated viral suspension was emulsified with mineral oil (Montanide ISA 740, Seppic, Paris, France) in a 2:1 ratio (two parts inactivated viral suspension, one part mineral oil).

One milliliter of vaccine was administered intramuscularly in the thigh of the animals, after they had been immobilized with 5 mg/kg ketamine (Ketavet, Gellini S.p.A., Via Nettunese 20 Aprilia -LT-, Italy) and 0.05 mg/kg medetomidine (Domitor, Vetem S.p.A., Porto Empedocle -AG-, Italy). After 30 days, five African wild dogs (B, D, G, H, and I) were vaccinated again using a blowpipe. Blood samples were obtained at the first vac-

ination and 20 days after the second vaccination (day 50). To reduce animal stress, no blood samples were obtained at day 30 after the first vaccination.

Serologic testing was conducted using serum neutralization (SN) tests in 96-well microplates. Serial twofold dilutions starting at 1:5 of each serum sample were mixed with 100 TCID₅₀ of the Onderstepoort strain and maintained at room temperature for 1 hr before the addition of 20,000 Vero cells per well. Plates were incubated for 4 days at 37 C. The SN titer was considered to be the highest serum dilution still neutralizing the virus.

Other than a mild transient limp of 3–4 days duration noted in three animals, there was no overt reaction to vaccination observed. All animal tested seronegative when first vaccinated. Antibodies (titers $\geq 1:40$ – $1:320$) were detected in all wild dogs except animal F (1:20) 50 days after vaccination. The highest antibody titers were detected in two animals vaccinated twice (H and I, 1:160 and 1:320, respectively).

African wild dogs are susceptible to infection with CDV and are best protected against the disease by vaccination (Appel, 1987). Vaccination with MLV has been suspected of causing the deaths of African wild dogs (Van Heerden et al., 1989; Durchfeld et al., 1990), so it is necessary to determine whether inactivated vaccine may represent a viable alternative. Although killed CDV vaccines are safe, many animals immunized with inactivated CDV vaccines develop low or no humoral immunity (Montali et al., 1983; Williams et al., 1996; van Heerden et al., 2002), and only a minority of the animals vaccinated may seroconvert (Visee, 1996). On the other hand, Franke et al. (1989) described safety and efficacy of an inactivated CDV vaccine in >100 captive wild animals of various species, including the African wild dogs.

A recombinant virus-vectored vaccine for CD is now available (Purevax, Merial Inc., Athens, Georgia, USA) and this

might represent a safe alternative for vaccination of wild carnivores. However, to our knowledge, the vaccine has not been tested in African wild dogs. Therefore, in the absence of effective tools for the prevention of CD, the use of killed vaccine with oil adjuvant to induce high and persistent neutralizing antibodies in African wild dogs was the target of our investigation.

The antibody titers indicative of protection against CD in African wild dogs have not been evaluated in detail; because of the high economic value and the threatened status of the animals, it was not possible to do a challenge infection. However, there is evidence that neutralizing antibody levels $<1:20$ are not protective against CDV, whereas titers $\geq 1:20$ may confer protection (van de Bildt et al., 2002).

Our previous observations (unpubl.) demonstrated that killed vaccines without adjuvants, as well as vaccines with aluminum hydroxide adjuvant, did not elicit neutralizing antibodies in African wild dogs. These findings led us to test other adjuvants; and the results of the present study suggest that oil adjuvants may be a good way to enhance the immune response to inactivated CDV vaccines. However, oil adjuvants may lead to unwanted adverse effects, such as tissue reactions at the injection site. Further investigations will evaluate the persistence of antibodies induced by this inactivated vaccine and will focus on the improvement of vaccine responses by use of other adjuvants.

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