



# First description of Canine circovirus infection in red foxes (*Vulpes vulpes*) in Italy

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Canine circovirus (CanineCV) has been firstly reported in 2012 in dogs and, in 2015, a closely related circovirus has been detected in red foxes (*Vulpes vulpes*) (Bexton et al., 2015; Dowgier et al., 2017). Phylogenetic analyses showed that circoviruses detected in dogs and red foxes share >80% nucleotide (nt) identity; consequently, it has been suggested that they belong to the same species (Zaccaria et al., 2016). Studies on the circulation of circoviruses in wild canids are lacking and these viruses have never been detected in red foxes in Italy; however, the possibility that these animals can act as a source of infection for dogs, or vice versa, underlines the need to increase surveillance activities using diagnostic methods able to detect all CanineCV strains circulating in domestic and wild canids (Zaccaria et al., 2016). Aim of this study is to assess the presence of circoviruses in Italian red foxes using a molecular assay targeting a genomic region highly conserved among all viral sequences from canids and to analyse the nucleotide sequences obtained.

## MATERIALS AND METHODS

The study was conducted on -20 ° C stored samples from 32 red foxes shot in 2011 during the regular hunting season in the province of Pisa (Tuscany, Italy). Faeces of all foxes were collected and, for 17 out of 32 of them, kidney and liver samples were also available. Total DNA was extracted and screened using a SYBR Green *Real-time* PCR assay (qPCR) targeting a fragment of 132 nt in the IR between the 3' ends of the two major ORFs highly conserved between all dog and fox circoviruses. Each sample was tested in duplicate and those with an exponential increase in the amplification plot and a specific melting peak in both replicates were considered positive. A rolling circle amplification (RCA) was performed on the positive samples to increase the amount of circular DNA. Subsequently, viral DNA was amplified by PCR using a DNA polymerase with 3'-5' exonuclease activity and amplicons were directly sequenced. The nucleotide sequences obtained were assembled, translated into amino acid, aligned with reference sequences and phylogenetically analysed.

## RESULTS

Circovirus DNA was detected in 1 out of 32 (3.1%) faecal samples (fox number 09-10F); kidney and liver of the positive subject was not available. None of the kidney and liver samples were positive. A full-genome sequence of 2063 nt in length was obtained; it shared from 96% to 86% nucleotide identity with the reference strains of CanineCV from dogs and 86% with those from foxes. The identity of the translated amino acid sequence was between 96% and 89% with dog strains and between 90% and 88% with fox strains for the putative replication associated protein, while, for the putative capsid protein, it ranged from 98% to 93% and 90% to 88% with the dog and fox strains respectively. Phylogenetic analysis showed that the virus 09-10F grouped with most of the sequences of CanineCV obtained from dogs, forming a monophyletic group with strains detected in Asia and clearly separated from those detected in Italy. CanineCV sequences from foxes formed a separate cluster with the canine strain UCD3 (Figure 1).

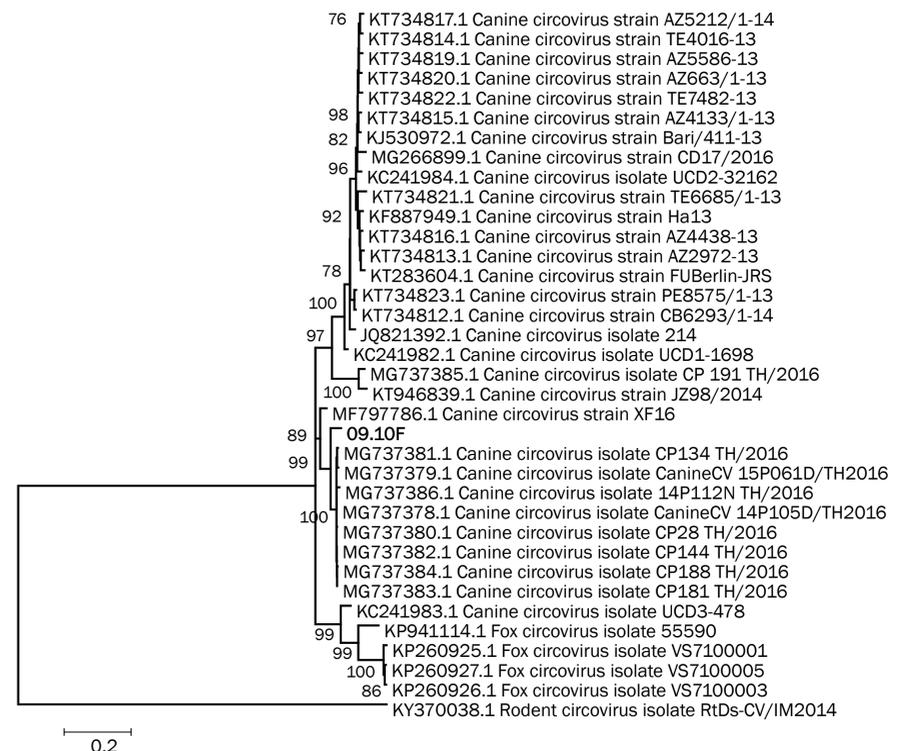


Fig. 1. Phylogenetic tree constructed by maximum likelihood method. The phylogenetic analysis includes the sequence 09.10F recovered from a red fox in the present study (in bold), the sequences of CanineCV reference strains obtained from the GenBank database and a reference sequence of Rodent circovirus from GenBank used as an outgroup. Bootstrap values greater than 70%, calculated on 1000 replicates, are indicated on the respective branches.

## CONCLUSIONS

This is the first report of circovirus circulation among red foxes in Italy. In the only survey previously conducted in the same country, 24 red foxes from the Abruzzi and Molise regions were tested negative using both a *Real-time* PCR assay specific for dog's strain and one for fox's strain (Zaccaria et al., 2016). The prevalence of the infection in red foxes in the present study is significantly lower to that reported in the United Kingdom (62,5%) in a survey carried out on serum samples of 32 red foxes with and without neurological signs (Bexton et al., 2015). The remarkable methodological differences in the two studies make it difficult to draw conclusions from these discrepancies, but further investigation should be conducted to better evaluate the link between circovirus infections and neurological diseases in canids. The present study suggests that foxes and dogs can share similar circovirus strains and probably act as a source of infection for each other. These findings confirm that the use of methods able to detect all CanineCV strains is of fundamental importance for surveillance and diagnostic activities in both domestic and wild canids.

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