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Identification and Characterization of *Fringilla coelebs* Papillomavirus 1 (FcPV1) in Free-living and Captive Birds in Italy

Alice Prosperi,[1] Mario Chiari,[1,3] Mariagrazia Zanoni,[1] Laura Gallina,[2] Giovanni Casà,[2] Alessandra Scagliarini,[2] and Antonio Lavazza[1] [1]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini," Via Bianchi 9, 25124 Brescia, Italy; [2]Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum Università di Bologna, Via Tolara di sopra 50, 40064 Ozzano Emilia (Bologna), Italy; [3]Corresponding author (email: mario.chiari@izsler.it)

ABSTRACT: A papillomavirus (PV) was identified by negative-staining electron microscopy in skin lesions of two bird species (Fringillidae) in Italy. Genetic analyses revealed an FcPV1 with a low genetic variability in the E6, E7, E1, E2, and L1 genes and the long control region when compared to the FcPV1 reference strain.

Papillomaviruses (PVs; *Papillomaviridae*; Van Regenmortel et al. 2000), are host-specific DNA viruses that induce mostly benign, but occasionally malignant, neoplastic lesions in mammals (including humans), reptiles, and birds (Rector and Van Ranst 2013). Virions are spherical, 55–60 nm in diameter, without an envelope, and have a typical, well-defined morphology, regardless of the site and the type of injury (Nasir and Campo 2008). Papillomaviruses have a circular, double-stranded DNA genome of about 8,000 base pairs (bp), which encodes for six early (E1, E2, E4, E6, E7, and E9), two late (L1 and L2) proteins and a regulatory region (LCR; Nasir and Campo 2008; Rector and Van Ranst 2013).

Compared to mammalian infections, only a few reports are available regarding avian papillomatosis and only four species of avian PV have been identified: *Fringilla coelebs* Papillomavirus 1 (FcPV1), *Psittacus erithacus* Papillomavirus 1, *Francolinus leucoscepus* Papillomavirus 1, and *Pygoscelis adeliae* Papillomavirus 1 (Varsani et al. 2014).

Papillomavirus-induced lesions were described in an African Grey Parrot (*Psittacus erithacus*; O'Banion et al. 1992), captive water birds (Zangger and Müller 1990), European Greenfinches (*Carduelis chloris*; Sironi and Gallazzi 1992), and a Northern Gannet (*Morus bassanus*; Daoust et al. 2000). Leg and foot epithelial proliferative lesions were most commonly described in Chaffinches (*Fringilla coelebs*) and rarely in related species, such as Bramblings (*Fringilla montifringilla*; Lina et al. 1973). Reports of PV lesions in Chaffinches and Bramblings date back to 1969 (Blackmore and Keymer 1969), but data, including genomic sequences, are available on the strains circulating in the Netherlands and Sweden (Lina et al. 1973; Moreno-Lopez et al. 1984), the Czech Republic and Germany (Literàk et al. 2003), and Portugal and Spain (Perez-Tris et al. 2011). We describe infectious leg papillomatosis in captive and free-ranging Chaffinches and Bramblings from Italy, and genetically characterize the FcPV1 strains circulating in Italy.

From 2012 to 2014 from Brescia Province (Italy), a wild Brambling and a Chaffinch found dead, along with four captive-reared Chaffinches, all with severe pododermatitis were referred to the diagnostic department of Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (Fig. 1). Diagnostic investigations excluded a correlation between the skin lesions and the causes of death, which were due to trauma in the two wild birds and enteritis in the captive birds. Macroscopically, the skin lesions were consistently hyperplastic and locally extensive and had proliferative papilloma-like nodules in the foot and tarsus-metatarsus, similar to descriptions of lesions induced by FcPV1 (Lima et al. 1973; Literàk et al. 2003).

Tissue samples collected from skin lesions of all six birds were examined by negative-staining electron microscopy (NaPT 2%, pH 6.8), using the Airfuge method (Lavazza et al.

FIGURE 1. Hyperplastic, locally extensive, and proliferative papilloma-like lesions, on the foot and tarsus-metatarsus of a wild Brambling (*Fringilla montifringilla*) from Brescia Province, northern Italy, 2012.

2015). Observations made using an FEI Tecnai G2 Spirit transmission electron microscope (FEI, Hillsboro, Oregon, USA) operating at 85 kV, revealed numerous PV-like virions.

Total DNA was extracted from the lesions of both the wild Chaffinch and Brambling as well as from one of the captive Chaffinches, using the NucleoSpin Tissue Kit (Macherey Nagel, Milan, Italy) in accordance with the manufacturer's instructions. The viral genome was amplified using isothermal rolling circle amplification (TempliPhi DNA Amplification Kit, GE Life Sciences, Milan, Italy), and then analyzed using the restriction enzyme HindIII (Roche, Milan, Italy). Restriction fragments of one of the Chaffinches' samples were cloned (Fast Cloning Pack, Thermo Scientific Fermentas, Milan, Italy), spread (One Shot TOP10, Invitrogen, Milan, Italy), and sequenced (Bio-Fab Research, Rome, Italy). The analysis of 6,844 bp of the genome was performed, aligned, and compared using BIOEDIT 7.2.0 (Ibis Biosciences, Carlsbad, California, USA). The sequences were assembled into two genomic fragments of 4,594 bp (GenBank KU504336) and 2,250 bp (KU504337). The two genomic regions sequenced included E6, E7, E1, E2, a portion of L2 and L1 genes, and LCR. With the exception of a single-nucleotide mutation at nucleotide position 85 of the E2 gene (causing the translation of glutamic acid instead of a glutamine), the strains showed a high similarity when compared with the reference FcPV1 sequence (GenBank AY057109) described by Terai et al. (2002). FcPV1 has been identified by molecular techniques and partially sequenced in Chaffinches in Madeira Island, Portugal, and Balearic Islands, Spain (Perez-Tris et al. 2011; GenBank FN424356 and FN424357). There are two additional sequences of isolates from Dutch and Swedish Chaffinches (Moreno-Lopez et al. 1984; GenBank K02020 and U89669). All belong to a fragment of the L1 gene and show very high similarity to FcPV1 (Terai et al. 2002) but with one change in FN424356 and a gap in K02020. The sequence of the entire L1 open reading frame (GenBank KU504337) from our samples was 100% identical to the reference FcPV1.

In contrast to several reports of the disease in Chaffinches from the UK and Netherlands, limited data are available from the other European countries, including Italy, despite a wide distribution of the species. In Italy, lesions associated with FcPV1 in Chaffinches and Bramblings have been reported only in captive-reared birds, causing economic losses due to the value of these birds, which are used as decoys during hunting practice (Sironi and Gallazzi 1992).

The transmission of FcPV1 was shown between infected birds and other fringillids living together in the same space (Sironi and Gallazzi 1992). In Europe, the FcPV1-induced lesions are not considered a threat to the conservation of Chaffinches because there is no evidence of an association of the disease with increased mortality (Erdélyi 2012). The incidence of this disease in wild birds seems to be low, but when it occurs in a community it establishes an endemic infection leading to a significant clustering of cases in some areas. Even if the transmission of PVs requires close cutaneous or mucosal contact (Rector and Van Ranst 2013), the species-specific nature of the virus, together with the social behavior of the affected species, may lead to local transmis-

sion. For this reason, the pathogenesis, prevalence, and effects on fitness of PV infection on affected populations of Chaffinches and Bramblings should be further investigated.

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