Evidence for swine and human papillomavirus in pig slurry in Italy

P. Di Bonito¹, L. Galati¹, A. Foca², M. Brambilla³, C. Bisaglia³, G. Bonanno Ferraro⁴, P. Mancini⁴, M. Iaconelli⁴, C. Veneri⁴ and G. La Rosa⁴

1 Viral Hepatitis, Oncoviruses and Retroviruses (EVOR) Unit, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
2 School of medicine, “Magna Graecia” University, Catanazor, Italy
3 Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria (CREA), Research Centre for Engineering and Agri Food Processing, Treviglio, BG, Italy
4 Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy

Keywords
environmental, diversity, identification, viruses, waste.

Abstract
Aims: The diversity and the geographical distribution of swine papillomaviruses (PVs) are virtually unknown. The occurrence and the diversity of swine PV were therefore investigated in pig slurry collected in Italy, to contribute towards filling this gap in knowledge.

Methods and Results: Twenty-two slurry samples underwent analysis by nested PCR and DNA sequencing using published and newly designed specific primer pairs for Sus scrofa papillomavirus (SsPV) type 1 and 2 (SsPV1 and 2), along with degenerate PV-specific primers targeting the major coat protein L1 and the helicase protein E1. Overall, three samples (13.6%) were positive for SsPV1 by specific primers, and nucleotide (nt) sequences showed 99–100% nt identity with SsPV1 variant a (EF395818), while SsPV2 was not found in any sample. Using generic primers, eight samples (36.4%) were tested positive for HPV, and were characterized as follows: β1-HPV8, β1-HPV14, β1-HPV206, β2-HPV113, β2-HPV120 and γ1-HPV173. Moreover, one unclassified γ-type was detected.

Conclusions: Both swine and human PVs were detected in pig slurry in this study. The unexpected presence of HPV in pig waste could be explained as the result of an improper use of the sewage collection pits and/or with improper procedures of the operators.

Significance and Impact of the Study: This study reports the first detection of SsPV1 in Italy, along with the first detection of HPVs in pig slurry samples in Italy, and expands our knowledge about PV diversity and geographic distribution.

Introduction
Papillomaviridae is a large family of viruses that infect the epithelium of skin and mucosa causing benign and malignant tumours in a wide variety of vertebrates such as bovines, canines, equines, lagomorphs, birds and humans. Phylogenetically, Papillomaviruses (PVs) group into 49 genera, each further divided into species and types (http://pave.niaid.nih.gov/). The rapid evolution of the PCR-based protocols and the high-throughput sequencing technologies has led to a continual discovery of new species and types (Arroyo et al., 2013; Bzhala et al., 2014; Bzhalava et al., 2015; Brancaccio et al., 2018). The Papillomavirus Episteme database (PaVE) (van Doorslaer et al., 2017), established to provide organized and curated PV genomics information and tools, currently contains 330 reference genomes of human PV (HPV) and 183 reference genomes of animal PVs (https://pave.niaid.nih.gov/#home, accessed on 14 March 2019). The number of characterized animal PVs increases...
continuously (Mengual-Chulia et al., 2012; Rector and Van Ranst 2013) to the extent that recently, the proposal to create an International Reference Center for Animal PVs has raised (van Doorslaer and Dillner, 2019) in addition to the Referent Centre for the human viruses (www.hpvcenter.se).

Currently, there are only two reference PV sequences within the Suidae family: Sus scrofa papillomavirus 1 (SpPV1), classified into the species Dyodeltapapillomavirus 1 (further divided into variants a and b) and Sus scrofa papillomavirus 2 (SpPV2), which is still unclassified. The first study on Swine PV was published in 2008 by Stevens and co-workers, who used degenerate primers to amplify novel PVs from the healthy skin of domestic pigs in Belgium and reported the complete genomic sequence of two variants: SpPV1a and 1b (Stevens et al., 2008). Eight years later, in 2016, a novel PV, named SpPV2, was isolated and sequenced from wild boar in Germany (Link et al., 2017). It was the first swine PV associated with a skin fibropapilloma. However, phylogenetic analysis was not able to relate SpPV2 definitely with other PV species or existing genera; therefore, it was suggested as the potential representative of a new PV genus (Link et al., 2017).

Swine PVs have not been described in other parts of the world: currently, the swine sequences of the two mentioned studies are the only available in the GenBank database.

Since SpPV diversity and geographical distribution are virtually unknown, with only two studies published on the topic (Stevens et al., 2008; Link et al., 2017), in the present work we investigated the occurrence and the diversity of SpPVs in pig slurry in Italy using both swine specific and broad-range PV primers, known to detect both human and animal PVs.

Materials and methods

Twenty-two slurry samples were collected in 2015 from 10 farms placed in Northern Italy (Emilia Romagna and Lombardy Regions) in the framework of a previous study (La Rosa et al., 2016). Briefly, 5 ml of slurry was diluted to 20 ml with sterile water and then treated with 2 ml of 2.5 mol l⁻¹ glycine pH 9.5 and incubated in ice for 30 min. Samples were then washed with chloroform twice and centrifuged after of vigorous vortexing. Viral nucleic acids extraction from chloroform-treated samples took place using the NucliSENS easyMAG (BioMerieux, Marcy l’Etoile, France) semi-automated extraction system.

Genomes underwent analysis for swine and human PVs using nested PCR with published and newly designed primers targeting the L1 (major capsid protein) and E1 regions (helicase protein involved in viral replication).

For the detection of SpPV1, the first PCR cycle foresaw the use of published primers (Stevens et al., 2008) while newly designed primers were designed for the nested reaction. For SpPV2 detection, novel primer pairs (designed on the unique available sequence in GenBank) were used to amplify L1 and E1 fragments corresponding to those of SpPV1 primers. However, broad-range sets of primers were also used to detect both human and animal PV. These targeted the L1 (MY09/MY1-GP5/GP6, FAP59/64-FAP6085/6319, CP65/70-CP66/69, AR-L1F1/AR-L1R3 and AR-L1F8/AR-L1R9) and E1 regions (PM-A/PM-B) according to previously validated protocols for the detection of mucosal and cutaneous HPV genotypes (La Rosa et al., 2013). Table 1 shows the list of primers and PCR used in this study, along with the amplicon length and the references.

PCR reactions with broad-range primers were performed in 25 μl of reaction mix using 2 μl of DNA and 1 μl (10 pmol) of primer, using the TagGreen Mix PCR (Promega, Mannheim, Germany). Reaction conditions were as follows: 94°C (10 min) as first denaturation step, 45 cycles of: denaturation at 94°C (1 min), annealing at 50°C (1 min) and extension at 72°C (1 min) with a final extension step at 72°C (5 min). Nested PCR was performed using 2 μl of the first PCR product at the same conditions of the first amplification.

PCR reactions were carried out in a 96-well thermocycler (Applied Biosystems, Life Technologies, Carlsbad, CA) and analysed on agarose gel (1%) electrophoresis stained with ethidium bromide. PCR products of expected length were purified by a Montage PCRm96 Microwell filter plate (Millipore, Burlington, MA) and subjected to direct automated Sanger sequencing on both DNA strands (Bio-Fab Research, Rome, Italy). The forward and reverse sequences were edited and assembled into contigs using MEGA software ver. 6.0, and consensus sequences were compared to prototype sequences of the PaVE (http://pave.niaid.nih.gov/#home) and of GeneBank database using the Basic Local Alignment Search Tool (BLAST) at http://blast.ncbi.nlm.nih.gov/, to find the most related reference types.

Phylogenetic analysis was performed using MEGA software ver. 6.0. The alignment of nucleotide (nt) sequences took place using the CLUSTAL W algorithm. The phylogenetic tree was constructed using the maximum likelihood method based on the general time reversible model, integrated into the MEGA software.

Results

Samples were analysed using nested PCR with the published and newly designed primer shown above. High-quality DNA sequences were always obtained by direct
amplicon sequencing. Table 2 reports the sequencing results: three samples (ID17, ID19 and ID20), collected from two different farms, tested positive with the SsPV1 L1 nested assay. Upon sequencing, they showed 99–100\% nt identity with the GenBank sequence EF395818, classified as SsPV1 variant a. Only one of these positive samples could be further amplified with primers targeting the E1 coding region, which resulted in 99% nt identity with EF395818. No amplification was obtained with the newly designed primers specific for SsPV2.

Eight samples tested positive using the broad-range FAP6085/6319 primers and one of these also proved to be positive with CP65/69 primers, while no amplicons were produced with the other tested oligos. All positive samples were characterized as human PVs. Six different types were identified: HPV8, HPV14, HPV206 (Betapapillomavirus 1), HPV113 and HPV120 (Betapapillomavirus 2), and HPV173 (Gammapapillomavirus 1). Moreover, one sequence could not be assigned to any classified HPV since the most related reference sequence in PaVE database was γ19-HPV166, with only 80-7% of nt identity (coverage 74-7%). However, it showed 99% nt identity with two unclassified γ-types in GenBank: isolate HPV-mSK_054 (MH777199) found in skin swab in DOCK8-deficient patients (Tirosh et al., 2018) and isolate KC31 (KC752034) found in skin samples collected from healthy individuals (Li et al., 2013).

One sample contained two HPV sequences: the amplicon obtained with FAP6085-6319 primers was characterized as β2-HPV113, while the amplicon found with CP65/70 primers was characterized as β2-HPV120.

Figure 1 depicts the results of the phylogenetic study, performed on the sequences obtained in the L1 region. The tree includes the sequences obtained in this study, in addition to the prototype sequences from the PaVE

| Table 1 Primers used in the present study for PCR and sequencing |
|---------------------------------------------------------------|
| Primer name        | Region | Amplicon bp          | Primer sequence 5’-3’                      | References |
| SsPV-L1F          | L1     | 570 bp (first cycle) | CACGGACGAATATGTACAGC                         | Stevens et al. (2008) |
| SsPV-L1R          | L1     | 414 bp (nested)      | GTCTCGAAATCCCTAGTACC                        |              |
| 2158-SsPV-L1F-nest|        | 574 bp (first cycle) | CGAATATGCAACGGCCACCC                       | This study   |
| 2159-SsPV-L1R-nest|        | 409 bp (nested)      | GGTGTCAGCCAAATAGGAA                       | Stevens et al. (2008) |
| SsPV-E1F          | E1     | 574 bp (first cycle) | GTACAGAAGGTTGAGATGCC                       | This study   |
| SsPV-E1R          | E1     | 409 bp (nested)      | GTAATCTGGTCATACCC                         |              |
| 2160-SsPV-E1F-nest|        | 458 bp (first cycle) | CGCAGGGCAGACGAGATGAGATGCC                 | This study   |
| 2161-SsPV-E1R-nest|        | 435 bp (nested)      | AAGCAGGCGGAGACGATATGAG                    |              |
| 2231- SsPV2-L1F   | L1     | 458 bp (first cycle) | TGTGAAACGTCCTAAGGTCTCT                     | This study   |
| 2232-SsPV2-L1R    | L1     | 501 bp (first cycle) | CGTCAATTTCCAAGCCGCTG                      |              |
| 2233-SsPV2-L1R-nest|      | 478 bp (nested)      | ACAATATGCCACATCTTCTTCT                    |              |
| 2234-SsPV2-E1F    | E1     | 449 bp (first cycle) | CGMCAGGGGWCATATAAGTTG                     | Manos et al. (1989) |
| 2235-SsPV2-E1R    | E1     | 377 bp (nested)      | AGGCTGAAATGAAATGAAGATGAGTTC               | de Roda Husman et al. (1995) |
| 2236-SsPV2-E1F-nest|      | 452–467 bp (first cycle) | CARGGTCAYAAYAATGGYAT                     | Forslund et al. (1999) |
| 2237-SsPV2-E1R-nest|      | 377 bp (nested)      | GATGTCTACGTGCCTGGAGA                       | Forslund et al. (2003a); Forslund et al. (2003b) |
| MY11              | L1     | 449 bp (first cycle) | GCACCATACATCTTCTCTC                       | Berkhout et al. (1995) |
| MY09              | L1     | 138 bp (nested)      | AATCARMGTTTTRTTACCGT                      |              |
| GP5+              |        | 484 bp (first cycle) | GTTCGACCCCCAGAGATGAC                       |              |
| GP6+              |        | 238 bp (nested)      | TTAATGTGAAATGAAATGA                       |              |
| FAP59             |        | 452–467 bp (first cycle) | CARGGTCAYAAYAATGGYAT                     | Berkhout et al. (1995) |
| FAP64             |        | 377 bp (nested)      | GATGTCTACGTGCCTGGAGA                       |              |
| FAP6085           |        | 452–467 bp (first cycle) | CARGGTCAYAAYAATGGYAT                     | Berkhout et al. (1995) |
| FAP6319           |        | 377 bp (nested)      | GATGTCTACGTGCCTGGAGA                       |              |
| CP65              |        | 599 bp (first and second cycle) | TTDCAAGATGGCNGGTNGGCT                     | Rector et al. (2005) |
| CP70              |        | 704 bp (first and second cycle) | TTDCAAGATGGCNGGTNGGCT                     | Rector et al. (2005) |
| CP66              |        | 704 bp (first and second cycle) | TTDCAAGATGGCNGGTNGGCT                     | Rector et al. (2005) |
| CP69              |        | 704 bp (first and second cycle) | TTDCAAGATGGCNGGTNGGCT                     | Rector et al. (2005) |
| AR-L1F1           |        | 704 bp (first and second cycle) | TTDCAAGATGGCNGGTNGGCT                     | Rector et al. (2005) |
| AR-L1R3           |        | 704 bp (first and second cycle) | TTDCAAGATGGCNGGTNGGCT                     | Rector et al. (2005) |
| AR-L1F8           |        | 704 bp (first and second cycle) | TTDCAAGATGGCNGGTNGGCT                     | Rector et al. (2005) |
| AR-L1R9           |        | 704 bp (first and second cycle) | TTDCAAGATGGCNGGTNGGCT                     | Rector et al. (2005) |
| PM-A              | E1     | 570 bp (first and second cycle) | AATCARMGTTTTRTTACCGT                      | de Koning et al. (2006) |
| PM-B              | E1     | 449 bp (first and second cycle) | AATCARMGTTTTRTTACCGT                      | de Koning et al. (2006) |

Journal of Applied Microbiology 127, 1246–1254 © 2019 The Society for Applied Microbiology
database and from GeneBank. Sequences from slurry samples are grouped into five different clusters corresponding to Betapapillomavirus 1 and 2, Gammapapillomavirus 1 and 19, and Dyodeltapapillomavirus 1, according to the genotyping results by BLAST analysis.

### Discussion

Papillomaviruses affect all domestic animals and many wild vertebrates. This study reports the first detection of swine PV in Italy, specifically SsPV1 variant a. SsPV1 is the prototype virus for the species Dyodeltapapillomavirus 1, the single representative of the genus Dyodeltapapillomavirus, detected from swab samples taken from the healthy skin of two female domestic pigs in Belgium (Stevens et al., 2008). Little is known about the diseases that SsPV causes. A few studies documented fibropapillomatosis in piglets and transmissible genital papilloma in pigs (Rang and Dimigen 1971; Rieke 1980; Vitovec et al., 1999). However, these were before molecular techniques made it possible to sequence the genome and therefore, the infectious agent could not be characterized precisely. More recently, Nishiyama et al. (2011) examined a cutaneous fibropapillomatosis on the head and the back skin of a 4-month-old piglet, but PV antigen and DNA were not detected in these lesions by immunohistochemistry and PCR, therefore the fibropapillomatosis was considered hamartomatous rather than infectious. However, the generic MY09/11 primers used in that study might have failed to detect swine PV. In the present study, a wide panel of broad-range primers has been used, including MY09/11 and FAP primers, the latter previously proved to allow the amplification of different animal PVs (Antonsson and Hansson, 2002; Li et al., 2013; Silva et al., 2013). However, only specific primers for SsPV provided positive results. It is likely that esophytic or endophytic lesions such wart papillomas and venereal papillomas are more common than known in the swine population. Since animals with lesions are rapidly removed from the herds, the identification of PV is rarely performed. As consequence, the only available SsPV sequences in GeneBank and in PAVE database are the SsPV1 variants a and b (EF395818 and EF395819) found in healthy skin of domestic pigs (Stevens et al., 2008), and the SsPv2 sequence (KY817993) found in a wild boar with esophytic papillomatosis (Link et al., 2017).

In our study, positive results for SsPv2 were not found. However, the newly designed primers for SsPv2 were engineered on the basis of the unique available sequence in GeneBank, and we cannot be certain about the proper functioning of such primers since the genetic variability of SsPv2 is still unknown. Seven HPV genotypes were detected in pig slurry samples: HPV8, HPV14, HPV113, HPV120, HPV173, HPV206 (mRTRX7nr) and an unclassified c-type. Table 3 reports a summary of the clinical conditions associated with them.

In one sample, two different types were detected, demonstrating that slurry samples, like wastewater samples may contain multiple viral strains and can be therefore a good matrix to explore viral diversity.

The occurrence of human PVs in swine slurry was unexpected. It is conceivable that the presence of HPVs in pig waste might result from improper use of the sewage collection pits or from operators negligence to comply with the biosafety regulations. However, the possibility that human PVs infect and multiply in pigs cannot be excluded in the absence of specific studies.

In conclusion, this study reports the first detection of SsPV1 in Italy, along with the first detection of HPVs in

### Table 2: Sequencing results for swine and human PVs

| Sample ID | Most closely related reference type in PAVE database | Nt identity | Most closely related sequence in GenBank | Nt identity |
|-----------|-----------------------------------------------------|-------------|----------------------------------------|-------------|
| Swine papillomavirus | | | | |
| ID17 | SsPV1 (Dyodeltapapillomavirus 1); EF395818 | 100% | EF395818 | 100% |
| ID19 | 99% | FM955842 | 95.1% |
| ID20 | 99% | JQ963500 | 98.2% |
| Human papillomavirus | | | | |
| ID2 | HPV113 (Betapapillomavirus 2); FM955842 | 95.1% | JQ963500 | 98.2% |
| ID3 | HPV120 (Betapapillomavirus 2); JQ963500 | 98.9% | X74467 | 98.9% |
| ID7, ID12, and ID23 | HPV206 (Betapapillomavirus 1); U85660 | 98.1% | U85660 | 100% |
| ID11 | HPV173 (Gammapapillomavirus 1); X74467 | 91.2% | AF121431 | 100% |
| ID21 | HPV166 (Gammapapillomavirus 19); M12737 | 80.7% (QC 74.7%) | MH777199 and KC752034 | 99% |

QC, query coverage.
Figure 1 Phylogenetic tree constructed with the sequences obtained in the L1 region. The tree was constructed using the maximum likelihood method based on the general time reversible (GTR) model. The sequences identified in this study are in bold.
pig slurry, fostering the need of further studies to expand our knowledge about swine PV geographic distribution dynamics. The results of the present study suggest that these PVs could be more widespread than previously considered. Also, swine PV diversity needs to be studied: the lack of sequence information, with only three SPV sequences currently available, hampers the design of novel PCR primers capable to successfully detect SPVs. The next-generation sequencing in swine and slurry samples be considered among the best available techniques recommended for pig rearing (Giner Santonja et al., 2017). As for human PVs, their presence in pig slurry opens issues on their interspecies transmission of viruses (e.g influenza and Coronavirus) involving pigs have been demonstrated (Yassine et al., 2013; Rajao et al., 2018). As for human PVs, their presence in pig slurry opens issues on their potential harmfulness for humans with particular reference to both occupational health risks for animal farmers (because of the direct contact they have with pigs and their wastes, e.g. during the periodical cleaning barns undergo) and the potential environmental pollution resulting from manure spreading on the soil. As a matter of fact, Ngabo et al. (2016) pointed out that, being farmer was among the secondary significant risk factors for HPV positivity (besides HIV-positivity) in the population they studied, even though they do not relate this with the exposure to farm effluents (Ngabo et al., 2016).

The seasonal temperature transitions manures experience has slight or negligible effects on manure characteristics and virus persistence (Auffret et al., 2019). However, acting on chemical manure properties may represent a potential improvement. Increasing manure pH, on the one hand, affects virus persistence (Hurst et al., 1980; Stevens et al., 2018) but, on the other, it causes ammonia emission from manures into atmosphere to increase (Petersen and Sommer, 2011) so that it cannot be considered among the best available techniques recommended for pig rearing (Giner Santonja et al., 2017). Therefore, further investigation of HPV dynamics in manures could allow the set-up of effective in-farm manure management methods and practices aimed at improving the safety of exposed workers and lowering virus persistence in farm effluents and soils, in compliance with environmental and rearing regulations.

Acknowledgements

We gratefully acknowledge Mr. Alex Filisetti (CREA-IT) for swine slurry sample collection.

Conflict of Interest

The authors declare that there are no conflict of interest.

References

Antonsson, A. and Hansson, B.G. (2002) Healthy skin of many animal species harbors papillomaviruses which are

Table 3 Clinical conditions associated with the PVs detected in pig slurry in this study

| PV type       | Lesion type, isolation site and source | Main references                                      |
|---------------|---------------------------------------|-----------------------------------------------------|
| SsPV1 Variant a | Healthy skin of domestic pigs          | Stevens et al. (2008)                                 |
| β1-HPV8       | Healthy skin, eyebrow hair, EV lesions, mouth, anal canal, AK, SCC | Bzhalava et al. (2014); Donà et al. (2019); Ekstrom et al. (2013); Fuchs et al. (1986); Rollison et al. (2019); Torres et al. (2015) |
| β1-HPV14D     | Healthy skin, eyebrow hair, oral rinse samples, oral cancer, oropharyngeal cancer, EV lesions, anal canal, KS lesion, AK, KA, SCC | de Oliveira et al. (2004); Donà et al. (2019); Donà et al. (2015); Murahwa et al. (2014); Rollison et al. (2019); Sabol et al. (2016); Torres et al. (2015); Winer et al. (2018) |
| β1-HPV206     | Healthy skin, SCC, AK                  | Hopfi et al. (1997); Tirosh et al. (2018)             |
| β2-HPV113     | Healthy skin, eyebrow hair, oral rinse samples, wart, anal canal, SCC, BCC, HNC, oral cancer, oropharyngeal cancer | de Villiers and Gunst (2009); Donà et al. (2019); Donà et al. (2015); La Rosa et al. (2013); Rollison et al. (2019); Sabol et al. (2016); Torres et al. (2015); Winer et al. (2018) |
| β2-HPV120     | Healthy skin, eyebrow hair, oral cavity, SSC, stool samples, anal canal oral cancer, oropharyngeal cancer | Bottalico et al. (2012); Donà et al. (2019); Donà et al. (2015); Ma et al. (2014); Rollison et al. (2019); Sabol et al. (2016); Torres et al. (2015); Winer et al. (2018) |
| γ1-HPV173     | Healthy skin; oral rinse samples, eyebrow hair, HNC, SPA/pool | Di Bonito et al. (2017); Martin et al. (2014); Rollison et al. (2019); Winer et al. (2018) |
| Unclassified γ-type | Healthy skin                         | Tirosh et al. (2018)                                 |

SCC, squamous cell carcinoma; AK, actinic keratosis; BCC, basal cell carcinoma; KA, keratoacanthoma; HNC, head and neck carcinoma; EV, epidermodysplasia verruciformis; KS, Kaposi sarcoma.
closely related to their human counterparts. J Virol 76, 12537–12542.
Arroyo, L.S., Smelov, V., Bzhalava, D., Eklund, C., Hultin, E. and Dillner, J. (2013) Next generation sequencing for human papillomavirus genotyping. J Clin Virol 58, 437–442.
Auffret, M.D., Brassard, J., Jones, T.H., Gagnon, N., Gagné, M.-J., Muelhauser, V., Masse, L., Topp, E. et al. (2019) Impact of seasonal temperature transition, alkalinity and other abiotic factors on the persistence of viruses in swine and dairy manures. Sci Total Environ 659, 640–648. https://doi.org/10.1016/j.scitotenv.2018.12.306.
Berkhout, R.J., Tieben, L.M., Smits, H.L., Bavinck, J.N., Vermeer, B.J. and ter Schegget, J. (1995) Nested PCR approach for detection and typing of epidermodysplasia verruciformis-associated human papillomavirus types in cutaneous cancers from renal transplant recipients. J Clin Microbiol 33, 690–695.
Bottalico, D., Chen, Z., Kocjan, B.J., Seme, K., Poljak, M. and Burkh, R.D. (2012) Characterization of human papillomavirus type 120 (HPV120): a novel Betapapillomavirus with tropism for multiple anatomic niches. J Gen Virol 93, 1774–1779.
Branaccio, R.N., Robitaille, A., Dutta, S., Cuenin, C., Santare, D., Skenders, G., Leja, M., Fischer, N. et al. (2018) Generation of a novel next-generation sequencing-based method for the isolation of new human papillomavirus types. Virology 520, 1–10.
Bravo, I.G. and Felez-Sanchez, M. (2015) Papillomaviruses: viral evolution, cancer and evolutionary medicine. Evol Med Public Health 2015, 32–51.
Bzhalava, D., Muhr, L.S., Lagheden, C., Ekstrom, J., Forslund, O., Dillner, J. and Hultin, E. (2014) Deep sequencing extends the diversity of human papillomaviruses in human skin. Sci Rep 4, 5807.
Bzhalava, D., Eklund, C. and Dillner, J. (2015) International standardization and classification of human papillomavirus types. Virology 476, 341–344.
Di Bonito, P., laconelli, M., Gheit, T., Tommasino, M., Della, L.S., Bonadonna, L. and La Rosa, G. (2017) Detection of oncogenic viruses in water environments by a Luminex-based multiplex platform for high throughput screening of infectious agents. Water Res 123, 549–555.
Donà, M.G., Gheit, T., Latinu, A., Benevolo, M., Torres, M., Smelov, V., McKay-Chopin, S., Giglio, A. et al. (2015) Alpha, beta and gamma human papillomaviruses in the anal canal of HIV-infected and uninfected men who have sex with men. J Infect 71, 74–84.
Donà, M., Chiantore, M., Tari, G., Fiorucci, G., Vescio, M., La Rosa, G., Accardi, L., Giuliani, M. et al. (2019) Comprehensive analysis of beta and gamma Human Papillomaviruses in actinic keratoses and apparently healthy skin of elderly patients. Br J Dermatol. https://doi.org/10.1111/bjd.17836
van Doorslaer, K. and Dillner, J. (2019) The launch of an international animal papillomavirus reference center. Viruses 11, 55.
van Doorslaer, K., Li, Z., Xirasagar, S., Maes, P., Kaminsky, D., Liou, D., Sun, Q., Kaur, R. et al. (2017) The Papillomavirus Episteme: a major update to the papillomavirus sequence database. Nucleic Acids Res 45, D499–D506.
Ekstrom, J., Muhr, L.S., Bzhalava, D., Soderlund-Strand, A., Hultin, E., Nordin, P., Stenquist, B., Paoli, J. et al. (2013) Diversity of human papillomaviruses in skin lesions. Virology 447, 300–311.
Forslund, O., Anttonsson, A., Nordin, P., Stenquist, B. and Hansson, B.G. (1999) A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. J Gen Virol 80, 2437–2443.
Forslund, O., Ly, H. and Higgins, G. (2003a) Improved detection of cutaneous human papillomavirus DNA by single tube nested ‘hanging droplet’ PCR. J Virol Methods 110, 129–136.
Forslund, O., Ly, H., Reid, C. and Higgins, G. (2003b) A broad spectrum of human papillomavirus types is present in the skin of Australian patients with non-melanoma skin cancers and solar keratoses. Br J Dermatol 149, 64–73.
Fuchs, P.G., Iftner, T., Wening, J. and Pfister, H. (1986) Epidermodysplasia verruciformis-associated human papillomavirus 8: genomic sequence and comparative analysis. J Virol 58, 626–634.
Giner Santonja, G., Georgitzikis, K., Scalet, B.M., Montobbio, P., Roudier, S. and Delgado Sancho, L. (2017) Best available techniques (BAT) reference document for the intensive rearing of poultry or pigs; EUR 28674 EN. Available at: http://eippcb.jrc.ec.europa.eu/reference/BREF/IRPP/JRC107189_IRPP_Bref_2017_published.pdf.
Gottschling, M., Goker, M., Stamatakis, A., Bininda-Emonds, O.R., Nindl, I. and Bravo, I.G. (2011) Quantifying the phylodynamic forces driving papillomavirus evolution. Mol Biol Evol 28, 2101–2113.
Hopfl, R., Bens, G., Wieland, U., Petter, A., Zelger, B., Fritsch, P. and Pfister, H. (1997) Human papillomavirus DNA in non-melanoma skin cancers of a renal transplant recipient: detection of a new sequence related to epidermodysplasia verruciformis associated types. J Invest Dermatol 108, 53–56.
Hurst, C.J., Gerba, C.P. and Cech, I. (1980) Effects of environmental variables and soil characteristics on virus survival in soil. Appl Environ Microbiol 1980, 1067–1079.
de Villiers, E.M. and Gunst, K. (2009) Characterization of seven novel human papillomavirus types isolated from cutaneous tissue, but also present in mucosal lesions. J Gen Virol 90, 1999–2004.
de Koning, M., Quint, W., Struijk, L., Keter, B., Wanningen, P., van Doorn, L.J., Weissenborn, S.J., Feltkamp, M. et al. (2006) Evaluation of a novel highly sensitive, broad-
spectrum PCR-reverse hybridization assay for detection and identification of beta-papillomavirus DNA. *J Clin Microbiol* **44**, 1792–1800.

de Oliveira, W.R., He, Q., Rady, P.L., Hughes, T.K., Neto, C.F., Rivitti, E.A. and Tying, S.K. (2004) HPV typing in Brazilian patients with epidermodysplasia verruciformis: high prevalence of EV-HPV 25. *J Cutan Med Surg* **8**, 110–115.

de Roda Husman, A.M., Walboomers, J.M., van den Brule, A.J., Meijer, C.J. and Snijders, P.J. (1995) The use of general primers GP5 and GP6 elongated at their 3’ ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* **76**, 1057–1062.

La Rosa, G., Fratini, M., Accardi, L., D’Oro, G., Della Libera, M., Muscillo, M. and Di Bonito, P. (2013) Mucosal and cutaneous human papillomaviruses detected in raw sewages. *PLoS ONE* **8**, e52391.

La Rosa, G., Della Libera, S., Brambilla, M., Bisaglia, C., Pisani, G., Ciccgazione, A.R., Bruni, R., Taffon, S. et al. (2016) Hepatitis E virus (genotype 3) in swine slurry samples from swine farming activities in Italy. *Food Environ Virol* **9**, 219–229.

Li, J., Pan, Y., Xu, Z., Wang, Q., Hang, D., Shen, N., Liu, M., Zhang, C. et al. (2013) Improved detection of human papillomavirus harbored in healthy skin with FAP6085/64 primers. *J Virol Methods* **193**, 633–638.

Link, E.K., Hoferer, M., Strobel, B., Riegber, K., Langenmayer, M.C., Sutter, G. and Fux, R. (2017) *Sus scrofa* papillomavirus 2 - genetic characterization of a novel suid papillomavirus from wild boar in Germany. *J Gen Virol* **98**, 2113–2117.

Ma, Y., Madupu, R., Karaoz, U., Nossa, C.W., Yang, L., Yooseph, S., Yachiminski, P.S., Brodie, E.L. et al. (2014) Human papillomavirus community in healthy persons, defined by metagenomics analysis of human microbiome project shotgun sequencing data sets. *J Virol* **88**, 4786–4797.

Manos, M.M., Ting, Y., Wright, D.K., Lewis, J., Broker, T.R. and Wolinsky, S.M. (1989) The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* **7**, 209–214.

Martin, E., Dang, J., Bzhalava, D., Stern, J., Edelstein, Z.R., Koutsksy, I.A., Kiviat, N.B. and Feng, Q. (2014) Characterization of three novel human papillomavirus types isolated from oral rinse samples of healthy individuals. *J Clin Virol* **59**, 30–37.

Mengual-Chulia, B., García-Perez, R., Gottschling, M., Nindl, I. and Bravo, I.G. (2012) Novel animal papillomavirus sequences and accurate phylogenetic placement. *Mol Phylogenet Evol* **65**, 883–891.

Murahwa, A.T., Muchemwa, F.C., Duri, K., Borok, M.Z., Kanyera, R.B., Manhanza, M.T., Mapungire, M.P. and Stray-Pedersen, B. (2014) Presence of betapapillomavirus in Kaposi sarcoma lesions. *J Med Virol* **86**, 1556–1559.

Nasir, L. and Campo, M.S. (2008) Bovine papillomaviruses: their role in the aetiology of cutaneous tumours of bovids and equids. *Vet Dermatol* **19**, 243–254.

Ngabo, F., Franceschi, S., Baussano, I., Umulisa, M.C., Snijders, P.J., Uytterlinde, A.M., Lazzarato, F., Tenet, V. et al. (2016) Human papillomavirus infection in Rwanda at the moment of implementation of a national HPV vaccination programme. *BMC Infect Dis* **16**, 225. https://doi.org/10.1186/s12879-016-1539-6.

Nishiyama, S., Akiba, Y., Kobayashi, Y., Shiga, A., Kamiie, J. and Shirotai, K. (2011) Congenital cutaneous fibropapilomatosis with no evidence of papillomavirus infection in a piglet. *J Vet Med Sci* **73**, 283–285.

Petersen, S.O. and Sommer, S.G. (2011) Ammonia and nitrous oxide interactions: roles of manure organic matter management. *Anim Feed Sci Technol* **166–167**, 503–513.

Rajao, D.S., Vincent, A.L. and Perez, D.R. (2018) Adaptation of human influenza viruses to swine. *Front Vet Sci* **5**, 347.

Rang, H. and Dimigen, J. (1971) [Papilloma in the pig (brief communication)]. *Dtsch Tierarztl Wochenschr* **8**, 637.

Rector, A. and Van Ranst, M. (2013) Animal papillomaviruses. *Virology* **445**, 213–223.

Rector, A., van Doors, K., Bertelsen, M., Barker, I.K., Olberg, R.A., Lemey, P., Sundberg, J.P. and Van Ranst, M. (2005) Isolation and cloning of the raccoon (*Procyon lotor*) papillomavirus type 1 by using degenerate papillomavirus-specific primers. *J Gen Virol* **86**, 2029–2033.

Rieke, H. (1980) [An extreme congenital papillomatosis of a piglet (author's trans)]. *Dtsch Tierarztl Wochenschr* **87**, 412–413.

Rollison, D.E., Schell, M.J., Fenske, N.A., Cherpelis, B., Messina, J.L., Giuliano, A.R., Epling-Burnette, P.K., Hampras, S.S. et al. (2019) Cutaneous viral infections across 2 anatomic sites among a cohort of patients undergoing skin cancer screening. *J Infect Dis* **219**, 711–722.

Roperto, S., Russo, V., Ozkul, A., Corteggi, A., Sepci-Dincel, A., Catoi, C., Esposito, I., Riccardi, M.G. et al. (2013) Productive infection of bovine papillomavirus type 2 in the urothelial cells of naturally occurring urinary bladder tumors in cattle and water buffaloes. *PLoS ONE* **8**, e62227. https://doi.org/10.1371/journal.pone.0062227.

Sabol, I., Smahelova, J., Klozar, J., Mravak-Stipetic, M., Gheit, T., Tommasino, M., Grce, M. and Tachezy, R. (2016) Beta-HPV types in patients with head and neck pathology and in healthy subjects. *J Clin Virol* **82**, 159–165.

Silva, M.A., Batista, M.V., Pontes, N.E., Santos, E.U., Coutinho, L.C., Castro, R.S., Bôlimo, V.Q. and Freitas, A.C. (2013) Comparison of two PCR strategies for the detection of bovine papillomavirus. *J Virol Methods* **192**, 55–58.

Silvestre, O., Borzacchiello, G., Nava, D., Iovane, G., Russo, V., Vecchio, D., D’Ausilio, F., Gault, E.A., et al (2009) Bovine papillomavirus type 1 DNA and E5 oncoprotein
expression in water buffalo fibropapillomas. *Vet Pathol* 46, 636–641. https://doi.org/10.1354/vp.08-VP-0222-P-FL.

Stevens, H., Rector, A., Van Der Kroght, K. and Van Ranst, R.M. (2008) Isolation and cloning of two variant papillomaviruses from domestic pigs: *Sus scrofa* papillomaviruses type 1 variants a and b. *J Gen Virol* 89, 2475–2481.

Stevens, E.E., Miller, D.N., Brittenham, B.A., Vitosh-Sillman, S.J., Brodersen, B.W., Jin, V.L., Loy, J.D. and Schmidt, A.M. (2018) Alkaline stabilization of manure slurry inactivates porcine epidemic diarrhea virus. *Swine Health Prod* 26, 95–100.

Tirosh, O., Conlan, S., Deming, C., Lee-Lin, S.Q., Huang, X., Su, H.C., Freeman, A.F., Segre, J.A. *et al.* (2018) Expanded skin virome in DOCK8-deficient patients. *Nat Med* 24, 1815–1821.

Torres, M., Gheit, T., McKay-Chopin, S., Rodriguez, C., Romero, J.D., Filotico, R., Dona, M.G., Ortiz, M. *et al.* (2015) Prevalence of beta and gamma human papillomaviruses in the anal canal of men who have sex with men is influenced by HIV status. *J Clin Virol* 67, 47–51.

Vitovec, J., Kursa, J., Kratochvil, P. and Skalova, A. (1999) Congenital fibropapillomatosis in a piglet. *Vet Pathol* 36, 83–85.

Winer, R.L., Gheit, T., Feng, Q., Stern, J.E., Lin, J., Cherne, S. and Tommasino, M. (2018) Prevalence and correlates of beta and gamma human papillomavirus detection in oral samples from mid-adult women. *J Infect Dis* 219, 1067–1075.

Yassine, H.M., Lee, C.W. and Saif, Y.M. (2013) Interspecies transmission of influenza a viruses between Swine and poultry. *Curr Top Microbiol Immunol* 370, 227–240.