Natural distemper infection in stone martens (Martes foina): From infection to neutralizing antibodies

Short Communication

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Declaration of Competing Interest

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Abstract

We report an outbreak of canine distemper virus (CDV) among stone martens (*Martes foina*) in Italy. After being rescued in Northern Italy between April and June 2018, six subjects were kept in a wildlife and exotic animal rescue center in Bologna province. Subjects have been monitored for 15 months in captivity. Within this time-lapse, two subjects died, while among the remaining four, only one showed clinical symptoms referable to distemper. Surviving subjects have been regularly tested for CDV by means of reverse transcriptase-PCR from conjunctival and oropharyngeal swabs for eleven months. The identified viruses belonged to the Wildlife-Europe CDV genetic subgroup. Neutralizing antibodies were detected at the end of the eleven months, when all subjects tested reverse transcriptase-PCR negative. Our findings confirm the circulation of the Wildlife-Europe CDV genetic subgroup (Europe 1/South America 1 lineage) within the Italian wildlife, and improve knowledge on viral infection in stone martens.

Keywords: distemper; phylogenetic analysis; serology; stone marten; wildlife

Canine distemper is distributed worldwide and constitutes one of the most serious contagious diseases of carnivores, fluctuating in disease presentation (Greene and Vandevelde, 2012) and causing high lethality rate. Mustelids are a diverse group of carnivorous mammals known to be very susceptible to distemper (Philippa et al., 2008), displaying clinical signs similar to those in canids including anorexia, oculo-nasal mucopurulent discharge, multifocal dermal hyperkeratosis, emaciation, severe pruritus, intermittent diarrhoea, pneumonia, and rapid death (Mos et al., 2003; Williams et al., 1988). The stone marten (*Martes foina*) is widely distributed in Eurasia (IUCN, 2015), where is considered as possible canine distemper virus (CDV) wildlife reservoir (Myers et al., 1997; Philippa et al., 2008). Nevertheless, no information is available on spontaneous infection within this species. We report an outbreak of canine distemper in six stone martens held in Monte Adone wildlife rescue center in Bologna province.

This was a retrospective study; no stone martens were sampled exclusively for the purposes of this study. Only samples taken to diagnose the aetiology of the clinical disease and to monitor viral shedding and immunity before reintroduction into the wild, were used. Subjects were rescued in Northern Italy in 2018: two (#110A, #110B) at the end of April, three at the beginning of June (#246A, #246B, #246C) and one (#298) at the end of the same month (**Appendix A: Supplementary material**). All the stone martens were young, found in a state of apparent abandonment in anthropised areas (near human dwellings), and entered the structure in good general conditions, with the exception of #298, a male that showed mild sensory depression, which led to suspect to a car collision. Nonetheless, the subject fully recovered in five days. At the entrance of the subjects in the facility, an observation period (ranging from 2 to 30 days) was carried out in a separate isolation area before moving them to a fenced outdoor common area. In July 2018, on day 21 from rescue, subject #246A (female) showed hyperthermia and violent convulsions and died after 19 days (day 40 from rescue). One week later, subject #110A (female) was found dead, without showing any pathological sign in the previous days. On both subjects #246A and #110A, a post mortem CDV one-step real-time reverse transcription-PCR (RT-qPCR)

98 was performed on the RNA extracted from foot pads (**Appendix A: Supplementary material**,
99 Scagliarini et al., 2007) sampled in July 2018. Histological analysis was performed only on subject
100 #246A. In addition, qualitative two-step reverse transcription-PCR (RT-PCR) was performed to
101 amplify the hemagglutinin (H) gene of CDV identified in animals #246A and #110A (**Appendix A:**
102 **Supplementary material,** Demeter et al., 2007); these were subsequently sequenced, analysed by
103 BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and aligned with 116 CDV reference strains
104 (**Appendix A: Supplementary material**) retrieved from GenBank
105 (https://www.ncbi.nlm.nih.gov/genbank/). Phylogeny was reconstructed by MEGA X version
106 10.1.7 (Kumar et al., 2018).
107 Ten days after death of subject #110A, at day 43 from its rescue, subject #298 showed visual
108 impairment, ocular nasal discharge, as well as lip ulcers and was quarantined. No symptoms were
109 reported during the following period for the remaining three subjects.
110 The four surviving animals were quarantined and viral RNA shedding monitored to identify when
111 they were no longer potentially infectious. Starting from the end of August 2018, all subjects were
112 tested seven times within nine months for CDV RNA by one-step RT-PCR from conjunctival and
113 oropharyngeal swabs (**Appendix A: Supplementary material**, Frisk et al., 1999). At the end of the
114 isolation period (June 2019), presence of neutralizing antibodies was also investigated to assess
115 whether animals were protected from reinfection before reintroduction into the wild (**Appendix A:**
116 **Supplementary material**, Confer et al., 1975).
117 Histology on entrails from subject #246A revealed the presence of both enteritis with de-
118 epithelialized, shortened and fused intestinal villi as well as interstitial pneumonia. Post-mortem
119 RT-qPCR for CDV from foot pads of subjects #246A and #110A tested positive with a quantity of
120 17 and 2.8 x 10^3 viral RNA copies/mg of tissue, respectively. Analyses performed on complete
121 CDV H gene nucleotide sequences (1824 bp) obtained from #246 and #110A (GenBank ID
122 MW015089 and MW015090) showed a unique non-synonymous nucleotide mutation in position
123 268 (corresponding to amino acid residue 90) that distinguished them with an overall nucleotide

identity >99,8% to the closest strain available in GenBank (ID MN044701). This strain was identified in a fox in North-Eastern Italy in 2018 (Bianco et al., 2020). Phylogeny revealed that the infection was caused by viruses belonging to the Wildlife-Europe CDV genetic subgroup, Europe 1/South America 1 lineage (Bianco et al., 2020; Panzera et al., 2015) (**Figure 1**), closely related to other CDV recently identified in wildlife and dog.

In **Table 1** the results of RT-PCR on oropharyngeal and conjunctival swabs sampled from survived animals are reported. Stone martens #298 and #110B tested positive until the third month (October 2018), while the remaining two subjects #246B and #246C tested positive until the fifth month (December 2018). Virus neutralization (VN) test revealed positive antibody titres of: 1:32 for subject #246C, 1:64 for both #110B and #298, and 1:128 for #246B.

Here we report a natural outbreak of canine distemper in six stone martens that showed different courses: two of them died, one showed mild signs while the remaining three displayed no symptoms. For all the animals the exact time of exposure is unknown. Nevertheless, it can be speculated that subject #246A might have been the source of infection, entering the structure during an incubation phase of the disease and developing convulsive crisis in July 2018, after 21 days, and dying 19 days later, in line with the data described in ferrets (Perpiñán et al., 2008). Furthermore, a concurrent subclinical infection of subject #298 cannot be excluded. Indeed, the mental dullness presented by the subject, initially ascribed to a car collision, might have been caused by the transient fever that occurs within the first days post CDV infection. Subsequent spread of the virus in the organism would have produced nasal and ocular discharge and lip ulcers 38 days later. As a matter of fact, asymptomatic carriers have been documented among other mustelids such as minks (Zhao et al., 2015). Also in dogs, it is estimated that at least 50% of CDV infections are subclinical (Peterson and Kutzler, 2011). The intermittent but protracted detection of the viral RNA (attributable to the sampling technique, the storing procedure between sampling and testing and the RT-PCR assay sensitivity) suggests that viral RNA excretion long lasted in all subjects. More specifically, in subjects #298 and #110B the viral excretion lasted at least three months while in the

150 remaining two subjects at least five months. This persistence recalls the pathogenesis described in
151 dogs, where shedding of virus can continue for as long as three to four months (Sykes, 2014).
152 Animals shed the virus in all secretions and since CDV is known for not persisting long in the
153 environment, mildly affected and recovering infected animals may play an important role in
154 maintaining transmission cycles in shelters (Miller and Hurley, 2009). This is particularly important
155 within rescue centres, housing animals of unknown medical histories and endangered wildlife. In
156 fact, CDV is considered of growing concern due to its propensity for host-switching and emergence
157 in new species, including endangered species (Feng et al., 2016; Sulikhan et al., 2018)..
158 Clinical manifestations and histological results confirmed that the gastrointestinal tract, the
159 respiratory and nervous systems were the most affected in stone martens, as already documented in
160 other mustelids (Perpiñán et al., 2008).
161 Morbilliviruses induce long-term immunity, in particular, immune response towards CDV infection
162 is considered lifelong (Schultz et al., 2010), thus detection of seropositive animals implies
163 protection against the virus. Sero-conversion of mink, in experimental conditions, has been
164 described to take place at 14 days post infection (dpi) reaching the highest titres at 21 dpi (Zhao et
165 al., 2015). Since we did not test subjects so early we cannot compare our results, nevertheless, it is
166 assumable that, as described in dogs, clinical signs resolve and the virus is cleared from most body
167 tissues when antibody titre increases (Greene and Vandevelde, 2012). Indeed, the neutralization test
168 is still considered the gold standard for measuring protection against infection, and antibody titres
169 correlate with the level of protection (Appel and Robson, 1973).
170 The identified viruses belonged to the Wildlife-Europe CDV genetic subgroup (Europe 1/South
171 America 1 lineage). This wildlife well-adapted CDV genetic group has been detected in Europe
172 (Monne et al., 2011). In Italy, it also caused the first spillover event, from wildlife to a non-
173 vaccinated domestic dog (Bianco et al., 2020). Coevolution and phylogenetic clustering of CDV
174 identified in both wildlife and domestic dogs have previously been reported (Piewbang et al., 2020),
175 describing an intricate epidemiological dynamics characterised by multiple host infections and

interspecies transmission (Duque-Valencia et al., 2019). In this light, the central role of wildlife in the disease epidemiology should be exploited (Anis et al., 2020), enhancing passive surveillance of the wide range of susceptible animal species to broaden the evolutionary analysis of CDV, a pathogen that exhibits a high mutation rate, to undertake reliable wildlife conservation strategies.

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Appendix A: Supplementary material

Supplementary data to this article can be found online at doi…

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Figure captions

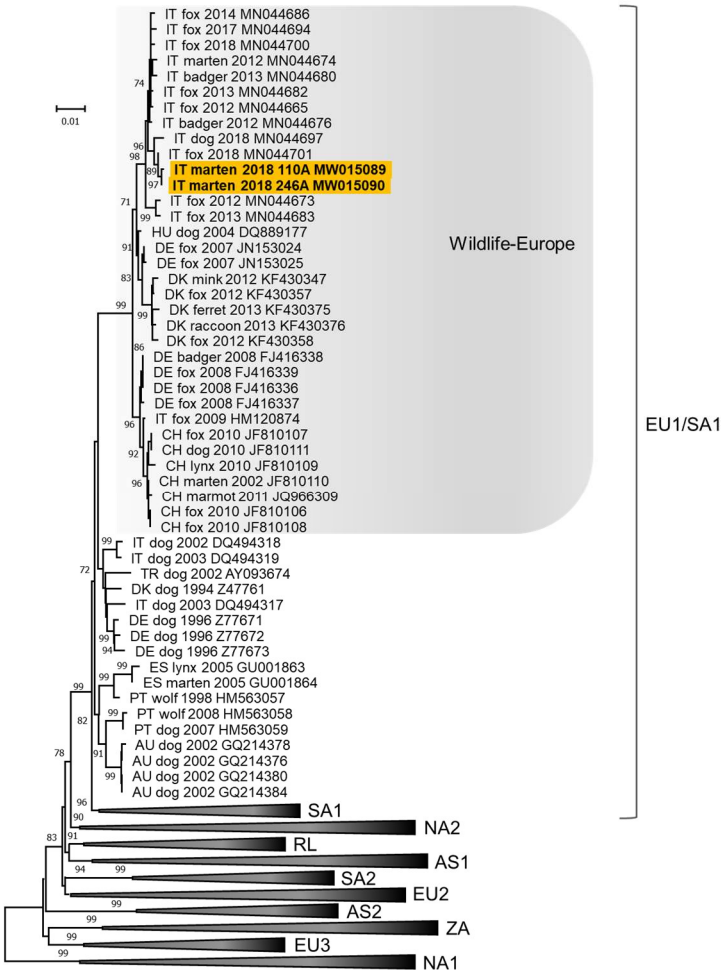

Figure 1. Unrooted phylogenetic tree constructed on the canine distemper virus (CDV) hemagglutinin (H) gene nucleotide sequences.

Phylogeny was carried out on the CDV H gene nucleotide sequences generated in this study (1824 nucleotides) and with sequences of 116 CDV reference strains obtained from the GenBank database (**Appendix A: Supplementary material**) using the software MEGA X version 10.1.7 (Kumar et al., 2018). Phylogenetic tree was constructed using Neighbor-Joining method and the best-fit model of nucleotide substitution was determined using the Find Best DNA/Protein Model function implemented in MEGA X. The Tamura 3-paremeter (T92) model with gamma distribution resulted optimal for the nucleotide alignment. The robustness of individual nodes on the phylogeny was estimated using 1000 bootstrap replicates and bootstrap values >70 were indicated at the corresponding node. The CDV strains included in the phylogenetic analysis are named with:

307 acronym of nation, host species, year of identification, lab numbers only for sequences generated in
308 this study, and the GenBank accession number. When the year of identification was not available,
309 the year of deposition of the nucleotide sequence in the GenBank database was indicated. Canine
310 morbillivirus genetic lineages: America 1 (NA1), America 2 (NA2), Europe 1/South America 1
311 (EU1/SA1), Europe 2/Europe-Wildlife (EU2), Europe 3/Arctic like (EU3), Asia 1 (AS1), Asia 2
312 (AS2), South Africa (ZA), South America 2 (SA2) and Rockborn like (RL). The genetic lineages
313 not containing CDV sequences obtained in this study are compressed. In grey: Wildlife Europe
314 genetic subgroup. Highlighted: sequences generated in this study.
315

316 **Table 1.** Results of reverse transcriptase-PCR (RT-PCR) on oropharyngeal and conjunctival swabs

317 sampled from survived animals

Subject and samples	RT-PCR results at different sampling times						
	August 24, 2018	**September 12, 2018**	**October 16, 2018**	**December 19, 2018**	**February 20, 2019**	**May 27, 2019**	**April 17, 2019**
#110B							
OFS	N	N	N	N	N	N	N
CS	P	N	P	N	N	N	N
#246B							
OFS	P	N	N	N	N	N	N
CS	N	N	N	P	N	N	N
#246C							
OFS	N	N	N	N	N	N	N
CS	P	N	N	P	N	N	N
#298							
OFS	P	P	P	N	N	N	N
CS	P	P	P	N	N	N	N

318

319 CS: conjunctival swab. N: negative. OFS: oropharyngeal swab. P: positive.