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ANTIBODIES TO ALEUTIAN MINK DISEASE PARVOVIRUS IN FREE-RANGING EUROPEAN MINK (*MUSTELA LUTREOLA*) AND OTHER SMALL CARNIVORES FROM SOUTHWESTERN FRANCE

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ABSTRACT: Owing to the rapid decline of the European mink (*Mustela lutreola*) in France, a national conservation action plan has been initiated, in which scientific research to improve understanding of the causes of the decline is one of the primary objectives. In order to investigate the possible role of Aleutian disease parvovirus (ADV) in decline of the species, a serologic survey was conducted from March 1996 to March 2002 in 420 free-ranging individuals of six species of small carnivores distributed in eight départements of southwestern France. Antibodies to ADV were detected in 17 of 75 American mink (*Mustela vison*), 12 of 99 European mink, 16 of 145 polecats (*Mustela putorius*), four of 17 stone martens (*Martes foina*), one of 16 pine martens (*Martes martes*), and three of 68 common genets (*Genetta genetta*). Seroprevalence was significantly higher in American mink than in other species. Seropositive individuals with gamma globulin levels >20% were observed in four European mink, four American mink, two stone martens, and one pine marten. Geographic distribution of positive animals indicates the virus has spread to all areas where European mink are found. Furthermore, a trend of increasing prevalence seems to appear in *Mustela* sp. sympatric with American mink. Although further investigations are necessary to evaluate the role of ADV in decline of European mink, evidence of the virus in the wild at the levels found in our study has implications for conservation of this species.

Key words: Aleutian mink disease parvovirus, *Genetta genetta, Martes foina, Martes martes, Mustela lutreola, Mustela putorius, Mustela vison,* serologic survey.

INTRODUCTION

The European mink (*Mustela lutreola*) is one of the 15 species of carnivores listed by the International Union for the Conservation of Nature and Natural Resources (IUCN, 2003) as threatened with extinction. This species retracted from most of its range in the last century (Youngman, 1982; Saint-Girons, 1991; Rozhnov, 1993; Maran and Henttonnen, 1995) and its distribution is still dramatically decreasing. In France, the species has lost nearly half of its range during the last 20 yr and is now only present in seven départements in southwestern France (Maizeret et al., 2002). Owing to this rapid decline, the French Ministry of the Environment initiated a conservation action plan for the period 1999–2004. The objectives were to stop the decline of the species in France and to assist recovery in part of the area where it recently was found. Among the main objectives of this plan is scientific research to improve knowledge of the causes of regression of French populations of mink.

Excessive trapping, change or loss of habitat, and interspecies competition by

the larger American mink (*Mustela vison*) are generally mentioned as factors implicated in decline of European mink (Saint-Girons, 1991; Rozhnov, 1993; Maran and Henttonen, 1995; Lodé, 2001). The role of diseases is an obvious possible factor in the decline of the European mink population (Maran and Henttonen, 1995), and introduction of the Aleutian mink disease virus (ADV) by the exotic American mink has been suggested (Mañas et al., 2001). Aleutian disease, a persistent and progressive parvovirus infection, characterized by plasmacytosis and resulting hypergammaglobulinemia followed by immune complex formation (Aasted, 1985), could lead to population decline (Mañas et al., 2001).

In France, three feral American mink populations have been identified (Léger and Fournier, unpubl. data), and the distribution of the southwestern population overlaps European mink distribution. In order to investigate prevalence and diffusion of ADV in the French range of European mink, a serologic survey was conducted in several small carnivore populations, including European mink, American mink, polecat (*Mustela putorius*), stone marten (*Martes foina*), pine marten (*Martes martes*), and common genet (*Genetta genetta*).

MATERIALS AND METHODS

Serum samples were collected from 99 European mink, 75 American mink, 145 polecats, 17 stone martens, 16 pine martens, and 68 common genets trapped in eight départements of southwestern France (42°47′N to 46°22′N, 0°54′W to 4°7′W) between March 1996 and March 2002 (Fig. 1). Capture in March 2001 of a European mink in the Gers département meant the Gers had to be included in the study area initially composed of the seven départements defined by Maizeret et al. (2002).

The animals were caught in live traps during several studies, including a survey of European mink distribution conducted between 1991 and 1997 (Maizeret et al., 2002) and started again in 2000, a behavioral study of European mink conducted from March 1996 to August 1999 in the Landes de Gascogne region (Maizeret et al., unpubl. data), and control of American mink conducted since August 2001 (Fournier et al., unpubl. data). Trapping was conducted from September to April to avoid birth and nursing periods. Some animals were also incidentally captured during pest control.

Animals were transferred from traps to a tubular opaque canvas bag and were given an intramuscular injection in the thigh of medetomidine (Domitor® 1 mg/ml, Pfizer Santé Animale, Paris, France) and ketamine (Ketamine UVA 500® 50 mg/ml, Laboratoires UVA, Ivry-sur-Seine, France). For mustelids, doses were reduced compared to doses used for surgical procedures (Fournier-Chambrillon et al., 2003), and a combination of 150 μg/kg medetomidine and 7.5 mg/kg ketamine was sufficient. Common genet received a combination of 150 μg/kg medetomidine and 10 mg/kg ketamine. Manipulations included a detailed clinical exam. Animals were weighed using an electronic letter scale (Maultronics 151 20, Maul®, Bad König, Germany), measured (total length, head and body, foot), and the sex determined. Age was defined as juvenile (milk teeth), subadult (adult teeth without abrasion and tartar), adult (teeth partly abraded and with tartar), and old adult (teeth largely abraded with much tartar). Physical condition was defined as very good (particularly corpulent animals with developed muscles and glossy hair), good (animals apparently clinically healthy), and poor (thin animals with muscle atrophy, dull hair, sometimes dehydrated). Reproductive status was determined as gestation, lactation, and apparent or nonapparent testicles. All animals were marked by a cut on the ear and received a subcutaneous electronic identification system tag (Injectable Trovan® sterilized glass transponder, in a needle, Eid Aalten B.V., Aalten, the Netherlands) between the shoulders. A blood sample of up to 2.5 ml was taken from a jugular vein with a disposable plastic syringe with a 0.6×25 mm disposable needle (Terumo®, Terumo Europe N.V., Leuven, Belgium). Blood was transferred into a plain silicone coated glass tube (Venoject, Terumo). When the procedures were completed, animals were reversed with 750 μg/kg atipamezole (Antisedan®, 1 mg/ml, Pfizer Santé Animale), placed back in the trap to recover, and released 2–3 hr after recovering at the capture site. Blood was transferred the same or the next day to the Laboratoire départemental des Landes (Mont-de-Marsan, France). It was centrifuged at 3,000 × G for 5 min, and the serum was drawn off and stored at −20 C in 100 μl aliquots. Serum samples were submitted to the Immunological Laboratory of the Royal Veterinary and Agricultural University of Copenhagen (Copenhagen, Denmark) to be tested for the presence of antibodies to ADV. We used countercurrent

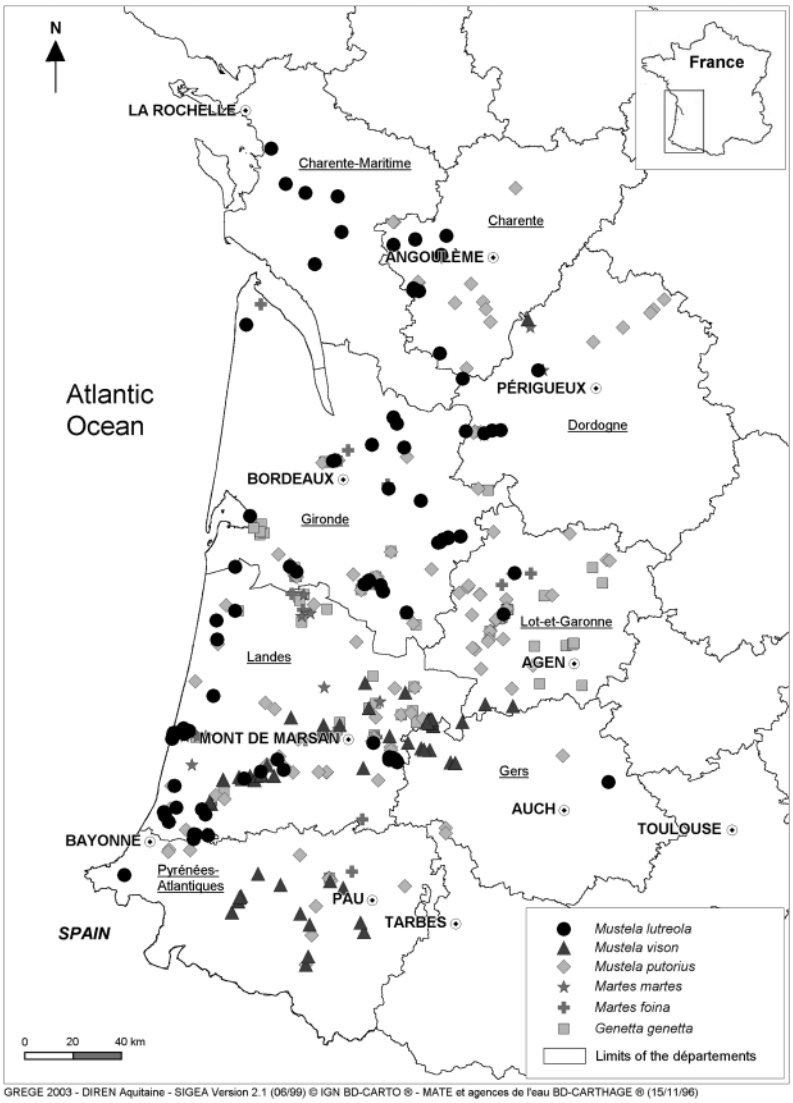

FIGURE 1. Geographic distribution of 420 free-ranging animals tested for antibodies to Aleutian disease virus in southwestern France.

immunoelectrophoresis and countercurrent line absorption immunoelectrophoresis (Aasted et al., 1986). This second test has the advantage of being one of the most sensitive electrophoretic methods and offers specific testing of a given precipitate (Alexandersen et al., 1985a; Aasted et al., 1986). Animals were classified positive when they were positive on one of the tests. Serum electrophoresis was performed on all positive samples (except one positive stone marten) in order to quantify gamma globulin (Svendsen et al., 1983; Aasted, 1989). Moreover, in order to have a reference of noninfected mink, the serum gamma globulins were quantified for 16 seronegative European mink and 16 seronegative American mink.

We used logistic regression models (Agresti, 1990) to analyze the association of species and sex with the probability of ADV infection. The model relates the logit of the probability for an individual of being ADV positive to the predictor variables, species, and sex. Second, we split animals of the European species into two groups: group 1 animals were located in the range of the feral population of American mink; group 2 animals were located outside this area. To test whether or not the prevalence of ADV was greater in animals living in the vicinity of

TABLE 1. Model selection for seroprevalence data of antibodies to Aleutian disease virus in six free-ranging small carnivores from southwestern France for all data combined and for data according to group. The best models according to Akaike information criterion (AIC) are indicated in bold.

Model	Deviance	Degrees of freedom	AIC
All data combined			
Full model	0	0	24
S[a] + Sp[b]	6.47	5	20.47
Sp	**7.11**	**6**	**19.11**
Null model	21.32	11	23.32
By group			
Full model	0	0	20
G[c] + Sp	3.47	4	15.47
G	9.86	8	13.86
Null model	**10.80**	**9**	**12.80**

[a] Sex.
[b] Species.
[c] Group (group 1 = animals located in the range of American mink; group 2 = animals located outside the range of American mink).

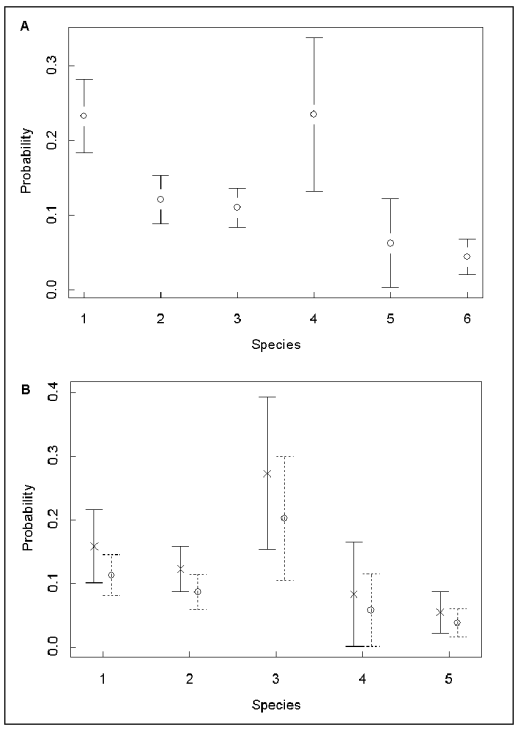

FIGURE 2. Predicted seroprevalence±standard error (95%) of antibodies to Aleutian disease virus in six free-ranging small carnivores from southwestern France. (A) by species (1. *Mustela vison,* 2. *Mustela lutreola,* 3. *Mustela putorius,* 4. *Martes foina,* 5. *Martes martes,* 6. *Genetta genetta*); (B) by group (solid lines, group 1 [animals located in the distribution area of the feral population of American mink]; dashed lines, group 2 [animals located outside the distribution area of the feral population of American mink]) and species (1. *Mustela lutreola,* 2. *Mustela putorius,* 3. *Martes foina,* 4. *Martes martes,* 5. *Genetta genetta*).

American mink, we applied a logistic regression with species and group as predictor variables. We also tested for positive or negative trends using a binomial test. Selection of the most parsimonious models was carried out using the Akaike information criterion (AIC, Burnham et al., 1995). Interpreting the selected statistical model was done using predicted values of the selected model, which are based on the sum of logarithms of odds ratios, back transforming confidence intervals calculated on the original logit scale using a normal approximation (Agresti, 1990). All statistical analyses were done with S-Plus (Venables and Ripley, 1999). $P \leq 0.05$ was considered significant.

RESULTS

Individuals with positive reaction for antibody to ADV were observed in all species, that is, 17 of 75 American mink, 12 of 99 European mink, 16 of 145 polecats, four of 17 stone martens, one of 16 pine martens, and three of 68 common genets. The model selected included only the species, and no difference in prevalence of ADV between sexes was detected (Table 1). The occurrence of positive animals was significantly higher in the American mink than in the other fissiped species (Fig. 2). Only the stone marten had ADV prevalence as high as American mink, but the sample size was too small to confidently assess the ADV prevalence in the stone marten.

Antibody-positive animals were mostly subadult or adult. However, one positive European mink and one positive common genet were juveniles, although the sample size in this age class was low (two European mink, 25 common genets, three polecats, and one pine marten). Older animals were negative, but the sample size in this age class was also low (five European mink, two polecats, and one stone marten).

TABLE 2. Mean gamma globulin levels of the total serum protein ±SD (range) in six free-ranging small carnivores from southwestern France positive for antibodies to Aleutian disease virus (ADV). In order to have a reference on noninfected minks, the serum gamma globulins were quantified for 16 seronegative European mink and 16 seronegative American mink.

Species	Positives for antibodies to ADV		Negatives for antibodies to ADV
	Gamma globulin level >20%	Gamma globulin level <20%	
Mustela lutreola	21.9±2.0 (20.5–24.8%) n=4	12.7±3.3 (8.9–18.7%) n=8	9.5±4.0 (4.3–17.3%) n=16
Mustela vison	30.3±4.7 (23.4–33.8%) n=4	9.0±4.2 (3.5–19.9%) n=13	6.6±2.1 (4.1–11.4%) n=16
Mustela putorius	n=0	10.4±3.3 (4.2–16.0%) n=16	
Martes foina	20.4–22.0% n=2	16.40% n=1	
Martes martes	20.4% n=1		
Genetta genetta	n=0	8.2±1.7 (6.7–10.0%) n=3	

Antibody-positive individuals in poor physical condition were only observed among European and American mink (three of 14 and two of five, respectively), but the prevalence of antibodies in mink in poor condition was not higher (chi-square test) than in animals in good condition (nine of 70 and nine of 42, respectively). Six of 19 American mink and one of 33 polecats in very good physical condition were also positive for antibodies to ADV.

Gamma globulin levels >20% of the total serum proteins, considered to be hypergammaglobulinemia, were observed in individuals of all species except the polecat and the common genet (Table 2). Among these, one European mink and one American mink were in poor condition; all others were in good condition.

The geographic distribution of the positive animals shows that the virus has spread in the eight départements of the study area and that indigenous animals are found ADV seropositive outside the range of the American mink (Fig. 3). We did not detect any difference in ADV prevalence between groups 1 and 2 (Tables 1 and 3). However, predicted prevalences suggested an increase could occur when species live in the vicinity of American mink (Fig. 2). This trend was significant using the binomial test (P=0.03).

DISCUSSION

The prevalence and significance of ADV in free-ranging carnivores is largely unknown (Steinel et al., 2001). In Europe, Yamaguchi and Macdonald (2001) and Mañas et al. (2001) recently reported ADV in a feral population of American mink in southern England and in riparian carnivores in Spain, respectively. Our study is the first investigation on ADV in the wild in France.

Our results confirm the large spectrum of species positive for antibodies to ADV (Alexandersen et al., 1985b) and the large geographic spread of the virus in the wild. The spread of ADV may be related to the characteristics and modes of transmission of the virus and to the territorial behavior of the species studied. Aleutian disease virus is highly persistent in the environment

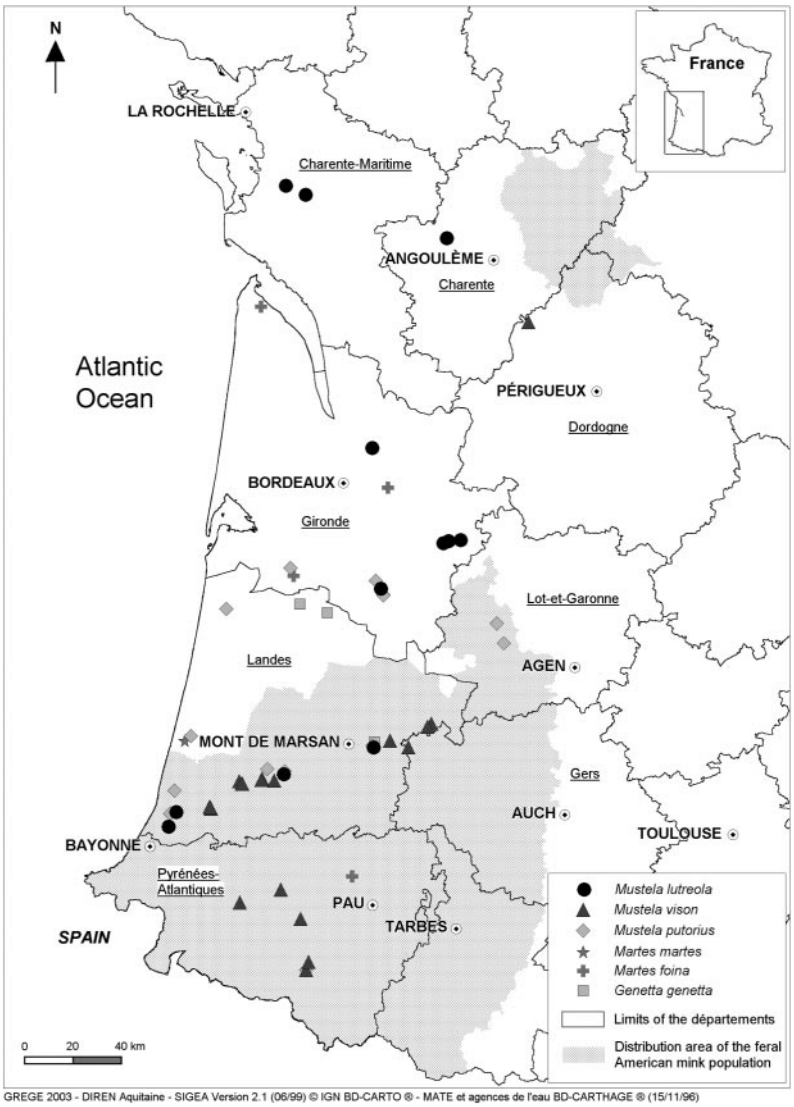

FIGURE 3. Geographic distribution of 53 free-ranging animals seropositive for antibodies to Aleutian disease in southwestern France.

TABLE 3. Occurrence of seropositive animals for antibodies to Aleutian disease virus in six free-ranging carnivores from southwestern France in relation to their location in the distribution area of the feral population of American mink (group 1) or outside this area (group 2).

	Group 1		Group 2	
	Positive	Negative	Positive	Negative
Mustela lutreola	4	14	8	73
Mustela putorius	9	59	7	70
Martes foina	1	7	3	6
Martes martes	—	3	1	12
Genetta genetta	1	24	2	41

(Aasted, 1985); is present in urine, feces, and saliva (Kenyon et al., 1963; Gorham et al., 1964); and may be spread by asymptomatic carriers (Gorham et al., 1964, 1976; Aasted and Hauch, 1988). In addition to horizontal transmission by direct or indirect contact, vertical transmission has been shown (Padgett et al., 1967; Haagsma, 1969; Bazeley, 1976). Moreover, exceptional movements of about 35 km have been observed in male European mink going from one river valley to another (Maizeret and Fournier, unpubl. data), and such behavior could contribute to the spread of the virus.

The 23% seroprevalence of ADV in American mink observed in our study is lower than the 52% prevalence ($n=27$) reported by Yamaguchi and Macdonald (2001) in southern England, but the geographic distribution of their sample was restricted to 24 km of river. Our comparative study shows the seroprevalence in American mink, the possible initial host of the virus, is higher than in other species. Therefore, feral American mink represent an important source of excretion and spread of the virus and there is a trend of increasing seroprevalence in *Mustela* sp. living in the range of American mink. The role of the American mink in the spread of the virus to European mink is expected from the statistical model and might be confirmed if more animals were tested. To our knowledge, the present study is the first report of detection of antibodies to ADV in the family Viveridae. However, the common genet seldom seems to be in contact with the virus in the wild.

In American mink, the first clinical signs of ADV are usually anorexia and weight loss, dehydration, anemia, and hemorrhage (Aasted, 1985). However, both the virus and host genotype influence severity of the disease (Henson et al., 1976; Bloom et al., 1994), and inapparent infections have been described (An and Ingram, 1977, 1978). In our study, seropositive animals were not in worse physical condition than seronegative animals. Hypergammaglobulinemia appears before clinical signs (Haagsma, 1969), and the level of gamma globulins is an indication of a progressive infection. Gamma globulin levels >20% were observed in all species except common genet and polecat. Considering the large number of polecats studied, this suggests that viral strains in the wild are not pathogenic for this species. On the other hand, occurrence of seropositive animals showing gamma globulin levels >20% was similar between European mink and American mink and suggests similar pathogenic potential of ADV in the two mink species.

The impact of ADV on free-ranging mink populations is difficult to estimate. In spite of the high seroprevalence of ADV observed in American mink, the population is still expanding. The contribution of ADV infection to the decline of European mink could be significant because of its persistent nature and of its potential negative effects. Aleutian disease virus has a negative influence on reproduction (Haagsma, 1969; Gohram et al., 1976; Hansen and Lund, 1987; Bloom et al., 1994) and can increase susceptibility to other diseases (Bloom et al., 1994). Further studies of carcasses and identification of virus strains would provide more information on the pathogenicity of the virus in free-ranging mustelids and on its possible role in the decline of European mink.

The fact that ADV is widespread in the wild has several implications for conservation of European mink. Vaccination against ADV is not effective (Aasted, 1985), so there is no way of preventing spread of the disease in the wild by this means. Thus, it is urgent to limit the other factors of decline in order to effectively support recovery of the species. Because the feral American mink population represents a major source of virus and has a great colonizing potential, control of their populations should be a priority of the national conservation action plan. Effective measures to prevent animals from escaping from fur farms should be established.

Strict protocols for disinfection of equipment during trapping programs should be implemented. And if a captive breeding program proves necessary because of decline of the species, strict sanitary protocols must be applied on the breeding farms to prevent contamination by this virus.

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