Occurrence of different Canine distemper virus lineages in Italian dogs

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Currently, 9 genetic lineages of Canine distemper virus (CDV) are recognized throughout the world: America 1, America 2, Asia 1, Asia 2, Europe Wildlife, Arctic-like, South Africa, Europe 1/South America 1 and South America 2. In addition, new CDV lineages were recently proposed to include viruses circulating in Asia (Asia 3) and viral strains related to Rockborn vaccine strain (Calderon et al. 2007, Martella et al. 2011, Panzera et al. 2012, Zhao et al. 2010). The attachment/haemagglutinin (H) is the most variable CDV protein and has been used throughout the years to distinguish the different genetic lineages and to classify the CDV circulating strains. Furthermore, the 3’ end of H gene codifies for important amino acid domains involved in interaction between virus and cellular receptor and it has been hypothesized that residues 530 and 549 might affect the viral tropism (McCarthy et al. 2007, Nikolin et al. 2012).

The CDV-related disease in canine population has generally been controlled by live attenuated vaccines, but outbreaks of CDV are still reported worldwide also in vaccinated animals (Martella et al. 2007). Moreover, sequencing of several CDV strains belonging to different genetic lineages has highlighted considerable genetic and antigenic diversities (especially in the H gene/protein). However the effects that these mutations may have on the virulence remain unclear, as well as the susceptibility of different carnivore hosts and the ability of the currently available vaccines to protect from infection (McCarthy et al. 2007, Nikolin et al. 2012, Sekulin et al. 2011).

Three CDV genetic lineages are currently circulating in Italy: the Europe 1 lineage (historically spread in the dogs as well as in wild animals), also named Europe Wildlife and sporadically reported in...
Canine distemper virus in Italian dogs

Table I. Details of 7 CDV strains identified in dogs in Italy between years 2002-2012.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sampling date</th>
<th>Breed</th>
<th>Sex</th>
<th>Age</th>
<th>Origin</th>
<th>Vaccination status</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>444</td>
<td>2002</td>
<td>Labrador retriever</td>
<td>M</td>
<td>2m</td>
<td>Emilia-Romagna</td>
<td>Yes</td>
<td>R + N</td>
</tr>
<tr>
<td>456</td>
<td>2003</td>
<td>German shepherd</td>
<td>F</td>
<td>2m</td>
<td>Emilia-Romagna</td>
<td>No</td>
<td>R + N</td>
</tr>
<tr>
<td>64</td>
<td>2004</td>
<td>Cane Corso</td>
<td>F</td>
<td>1y 3m</td>
<td>Emilia-Romagna</td>
<td>Yes</td>
<td>Gl + N + C</td>
</tr>
<tr>
<td>741</td>
<td>2006</td>
<td>Mixed-breed</td>
<td>Un</td>
<td>Un</td>
<td>Emilia-Romagna</td>
<td>Un</td>
<td>Un</td>
</tr>
<tr>
<td>99</td>
<td>2011</td>
<td>Mixed-breed</td>
<td>F</td>
<td>5m</td>
<td>Lazio</td>
<td>Un</td>
<td>Gl + N</td>
</tr>
<tr>
<td>319</td>
<td>2012</td>
<td>Mixed-breed</td>
<td>M</td>
<td>2m</td>
<td>Lazio</td>
<td>Un</td>
<td>Gl + N</td>
</tr>
<tr>
<td>352</td>
<td>2012</td>
<td>Mixed-breed</td>
<td>F</td>
<td>2m</td>
<td>Lazio</td>
<td>No</td>
<td>N</td>
</tr>
</tbody>
</table>

M = male; F = female; m = months; y = years; R = respiratory symptoms; N = neurological symptoms; Gl = gastrointestinal symptoms; C = cutaneous lesions; Un = unknown.

The nucleotide sequences demonstrate that the analysed CDV strains belonged to 2 clusters. The first cluster included 6 Arctic-like lineage viruses (444.2002, 456.2003, 64.2004, 99.2011, 319.2012 and 352.2012) that showed an identity of 97.7-99.8% among themselves and of 97.2-100% with other Arctic-like strains. In particular, 444.2002 showed a complete identity with 3 North Italian CDVs identified in dogs in 2000-2001 (HM443711, HM443719 and HM443720). The last identified CDV strain (741.2006) belonged to Europe 1 lineage, showing an identity of 93.2-94.8% with viruses of the first cluster and of 95.7-99.8% with Europe 1 strains. Notably, the nucleotide identity calculated by comparing the viruses of the first cluster with Arctic-like reference strains decreases progressively from oldest samples to samples collected more recently. Furthermore, the identities between 741.2006 and 99.2011, 319.2012 and 352.2012 were found to be lower than those calculated with the Arctic-like reference strains.

Figure 1. Rooted phylogenetic tree constructed on nucleotide sequences of the 3' fragment of H gene of the CDV genome (582 base pairs). The phylogenetic tree was constructed using the neighbor-joining method with the nucleotide sequences generated in this study and with sequences of 118 CDV reference strains obtained from the GenBank database and a Phocine distemper virus as outgroup. Bootstrap values greater than 80% are indicated on the respective branches. The CDV strains included in the phylogenetic analysis are named with: acronym of nation, host species and year of identification (with lab numbers for sequences generated in this study), plus the GenBank accession number. When the year of identification was not available, the year of deposition of the nucleotide sequence in the GenBank database was indicated. Highlighted in black: sequences generated in this study. Highlighted in gray: Wildlife Europe 2006-2009 subgroup.
These findings are further supported by the phylogenetic tree, which depicts 6 viruses clustering in the Arctic-like lineage and 741.2006 in the Europe 1/South America 1 lineage (Figure 1). Furthermore, 99.2011, 319.2012 and 352.2012, formed a monophyletic clade inside the Arctic-like lineages, strictly related with a North Italian CDV identified in 2008 (HM443706).

The H protein of the 6 Arctic-like CDVs exhibited residues asparagine (N) at position 530 and tyrosine (Y) at position 549, in accordance with all Arctic-like strains sequenced until now; whereas, in accordance with all Europe 1 strains identified in domestic dogs, 741.2006 exhibited residues glycine (G) at position 530 and tyrosine (Y) at position 549 (Martella et al. 2007, Monne et al. 2011). Some differences were detected in other amino acid positions, as shown in Figure 2. In particular, 99.2011, 319.2012 and 352.2012 exhibit methionine (M) at position 445 and serine (S) at position 559 which were never reported previously in other CDVs. Furthermore, these latter Arctic-like viruses exhibit threonine (T) at position 417 and asparagine (N) at position 435 previously reported in another North Italian CDV strain HM443706.

These data show a considerable spread of Arctic-like lineage in the sampled dogs, in agreement with previous reports on CDV strains collected in Italy which had revealed the presence of Arctic-like CDVs in dogs since 2000 and confirm the dissemination of this novel genotype in Italy (Martella et al. 2006, Martella et al. 2007, Monne et al. 2011). The Arctic-like lineage is native to the Arctic ecosystem and was usually related to infection of wild animals; it was only occasionally associated with outbreaks in domestic dogs from country geographically distant from Italy, such as Hungary, United States of America, China and Greenland (Blixenkrone-Möller et al. 1992, Demeter et al. 2007, Pardo et al. 2005). Subsequently to the arrival of Arctic-like CDVs in Italy, probably due to a significant movement of viruses from the East-Central Europe to Italy in consequence of intense trade of dogs (Martella et al. 2006), the increasing detection of these viruses might suggest that strains belonging to Arctic-like lineage are becoming progressively endemic in Italy. Furthermore, the 3 viruses more recently identified (99.2011, 319.2012 and 352.2012) showed 4 distinctive amino acid mutations compared to all other Arctic CDVs.

Although the study shows a high prevalence of Arctic-like CDV in sampled dogs, the number of viral

![Figure 2. Amino acids differences in the sequence tract of H gene between the 7 viral strains identified in dogs in Italy between years 2002-2012. The ruler at the top shows the amino acid positions corresponding to the entire H protein.](image-url)
strains analysed should be increased, extending the monitoring activities also to other geographical areas, to properly assess the incidence of Arctic-like CDV strains in Italian dogs. Further studies would be necessary to understand what benefits the new amino acid mutations can confer to the Arctic-like CDVs and in which way these may affect their spreading in Italy and in Europe.

References


