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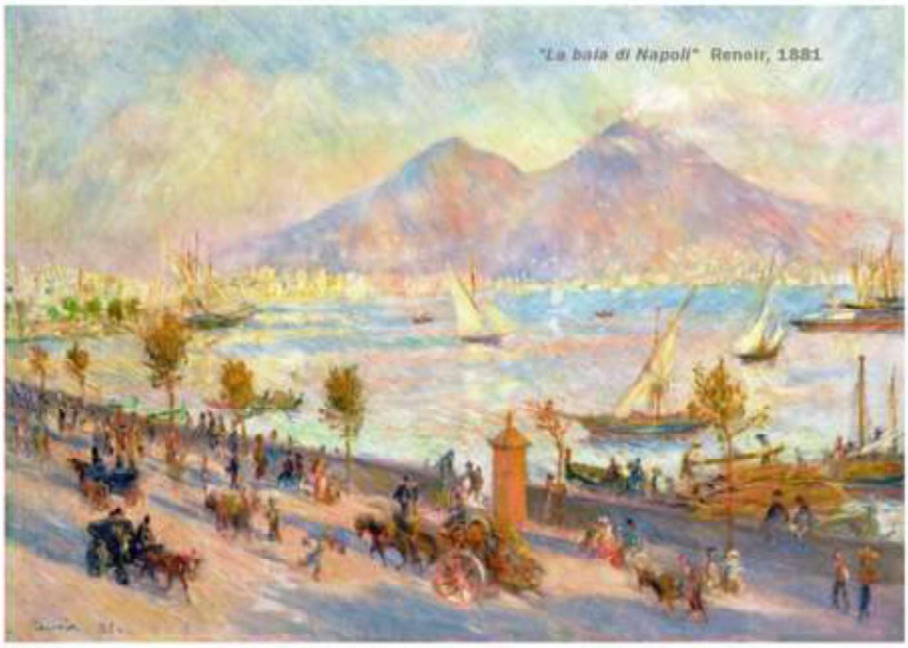

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PREVALENCE OF CANINE DISTEMPER VIRUS IN CANIDS IN CENTRAL ITALY AND FIRST IDENTIFICATION OF ARCTIC LINEAGE IN MARCHE AND UMBRIA REGIONS: A PRELIMINARY STUDY

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Canine distemper virus (CDV) is one of the most commonly virus implicated in outbreaks in wild and domestic carnivores. CDV causes severe systemic diseases which normally involves the respiratory, gastrointestinal and nervous systems. To our knowledge the literature about the real incidence of such disease is scarce, particularly in wild animals population. Recently, outbreaks of CDV have been documented in Italian grey wolves (*Canis lupus italicus*) [1], a least concerned species in IUCN Red List. Therefore, the surveillance of CDV is a priority for the conservation of the wolves and, more generally, for the protection of wild carnivores which are widespread in Central Italy, especially in the National Parks. In total, 215 samples, belonging to 148 canids for CDV presence, were analysed from November 2012 to December 2016 in the laboratory of IZSUM. Of these, 37.2% were dogs, 33% wolves and 29.8% foxes. Animals were collected in 12 different provinces of 6 Regions: Umbria, Marche, Emilia Romagna, Tuscany, Lazio and Apulia. All samples were collected from dead animals which were sent to the Diagnostic Units of Istituto Zoprofilattico Sperimentale Umbria e Marche and subjected to autopsy. The RNA was extracted from organ pools and swabs with a commercial kit, retrotranscripted to cDNA and amplified by the real time PCR with QuantiFast SYBR Green RT PCR kit (Qiagen GmbH, Hilden, Germany) using primers for a fragment of 278bp in CDV nucleoprotein (NP) gene [2]. Samples having a melting temperature (TM) value ±0.5°C versus TM value of positive control were considered positive. Moreover, samples were visualized by UV rays with GelRed TM (Biotium Inc.) after electrophoresis in agarose gels and bands of appropriate sizes were excised, extracted and sequenced. Sequences obtained (n=11) were aligned with NP gene sequences of CDV available in GenBank by MUSCLE. Molecular phylogenetic analysis (MEGA 7.0) was carried out by using Maximum Likelihood method based on the Tamura 3-parameter model. The CDV RNA was identified in 20.3% of the analysed animals. A high positivity rate was identified in dogs with 10.1% of 148 sample tested positive followed by wolves (6.08%) and foxes (5.11%). The Artic Lineage of CDV was identified in 9 out of 11 sequenced samples, in both wild and domestic canids. This strain was identified in 3 different provinces (PU, AP, PG), raising concerns given the vastness of the affected area. Two Onderstepoort strains were also identified. In conclusion, this study shows a wide CDV circulation involving different ecotypes and species in the investigated area. Further studies, based on epidemiological and genetic analysis, will be carried out in order to assess the phylogenetic correlation among the identified strains. This follow up will be important in order to highlight potential risk factors associated with the introduction of this new genotype and to better understand the role played by domestic and wild carnivores interactions in virus spreading. These additional studies should be carried out as soon as possible in order to prevent virus dissemination and to perform ad hoc vaccination campains.

[1] Di Sabattino et al., 2014;Arctic lineage-canine distemper virus as a cause of death in Apennine wolves (Canis lupus) in Italy. [2] A.L.Frisk et al., 1999;Detection of canine distemper virus nucleoprotein RNA by reverse tratranscription PCR using serum, Whole blood, and cerebrospinal fluid from dogs with distemper.