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Detection of bovine Deltapapillomavirus DNA in peripheral blood of healthy sheep (*Ovis aries*)

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Summary

Blood samples from 65 sheep were tested for the presence of bovine Deltapapillomavirus (δPVs) DNA. The sheep were divided into three groups. Sheep in groups 1 and 2 were from Sardinia and Campania, respectively, and were in contact with cattle and grazed on lands contaminated with bracken fern. Sheep in Group 3 lived in closed pens and had no contact with cattle. These sheep were fed hay that did not contain bracken fern. Bovine δPV E5 DNA was detected in blood from 24 of 27 (89%) sheep in Group 1. A single bovine δPV type was detected in the blood from nine (33%) sheep, including the detection of bovine δPV-1 DNA in four sheep, bovine δPV-2 in four and δPV-13 in one sheep. Two δPV types were detected in 33% of the sheep, and three bovine δPV types were detected in 22% of the sheep. Bovine δPVs were detected in 17 of 20 (85%) sheep from Group 2. The detection rate by a single δPV type was 40% with just δPV-1 DNA amplified from two, just δPV-2 DNA from four, and just δPV-13 DNA from two sheep. Two and three δPVs were detected in 30% and 15%, respectively. All sequenced amplicons showed a 100% identity with papillomaviral E5 DNA deposited in GenBank. Bovine δPV-14 DNA sequences were not detected from any sheep. No bovine δPV DNA was revealed in blood samples from sheep in Group 3. The detection of bovine δPV DNA in the blood of sheep means that sheep may be able to be infected by these PVs. This suggests that bovine δPVs could potentially be a previously unrecognized cause of disease in sheep. Furthermore, it is possible that sheep could act as a reservoir for these viruses.

KEYWORDS
bladder tumours, bovine Deltapapillomavirus, enzootic haematuria, peripheral blood, sheep

1 | INTRODUCTION

Papillomaviruses are small, non-enveloped double-stranded DNA viruses that have been detected in a variety of animals, including humans (IARC, 2007). While these viruses are most well recognized to infect mucosal and cutaneous epithelia, recent research has suggested that they may also be able to infect a wide range of tissues including peripheral blood mononuclear cells (PBMCs) (Melo et al., 2015; Roperto et al., 2008, 2011).

Infection by bovine Deltapapillomaviruses (δPVs) has biological properties that are characterized by inducing tumours of the skin in the respective hosts (De Villiers, Fauquet, Broker, Bernard, & zur Hausen, 2004). In addition, BPVs appear to be involved in oesophagus (Borzacchiello et al., 2003) and bladder tumours of cattle (Campo, Jarrett, Barron, O'Neil, & Smith, 1992).

Currently, 22 bovine papillomaviruses (BPVs) have been fully sequenced. Based on the degree of nucleotide sequence diversity of the L1 gene, BPVs are classified in five genera: Deltapapillomavirus (δPV) composed of four types (BPV-1, -2, -13, -14), Epsilonpapillomavirus (εPV) comprising BPV-5 and -8, Xipapillomavirus (χPV) composed of BPV-3, BPV-4, BPV-6, BPV-9, BPV-10, BPV-11, BPV-12, BPV-15, BPV-17 and BPV-20, Dyokappapapillomavirus (DyoκPV) comprising BPV-16, BPV-18 and BPV-22 and Dyoxipapillomavirus (DyoχPV) which contains only BPV-7. BPV-19 and BPV-21 have not

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yet been classified within any genus (Bauermann et al., 2017; http://pave.niaid.nih.gov).

Within papillomaviruses, the bovine δPVs are unique in their ability to infect multiple species. Bovine δPV DNA and gene expression have been detected in cutaneous sarcoids in horses (Brandt, Haralambus, Schoster, Kimbauer, & Stanek, 2008; Carr et al., 2001). Papillomaviral DNA and gene expression have also been seen in cutaneous fibropapillomas and bladder tumours of water buffaloes (Pangty, Singh, Goswami, Saikumar, & Somvanshi, 2010; Roperto et al., 2013; Silvestre et al., 2009; Somvanshi, 2011). In addition, bovine δPVs are thought to cause cutaneous sarcoids in African lions (Orbell, Young, & Munday, 2011), domestic cats (Munday et al., 2015), Cape mountain zebras, giraffes and sable antelopes (Williams et al., 2011). Bovine δPV types have also been detected within clinically normal samples of skin from many non-bovine wild ruminants (Savini, Dal Molin, Gallina, Casà, & Scagliarini, 2016).

In sheep, an ovine papillomavirus that appeared to be closely related to bovine δPV-1 and δPV-2 has been detected in precancerous aural lesions by restriction endonucleases (Trenfield, Spradbrow, & Vanselow, 1990). Additionally, the E5 oncoprotein of bovine δPVs (BPV-2 and BPV-13) was recently detected in a series of congenital neoplastic lesions of the skin and oral mucosa of lambs (Lucà et al., 2016). This suggested that sheep may also be infected by bovine δPVs and that this cross-species infection could potentially be a cause of reproductive wastage in sheep.

The purpose of this study was to investigate whether bovine δPV DNA could be detected in the peripheral blood of domestic sheep (*Ovis aries*). The detection of DNA within the blood of sheep would support a potential role of bovine δPVs in disease of sheep and would also demonstrate a potential novel route of bovine δPV spread.

2 | MATERIALS AND METHODS

2.1 | Blood collection

Peripheral blood samples were collected into EDTA-containing tubes from 65 healthy sheep that were subdivided into three groups. Group 1 was composed of twenty-seven 1- to 4-year-old sheep from flocks in Sardinia, an island in central Italy; Group 2 comprised twenty 1- to 12-year-old sheep from flocks in Campania, a peninsular region of southern Italy. All animals from groups 1 and 2 had close contact with cattle and grazed on lands contaminated by bracken fern. Cattle from the pastures, that the sheep grazed, had previously been reported to develop chronic enzootic haematuria and bovine δPV-associated bladder tumours (Russo, Inglese et al., 2017). Group 3 was composed of eighteen 2- to 8-year-old sheep from Campania that lived in a closed pen. These sheep were fed bracken fern-free hay, and they did not have contact with cattle.

2.2 | PCR analysis

Total DNA was extracted from whole blood using a DNeasy Blood & Tissue Kit (Qiagen TM, ME, DE) according to the manufacturer's instructions. PCR was performed with 100 ng of DNA using primers specifically designed to detect a section of the E5 gene from BPV-1, BPV-2, BPV-13 and BPV-14. The following primers were used: E5 BPV-1 forward 5′- GCCTTTTCTTCATCTGACTG-3′, reverse 5′- GCC AGTGATGTAAAGGCATT-3′; E5 BPV-2 forward 5′- TGGTTTCTATT GTTCTTGGGACT -3′, reverse 5′- GGAGCACTCAAAATGATCCCA-3′; E5 BPV-13 forward 5′- CACTGCCATTTGGTGTTCTT -3′, reverse 5′- AGCAGTCAAAATGATCCCAA-3′; E5 BPV-14 forward 5′- CTATG CCTTGTACTTTTACT -3′, reverse 5′-AATAACAACAAGATACGCAT -3′. Amplification conditions for PCR were as follows: 94°C for 5 min, 40 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 30s. Positive controls included the following: DNA extracted from a bovine δPV-1 E5-expressing equine fibroblast cell line EqSO2a (Yuan, Gault, Campo, & Nasir, 2011) and DNA extracted from a urinary bladder tumour that had previously shown to contain bovine δPV-2, δPV-13 and δPV-14 (Roperto, Munday, Corrado, Goria, & Roperto, 2016; Roperto, Russo et al., 2016; Roperto et al., 2010). No template DNA was added in negative controls.

2.3 | DNA sequencing

PCR products were purified by means of a Qiaquick® PCR purification Kit (Qiagen GmbH, Hilden, Germany) and bidirectionally PCR sequenced with a BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's recommendations. Sequences were dye-terminator removed by means of a DyeEx® 2.0 spin kit (Qiagen GmbH, Hilden, Germany) and then run on a 3130 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). Electropherograms were analysed using Sequencing Analysis v5.2 and Sequence Scanner v1.0 software (Applied Biosystems, Foster City, CA, USA). The sequences obtained from the amplicons related to BPV-1, BPV-2, BPV-13 and BPV-14 were compared with those reported in BLAST (http://blast.ncbi.nlm.nih.org). Phylogenetic analysis was carried out using the CLUSTAL Omega Multiple Sequence Alignment Program.

2.4 | Statistical analysis

The Fisher's exact test was used to compare the detection rates of bovine δPVs in sheep from groups 1, 2 and 3, while a Cochran's *Q* test was used to compare the distribution of δPV genotypes within the flocks. All statistical analyses were carried out using SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA), and a *p*-value $<.05$ was considered to be statistically significant.

3 | RESULTS

Papillomaviral DNA was amplified from positive controls as expected with 180-, 106-, 120- and 175-base pair (bp) sections of DNA amplified by the specific primers for bovine δPV-1, δPV-2, δPV-13 and δPV-14, respectively.

Bovine δPV DNA was amplified from 24 of 27 (89%) blood samples from sheep in Group 1. Nine samples (33%) contained DNA from just one bovine δPV type including four samples that contained only δPV-1 DNA, four samples that contained only δPV-2 DNA and one sample that contained only δPV-13 DNA. Fifteen (56%) of the samples contained DNA from multiple bovine δPV types with nine (33%) of samples containing two δPV types and six (22%) contained three δPV types. In total, 15 blood samples contained δPV-1 DNA, 18 contained δPV-2 DNA and 12 contained δPV-13 DNA. δPV-14 DNA was not detected in any sample.

Bovine δPV DNA was amplified from 17 of 20 (85%) blood samples from sheep in Group 2. Eight samples (40%) contained just one PV type including two samples that only contained δPV-1 DNA, four samples that contained only δPV-2 DNA and two samples that contained only δPV-13 DNA. Nine samples (45%) contained multiple δPV types including six that contained two δPV types and three that contained DNA from three δPV types. Overall, nine blood samples contained bovine δPV-1 DNA, 12 contained bovine δPV-2 DNA and eight contained bovine δPV-13 DNA. No blood samples contained bovine δPV-14 DNA. All data related to incidence and distribution of bovine δPVs are summarized in Table S1 and Table S2.

No bovine δPV DNA was amplified from sheep in Group 3.

To confirm that the amplified DNA was papillomaviral, the amplicons detected by the primers specific for bovine δPV-1, δPV-2 and δPV-13 were sequenced. This revealed a 100% identity with the sequences of bovine δPV-1 E5, δPV-2 E5 and δPV-13 E5 DNA deposited in GenBank under accession numbers X02346.1 (Figure 1), KT315735.1 (Figure 2) and JQ798171.1 (Figure 3), respectively.

There were no significant differences in the detection rate of bovine δPVs between sheep of Group 1 and Group 2 (δPV-1: p = .552; δPV-2: p = .548; δPV-13: p = .769). Furthermore, Cochran's Q test showed no statistically significant differences about the distribution of three δPV types between the two groups (Group 1—Sardinia p = .223; Group 2—Campania: p = .395).

4 | DISCUSSION

To the authors' knowledge, this is the first time that bovine δPV DNA has been detected in the peripheral blood of sheep. All sheep that were found to have blood that contained bovine δPV DNA had been in contact with cattle. In contrast, bovine δPVs were not detected in any sheep that had not had contact with cattle suggesting that cattle are the source of bovine δPVs in sheep. While the mechanism of transmission is uncertain, all infected sheep had been in contact with cattle from herds that had previously developed urinary bladder cancer. Such cancers contain active bovine δPV infections suggesting that sheep could have been infected after eating pasture contaminated by cattle urine containing infective virus. Bovine δPV DNA was detected in the blood of sheep from Sardinia, an island of central Italy, and sheep from Campania, a peninsular region in the south of Italy at approximately equal frequencies. This suggests that detection of bovine δPVs in sheep is not confined to one region and suggests that all sheep that have contact with cattle may be potentially infected by bovine δPVs.

Bladder cancer in cattle is also strongly associated with bracken fern, and all sheep that were found to have bovine δPV DNA in their blood had potentially ingested this plant. Ptaquiloside, the major toxic substance of bracken fern, known to impair the immune system, has recently been detected in healthy sheep and goats grazing on lands contaminated with bracken fern (Virgilio et al., 2015). As bracken fern has been shown to cause immunosuppression, it is possible that this is an additional factor promoting cross-species transmission from cattle to sheep. Further studies are required using blood from a wider geographic location to determine whether exposure to bracken fern is required to detect PV DNA in blood or whether infection of sheep by bovine δPVs is a worldwide occurrence.

As PVs typically infected differentiating epithelium, the detection of bovine δPV DNA in blood may seem surprising. However, bovine δPV genes and their expression have previously been found in peripheral blood mononuclear cells (PBMCs) both of healthy cattle and cattle with bladder tumours as well as in blood of healthy and

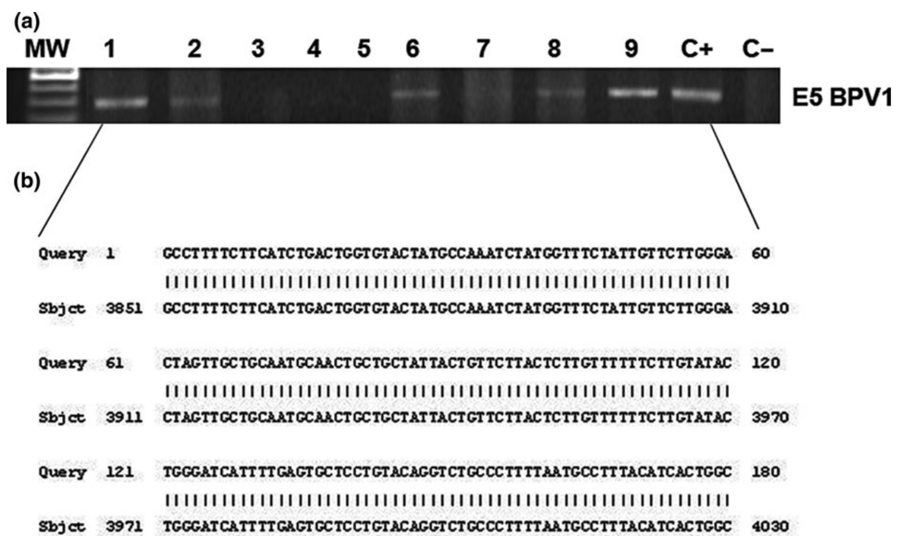

FIGURE 1 Electrophoresis of PCR products. (a) MW = DNA molecular weight marker (100-base pair (bp) ladder) Lanes 1–9: nine representative blood samples from healthy sheep; lane C+: bovine δPV-1 expressing equine fibroblast cell line EqSO2a as positive control; lane C−: PCR negative control (no DNA added). (b) Alignment of the sequences shows 100% identity between the sequence of the amplicons in lanes 1, 2, 6, 8, 9 and the sequences of bovine δPV-1 deposited in GenBank (Sequence ID: X02346.1)

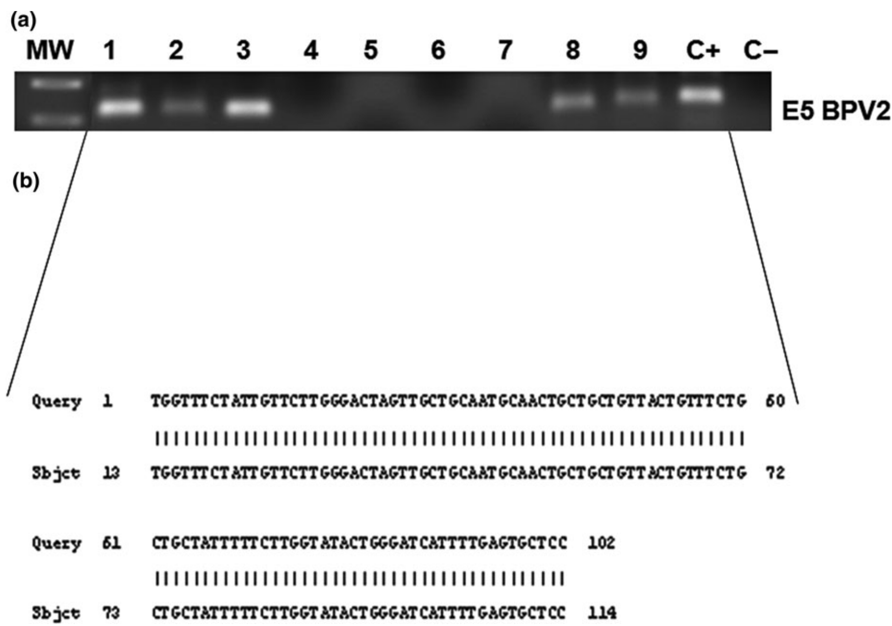

FIGURE 2 Electrophoresis of PCR products. (a) Lane MW = DNA molecular weight marker (100-base pair (bp) ladder) lanes 1–9: nine representative blood samples from healthy ewes; lane C+: bovine δPV-2 DNA associated with bladder tumour as positive control; lane C−: negative control (no DNA added). (b) Alignment of the sequences shows 100% identity between the sequence of the amplicons in lanes 1, 2, 3, 8, 9 and the sequences of bovine δPV-2 deposited in GenBank (Sequence ID: KT315735.1)

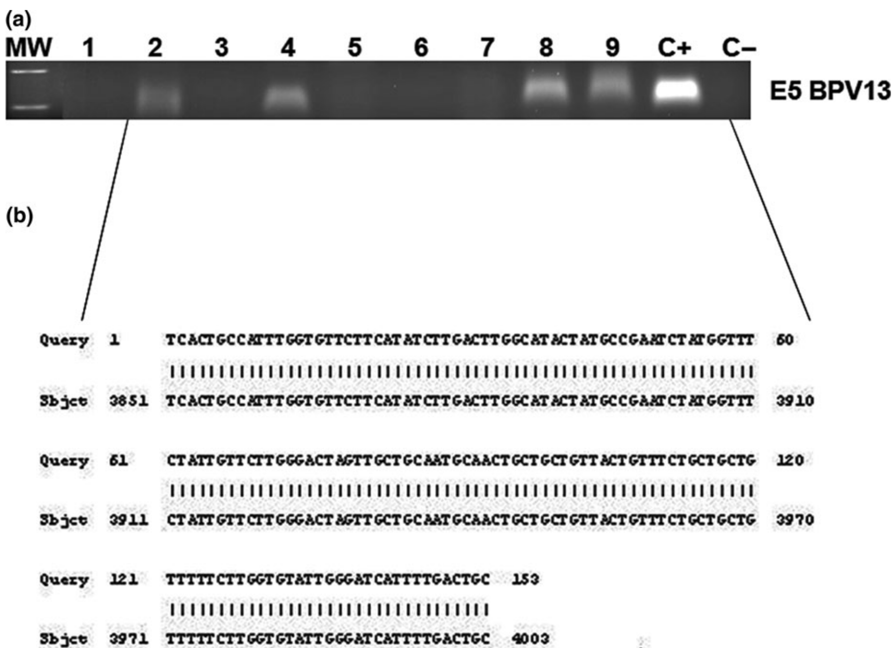

FIGURE 3 Electrophoresis of PCR products. (a) Lane MW = DNA molecular weight marker (100-base pair (bp) ladder) lanes 1–9: nine representative blood samples from healthy ewes; lane C+: bovine δPV-13 DNA associated with bladder tumour as positive control; lane C−: negative control (no DNA added). (b) The lower part of the figure shows 100% identity between the sequence of the amplicons in lanes 2, 4, 8, 9 and the sequence of bovine δPV-13 deposited in GenBank (Sequence ID: JQ798171.1)

sarcoid-affected horses (Brandt et al., 2008; Roperto et al., 2008, 2011). Additionally, DNA from 94 different viruses including many papillomaviruses has been amplified from the blood of healthy humans (Moustafa et al., 2017). This supports the earlier study that detected human alpha, beta or gamma papillomavirus DNA in the blood of 8.3% of healthy male blood donors (Chen et al., 2009). Furthermore, Felis catus papillomavirus type 2 belonging to Dyothetapapillomavirus genus (dyoθFcaPV) was recently detected in the blood of healthy cats (Altamura, Jebara, Cardeti, & Borzacchiello, 2017). Overall, there is increasing evidence that PVs can infect blood cells and the presence of PV DNA in the blood may more common than has historically been recognized.

While the significance of the detection of PV DNA in peripheral blood is still uncertain, it is possible that PVs are able to spread through the body by this route. Therefore, passage through the blood may be a significant cause of disease within the host and an important route of spread among animals. Our results suggest that clinically healthy domestic sheep may represent a reservoir for bovine δPVs. Therefore, it is conceivable that healthy sheep may have a role in intra- and interspecies bovine δPV transmission.

Several viruses can pass the maternal–foetal barrier. As transplacental transmission of human papillomavirus through blood has been reported (Freitas, Mariz, Silva, & Jesus, 2013; Rombaldi, Serafini, Mandelli, Zimmermann, & Losquiavo, 2008), it is possible

that transplacental spread is important in the transmission of human papillomavirus, not unlike several other viruses (Koskimaa et al., 2017). Similarly, transplacental transmission of bovine δPVs has been reported in cattle (Roperto et al., 2011, 2012) and neoplastic lesions of the placenta that were associated with bovine δPV infection have been reported in water buffaloes (Russo, Paciello et al., 2017). Bovine δPV-2 and δPV-13 were detected in congenital neoplastic lesions of lambs and in blood of their mothers. This virus concordance allows us to believe that haematogenous transplacental transmission can occur also in sheep (Roperto et al., manuscript in preparation). There is some evidence that placental infection by common viruses in humans, including viruses thought to be non-pathogenic or to have low pathogenicity, may contribute to placental dysfunction leading to poor pregnancy outcome including spontaneous miscarriage, foetal anomalies and persistent post-natal infection (Arechavaleta-Velasco, Koi, Strauss, & Parry, 2002; Koi, Zhang, & Parry, 2001). If bovine δPVs can spread through the placenta from the blood of sheep and cause foetal or placental disease, it is possible that infection of sheep with bovine δPVs could be a significant cause of decreased foetal reproductive performance of sheep. If this is proven, this would suggest additional control methods may be required to ensure sheep are not infected by bovine PVs.

If bovine δPVs do influence placental and foetal development, this may be due to the expression of the E5 oncoprotein, which has previously been shown to activate the platelet-derived growth factor β receptor (PDGFßR) in the placenta epithelium of pregnant cows (Roperto et al., 2012). As PDGFßR is known to have a role in organogenesis and placenta development (Andrae, Gallini, & Betsholtz, 2008; Chen, Liu, Chen, Chen, & Chen, 2015), PV-induced aberrant PDGFßR activation could conceivably cause dysregulation of the placenta homeostasis leading to organ defects and placental abnormalities (Arechavaleta-Velasco et al., 2002; Hoch & Soriano, 2003).

While sheep in this study were commonly infected by bovine δPV-1, δPV-2 and δPV-13, none of the sheep had blood that contained bovine δPV-14 DNA. δPV-14 is currently thought to be the cause of sarcoids in domestic and exotic cats. Interestingly, this PV has never been reported in an equine sarcoid suggesting that the host range of this PV may be restricted to cows and cats. In contrast, bovine δPV-1, δPV-2 and δPV-13 have been reported to infect a number of ungulate species. The failure to detect bovine δPV-14 in sheep supports the hypothesis that δPV-14, in contrast to the other bovine δPVs, may infect a more limited range of non-bovine species (Munday et al., 2015). Evidence from the present study suggests that δPV-14 is unable to cause cross-species infections in sheep.

In conclusion, the results of this study show that a high proportion of sheep carry bovine δPV DNA within their blood. All the sheep that had bovine δPV DNA had contact with cattle suggesting that the infection occurred due to contact with cattle. Whether or not exposure to bracken fern also promotes infection of sheep by bovine δPVs remains unknown. While none of the sheep in the present study showed clinical signs attributable to bovine δPV infection, it is possible that bovine δPVs could be a recognized cause of disease in sheep.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

SR and FR conceived and designed the experiments; VR and FDF performed the experiments; SR, VR, FC and FDF analysed the data. SR, JSM and FR wrote the manuscript.

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REFERENCES

Altamura, G., Jebara, G., Cardeti, G., & Borzacchiello, G. (2017). Felis catus papillomavirus type-2 but not type-1 is detectable and transcriptionally active in the blood of healthy cats. *Transboundary & Emerging Diseases*. https://doi.org/10.1111/tbed.12732

Andrae, J., Gallini, R., & Betsholtz, C. (2008). Role of platelet-derived growth factors in physiology and medicine. *Genes & Development*, *22*, 1276–1312. https://doi.org/10.1101/gad.1653708

Arechavaleta-Velasco, F., Koi, H., Strauss, J. F., & Parry, S. (2002). Viral infection of the trophoblast: Time to take a serious look at its role in abnormal implantation and placentation? *Journal of Reproductive Immunology*, *55*, 115–121.

Bauermann, F. V., Joshi, L. R., Mohr, K. A., Kutish, G. F., Meier, P., Chase, C., … Diel, D. G. (2017). A novel bovine papillomavirus type in the genus *Dyokappapapillomavirus*. *Archives of Virology*. https://doi.org/10.1007/s00705-017-3443-9

Borzacchiello, G., Ambrosio, V., Roperto, S., Poggiali, F., Tsirimonakis, E., Venuti, A., … Roperto, F. (2003). Bovine papillomavirus type 4 in oesophageal papillomas of cattle from the South of Italy. *Journal of Comparative Pathology*, *128*, 203–206. https://doi.org/10.1053/jcpa.2002.0626

Brandt, S., Haralambus, R., Schoster, A., Kimbauer, R., & Stanek, C. (2008). Peripheral blood mononuclear cells represent a reservoir of bovine papillomavirus DNA in sarcoid-affected equines. *Journal of General Virology*, *89*, 1390–1395. https://doi.org/10.1099/vir.0.83568-0

Campo, M. S., Jarrett, W. F. H., Barron, R. J., O'Neil, B. W., & Smith, K. T. (1992). Association of bovine papillomavirus type 2 and bracken fern with bladder cancer in cattle. *Cancer Research*, *52*, 6898–6904.

Carr, E. A., Theon, A. P., Madewell, B. R., Hitchcock, M. E., Schlegel, R., & Schiller, J. T. (2001). Expression of transforming gene (E5) of bovine

papillomavirus in sarcoids obtained from horses. *The American Journal Veterinary Research*, *62*, 1212–1217. https://doi.org/10.2460/ajvr.2001.62.1212

Chen, A. C. B., Keleber, A., Kedda, M. A., Spurdle, A. B., McMillan, N. A. J., & Antonnsson, A. (2009). Human papillomavirus DNA detected in peripheral blood samples from healthy Australian male blood donors. *Journal of Medical Virology*, *81*, 1792–1796. https://doi.org/10.1002/jmv.21592

Chen, C. Y., Liu, S. H., Chen, C. Y., Chen, P. C., & Chen, C. P. (2015). Human placenta-derived multipotent mesenchymal stromal cells involved in placental angiogenesis via the PDGF-BB and STAT3 pathways. *Biology & Reproduction*, *93*, 103.

De Villiers, E. M., Fauquet, C., Broker, T. R., Bernard, H. U., & zur Hausen, H. (2004). Classification of papillomaviruses. *Virology*, *324*, 17–27. https://doi.org/10.1016/j.virol.2004.03.033

Freitas, A. C., Mariz, M. C., Silva, M. A. R., & Jesus, A. L. S. (2013). Human papillomavirus vertical transmission: Review of current data. *Clinical Infectious Diseases*, *56*, 1451–1456. https://doi.org/10.1093/cid/cit066

Hoch, R. V., & Soriano, P. (2003). Roles of PDGF in animal development. *Development*, *130*, 4769–4784. https://doi.org/10.1242/dev.00721

International Agency for Research on Cancer (IARC). (2007). *Human papillomavirus* - Vol. *90*, (p. 48), ed. Lyon, France: World Health Organization.

Koi, H., Zhang, J., & Parry, S. (2001). The mechanism of placental viral infection. *Annals of New York Academy of Sciences*, *943*, 148–156. https://doi.org/10.1111/j.1749-6632.2001.tb03798.x

Koskimaa, H. M., Paaso, A., Welters, M. J., Grénmanm, S., Syrjänen, K., van der Burg, S. H., & Syrjänen, S. (2017). The presence of human papillomavirus (HPV) in placenta and/or cord blood might result in Th2 polarization. *European Journal of Clinical Microbiology & Infectious Diseases*, *36*, 1491–1503. https://doi.org/10.1007/s10096-017-2958-z

Lucà, R., Pintus, D., Ligios, C., Roperto, F., Leonardi, L., Di Guardo, G., . . . Roperto, S. (2016). E5 oncoprotein of bovine Deltapapillomaviruses is expressed in congenital carcinosarcomatosis of lambs. *Proceedings of LXX Meeting of Italian Society of Veterinary Sciences*, *70*, 263.

Melo, T. C., Carvalho, R. F., Mazzucchelli-deSouza, J., Diniz, N., Vasconcelos, S., Assaf, S. L. M. R., . . . Stocco, R. C. (2015). Bos taurus papillomavirus activity in peripheral blood mononuclear cells: Demonstrating a productive infection. *Genetics and Molecular Research*, *14*, 16712–16727. https://doi.org/10.4238/2015.December.11.19

Moustafa, A., Xie, C., Kirkness, E., Biggs, W., Wong, E., Turpaz, Y., . . . Talenti, A. (2017). The blood DNA virome in 8,000 humans. *PLoS Pathogens*, *13*(3), e1006292. https://doi.org/10.1371/journal.ppat.1006292

Munday, J. S., Thomson, N., Dunowska, M., Knight, C. G., Laurie, R. E., & Hills, S. (2015). Genomic characterisation of the feline sarcoid-associated papillomavirus and proposed classification as Bos taurus papillomavirus type 14. *Veterinary Microbiology*, *177*, 289–295. https://doi.org/10.1016/j.vetmic.2015.03.019

Orbell, G. M. B., Young, S., & Munday, J. S. (2011). Cutaneous sarcoids in captive African lions associated with feline sarcoid-associated papillomavirus infection. *Veterinary Pathology*, *48*, 1176–1179. https://doi.org/10.1177/0300985810391111

Pangty, K., Singh, S., Goswami, R., Saikumar, G., & Somvanshi, R. (2010). Detection of BPV-1 and -2 and quantification of BPV-1 by real-time PCR in cutaneous warts in cattle and buffaloes. *Transboundary & Emerging Diseases*, *57*, 185–196. https://doi.org/10.1111/j.1865-1682.2009.01096.x

Rombaldi, R. L., Serafini, E. P., Mandelli, J., Zimmermann, E., & Losquiavo, K. P. (2008). Transplacental transmission of human papillomavirus. *Virology Journal*, *5*, 106. https://doi.org/10.1186/1743-422X-5-106

Roperto, S., Borzacchiello, G., Brun, R., Leonardi, L., Maiolino, P., Martano, M., . . . Roperto, F. (2010). A review of bovine urothelial tumors and tumor-like lesions of the urinary bladder. *Journal of Comparative Pathology*, *142*, 95–108. https://doi.org/10.1016/j.jcpa.2009.08.156

Roperto, S., Borzacchiello, G., Esposito, I., Riccardi, M., Urraro, C., Lucà, R., . . . Roperto, F. (2012). Productive infection of bovine papillomavirus type 2 in the placenta of pregnant cows affected with urothelial bladder tumors. *PLoS ONE*, *7*(3), e33569. https://doi.org/10.1371/journal pone.0033569.

Roperto, S., Brun, R., Paolini, F., Urraro, C., Russo, V., Borzacchiello, G., . . . Venuti, A. (2008). Detection of bovine papillomavirus type 2 in the peripheral blood of cattle with urinary bladder tumours: Possible biological role. *Journal of General Virology*, *89*, 3027–3033. https://doi.org/10.1099/vir.0.2008/004457-0

Roperto, S., Comazzi, S., Ciusani, E., Paolini, F., Borzacchiello, G., Esposito, I., . . . Roperto, F. (2011). PBMCs are additional sites of productive infection of bovine papillomavirus type 2. *Journal General Virology*, *92*, 1787–1794. https://doi.org/10.1099/vir.0.031740-0

Roperto, S., Munday, J. S., Corrado, F., Goria, M., & Roperto, F. (2016). Detection of bovine papillomavirus type 14 DNA sequences in urinary bladder tumors in cattle. *Veterinary Microbiology*, *190*, 1–4. https://doi.org/10.1016/j.vetmic.2016.04.007

Roperto, S., Russo, V., Leonardi, L., Martano, M., Corrado, F., Riccardi, M. G., & Roperto, F. (2016). Bovine papillomavirus type 13 expression in the urothelial bladder tumors of cattle. *Transboundary & Emerging Diseases*, *63*, 628–634. https://doi.org/10.1111/tbed.12322

Roperto, S., Russo, V., Özkul, A., Sepici-Dincel, A., Maiolino, P., Borzacchiello, G., . . . Roperto, F. (2013). Bovine papillomavirus type 2 infects the urinary bladder of water buffalo (Bubalus bubalis) and plays a crucial role in bubaline urothelial carcinogenesis. *Journal of General Virology*, *94*, 403–408. https://doi.org/10.1099/vir.0.047662-0

Russo, V., Inglese, C., Avallone, L., Roperto, F., Abate, C., Zizzo, N., . . . Roperto, S. (2017). Sigma 2 receptor expression levels in blood and bladder from healthy and bladder cancer cattle. *Veterinary Comparative Oncology*, *15*, 1503–1512. https://doi.org/10.1111/vco.12295

Russo, V., Paciello, O., Zicarelli, G., Urraro, C., Ceccarelli, D. M., De Falco, F., . . . Roperto, S. (2017). Placental papillomatosis in water buffalo associated with bovine deltapapillomavirus. *Proceedings of LXXI Meeting of Italian Society of Veterinary Sciences*, *71*, 155.

Savini, E., Dal Molin, E., Gallina, L., Casà, G., & Scagliarini, A. (2016). Papillomavirus in healthy skin and mucosa of wild ruminants in the Italian Alps. *Journal of Wildlife Diseases.*, *52*, 82–87. https://doi.org/10.7589/2015-03-065

Silvestre, O., Borzacchiello, G., Nava, D., Iovane, G., Russo, V., Vecchio, D., . . . Paciello, O. (2009). Bovine papillomavirus type 1 DNA and E5 oncoprotein expression in water buffalo fibropapillomas. *Veterinary Pathology*, *46*, 636–641. https://doi.org/10.1354/vp.08-VP-0222-P-FL

Somvanshi, R. (2011). Papillomatosis in buffaloes: A less-known disease. *Transboundary & Emerging Diseases*, *58*, 327–332. https://doi.org/10.1111/j.1865-1682.2011.01211.x

Trenfield, K., Spradbrow, P. B., & Vanselow, B. A. (1990). Detection of papillomavirus DNA in precancerous lesions of the ears of sheep. *Veterinary Microbiology*, *25*, 103–116. https://doi.org/10.1016/0378-1135(90)90070-C

Virgilio, A., Sinisi, A., Russo, V., Gerardo, S., Santoro, A., Galeone, A., . . . Roperto, F. (2015). Ptaquiloside, the major carcinogen of bracken fern, in the pooled raw milk of healthy sheep and goats: An underestimated, global concern of food safety. *Journal of Agricultural and Food Chemistry*, *63*, 4886–4892. https://doi.org/10.1021/acs.jafc.5b01937

Williams, J. H., van Dyk, E., Nel, P. J., Lane, E., Van Wilpe, E., Bengis, R. G., . . . van Heerden, J. (2011). Pathology and immunohistochemistry of papillomavirus-associated cutaneous lesions in Cape mountain zebra, giraffe, sable antelope and African buffalo in South Africa. *Journal of the South African Veterinary Association*, *82*, 97–106.

Yuan, Z. O., Gault, E. A., Campo, M. S., & Nasir, L. (2011). Different contribution of bovine papillomavirus type 1 oncoproteins to the

transformation of equine fibroblasts. *Journal of General Virology*, *92*, 773–783.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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