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## Serology and protein electrophoresis for evidence of exposure to 12 mink pathogens in free-ranging American mink (*Neovison vison*) in Argentina

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### ABSTRACT

**Background:** Basic pathologic characteristics for farmed minks were previously reported worldwide. However, its status in the wild has not been studied in detail.

**Objective:** Serology and electrophoresis were carried out for evidence of exposure to 12 mink pathogens on two different locations.

**Animals and methods:** Serology was done in 87 wild minks by reference techniques against *Toxoplasma gondii*, *Encephalitozoon cuniculi*, *Neospora caninum*, *Brucella abortus*, *Mycobacterium bovis*, *Leptospira interrogans*, canine distemper virus (CDV), canine adenovirus (CAV), canine parvovirus (CPV), rabies virus (RV), Influenza A virus (FLUAV) and Aleutian disease virus (ADV). Hypergammaglobulinemia, the ADV main clinical feature, was determined by conventional electrophoresis.

**Results:** Seventy-one percent of the 87 sera had antibodies against one or more pathogens. ADV accounted for the highest seroprevalence (29%), followed by *T. gondii* (26%), *L. interrogans* (14%), *M. bovis* (12%), *B. abortus* (9%), *N. caninum* (3%), CPV (3%) and CDV (2%). Seroprevalence was influenced by location but not sex or age. Additionally, 16% of the seropositive samples for ADV had gammaglobulin levels >40.0 g/L. Antibody titers for CDV and CPV were low and difficult to interpret as almost all these cases had borderline concentrations.

**Conclusion:** A cautious interpretation of the results is urged as the epidemiological role of the wild mink is largely unexplored for most of these agents. Nevertheless, the information may be clinically relevant.

### ARTICLE HISTORY

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### KEYWORDS

Mink; *Neovison vison*; serology; wildlife

## 1. Introduction

The American mink (*Neovison vison*) was deliberately introduced as a valuable fur-bearing mammal for commercial production in South America in 1930, then it subsequently escaped or had been released from farms (Martino & Villar 1991; Sepúlveda et al. 2011). It is considered a pest in the region and classed as Least Concern by the IUCN (Reid & Helgen 2008; Barros et al. 2014). According to the local people and hunters, mink numbers recently declined in some areas and the specific causes of non-hunting mortality remain unknown. Whether minks could be alternate hosts and are involved in the transmission and maintenance of diseases in wildlife is largely unexplored.

Our objective was to determine the antibody prevalence to a select group of pathogens in two mink populations which may aid in understanding the role of free-ranging minks in disease ecology.

## 2. Material and methods

Capture fieldwork was carried out between June 2013 and July 2015 on two sites: area 1 (the vast Buenos Aires province, 38° 50' S, 57° 34' W, a subtropical rural region with an abundant native fauna and domestic animals) and area 2 (the cold patagonian grasslands of the Santa Cruz Province, 46° 36' S, 72° 24' W and 46° 48' S, 67° 58' O).

Animals were visually located and captured either manually using a drive net system or with single-door box-traps set every 250–500 m. Blood collection was done as already detailed (Philippa et al. 2008). Age and sex were estimated primarily from dentition and genital development (Fournier-Chambrillon et al. 2004; Harrington et al. 2012). After sampling, the animals were then released.

Serum was tested for antibodies against the following disease agents: *Toxoplasma gondii*, *Encephalitozoon cuniculi*, *Neospora caninum*, *Brucella abortus*, *Mycobac-*

*terium bovis*, *Leptospira interrogans*, canine distemper virus (CDV), canine adenovirus (CAV), canine parvovirus (CPV), rabies virus (RV), Influenza A virus (FLUAV) and Aleutian disease virus (ADV). Briefly, antibodies to *T. gondii* were identified by the modified direct agglutination test and titers  $\geq 1:32$  were considered positive (Sepúlveda et al. 2011). Exposure to *E. cuniculi* was determined by the enzyme-linked immunosorbent assay (ELISA), and the test was considered positive if the optical densities of the coated well was more than 0.394 (Hersteinsson et al. 1993). Specific antibodies to *N. caninum* using the indirect fluorescent antibody test with a canine conjugate were investigated and titers  $\geq 1:25$  were taken as positive (Stuart et al. 2013). A commercial indirect ELISA (CHEKIT<sup>®</sup>, Bern, Switzerland) was used as a screening test to detect animals with *B. abortus* antibodies and titers  $\geq 1:32$  were taken as positive. Because of the known high sensitivity and the limited specificity of this ELISA, all samples found positive or suspect were subjected to subsequent complement fixation testing (CFT) and  $>20$  U/mL was considered indicative of exposure (OIE 2005). For *M. bovis* testing, a commercial *M. bovis* antibody ELISA (IDEXX Laboratories, Westbrook, ME, USA) was used and a cut-off value was an OD ratio of  $\geq 0.3$  (Buddle et al. 2013). Thirteen serovars of *L. interrogans* (*bataviae*, *pomona*, *grippothyphosa*, *hardjo*, *icterohaemorrhagiae*, *sejroe*, *autumnalis*, *copenhageni*, *australis*, *grippothyphosa*, *bratislava*, *pyrogenes* and *canicola*) were investigated by microscopic agglutination and screened at a serum dilution of 1:100 (OIE 2005). Serum neutralization (SN) test for CDV and CAV was performed; neutralizing titers of 1:20 or higher were considered positive for CDV and  $\geq 1:15$  for CAV (Philippa et al. 2008; Santos et al. 2009).

Indirect immunofluorescence antibody test (IFAT) was used for the presence of antibodies to CPV, with titers of  $\geq 1:15$  considered indicative of previous exposure (Helfer-Baker et al. 1980). Rapid fluorescent focus inhibition test with reciprocal titer of  $\geq 10$  in a 50% plaque reduction was employed for RV (OIE 2005). For FLUAV antibody testing, a commercial ELISA (IDEXX AI MultiS-Screen Ab Test, Westbrook, ME, USA) was

performed and samples with a sample-to-negative control ratio value below 0.50 were considered positive (Lebarbenchon et al. 2012). Counter-current electrophoresis (CIEP) was performed for the presence of antibodies to ADV using a commercial antigen (United Vaccines Inc., Madison, WI, USA) with 10 mL of 0.8 standard low agarose in barbital buffer, pH 8.6–9.0 (Martino & Villar 1991). Gammaglobulin levels (percent gamma) were determined by conventional electrophoresis using agarose Electrophoresis System; hypergammaglobulinemia was considered when gamma globulin values exceeded 40.0 g/L (Sánchez-Migallón Guzmán et al. 2008).

Chi-square tests using  $p < 0.05$  as the level of significance, relative risk (odds ratio, OR), and the Taylor series 95% confidence limits (CFs) were calculated with the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA, version 6.03, 1988) and were used to evaluate differences on the seroprevalences and relationships to locations, age and gender. Bonferroni correction was performed to adjust  $p$ -values.

### 3. Results

Blood samples were collected from 24 adult (10 males, 14 females) and 19 juvenile (12 males, 7 females) samples of area 1, meanwhile 21 adult (8 males, 13 females) and 23 juvenile samples (15 males, 8 females) came from area 2. Most of the animals were in apparent good health on body examination, except for four cases that showed emaciation.

Table 1 shows seroprevalence and reciprocal titers against the 12 pathogenic agents investigated.

Sixty-two animals (71%) were antibody positive for at least one of the pathogenic agents, with 33 being seropositive for 1 agent, 26 for 2 agents and 3 animals for 3 agents.

Both *T. gondii* and ADV titers combined were the most common association ( $n = 14$ ). *B. abortus*, CDV and CPV have been identified in area 1 only. Although less animals were captured in area 1, general seroprevalence rate was significantly higher than in area 2

**Table 1.** Seroprevalence and titers of 12 selected agents among the 87 minks.

Pathogen	Number of positive sera			Titers		
	Area 1 ( $n = 44$ ) (Buenos Aires)	Area 2 ( $n = 43$ ) (Patagonia)	Total (%), OR, CF <sup>a</sup>	Positive cut-off value	Minimum	Maximum
<i>T. gondii</i>	10	13	23 (26.4), 0.92, 0.55–1.18	$\geq 1:32$	01:32	1:544
<i>E. cuniculi</i>	0	0	0	$\geq 0.394$		
<i>N. caninum</i>	4	6	3 (3.4), 0.41, 0.05–0.64	$\geq 1:25$	1:25	1:320
<i>B. Abortus</i> <sup>b</sup>	8	0	8 (9.2), 2.20, 1.02–3.98	$\geq 1:32$	1:32	1:160
<i>M. Bovis</i>	9	2	11 (11.5), 1.68, 0.88–2.59	$\geq 0.3$	0.3	0.6
<i>L. Interrogans</i>	5	7	12 (13.8), 0.12, 0.05–0.77	1:100	1:40	1:800
CDV	2	0	2 (2.3), 1.12, 0.76–1.45	$\geq 1:20$	1:20	1:80
CAV	0	0	0	$\geq 1:15$		
CPV	3	0	3 (3.4), 1.71, 0.66–2.56	$\geq 1:15$	0:15	0:15
RV	0	0	0	$\geq 10$		
FLUAV	0	0	0	0.50		
ADV	18	7	25 (29.0), 1.37, 1.09–2.88			

<sup>a</sup>Percentage positive; OR, odds ratio (relative risk) and CF, 95% confidence limits

<sup>b</sup>An insufficient amount of sera prevented one case with ELISA antibody titer  $\geq 1:32$  to be subject to CFT and correlation analyses.

( $p < 0.05$ , OR = 0.8, 95% CF = 0.6–1.1). Particularly, seroprevalences for *B. abortus*, *M. bovis* and AMVD on area 1 were significantly higher than on area 2 ( $p < 0.01$ , OR 1.6 95% CF = 1.3–1.9;  $p < 0.05$ , OR = 0.27, 95% CF = 0.2–0.6 and  $p < 0.05$ , OR = 1.5, 95% CF = 1.3–1.9, respectively). Conversely, no major differences in seroprevalence rates were observed among age or sex categories for animals from both areas, except for evidence of exposure against *T. gondii*, which was more significantly prevalent on adult than on juvenile minks ( $p < 0.05$ , OR = 0.8, 95% CF = 0.4–1.1). Ten samples with positive antibody titer  $\geq 1:32$  for *B. abortus* by ELISA were also tested by CFT, with results correlating well between them (8/10,  $r = 0.86$   $p < 0.05$ , Kappa 0.93). One of these cases, an adult male showed emaciation.

Twelve animals (14%) were positive for the *Leptospira* serovars tested: *icterohaemorrhagiae* ( $n = 7$ ), *canicola* ( $n = 2$ ), *sejroe* ( $n = 1$ ), and *icterohaemorrhagiae* and *sejroe* combined ( $n = 2$ ). Mean antibody titer observed was 1:200, and the maximum titer of 1:800 belonged to a juvenile emaciated female from area 1. None of the sera reacted against the other serovars.

Among the viral agents, CDV and CPV were present in only one geographical location (area 1), both with low prevalences and titers, meanwhile, ADV comprised the greatest number of seropositive minks. The two CDV seropositive minks and two of the three minks positive for CPV had also seropositivity for ADV. Regarding ADV exposure, four adult minks out of the 25 seropositive animals by CIEP showed gammaglobulin levels exceeding the 20% of the total plasma protein ( $p > 0.05$ , OR = 3.2, 95% CF = 2.4–3.9). Mean hypergammaglobulinemia for all these four positive animals was  $58.9 \pm 3.2$  CF = 42.0–66.3%, meanwhile, two minks of the four presented with emaciation ( $p > 0.05$ , OR = 2.3, 95% CF = 1.4–2.8).

#### 4. Discussion

Significant information about known diseases within wildlife populations has been provided from serologic surveys (Philippa et al. 2008; Harrington et al. 2012). Captive minks are susceptible to a wide array of infectious and parasitic diseases (Martino & Villar 1991; McDonald & Lariviere 2001), but unfortunately, essential knowledge about the fundamental biology or population ecology of free-ranging minks is lacking. This is a cross-sectional survey of wild minks for serologic evidence of exposure to several pathogenic agents on two different locations. Here, the majority of the captures were healthy except for four minks commonly adults with clinical signs of starvation.

Overall, 71% of the samples had seropositivity. Our data suggest that there was significant difference in the prevalence of most of the agents between the locations covered by this survey. This is difficult to

explain. Some epidemiological factors (i.e. reservoirs, food habits, immunological status and demography) can obviously influence these results. The humid rural Buenos Aires with a major presence of livestock and humans is in sharp contrast to the large arid Patagonia. Also, both sites were far enough apart that individual mink was unlikely to move directly from one region to the other. Alternatively, there may be differences in fur-farming history: the Buenos Aires mink ranches continued to liberate animals even in the last decade, but the Patagonian farms closed a long time ago. That would explain perhaps higher prevalences of, for example, ADV or CDV, which are two of the most important diseases in captivity (Martino & Villar 1991; McDonald & Lariviere 2001).

Distribution of age and sex groups within the seroprevalence rates was similar for the bulk of the accessions. The lack of similar surveys in the literature preclude us to make a comparison of the data. *T. gondii* was the most prevalent parasite investigated and the only pathogen that showed a significant difference with respect to older age class. Previous studies on feral minks from southern Chile, England and Ireland reported higher seroprevalences (>50% against our 26%) and higher proportion of adults like in our survey (O’Crowley & Wilson 1991; Sepúlveda et al. 2011; Harrington et al. 2012). The presence of antibodies to *T. gondii* on both regions suggests that this organism may be active in the wild.

Regarding the other two parasites tested, all samples were negative for *E. cuniculi* and there was a low prevalence of *N. caninum* (3%), suggesting that the host range of *N. caninum* is more limited or does not persist as efficiently as *T. gondii* within the environment. On the first time that mustelids have tested positive for bovine neosporosis, only 1% of mink were seropositive by IFAT (Stuart et al. 2013). Eight percent of mink from Iceland were found seropositive for *E. cuniculi* (Hersteinsson et al. 1993); this finding contrasts with observations from other arctic areas and the absence of rodents in the diet of minks or foxes is the most likely explanation for the absence of *E. cuniculi*.

Eight of the 10 samples classified as positive for *B. abortus* by the ELISA were positive by the CFT test and, thus, correlation agreement between the tests was high. Not surprisingly, all cases came from area 1 where cattle brucellosis is enzootic. Moreover, mink is a well-known wild scavenger that often consumes carcasses in the area. Other authors reported no evidence of brucellosis in 15 feral minks surveyed (O’Crowley & Wilson 1991).

The infection of both captive and free-ranging wildlife species with *M. bovis* represents a zoonotic risk and continues to cause challenges for the livestock industry, zoos and governments around the world (Chambers 2013). Bovine tuberculosis is another well-known infection in minks under captivity (MacDonald &

Lariviere 2001). In 2007, we reported two fatal cases of TB in adult feral minks (Martino et al. 2007) but there are no other studies regarding wildlife. The average seroprevalence here was 11% and most of the positive samples came from area 1, where bovine tuberculosis is also enzootic.

Herein, three leptospira serovars were only tested positive among 12 minks from both regions, all with moderate titers. On examination of these samples, no four-fold rise in titer was seen except in one juvenile female. Probably, infecting serovars may vary regionally depending on exposure to other infected wild or domestic animal reservoir hosts (Barros et al. 2014). Also, there is an incomplete understanding of leptospirosis in wild animals as most of the published studies usually do not include isolation efforts or test a limited panel of serovars (Sykes et al. 2011).

Minks are susceptible to a variety of viral diseases (MacDonald & Lariviere 2001; Santos et al. 2009). Among the viruses tested, ADV accounted for the highest seroprevalence, followed by CPV (3.4%) and CDV (2.3%), meanwhile, all samples were negative for CAV, RV and FLUAV. Our data can match previous European surveys on feral minks which provided seropositivity for CDV (range 0%–3.4%), CAV (5.4%) and ADV (range 22.6%–51.8%) (Yamaguchi & Macdonald 2001; Fournier-Chambrillon et al. 2004; Philippa et al. 2008; Persson et al. 2015). Conversely, seronegativity was reported for CPV and CDV (Santos et al. 2009), for RV (Philippa et al. 2008) and for ADV (Sánchez-Migallón Guzmán et al. 2008). Our results for CDV and CPV should be interpreted prudently, as both were detected in few animals, mostly have low, borderline antibody titers, and even possibly non-specific (Philippa et al. 2008).

AD is a chronic viral disease in farmed minks and the virus has been found in many free-ranging mink populations in Europe and North America (Mañas et al. 2001; Persson et al. 2015). It accounted here for the highest prevalence of all the agents; most of the seropositive minks were from the Buenos Aires province, which has a more recent feral population due to deliberate releases. AD is also the most important cause of death in Argentinean mink farms since decades (Martino & Villar 1991). In addition, hypergammaglobulinemia was present in 16% of the CIEP positive samples. Likewise, other authors found 22.6% of CIEP positives, meanwhile, 23% of them had gammaglobulin levels higher than 40 g/L (Fournier-Chambrillon et al. 2004). The demonstration of the occurrence of this viral disease in a feral American mink population suggests that not only AD may represent a serious cause in the apparent decline of local mink populations but it may also threaten other mustelid species (Mañas et al. 2001; Yamaguchi & MacDonald 2001).

The lack of detectable antibodies to *E. cuniculi*, CAV, RV and FLUAV apparently suggests no prior exposure to this agent, but sometimes serological tests do not

become positive until an infected animal has had time to develop antibodies, or might be a remote exposure with following loss of detectable titers. Further analyses to identify seroconversions are strongly recommended. Likewise, positive results may be indicative of recent or past infection, but they also may be due to non-specific reactions.

In conclusion, the implications of the presence of the agents investigated in the wild minks are poorly known and more studies are needed to determine if this species could serve as a reservoir or may play an important role in their epidemiology. Nevertheless, the information resulting from a representative sample of wildlife tested is useful because of the paucity of information on diseases of free-ranging animals in South America.

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No potential conflict of interest was reported by the authors.

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