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Absence of Antibodies Against Canine Distemper Virus in Free-ranging Populations of the Eurasian Badger in Great Britain

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ABSTRACT: Canine distemper virus (CDV) is a serious disease of wild carnivores throughout the world. In Europe, infection has been detected in several carnivores including the Eurasian badger (*Meles meles*). In the present study 182 badger blood samples were collected from an intensively studied population of wild badgers in southwestern England (January–July, 1997), and a further 286 from throughout southern Britain (June 1996–November 1998). A neutralizing peroxidase-linked antibody test was used for the detection of antibodies against CDV. All the samples were negative for CDV antibodies, suggesting that in contrast to mainland Europe, the disease may be either absent or maintained at low levels in British badgers.

Key words: Canine distemper virus, Eurasian badger, *Meles meles*, sero-survey.

Canine distemper virus (CDV) is classified in the Morbillivirus genus of the family Paramyxoviridae and is the causative agent of a serious infectious disease in domestic and free-living carnivores throughout the world (Appel, 1987). Canine distemper is an acute or subacute highly contagious febrile disease that may be manifested by signs of generalized infection, respiratory disease, hyperkeratosis, disruption of the central nervous system or a combination of these (Budd, 1981; Appel and Summers, 1995).

Canine distemper virus infection has been diagnosed in wild-living members of the Canidae, Mustelidae, Hyaenidae, Procyonidae, Ailuridae, Ailuropodidae, Viverridae, Ursidae, and Felidae (Montali et al., 1987). Epizootics have been recorded in several species including gray foxes (*Urocyon cinereoargenteus*), raccoon dogs (*Nyctereutes procyonoides*), raccoons (*Procyon lotor*), and striped skunks (*Mephitis mephitis*) (Appel, 1987; Machida et al., 1993). Canine distemper virus affects susceptible animals of all ages. Morbidity and mortality rates vary among species and can be high in susceptible host populations (e.g., Machida et al., 1993; Appel and Summers, 1995). However, other wildlife species show little evidence of an effect at the population level despite widespread exposure to CDV (Creel et al., 1997). In Germany an interaction between wildlife and domestic dogs has been suggested as the prevalence of CDV in martens (*Martes* spp.) was positively correlated with that in domestic dogs (Steinhagen and Nebel, 1985).

In the last two decades several dramatic and unexpected distemper episodes have drawn attention to the importance of CDV in wildlife species (Appel and Summers, 1995). The first confirmed case of CDV in a Eurasian badger (*Meles meles*) was reported by Armstrong and Anthony (1942). Fischer (1965) described lesions of the CNS in four free-ranging badgers from Switzerland, in which CDV was suggested as a possible cause. Canine distemper virus infection has also been detected in free-ranging badgers in Austria (Kolbl et al., 1990) and southwestern Germany (van Moll et al., 1995). In the British Isles badger numbers have increased substantially in recent years (Wilson et al., 1997). Furthermore, the intimate communal behaviour of high density badger populations would facilitate spread of CDV within social groups by inhalation (Appel, 1987), and the cool, dark conditions in their underground burrow systems (setts) might allow the virus to persist outside the host (Shen and Gorham, 1980). However, there is currently no published information on CDV in wild badgers in the British Isles. Therefore, our objective was to determine if free-ranging badgers in Britain were naturally infected with CDV.

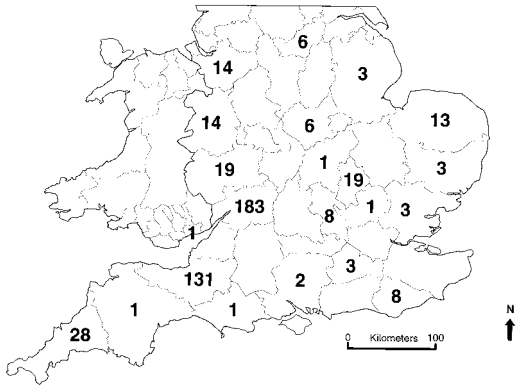

FIGURE 1. The distribution of blood samples collected for a serosurvey of antibodies to canine distemper virus in free-living badgers in southern Britain. Sample sizes are given by county.

In the present study serological tests were used to detect antibodies to CDV in 468 blood samples collected from badgers from southern England and Wales (Fig. 1). Badger serum came from two different sources. Firstly, blood samples were obtained from 182 badgers from a free-living population under intensive study at Woodchester Park, Gloucestershire, in southwestern England (approximately 51°7′N, 2°3′W). The badgers were captured between January and July of 1997, using cage traps baited with peanuts (Cheeseman and Mallinson, 1979). Trapped badgers were anaesthetised with ketamine hydrochloride (Vetalar 20 mg/kg^{-1}; Pharmacia and Upjohn Ltd, Crawley, UK) (MacKintosh et al., 1976), aged (adult, cub or yearling), sexed and a sample of jugular blood was taken. A further 286 blood samples were routinely collected by the UK Veterinary Laboratories Agency from badgers culled as part of an official UK Ministry of Agriculture badger management policy to control the transmission of bovine tuberculosis (*Mycobacterium bovis*) from badgers to cattle. Samples of jugular blood originated from badgers culled during the period June 1996 to November 1998. Serum from both sources was obtained by centrifuging whole blood and decanting the supernatant, and was subsequently frozen at −20 C prior to analysis.

The presence of antibodies against CDV was assessed using a neutralizing peroxidase-linked antibody (NPLA) test. The neutralization protocol followed was a modified version of that described by Appel and Robson (1973), and has been validated by Zaghawa et al. (1990). Briefly, each tested serum sample was serially twofold diluted in cell culture medium with the initial dilution of 1:5. The virus strain used was Onderstepoort (Haig, 1948). Viral suspensions were diluted in cell culture medium to contain 100 TCID50 per 50 μl prior to mixing with the diluted sera in the microtitre wells. Virus-serum mixtures including positive control sera and blank culture medium for cell growth control were set up. Positive control sera were obtained from another member of the mustelid family, the stone marten (*Martes foina*), and from the red fox (*Vulpes vulpes*). The virus-serum mixtures were incubated in CO_2 at 37 C for 1 hr, after which each of the mixtures (wells) was seeded with 180,000 Vero cells per ml. Vero cells were grown in Dulbecco's modified eagle medium (EDM; Life Technologie, 13435 Berlin, Germany) with 5% fetal bovine serum and were used for the propagation of CDV. The plates were incubated for 3 days at 37 C in CO_2 and thereafter washed once with PBS and fixed at 80 C in an oven overnight. Next, a mouse anti-CDV monoclonal antibody (CDPX 4/2 directed against the P protein) and a goat anti-mouse immunoglobulin G (IgG) peroxidase conjugate were applied. The substrate used was 3-amino-9-ethylcarbazole (Graham et al., 1965). Titers exceeding 1:10 were considered positive.

All 468 serum samples were negative to the presence of antibodies to CDV by NPLA test. This survey represents the first published data on the CDV status of badgers from Great Britain. The negative results from the intensively sampled study population at Woodchester Park provide compelling evidence that this population

has not experienced widespread exposure to CDV. The sample provided broad coverage of age ($n = 67$ cubs, 12 yearlings and 103 adults) and sex classes ($n = 75$ males, 107 females). In this high density population the aggregation of individual animals into territorial social groups could mitigate against the rapid dissemination of infectious disease throughout the population and contribute to the persistent concentration of infection within certain groups (Cheeseman et al., 1988). However, the blood samples obtained in the present study came from badgers resident in 28 contiguous social groups, thus providing broad coverage. Results from the smaller number of samples obtained from elsewhere in southern Britain, provide less compelling evidence for the absence of infection (particularly if it were highly aggregated), but are consistent with either absence or relatively low levels of exposure. Furthermore, the absence of positive results from badgers in Great Britain is in stark contrast to those obtained from the limited sampling of badgers carried out on the European mainland (e.g., Kolbl et al., 1990; van Moll et al., 1995).

In Britain the CDV status of wildlife species is unknown, but in domestic dogs infection is rare owing to a widespread and successful vaccination program (H. Thompson, pers. comm.). Nevertheless, the potential for transmission between unvaccinated domestic dogs and wildlife may exist in areas where interactions are most frequent (e.g., suburbs). Unfortunately, in the present study we could not explicitly test for aggregations of CDV infection in badgers in such areas, as samples were predominantly rural in origin.

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