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## EFFECTS OF A MODIFIED-LIVE VIRUS CANINE DISTEMPER VACCINE ON CAPTIVE BADGERS (*TAXIDEA TAXUS*)

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**ABSTRACT:** We vaccinated six captive badgers housed with five controls, and monitored blood antibody titers and white cell counts of both groups for 63 days postvaccination between 29 August and 3 December 1992. Five vaccinated badgers responded with antibody titers ranging from 1:64 to 1:1024 by 63 days postvaccination, whereas the sixth badger did not respond. Treatment badgers also had significant ( $P < 0.05$ ) decreases in lymphocytes on days 16, 29, and 63. No badgers developed clinical signs of distemper. Control badgers did not produce antibodies against CD virus; thus, the vaccine virus probably was not transmitted between treatment and control animals. The vaccine appears safe for use in healthy badgers, but additional safety and efficacy study is needed.

**Key words:** *Taxidea taxus*, North American badger, black-footed ferret, *Mustela nigripes*, canine distemper, modified-live virus vaccine, Wyoming.

### INTRODUCTION

Canine distemper (CD) virus has caused epizootics in many carnivores including raccoons (*Procyon lotor*), gray foxes (*Urocyon cinereoargenteus*), and striped skunks (*Mephitis mephitis*) (Appel, 1987). Resulting mortality can be extremely high and can have conservation implications for some species. The black-footed ferret (*Mustela nigripes*; BFF), a critically endangered North American carnivore, is highly susceptible to CD (Williams et al., 1988). An epizootic of CD decimated the last wild BFF population in 1985 (Thorne and Williams, 1988), but successful captive propagation has led to BFF reintroductions to the wild, which began in 1991 (Thorne and Oakleaf, 1991).

North American badgers (*Taxidea taxus*) may be important in the epizootiology of CD in BFF-reintroduction areas. Badgers occur sympatrically with BFF, and both species are nocturnal, fossorial, and prey mostly on burrowing rodents (Messick, 1987; Fagerstone, 1987). Also, badgers attempt to prey on BFF (Forrest et al., 1988). Although CD virus is transmitted primarily via aerosol or direct contact, fomite transmission is possible in burrows because CD virus may survive several days in low

light and cool temperatures (Shen and Gorham, 1980). Therefore, badgers come into close contact with BFF and are likely transmitters of CD; this has important implications for how both species should be managed on BFF release sites.

The clinical course of CD in badgers is known only anecdotally, but affects the likelihood of transmission in the wild. In all reported cases, infection of captive badgers with CD virus resulted in death (Armstrong, 1942; Farrell, 1957; Keppner, 1970); thus, badgers are highly susceptible. Disease progression was not well studied in any of these cases and studies of CD vaccination of badgers have not been reported.

Badgers can survive CD infection. Among serologic surveys of four wild populations on potential and actual BFF release sites in Wyoming and South Dakota (USA), low percentages (2 to 15%) of badgers were seropositive; all positive animals had low antibody titers (Williams et al., 1988, 1992; Goodrich and Buskirk, 1993). Higher percentages of seropositive badgers were expected because 50 to 57% of coyotes (*Canis latrans*) in the same areas had antibodies and coyotes and badgers frequently have physical contact (Minta et al., 1992). Also, CD was active within 1 yr

of the surveys in two coyote populations (E. S. Williams, unpubl.). However, badger population density and age structure in one area was not consistent with the recent high mortality we would have expected if CD had recently been epizootic (Williams et al., 1992). This difference in antibody prevalence between badger and coyote populations is poorly understood, but could be related to differences in susceptibility to CD and behavior (Williams et al., 1992).

Our objective was to determine the immune and clinical responses of badgers vaccinated with modified-live CD virus to better understand CD virus infection in badgers. We also wished to test the potential utility of a vaccine for badgers.

#### MATERIALS AND METHODS

Eleven wild badgers, including four adult (>1-yr-old) males (AM), three adult females (AF), two juvenile (5- to 6-mo-old) females (JF), and two juvenile males (JM), were captured in southeastern Wyoming (41°43'N, 106°18'W) between 29 August and 5 September 1992. Badgers were held in three banks of four individual cages each in a two by two arrangement. Cages within each bank were about 1 m<sup>3</sup> and separated on the sides by a removable steel partition, while cages above and below were separated by a wire floor, catch pan, and steel roof. We provided dry cat food (Cat Stars, Hubbard Milling Company, St. Paul, Minnesota, USA) and water twice a day, and meat scraps when available.

On 30 September 1992, after ≥25 days quarantine, with no clinical disease, we injected each of six badgers (AF75, AM31, AM72, JF74, AF76, and JM73) subcutaneously with 1 ml of a modified-live CD vaccine (Onderstepoort strain of CD virus attenuated in cultured chicken cells; Fromm® D, Solvay Animal Health, Inc., Mendota Heights, Minnesota). All badgers were given vaccine from the same lot. The remaining five animals served as controls.

Badgers were restrained with a noose pole and immobilized with an intramuscular injection of approximately 15 mg/kg ketamine hydrochloride (Vetalar, Aveco Company, Inc., Fort Dodge, Iowa, USA) and 1.5 mg/kg xylazine hydrochloride (Rompun, Cutter Laboratories, Inc., Shawnee, Kansas, USA) prior to vaccination and bleeding. We drew 10 ml of blood by cephalic venipuncture immediately prior to vaccination and six additional times between 30 September and 1 December. Blood was placed in ethylenediaminetetraacetic acid (EDTA) tubes and

clot tubes, and smears were made at the time of collection.

Blood in EDTA was used for determination of packed cell volume and total white blood cell counts (Coulter Counter, Coulter Electronics, Inc., Hialeah, Florida, USA). Differential white cell counts were conducted on Wright's stained smears (Jain, 1993) and absolute leukocyte counts were calculated. Serum neutralization tests for antibodies against CD virus were run on each blood sample except on day 7 postvaccination because we did not expect badgers to possess antibodies at this time based on vaccination studies we have conducted in other mustelids. Virus neutralization tests were conducted using a minor modification of the technique of Appel and Robson (1973) using Onderstepoort strain CD virus on Vero maru cells (Middle America Research Unit, Ancon, Canal Zone, Panama). Sera were heated at 56 C for 30 min and 12 duplicate serial twofold dilutions were made in 96 well microtiter plates. A constant volume of CD virus containing about 100 TCID<sub>50</sub> and medium (199 Earle's, Gibco, Grand Island, New York, USA with 10% calf serum) was added to all wells. Plates were incubated at 37 C in 5% CO<sub>2</sub> and 95% air for 1 hr. Vero maru cells were added at a concentration of  $1 \times 10^4$  per well. Antibody titer against CD was determined as the dilution of serum that resulted in complete protection of the cell monolayer. Antibody titers of ≥1:16 were considered positive.

Badgers were observed daily and examined at the time of bleeding for clinical signs of CD. Animals were euthanized by pentobarbital overdose (Sleepaway, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) on 1 December and examined post-mortem for gross lesions associated with CD infection. Lung, lymph node, stomach, liver, spleen, small and large intestine, urinary bladder, kidney, heart, conjunctiva, and brain tissues were collected at necropsy, fixed in 10% buffered formalin, and prepared for light microscopy. Tissues were embedded in paraffin, sectioned at 5 to 6 μm, and stained with hematoxylin and eosin.

Student's *t*-test (Sokal and Rohlf, 1981) was used to test for differences in mean white cell counts and weight gain between treatment and control badgers. All *P* values ≤0.05 were considered significant.

#### RESULTS

No badgers showed clinical signs or gross or pathologic lesions of CD. Both groups gained weight at rates that did not differ significantly (*P* < 0.1) during the trials ( $\bar{x}_{\text{treatment}} = 1.8 \pm 1.4$  (SD) kg and  $\bar{x}_{\text{control}} =$

TABLE 1. Serum neutralizing antibody titers to canine distemper (CD) virus in six North American badgers following inoculation with a modified-live virus CD vaccine.

| Day post-vaccination | Badger number |       |       |         |       |      |
|----------------------|---------------|-------|-------|---------|-------|------|
|                      | AM31*         | AM72  | JM73  | JF74    | AF75  | AF76 |
| 0                    | <1:4          | <1:4  | <1:4  | <1:4    | <1:4  | <1:4 |
| 14                   | <1:4          | <1:4  | 1:4   | 1:64    | <1:4  | <1:4 |
| 21                   | 1:128         | 1:32  | 1:64  | 1:512   | 1:16  | <1:4 |
| 29                   | 1:128         | 1:128 | 1:64  | 1:512   | 1:64  | <1:4 |
| 43                   | 1:256         | 1:64  | 1:256 | 1:512   | 1:512 | <1:4 |
| 63                   | 1:512         | 1:64  | 1:256 | 1:1,024 | 1:512 | <1:4 |

\* A = adult (>1 year); J = juvenile; M = male; F = female.

1.9  $\pm$  1.3 kg weight gained). However, AM2, a control, did not eat or drink and died on 8 October and was excluded from analysis. We believe that his death was unrelated to CD inasmuch as clinical signs began during quarantine and there was no clinical, pathologic, or serologic evidence of CD.

All badgers were seronegative on day 0. One badger (JF74) had seroconverted by day 14, four more tested positive by day 21, and the remaining badger (AF76) failed to seroconvert (Table 1). Maximum reciprocal titers for individuals that responded ranged from 128 to 1024. However, the trials may have ended before maximum titers were reached inasmuch as two badgers showed increased titers between day 43 and day 62. All control badgers remained seronegative. Mean lymphocyte

counts decreased and were significantly lower ( $P = 0.03$ ,  $P = 0.02$ ,  $P = 0.001$ ) in treatment badgers on days 14, 29, and 62, respectively (Table 2).

#### DISCUSSION

The modified-live CD vaccine was immunogenic and did not cause clinical CD in test badgers over 5-mo-old. We expected lymphopenia because CD virus infects lymphocytes (Krakowka et al., 1980) and lymphopenia has been documented in domestic ferrets (*M. putorius*) and BFF  $\times$  Siberian polecat (*M. eversmanni*) hybrids vaccinated with the same vaccine (E. S. Williams, unpubl.). Why AF76 did not produce CD antibodies is unclear. The vaccine may have been ineffective or incorrectly administered, but this is unlikely considering the response of the other animals. Lack of response to modified-live virus CD vaccines has been reported in river otters (*Lutra canadensis*) (Hoover et al., 1985), but the cause was not determined.

However, there are several potential vaccine-related problems that we did not detect, but that may still occur. First, the vaccine could cause death in young animals although not in adults as has been observed in gray foxes (Swango, 1985). Second, vaccinated badgers became lymphopenic and the same vaccine has been suspected of causing immunosuppression that resulted in death from opportunistic diseases in gray foxes (Halbrooks et al., 1981) and red pandas (*Ailurus fulgens*)

TABLE 2. Mean absolute lymphocyte counts (lymphocytes/ $\mu$ l) of six badgers inoculated with a modified-live virus canine distemper vaccine (treatment) and five uninoculated North American badgers (control).  $P$  values are based on the  $t$ -test.

| Day post-vaccination | Treatment |                    | Control |                    | $P$   |
|----------------------|-----------|--------------------|---------|--------------------|-------|
|                      | Mean      | Standard deviation | Mean    | Standard deviation |       |
| 0                    | 1,120     | 375                | 1,253   | 604                | >0.25 |
| 7                    | 1,781     | 1,122              | 2,521   | 2,239              | >0.25 |
| 14                   | 836       | 418                | 1,934   | 1,134              | 0.03  |
| 21                   | 1,517     | 831                | 2,573   | 1,577              | 0.15  |
| 29                   | 1,387     | 559                | 2,824   | 1,172              | 0.02  |
| 43                   | 1,056     | 684                | 1,559   | 736                | 0.16  |
| 63                   | 1,339     | 213                | 2,638   | 599                | 0.001 |

(Montali et al., 1983). Third, some species may shed modified-live vaccine viruses allowing transmission to other animals (Halbrooks et al., 1981). Although we did not directly test for vaccine virus shedding, no transmission occurred between treatment and control badgers housed in the same cage banks where animals could come within 2 cm of nose to nose contact and actually touch by reaching forefeet through the bars of the cages. Also, vaccinated and unvaccinated badgers escaped several times during the trials and could establish direct contact with other badgers through cage bars. Halbrooks et al. (1981) found that gray foxes readily transmitted vaccine virus under these conditions. Fourth, further study is needed to address immune competency via challenge with virulent CD virus. Lastly, the number of badgers may have been too small to always detect statistical differences in blood cell counts.

Antibody titers in our badgers were lower than those produced in BFF  $\times$  Siberian polecat hybrids inoculated with the same vaccine (E. S. Williams, unpubl.). Response of domestic and hybrid ferrets to the vaccine used in the badgers was greater than those reported in domestic ferrets following canine cell origin CD vaccine (E. S. Williams, unpubl.). The modified-live virus vaccine induced prolonged lymphopenia in badgers similar to that observed in BFF hybrids (E. S. Williams, unpubl.). This contrasts with a 7- to 14-day period of postvaccination lymphopenia in domestic ferrets and is evidence that badgers are highly susceptible to the effects of CD virus infection. Thus, it is likely that virulent CD virus causes death in most infected badgers because of the similarity of response between badgers and species that suffer essentially 100% mortality following infection. This would help explain why wild seropositive badgers are rare. Additionally, rate of transmission among badgers may be low because territoriality and mutual avoidance (Minta, 1993) result in a low rate of intraspecific interaction.

Our data are consistent with the view

that badgers are highly susceptible to CD virus infection, and these data provide insights as to why this pathogen may not cause high mortality in badger populations. However, because badgers do contract CD and have a high probability of coming into contact with BFF's, they should still be considered potential transmitters of the virus.

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