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Evidence of a novel cross-species transmission by ovine papillomaviruses

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Abstract

Ovine papillomavirus (OaPV) comprises four genotypes; OaPV1, OaPV2 and OaPV4 are fibropapillomaviruses within the genus *Deltapapillomavirus*, whereas OaPV3 is an epitheliotropic virus that belongs to the genus *Dyokappapapillomavirus*. To date, all of them have been known to infect sheep only. OaPV1, OaPV2 and OaPV4 have been associated with ovine cutaneous and mucosal fibropapillomas, whereas OaPV3 is a key factor in the squamous cell carcinoma pathway of the sheep skin. Whole blood samples obtained from 128 cattle at public slaughterhouses were investigated using droplet digital polymerase chain reaction (ddPCR). ddPCR is a new-generation PCR technique that enables an accurate and absolute quantification of target molecules with high sensitivity and specificity. All OaPVs were detected by identification and quantification of nucleic acids using specific fluorescent probes. Of 128 blood samples, 100 (~78%) showed OaPV infections. Further, 42, 35 and 23 blood samples showed single, double and triple OaPV infections, respectively. OaPV1 was responsible for 22 single infections, OaPV2 caused 16 single infections and OaPV3 and OaPV4 caused two single infections each. OaPV1 and OaPV2 were the most frequent ovine viruses in dual and triple infections. In many blood samples, both ovine *deltapapillomavirus* and *dyokappapapillomavirus* were found to be transcriptionally active, as shown by the detection and quantification of E5 oncogene transcripts for OaPV1, L1 transcripts for OaPV2, E6 and E7 transcripts for OaPV3 and E6 for OaPV4. OaPVs were found in the blood samples from cattle that shared grasslands rich in bracken ferns known to contain immunosuppressant substances. Furthermore, OaPVs were also found in cattle from intensive livestock farming without any contact with sheep. Because OaPV DNA was detected in both grass hay and corn silage, it is conceivable that these feed may be the viral sources.

KEYWORDS
blood, cattle, corn silage, cross-species transmission, grass hay, ovine papillomaviruses

1 | INTRODUCTION

Papillomaviruses are small, non-enveloped, double-stranded DNA viruses infecting mucosal and cutaneous epithelia of mammals, reptiles, birds and fish (IARC, 2007; Willemsen et al., 2020). As part of the commensal flora, these viruses can be found in the healthy skin and mucosa in a latent state; reactivation occurs following the loss of immunity, resulting in a persistent infection which poses oncogenic

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risk, with occurrence of tumours at several body sites (Sichero et al., 2019; Strickley et al., 2019).

Ovine papillomavirus (OaPV) infections occur in sheep and are caused by four oncogenic genotypes. OaPV1, OaPV2 and OaPV4 belong to the genus *Deltapapillomavirus*, whereas OaPV3 belongs to the genus *Dyokappapapillomavirus* (http://pave.niaid.nih.gov/). Ovine *deltapapillomavirus* is characterized by marked tropism for both mesenchymal and epithelial cells (Tore et al., 2017), whereas OaPV3 exclusively infects epithelial cells (Alberti et al., 2010). OaPVs have sporadically been associated with ruminal fibropapillomas, papillomas, papillomatosis and fibropapillomas of the skin (Gibbs et al., 1975; Hayward et al., 1993; Norval et al., 1985; Tilbrook et al., 1992; Trenfield et al., 1990; Uzal et al., 2000; Vanselow et al., 1982). Although it has been suggested that OaPVs may be responsible for the progression of cutaneous papillomas to squamous cell carcinomas (SCCs) in sheep (Vanselow et al., 1982), a novel OaPV, namely OaPV3, was only recently identified in a high number of SCCs in sheep, suggesting that OaPV3 could represent a key infectious agent in the onset of SCC in ovine species (Alberti et al., 2010; Vitiello et al., 2017). OaPV3 and OaPV4 are well characterized molecularly, as they are the only OaPVs identified in tumour samples from sheep. Indeed, it has been shown that the E6 and E7 oncogenes of OaPV3 and OaPV4 can immortalize primary sheep keratinocytes and regulate the levels of proliferative proteins such as cyclin A and cyclin-dependent kinases. However, it has been suggested that only OaPV3 E7 can strongly promote the cleavage and degradation of ovine retinoblastoma protein (pRb) (Tore et al., 2019). Calpain-mediated cleavage of pRb may result in the dysregulation of E2F transcription factors, which play crucial roles in the cell cycle, cell proliferation and viral replication (Darnell et al., 2007; Scarth et al., 2021). OaPV1 and OaPV2 are not well characterized molecularly so far; however, it has been postulated that they could be associated with tumours in sheep. DNA sequences related to OaPV2 E5 have been found in the mass of the buccal cavity of a pig, suggesting that similar to bovine *deltapapillomaviruses*, ovine fibropapillomaviruses may also be responsible for cross-species transmission (Munday et al., 2020). Unlike OaPV3 that induces cell transformation by the E6 and E7 oncoproteins (Tore et al., 2019), ovine *deltapapillomaviruses* may exert their main oncogenic activity through the oncoprotein encoded by the E5 gene, as verified in most artiodactyl fibropapillomaviruses (Munger & Howley, 2002). OaPV1, OaPV2 and OaPV4 are fibropapillomaviruses and belong to the *Deltapapillomavirus* clade, which is known to encode the most highly conserved E5 oncoproteins (Van Doorslaer, 2013); this is likely because of the integration of the E5 ORF in the genome of an ancestor of the genus *Deltapapillomavirus* occurring between 65 and 23 million years ago (Garcia-Vallvé et al., 2005).

Recently, the first systematic research on the molecular epidemiology of OaPV infection was conducted in sheep and revealed a divergent geographical prevalence of OaPV genotypes. Furthermore, this survey showed a high prevalence of OaPV infection as OaPV DNA was found in up to 76.4% of the peripheral blood of apparently healthy sheep (De Falco et al., 2021b).

This study aimed to provide evidence of a novel cross-species transmission and infection by OaPVs which were detected, quantified and found to be expressed in blood of cattle.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

We did not perform any animal experiments in this study. All samples were collected post-mortem from slaughterhouses, and hence, ethics approval was not required.

2.2 | DNA extraction from blood and matrix samples

Blood samples of 128 healthy cattle aged 2–14 years were collected from regions of southern Italy (Basilicata, Calabria, Campania). All animals were free at pasture, except for 30 cattle from intensive dairy livestock farming. Whole blood samples harvested in ethylenediaminetetraacetic acid-containing vacutainers were obtained from healthy cattle at public slaughterhouses. Total DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Wilmington, DE, USA) according to the manufacturer's instructions. Furthermore, DNA was extracted from 10 samples of grass hay and 10 maize silage (used as feed for cattle) obtained from intensive dairy livestock farming. The DNeasy plant Mini Kit (Qiagen, Wilmington, DE, USA) was used for the extraction of hay and maize DNA, according to the manufacturer's instructions.

2.3 | Environmental DNA (eDNA) extraction

The environmental DNA was harvested by the sponge Whirl-Pak method. Samples from the intensive farm were obtained from the wheels of tractors, a floor plan of the pickup used to transport hay, a chopping and mixing wagon. Samples were also obtained from hand surfaces of people working at this farm. Sponge swipe was performed using a $3.8 \times 7.6 \times 1.5$ cm^3 sterile sponge (VWR International, Radnor, PA, USA) moistened with sterile peptone water (LabRobot products, Stenungsund, Sweden) as previously described (Cardinale et al., 2022). Transport to the laboratory for analysis was completed within 24 h of sampling. A fragment of about 300 mg was cut from the sponges through sterile scalpels and immersed in 80 μL Proteinase K solution (20 mg/ml) and 720 μL of ATL buffer (Qiagen, Hilden, Germany). Samples were incubated at 37°C for 60 min, followed by 15 min at 60°C, and all liquid was recovered squeezing the sampling sponge. Finally, after centrifugation at $3000 \times g$ for 5 min to sediment debris, the liquid was collected, measured (on average ~600 μL) and were placed on the columns of the kit, and the DNA extraction was continued with DNeasy

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Blood & Tissue Kit (Qiagen, Wilmington, DE, USA), according to the manufacturer's instructions.

2.4 | Positive controls

The positive controls for ovine *Delta* and *Dyokappa Papillomaviruses* were obtained as previously reported (De Falco et al., 2021b).

2.5 | Droplet digital polymerase chain reaction (ddPCR)

Table 1 lists the primers and probes used for droplet digital polymerase chain reaction (ddPCR). Primers and probes were obtained as a mixture containing a primer-to-probe ratio of 3.6 (final concentration of 900 nM of each primer and 250 nM of probe). For ddPCR, a Bio-Rad QX100 ddPCR system was used according to the manufacturer's instructions. The reaction was performed in a final volume of 22 μL and contained 11 μL of ddPCR supermix for probes (2X; Bio-Rad Laboratories, Hercules, CA, USA), 1 μL of OaPVs primer and probe mixture, 7 μL of DNA samples (corresponding to 100 ng) and 3 μL of DNAase-free water. The plate containing the reactions was subsequently transferred to an automated droplet generator (AutoDG; Bio-Rad Laboratories, Hercules, CA, USA). AutoDG added 70 μLof droplet generation oil to each well, and each sample was partitioned into approximately 20,000 stable nanodroplets. The droplet generator transferred each row of 8 droplet emulsion (40 μL) champions into a new 96 well PCR plate, which was subsequently coated with a pierceable film heat-sealed using a PX1 PCR Plate Sealer (Bio-Rad Laboratories, Hercules, CA, USA). PCR amplification was performed using a T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) with the following thermal profile: hold at 95°C for 10 min, 40 cycles of 94°C for 30 s, 58°C for 1 min, 1 cycle at 98°C for 10 min and ending at 4°C. After amplification, the plate was loaded onto a droplet reader (Bio-Rad Laboratories, Hercules, CA, USA), and the droplets from each well of the plate were read automatically. The data were analysed using the QuantaSoft analysis tool (Bio-Rad Laboratories, Hercules, CA, USA). Poisson statistics were used to calculate the absolute concentration of the OaPV DNA in each sample. A manual threshold line was used to discriminate between positive (blue) and negative (grey) droplets. There were also differences in the fluorescence amplitude range of the background (negative) droplets among the OaPV samples: 1000–2500 for OaPV1 E5, 3500–7000 for OaPV2 L1, 500–2700 for OaPV3 E6, 2000–7000 for OaPV3 E7 and 1000–4000 for OaPV4 E6. Therefore, the ddPCR results could be directly converted into copies/μl in the initial samples simply by multiplying them by the total volume of the reaction mixture (22 μL) and then dividing that number by the volume of the DNA sample added to the reaction mixture (7 μL) at the beginning of the assay. Each sample was analysed in duplicate. According to previous studies on papillomavirus detection and quantification using ddPCR (De Falco et al., 2021a; De Falco et al., 2021b; Jeannot et al., 2016, 2021), blood samples were considered OaPV-positive in

TABLE 1 Primers and probes used for the detection and quantification of OaPVs

	Forward 5′ 3′	Reverse 5′ 3′	Probe	Region	Size-bp	Ref
OaPV1	CCTGATTCTATGACTGTAAGAGGC	CTCCCCACAGAAGTCCAAG	TGCAACAGCAGAGTCCCATCAGAAG FAM	E5 5′UTR/ORF E5	119	Sichero et al. (2019)
OaPV2	AGTTCCCGCTCTGATTTACC	ATGGCGGACGTATACTTGTTC	ATTGCCAGCAGTCTCCTCAGTCATTC FAM	Major capsid protein	134	
OaPV3 E7	AGCCCACACTCCCTGATATAG	TTCAGTCTTTGACAGCACCTC	AGCAACCAGCACTGTACACGCTAT FAM	E7	145	
OaPV3 E6	AACTATGCAGGAATGTACGAGG	AGTTTCTCTGACAGGTTGCAC	TTGAGCTGGATGTGAGGTGTGTGAC FAM	E6	145	Sichero et al. (2019)
OaPV4	GGGTTCTATGGTGTCTGCTTAG	GCTCAAAATGGTCTACTGTTGC	CAGGAATGCTCTGTGCAGGGTATAGTG FAM	E6	102	Sichero et al. (2019)

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the presence of at least three positive droplets at the same amplitude as positive controls. A sample was considered OaPV-negative when fewer than three droplets or no droplets containing OaPV amplicons were observed.

2.6 | Limit of detection (LoD) determination

The four OaPV genes were detected using ddPCR standard curves of the positive controls used in the serial dilutions. A calibration curve of the positive sample dilutions (log 10) was plotted against the number of PCR cycles. The linear range was determined by diluting the positive controls from 10^5 to 10^{-1} copies/μL, detecting each dilution thrice, taking the average value and correlating the result with the theoretical value. The lower detection limit obtained by ddPCR, with values <1 copies/μL, indicated high sensitivity.

2.7 | PCR

PCR was performed with DNA isolated from the blood, grass hay and maize silage samples using EconoTaq PLUS (Lucigen, WI, USA) and the OaPV primers used for ddPCR (see Table 1) according to the manufacturer's instructions. Conditions for PCR were as follows: 94°C for 2 min; 35 cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 20 s; final extension at 72°C for 5 min. All the amplicons were sequenced.

2.8 | Statistical analysis

To evaluate the actual differences in the prevalence of the four types of Papillomaviruses in the same animals, the Cochran–Armitage test was performed. $p < .05$ was considered to be statistically significant.

2.9 | RNA extraction and reverse transcription–polymerase chain reaction

Total RNA was extracted from 34 blood samples using an RNeasy Plus Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Genomic DNA was removed from the RNA samples using RNase-free DNase I (Fermentas Life Sciences, Thermo Fisher Scientific, MA, USA). One microgram of the Total RNA was used to generate a single strand of cDNA using the QuantiTect Reverse Transcription Kit (Qiagen TM, Germany), according to the manufacturer's instructions. PCR was performed on samples with and without reverse transcriptase (RT) added to the reaction mix, using the same primers reported in Table 1. The conditions used for PCR were 94°C for 5 min, followed by 35 cycles at 95°C for 30 s, 58°C for 30 s and 72°C for 30 s. All the amplicons were sequenced.

2.10 | One-step reverse transcription (RT)–ddPCR

Total RNA was extracted from 34 healthy cows (as negative controls) as previously reported. Total RNA of 100 ng was used for one-step RT–ddPCR Advanced Kit for Probes (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The reaction was performed in a final volume of 22 μL, containing 11 μL of ddPCR SuperMix 2X for probes, 1 μL of primer and probe mix for OaPVs (Table 1), 2 μL RT and 1 μL DTT. The plate was transferred to an automated droplet generator (AutoDG, Bio-Rad Laboratories, Hercules, CA, USA) as described above. PCR amplification was carried out on a T100 Thermal Cycler (Bio-Rad Laboratories Hercules, CA, USA) with the following thermal profile: 50°C for 60 min, 95°C for 10 min, 40 cycles of 94°C for 30 s, 58°C for 1 min, 1 cycle at 98°C for 10 min and ending at 4°C. After amplification, the plate was loaded onto a droplet reader (Bio-Rad Laboratories, Hercules, CA, USA), and the droplets from each well of the plate were read automatically. Therefore, the ddPCR results could be directly converted into copies/μL in the initial samples simply by multiplying them by the total volume of the reaction mixture (22 μL) and then dividing the number by the volume of the RNA sample added to the reaction mixture (5 μL) at the beginning of the assay. Each sample was analysed in duplicate.

2.11 | Sequence analysis

PCR products from DNA and cDNA were purified using a QIAquick PCR Purification Kit (Qiagen TM, ME, DE) and bidirectionally sequenced using a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) following the manufacturer's recommendations. Sequences were purified with a DyeEx_2.0 spin kit (Qiagen TM, DE) and run on a SeqStudio Genetic Analyzer (Thermo Fischer Scientific, CA, USA). Electropherograms were analysed using Sequencing Analysis v5.2 and sequence scanner v1.0 software (Thermo Fischer Scientific, CA, USA). The obtained sequences were compared to other sequences in GenBank using BLAST.

3 | RESULTS

OaPV DNA was detected in 100 of 128 whole blood samples (~78%) examined through ddPCR. In particular, OaPV1 DNA quantification ranged from 0.22 to 34.4 copy number/μl, OaPV2 DNA from 0.23 to 14.92 copy number/μL, OaPV3 DNA showed a range from 0.25 to 6.22 copies/μl and finally OaPV4 DNA from 0.25 to 12.03 copies/μL. These detailed results are listed in Figure S1.

Among 100 OaPV positive samples, single infections were detected in 42 (42%). OaPV1 and OaPV2 infections were the most representative being found in 22 (52.4%) and 16 (38%) single infections. OaPV3 and OaPV4 DNAs were found in two single infections (4.8%). Figure 1 summarizes these results. Differences in OaPV DNA genotype

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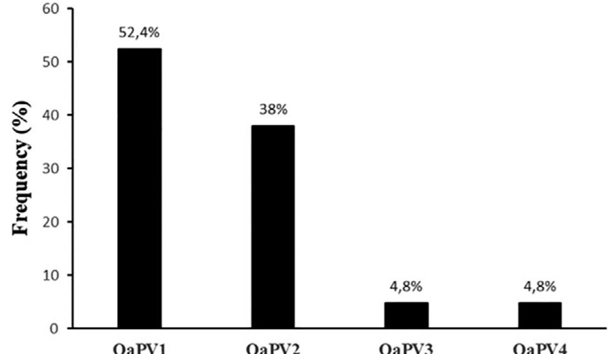

FIGURE 1 Detection rates of OaPV single infections using droplet digital polymerase chain reaction (ddPCR). Differences were significant after the Cochran–Armitage test ($p < .05$).

TABLE 2 Genotype coinfections evaluated by droplet digital polymerase chain reaction (ddPCR) with number and related percentages of their combination.

Coinfections	Genotype combination	Number
Double (35%)	OaPV1/OaPV2	23
	OaPV1/OaPV3	6
	OaPV2/OaPV3	4
	OaPV2/OaPV4	1
	OaPV3/OaPV4	1
Triple (23%)	OaPV1/OaPV2/OaPV3	18
	OaPV1/OaPV2/OaPV4	5

detection were statistically significant, using the Cochran–Armitage test ($p < .05$).

Table 2 summarizes the results of the coinfection we detected. Coinfections were observed in 58 out of 100 (58%) positive samples. In particular, 35 (35%) were dual infections, and 23 (23%) were triple infections. Dual coinfections by OaPV1/2 were the most frequent being observed in 23/35 (~66%); coinfections by OaPV1/3, OaPV2/3, OaPV2/4 and OaPV3/4 were more rarely detected. Coinfections with OaPV1/2/3 were the most frequent triple infections, as observed in 18/23 samples (~78%). OaPV1/2/4 infections have also been reported. However, OaPV1 was the most prevalent genotype in multiple coinfections being detected in 52 of them. OaPV2, OaPV3 and OaPV4 were detected in 51, 29 and 7 samples, respectively.

PCR analysis, using DNA isolated from blood samples, detected amplicons using primers specific to all OaPV genotypes. Sequencing revealed the presence of DNA fragments with 100% identity with OaPV1 E5, OaPV2 L1, OaPV3 E7 and OaPV4 E6 DNAs reported in GenBank (accession number: U83594.1., U83595.1., NC_038516.1 and KX954121.1, respectively) (Figure S2).

One-step RT–ddPCR was performed on 34 randomly selected positive samples. We detected and quantified the transcripts of OaPV1 E5 and OaPV2 L1 as well as transcripts of OaPV3 E6, E7 and OaPV4 E6, which showed that all OaPV genotypes can be transcriptionally active in healthy cattle. The Bio-Rad system quantified mRNA in copies/μL. Samples were considered positive if they had at least three or more positive droplets at the same amplitude as the positive control (Figure S3). Details of this investigation are reported in Figure S4.

Furthermore, we performed RT–PCR analysis of RNA from the whole blood samples. We detected amplicons, the sequencing of which showed 100% identity with OaPV1 E5, OaPV2 L1, OaPV3 E6 and E7 and OaPV E6 mRNAs reported in GenBank, thus validating the one-step RT–ddPCR results (Figure S5).

OaPV coinfections were most prevalent in cattle that shared grasslands with sheep by ddPCR. In cattle from intensive dairy farms without any apparent contact with sheep, double coinfections with OaPV1 and OaPV2 were also observed (detected in 11 out of 30 examined blood samples). OaPV1 E5 and OaPV2 L1 DNAs were found in feed composed of grass hay and corn silage. ddPCR detected and quantified high copy numbers of OaPV1 DNA as it was found in three grass hay samples (from 4.2 to 7.7 copies/μL) as well as in three feed composed of maize silage (from 3.43 to 5.7/μL copy number). OaPV2 DNA was also detected in grass hay (up to 14.4 copies/μL) and corn silage samples (up to 10.9 copies/μL) (Figure S6A). PCR analysis performed on all these matrix samples revealed amplicons, and the sequencing of obtained DNA fragments showed 100% identity with OaPV1, and OaPV2 DNA deposited in GenBank (Figure S6B).

DNA of OaPV1 as well as OaPV2 was also detected on vehicles, farming equipment and workers.

4 | DISCUSSION

Oncogenic OaPVs have so far been found to infect only ovine species. Accordingly, all benign and malignant OaPV-related tumours have been described exclusively in sheep. OaPVs have never been associated with any pathology in other domestic animals. This study shows, for the first time, that both ovine *Delta* and epitheliotropic *Dyokappa Papillomaviruses* can be found also in cattle (*Bos taurus*). All OaPV genotypes were detected through both DNA detection and transcriptionally active forms in blood samples from healthy cattle. Transcripts of E oncogenes as well as the L1 gene were peculiar molecular findings of this study, which suggested that the biological properties of OaPVs might be characterized by inducing both abortive and productive infections in cattle. Persistent OaPV infection can result in cattle carcinogenesis (Roperto, manuscript in preparation).

The scant information on the epidemiology of OaPV infections is the reason for the poor understanding of the biological significance of these viruses in cattle. Papillomavirus transmission by blood in sheep and other species appears to be a key event in their pathology and epidemiology (Cutarelli et al., 2021). It has been suggested that the blood infected with papillomavirus yields infections at permissive sites with detectable viral DNA, RNA transcripts and viral proteins (Cladel et al., 2019; Syrjänen & Syrjänen, 2021). Similar to humans, it is conceivable that ecological factors can influence the virulence of several papillomavirus from different genera, and the concomitant ecological changes in different hosts linked to the human domestication of farm animals, including sheep, may have increased their susceptibility to OaPV

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cross-species transmission and/or simply increased the frequency of physical contact to grant OaPVs improved access to a potential new host.

PVs have a long history of co-divergence with their hosts, and hence, these viruses are relatively more host-specific than other viruses (Geoghegan et al., 2017). Indeed, to date, bovine *Deltapapillomaviruses* have been the only papillomavirus responsible for documented cases of natural cross-species transmission leading to carcinogenetic events via oncoproteins encoded by E genes (IARC, 2007; Roperto et al., 2013). Most OaPV-harbouring cattle in this study have in common highlands rich in bracken ferns with sheep that live in the same geographical zones. Close physical proximity and/or sharing of grazing lands may be a prerequisite for papillomavirus types to cross host-species barriers, as suggested by the detection of various BPVs in other hoofed domestic animals (Cutarelli et al., 2021; de Villiers et al., 2004; Roperto et al., 2021). It is conceivable that animal husbandry practices and/or mammalian sympatry may contribute towards the cross-species transmission of OaPVs. This is corroborated by the fact that a high prevalence of OaPVs was found in cattle sharing large enclosures with sheep, which facilitates direct and indirect contacts. Therefore, our study strengthens the assumption that cross-species transmission may occur among related hosts inhabiting the same geographic areas (Parrish et al., 2008). Successful cross-species transmission has been suggested to occur among phylogenetically related hosts, likely because they share fewer divergent cell receptors (Murthy et al., 2013). Indeed, it is believed that the closer the phylogenetic relationship between hosts, the more likely it is that a pathogen will be able to jump between them with appropriate exposure. Immunosuppressants of bracken coupled with more frequent exposure between sympatric hosts may help OaPVs jump host species, resulting in host switching.

OaPVs have also been detected in cattle from intensive dairy farms without any apparent contact with sheep. The cows were fed grass hay and corn silage prepared with grass and maize grown using irrigation water from the Volturno river. Papillomaviruses have recently been detected and quantified in surface water of rivers (Iaconelli et al., 2015). Papillomaviruses have been detected in vegetables and irrigation water, and a long-term consumption of HPV-polluted water can be associated with cell transformation (Ghaffar et al., 2018; Itarte et al., 2021). Several papillomavirus genotypes of different genera are known to be responsible for oral infection; however, the information on associated risk factors is still limited (Wong et al., 2018). It is possible that polluted irrigation water may have played an overlooked role in the OaPV epidemiology. The molecular findings of this study appear to strengthen our suggestions as OaPV DNA has been detected and sequenced in hay as well as corn silage samples. However, further studies on virus isolation from these feed should be conducted to better understand the actual risk of virus transmission from feed. It is well known that papillomaviruses can survive without significant loss of infectivity during desiccation, as well as at low pH and high temperatures, chemical features that characterize these feed (Nielsen et al., 2021; Roden et al., 1997). Furthermore, a recent scientific report by the European Food Safety Authority showed that some viruses, including African swine fever virus, can be transmitted through feed based on hay and maize (Nielsen et al., 2021). Finally, it is also conceivable that vehicles, farming equipment and farm resident workers may contribute to spread OaPVs. However, further studies are needed to better understand OaPV transmission mechanistic pathway(s).

As the number of cross-species transmissions continues to rise and viral diseases pose a continual threat to animal populations, understanding the ecological diversity of OaPV prevalence and genotype distribution among new host species in different geographical regions remains essential. The need to understand how papillomaviruses transmit within a given species, as well as to new host species, has become increasingly important as the cross-species transmission of viruses from one host species to another is responsible for the majority of emerging infections that can profoundly affect animal health (Geoghegan et al., 2017).

Finally, both circulating OaPV DNA and OaPV RNA have been reported in the blood samples of cattle found healthy by ante- and post-mortem anatomo-clinical observations, confirming that blood represents an important primary route of papillomavirus infection, and that OaPVs can disseminate to any organ via the bloodstream. We detected and sequenced OaPVs in the urinary bladder of healthy cattle (personal observations), which supports the hypothesis that OaPVs may contribute to the composition of the normal bladder microbiota of cattle. BPV and HPV DNA have also been reported in the blood of healthy cattle (De Falco, Corrado, et al., 2021) and asymptomatic blood donors (Vergara et al., 2019). It has been suggested that there is an actual likelihood that HPVs could reach epithelial target sites in the blood, which might explain how and why HPVs are associated with tumours of several organs (Cladel et al., 2019; Conceição Gomes Nascimento et al., 2021; Syrjänen & Syrjänen, 2021; Vergara et al., 2019). Accordingly, papillomavirus transmission by blood represents a conceptually novel idea, and if accepted more generally, this would change the current thinking about the modes of PV spread, including HPV within the host (Cladel et al., 2019; Roperto et al., 2013; Syrjänen & Syrjänen, 2021). In this context, a naturally occurring OaPV infection may be an additional animal model that sheds light on the papillomavirus biology, including issues related to viral tropism in the One Health approach.

AUTHOR CONTRIBUTIONS

Francesca De Falco and Anna Cutarelli: methodology; data curation; formal analysis and investigation; visualization; Bianca Cuccaro: formal analysis; investigation; Cornel Catoi and Esterina De Carlo: validation; writing – original draft; Sante Roperto: conceptualization, supervision, validation, visualization and data curation; funding acquisition, writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

DATA AVAILABILITY STATEMENT

All relevant data are within the manuscript and its Supporting Information files.

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