



Equine sarcoids from Southern Italy: Molecular and Histopathological characterization

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ARTICLE INFO

Keywords:

Equine sarcoid
BPV
Histopathology

ABSTRACT

This study investigated the presence of δ bovine papillomaviruses (BPV-1, BPV-2, BPV-13, BPV-14) in equine skin lesions from Southern Italy, focusing on equine sarcoids and their histopathological correlations. 63 equine skin samples were analysed using PCR and sequencing for BPV detection, and their histopathological features were assessed. BPV DNA was detected in 69.84% of the samples, with BPV-1 and BPV-2 being the most prevalent genotype, followed by BPV-13, while BPV-14 was not detected. BPV DNA was also found in non-sarcoid tumours and non-neoplastic conditions. Histopathological analysis revealed in 41 samples typical sarcoid features: fibroblastic atypia and extracellular matrix deposition. Despite no statistical correlation was found between BPV genotypes and histopathological features, BPV-1 infection was associated with more severe fibroblastic atypia and abundant extracellular matrix. This study provides insights into the prevalence and potential pathogenic roles of different BPV genotypes in equine sarcoids and other skin lesions, underscoring the critical need for further research to develop targeted therapies.

1. Introduction

Equine sarcoid represents the most diagnosed cutaneous tumour in horses and other equids, such as donkeys, mules, and zebras (Ragland et al., 1970; Cotchin, 1977; Marti et al., 1993; Lazary et al., 1985). It was first described by Jackson in 1936 and it is now observed worldwide, with variable incidence ranging from 13% to 90% among skin diseases and tumours (Marti et al., 1993; Bergvall, 2013; Knottenbelt, 2019), according to the geographical area of study and potentially linked to the presence of vectors or proximity to cattle (Jackson, 1936; Marti et al., 1993; Valentine, 2006).

While equine sarcoid does not meet all criteria for malignancy, as it is non-metastatic, it is locally invasive and recurrent. Its anatomical localization, particularly at the girth, bridle area, distal limbs, corners of the mouth, and eyelids causes significant welfare issues, often leading to functional impairment and in severe cases to euthanasia (Martens et al., 2001; Knottenbelt et al., 2019).

Young horses (3-6 years) and geldings were thought to be

predisposed (Marti et al., 1993; Torrontegui and Reid, 1994). While recent studies indicate no age or sex predisposition, certain breeds, such as Quarter Horses and French Montagne Horses, exhibit higher risk, while Thoroughbreds show medium prevalence, and Standardbreds, low prevalence (Ogłuszka et al., 2021). Notably, a strong correlation exists between equine sarcoid and genetic variation in some lineages, mainly associated with the ELA W13 allele linked to MHC II (Bostrom et al., 1995).

δ bovine papillomaviruses (BPV-1, BPV-2, BPV-13) are recognized as the causative agents of equine sarcoid (Chambers et al., 2003; Torrontegui and Reid, 1994; Bogaert, 2011). It is well known that BPV infection begins in the epidermis, where it can remain latent, with subsequent viral material detection in sub-epidermal fibroblasts, where full transformation occurs (Brandt et al., 2011a, 2011b). The productive nature of BPV infection in horses remains questionable, with some authors reporting abortive infection and others demonstrating productive infection (Ogłuszka et al., 2021; Bogaert, 2011; Brandt et al., 2011a, 2011b).

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<https://doi.org/10.1016/j.rvsc.2025.105777>

Received 25 March 2025; Received in revised form 17 June 2025; Accepted 18 June 2025

Available online 19 June 2025

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BPV DNA has been detected not only in sarcoids but also in normal equine skin, dermatitis, and other equine tumours such as fibrosarcomas, schwannomas, myxosarcomas, and fibromas (Oguszk et al., 2021; Bogaert et al., 2005; Brandt et al., 2011a, 2011b; Gaynor et al., 2016; Munday, 2014; Wobeser et al., 2012; Hill et al., 2012). It is well known that other factors, are implicated in equine sarcoid pathogenesis, among those chronic physical traumas could have a role in potentially reactivating latent BPV infection and leading to pathological wound healing, from which keloids and subsequently sarcoids may originate (Martano et al., 2016; Martano et al., 2018; Martano et al., 2020). Clinically, equine sarcoids present six diverse types: mild occult, verrucous, nodular lesions, ulcerated fibroblastic, mixed, and malevolent forms (Knottenbelt, 2005).

Although the macroscopic appearance of sarcoids is relatively characteristic, histopathological confirmation is essential for reliable diagnosis and differentiation from other infectious and non-infectious cutaneous diseases. Under the histopathological perspective, sarcoid alterations involve proliferation of dermal fibroblasts and epidermal keratinocytes, however different variations can be observed according to the sarcoid type. Typical epithelial changes include hyperkeratosis, parakeratosis, severe hyperplasia with rete pegs, and epidermal atrophy or ulceration. Dermal changes involve proliferation of spindle-shaped to fusiform neoplastic fibroblasts with variable nuclear atypia, forming whorls or interlacing bundles, often oriented perpendicular to the basement membrane ("picket-fence" pattern). Collagen amount and type (fibrous or myxoid) vary considerably (Martens et al., 2001; Oguszk et al., 2021).

Despite the established association between sarcoid clinical subtypes and BPV infection (Parkinson and Else, 2024; Chambers et al., 2003), limited data exist on the relationship between histopathological features and BPV genotypes. Thus, this study aimed to identify and characterize BPV-1, BPV-2, BPV-13, BPV-14 DNA in normal and pathological skin lesions (sarcoid and non-sarcoid tumours and non-neoplastic conditions) from horses raised in Southern Italy. Therefore, the main objective of this study was to examine the histological features of sarcoids and statistically identify any correlations with BPV genotypes.

2. Material and methods

2.1. Samples

Sixty-three equine skin samples were collected using a non-probabilistic sampling method (convenience sampling) from equine skin lesions. Each sample was obtained from a different individual horse.

Skin lesion samples (neoplastic and non-neoplastic) surgically excised from affected horses in Southern Italy (Campania and Calabria Regions), following best veterinary practices in accordance with Directive 2010/63/EU (Article 1) and processed for routine diagnostic and

treatment. Informed consent was obtained from each horse owner for the use of tissues in this research, adhering to the ethical guidelines of the Anatomic Pathology Diagnostic Service, Department of Veterinary Medicine and Animal Production, University of Naples Federico II. Additionally, three normal skin samples were obtained at necropsy from healthy horses with no evidence of skin disease, euthanized for unrelated non-neoplastic, non-inflammatory conditions.

Clinical data, including gender, anatomical location of the skin lesion (limb, head/neck, trunk), and geographical origin, were recorded for each sample. Animals were categorized into three age groups: ≤ 5 years (36.), 6–9 years (34.1%), and ≥ 10 years (29.3%). The sample consisted of 16 females (39.0%) and 25 males (61.0%).

Due to the retrospective nature of data collection, complete information for all parameters was not available for each horse. The data of the equine skin samples used in this study are summarized in Table 1, Table 2 and Table 3.

2.2. Histopathological analysis

All samples were fixed in 10% neutral buffered formalin for histological examination. Formalin fixed tissue samples were processed by standard paraffin wax techniques. Sections of 4 μm thickness were cut and stained with haematoxylin and eosin (H&E; Carlo Erba Italy).

All samples were examined by expert pathologists using light microscopy and classified according to the World Health Organization (WHO) histological classification of epithelial and melanocytic tumours of the skin of domestic animals (Goldschmidt et al., 1998).

Additionally, equine sarcoid samples were subsequently examined, and the presence or absence of specific epidermal and dermal changes was assessed and scored by two independent observers (MM, BR, PM) under blinded conditions. The following morphological aspects were evaluated:

Epidermal changes:

- Severe epidermal hyperplasia with rete pegs formation (>20 cells)

Dermal changes:

- Moderate or abundant extracellular matrix
- Picket fence at the junction of the epidermis
- Mild or severe fibroblastic atypia

2.3. Biomolecular analysis

- DNA extraction

DNA was extracted from three to four 5 μm thick formalin-fixed paraffin-embedded (FFPE) tissue sections for each sample using the

Table 1
Prevalence of BPV and risk factor analysis by age, gender and site of skin lesion in equine sarcoid.

Factor	n	BPV Positive	%	SE % [§]	95 %CI	χ^2	P
Total	41	31	75.61	13.15	62.46–88.75		
Age							
≤ 5 years old	15	11	73.33	22.38	50.95–95.71		
6–9 years old	14	11	78.57	21.49	57.08–100.00	0.015	0.9923
≥ 10 years old	12	9	75.00	24.50	50.50–99.5		
Gender							
Female	16	12	56.25	24.31	31.94–80.56		
Male	25	19	76.00	16.74	59.26–92.74	0.047	0.828
Site of the skin lesion							
Limbs	13	10	76.92	22.90	54.02–99.83		
Head/Neck	13	12	92.31	14.49	77.82–100.00		
Trunk	15	9	60.00	24.79	35.21–84.79	0.553	0.758
Location							
Campania	25	18	72.0	17.6	54.5–89.6		
Calabria	16	12	75.0	21.2	53.8–96.2	0.022	0.880

Table 2
Prevalence of BPV and risk factor analysis by age, gender and site of lesion in equine non sarcoid skin lesions.

Factor	n	BPV Positive	%	SE % [§]	95 %CI	χ^2	P
Total	22	13	59.1	20.6	38.6–79.6		
Age							
≤ 5 years old	9	6	66.7	30.8	35.9–97.5	1.43	0.6982
6–9 years old	9	4	44.4	32.5	11.9–76.9		
≥ 10 years old	4	3	75.0	42.4	32.5–100.0		
Gender							
Female	13	8	61.5	26.5	35.1–87.9	0.026	0.8726
Male	9	5	55.6	32.5	23.1–88.1		
Site of the skin lesion							
Limbs	3	1	33.3	53.3	0.0–86.7	1.58	0.4535
Head/Neck	6	3	50.0	40.0	9.9–90.0		
Trunk	13	9	69.2	25.1	44.1–94.3		
Location							
Campania	15	9	60.0	24.8	35.2–84.8	0.115	0.7350
Calabria	7	4	57.1	36.7	20.5–93.8		
Type of lesion							
Normal skin	3	0	–	–	–	5.21	0.0736
Inflammatory skin conditions	11	8	72.7	26.3	46.4–99.1		
Non-sarcoid tumours	8	5	62.5	33.5	28.9–96.1		

QIAamp DNA Blood Mini Kit (Qiagen), following the manufacturer's protocol. The concentration of the extracted DNA was quantified using a Nanophotometer® NP80 (Implen).

- PCR for detection of BPV-1, BPV-2, BPV-13, BPV-14 DNA

For each sarcoid sample, 100–150 ng of DNA was subjected to polymerase chain reaction (PCR) using GoTaq® G2 Hot Start Green Master Mix (Promega Corporation). BPVs L1 primers were employed to amplify a fragment of BPV-1, BPV-2, BPV-13, BPV-14 L1 gene by using primers set and the amplification protocol developed by Munday et al. (2021).

Samples with no DNA template were run as negative control and the specificity of PCRs were ensured by sequencing. The quality of DNA samples was checked by amplifying a fragment of Equine β -Actin (175 bp) by following the amplification protocol: 94 °C for 2 min followed by 30 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min. The final extension was a 72 °C for 5 min. PCR products were run through electrophoresis along with a 100-bp DNA ladder in a 2% agarose gel with TAE (Tris-Acetate-EDTA) buffer, stained with ethidium bromide and visualized under UV using the ChemiDoc MP Imaging System (Bio-Rad Laboratories).

2.4. Sequences analysis

PCR products from DNA 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) according to the manufacturer's protocol. Sequences were removed with a DyeEx 2.0 spin kit (Qiagen TM, DE) and run on SeqStudio™ Genetic Analyzer (Applied Biosystems, CA, USA). Electropherograms were analysed using Sequencing analysis v5.2 and sequence scanner v1.0 software (Applied Biosystems, CA, USA). The sequences obtained were compared to others in GenBank using the BLAST program.

2.5. Statistical analysis

The statistical analysis was performed with the statistical software MedCalc version 16.4.3 (MedCalc Software, Ostend, Belgium; www.medcalc.org; 2016). Chi-square tests were used to compare the proportions of positivity in relation to the categorical dependent variables and to determine statistical significance within each class (age, gender, geographical origin and location of skin lesion). The chi-square test was also used to determine the statistical significance between the presence of the different BPV serotypes and the type of histological lesions.

A p-value of less than 0.05 was considered statistically significant. Odds ratios (ORs) and the corresponding 95 % confidence intervals (CI) were calculated to determine the extent of significant differences between the groups.

3. Results

3.1. Histopathological results

According to histopathological findings, samples were diagnosed as equine sarcoids (41), other non-sarcoid tumours (8; including 1 squamous cell carcinoma, 2 melanomas, 2 fibromas, 2 papillomas, and 1 myxoma), or inflammatory skin conditions (11). All sarcoid samples included in this study were classified as fibroblastic, verrucous or mixed types, and displayed typical histological features, which include both epidermal and dermal changes.

Typical epithelial changes included epidermal acanthosis, hyperkeratosis, parakeratosis, and irregular epithelial cell proliferation, resulting in long rete pegs or rete ridges (> 20 cells), which extended deep into the dermis, that was observed in 33 out of 41 BPV-positive samples (Fig. 1a). A typical dermal change was characterized by an increase in dense or myxoid extracellular matrix, ranging from minimal in 18 out of 41 BPV-positive samples to moderate/abundant in 21 out of 41 BPV-positive samples. Moreover, dermal proliferation of spindle-shaped to fusiform fibroblasts (so-called "sarcoid fibroblasts") exhibiting variable hyperchromasia, anisokaryosis, and anisocytosis was observed. Sarcoid fibroblast showed mild atypia in 24 out of 41 BPV-positive samples, and severe atypia in 20 out of 41 BPV-positive samples (Fig. 1b).

In the deep dermis, sarcoid fibroblasts were arranged in a whorling pattern (Fig. 1c) or parallel to interlacing short bundles (Fig. 1b), while at the dermal-epidermal junction, they were oriented perpendicularly to the basement membrane, forming the so-called "picket fence" arrangement in 21 out of 41 BPV-positive samples (Fig. 1d).

3.2. Biomolecular results

BPV1, BPV2, BPV13 DNA were detected in 44 out of 63 (69.84%) analysed samples, as no samples resulted positive for BPV14 DNA. Among the 41 sarcoid samples, 31 tested positive for BPVs (31/41–76 %) Of the 8 samples from other non-sarcoid tumours, 5 tested positive for BPVs (5/8–63 %) and specifically 2 fibromas, 2 papillomas, 1 myxoma and 1 melanoma. Of the 11 samples from non-neoplastic lesions, 8 were positive for BPVs (8/11–73 %) (Fig. 2). Conversely, no BPVs infection was detected in the 3 samples of normal skin. PCR analyses

Table 3

Prevalence of BPV-1/BPV-2/BPV-13 DNA and risk factor analysis by age, gender and site of skin lesion in equine sarcoid.

Factor	n	BPV-1 Positive	%	SE % [§]	95 %CI	χ^2	P
Total	41	25	60.98	14.93	40.06–75.91	–	–
Age							
≤ 5 years old	15	9	60.00	24.79	35.21–84.79		
6–9 years old	14	9	64.29	25.10	39.19–89.39	0.106	0.9485
≥ 10 years old	12	7	58.33	36.66	30.44–86.23		
Gender							
Female	16	14	87.5	16.21	71.29–100.00	6.038	0.0140*
Male	25	11	28.00	17.60	10.4–45.6		
Site of the skin lesion							
Limbs	13	9	69.23	25.09	44.14–94.32		
Head/Neck	13	9	69.23	25.09	44.14–94.32		
Trunk	15	7	46.67	25.25	21.42–71.9	0.002	0.999
Location							
Campania	25	15	60.0	19.2	40.8–79.2	0.028	0.866
Calabria	16	10	62.5	23.7	38.8–86.2		

Factor	n	BPV-2 Positive	%	SE % [§]	95 %CI	χ^2	P
Total	41	11	26.83	13.56	13.27–40.39	–	–
Age							
≤ 5 years old	15	4	26.67	22.38	4.29–49.05		
6–9 years old	14	4	28.57	23.66	4.91–52.24	0.024	0.9879
≥ 10 years old	12	3	25.00	24.50	0.50–49.50		
Gender							
Female	16	3	18.75	19.13	0.00–37.88		
Male	25	8	32.00	18.29	13.71–50.29	0.134	0.7143
Site of the skin lesion							
Limbs	13	2	15.38	19.61	0.00–35.00		
Head/Neck	13	5	38.46	26.45	12.01–64.91		
Trunk	15	4	26.67	22.38	4.29–49.05	1.024	0.5994
Location							
Campania	25	8	32.0	18.3	13.7–50.3		
Calabria	16	3	18.8	19.1	0.00–37.8	0.328	0.566

Factor	n	BPV-13 Positive	%	SE % [§]	95 %CI	χ^2	P
Total	41	8	19.51	12.13	7.38–31.64		
Age							
≤ 5 years old	15	5	33.33	23.86	9.48–57.19		
6–9 years old	14	3	21.43	21.49	0.00–42.92	3.465	0.1769
≥ 10 years old	12	0	–	–	–		
Gender							
Female	16	5	31.25	27.71	8.54–53.96		
Male	25	3	12.00	12.74	0.00–24.74	0.700	0.402
Site of the skin lesion							
Limbs	13	3	23.08	22.9	0.17–45.98		
Head/Neck	13	3	23.08	22.9	0.17–45.98		
Trunk	15	2	13.33	17.2	0.00–30.54	0.397	0.820
Location							
Campania	25	7	28.0	17.6	10.4–45.6		
Calabria	16	1	6.3	11.9	0.00–19.1	1.717	0.190

\$ SE = Standard Error * = $P < 0.05$.

§Standard Error.

\$Reference category.

#Odds Ratio.

performed on these samples revealed the presence of amplicons of the expected size, and the sequences obtained from the DNA fragments showed 100 % identity with the BPV-1, BPV-2, and BPV-13 DNA sequences deposited in GenBank (Fig. 3).

3.3. Statistical results

A total of 41 sarcoid samples from horses from 2 regions of southern Italy were tested for BPV-1, BPV-2, BPV-13 and BPV-14. Information on age, gender, location of skin lesions and geographical location was recorded. Of the 41 equine skin samples, 61 % were from the Campania region and 39 % from the Calabria region. Overall, 75.61 % (31/41, 95 % confidence interval (CI): 62.46–88.75 %) of the equine skin lesions

tested were positive for at least one of the three BPVs (Table 1). 10 equine sarcoid samples were simultaneously positive for more than one BPV, corresponding to a co-infection rate of 24.4 % (CI 95 %: 11.3–37.5). 4.9 % (CI 95 %: 0.00–11.5 %) were positive for BPV-1, BPV-2 and BPV-13, 9.8 % (CI 95 %: 0.67–18.8 %) were positive for BPV-2 and BPV-3 and finally 9.8 % (CI 95 %: 0.67–18.8 %) were positive for BPV-1 and BPV13. The data analysis showed that there was no statistical correlation between BPV positivity, and the variables considered (Table 1).

BPV-1 was found to be the most common papillomavirus with a prevalence of 60.98 % (25/41, 95 % CI: 40.06–75.91 %) (Table 3).

The prevalence of BPV-1 observed in females was statistically significant (p -value = 0.0140) (Table 3) compared to that observed in males, with an odds ratio (OR) of 8.9 (CI 95 %: 1.66–47.8), while there

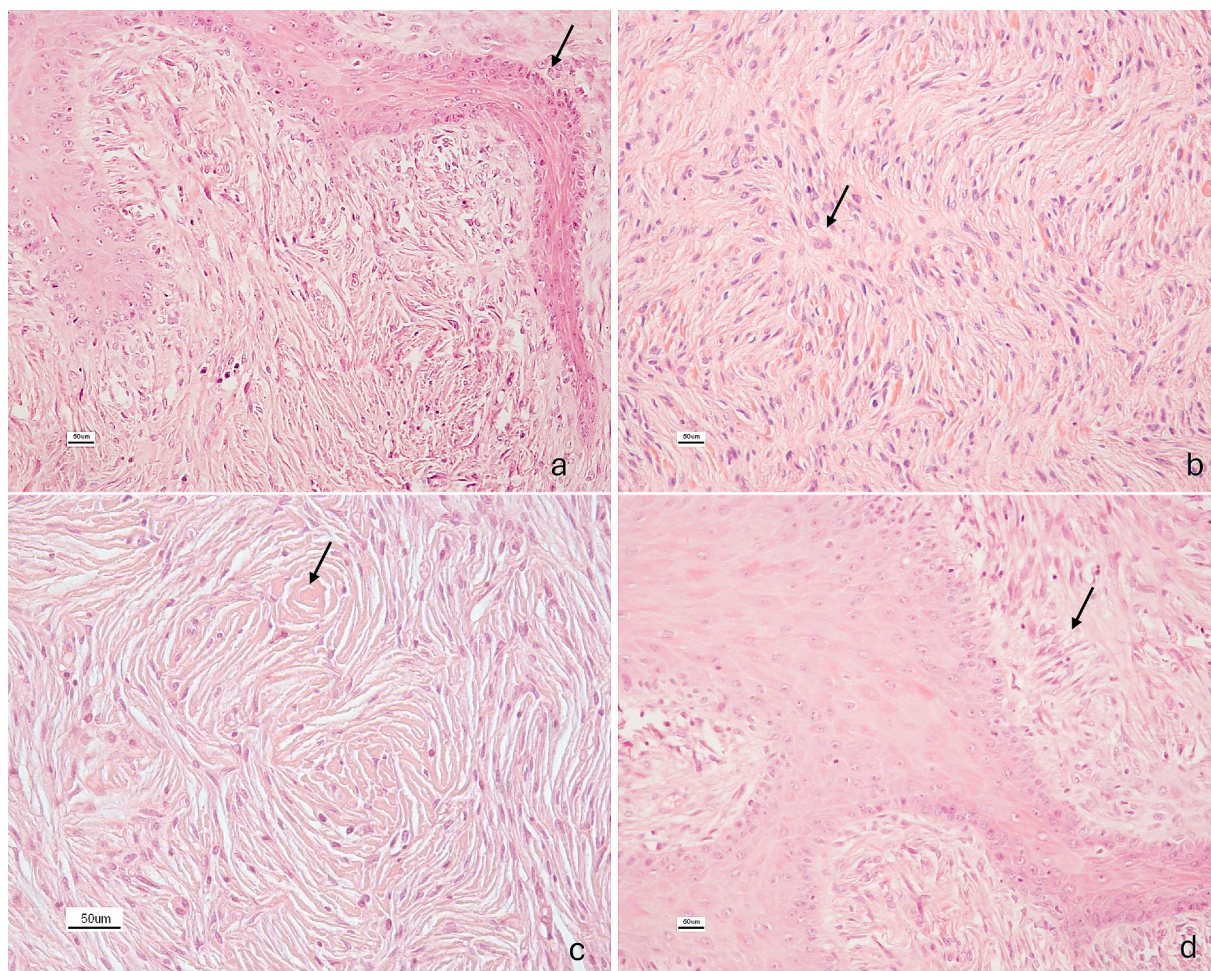


Fig. 1. Equine sarcoid. Haematoxylin and eosin, 200×; a) Hyperplasia with epithelial proliferations producing long rete peg (arrow), extending deep into the derma; b) Sarcoid fibroblasts with moderate to severe anisocytosis and anisokaryosis (arrow) organized in parallel rows and embedded in finely fibrillar to dense collagen; c) Proliferation of sarcoid fibroblasts whorling around a thick collagen bundle (arrow); d) Sarcoid fibroblasts oriented perpendicularly to the basement membrane at the dermal-epidermal junction, in the so-called “picket fence” arrangement (arrow).

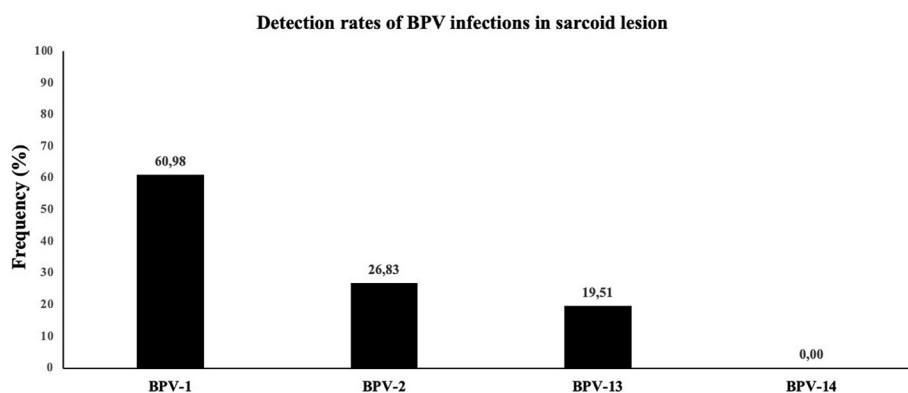


Fig. 2. Detection rates of BPV infections in tissues derived from sarcoid lesion using polymerase chain reaction (PCR).

was no statistical association between BPV-1 positivity and the other variables considered.

The BPV-2 genome was detected in 26.83 % (11/41, CI 95 % 13.27–40.39 %) of equine sarcoid samples. The statistical analyses showed that there was no statistically significant difference in prevalence in relation to the other variables considered (Table 3).

Finally, the genome of BPV-13 was detected in 19.51 % (8/41, CI 95 % 7.38–31.64 %) of equine sarcoid samples. No significant difference

was found in the molecular prevalence of BPV-13 in samples from horses of different age, gender, skin lesion location and geographical location (Table 3). BPV-14 genome was not detected in any sarcoid samples. Regarding the histopathological changes, the data analysis showed no statistical correlation between BPV infection, and the histopathological changes considered. However, severe fibroblastic atypia (78.6 %; 95 % CI: 57.1–100.0), abundant extracellular matrix (70.0 %; 95 % CI: 49.9–90.1) were the changes that occurred relatively frequently during

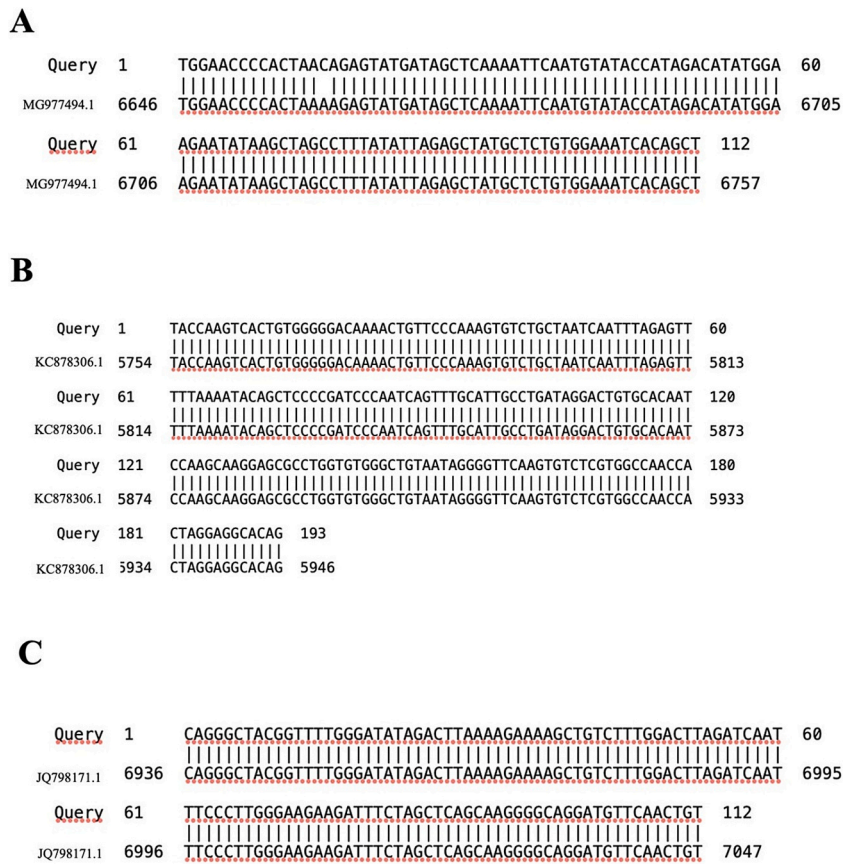


Fig. 3. Alignment: The amplicons (A) BPV-1 Seq, (B) BPV-2 Seq, (C) BPV-13 Seq, and the sequences reported in GenBank, respectively (accession number: MG977494.1; KC878306.1; JQ798171.1).

BPV-1 infection, followed by picket fence at the junction of the epidermis (60 %; 95 % CI: 38.5–81.5) and severe epidermal hyperplasia with rete pegs formation (54.5 %; 95 % CI: 33.7–75.4) (Table 4). In addition, severe epidermal hyperplasia with rete pegs formation (31.8 %; 95 % CI: 12.4–51.28) and picket fence at the junction of the epidermis (30 %; IC 95 %: 9.9–50.1) were the changes that occurred relatively frequently during BPV-2 infection, followed by mild

fibroblastic atypia (29.6 %; 95 % IC: 12.4–46.9) and moderate/severe fibroblastic atypia (21.4 %; 95 % IC: 0.00–42.9) (Table 4).

The BPV-13 genome was present in approximately 42.9 % (95 % CI: 16.9–68.8) of cases of mild fibroblastic atypia (Table 4), whereas the BPV-14 genome was not isolated in any of the samples analysed, neither in neoplastic nor in healthy skin.

Table 4
Histopathological features of BPV-1/BPV-2/BPV-13 positive equine sarcoids.

Histopathological features	BPV positive	BPV-1 Positive	%	SE % [§]	95 %CI	χ^2	P
Moderate/ severe epidermal hyperplasia with rete pegs	22	12	54.5	20.01	33.7–75.4	0.345	0.5571
Mild/moderate extracellular matrix	20	10	50.0	21.9	28.1–71.9	1.179	0.2776
Abundant extracellular matrix	20	14	70.0	20.1	49.9–90.1	0.147	0.7017
Picket fencing	20	12	60.0	21.5	38.5–81.5	0.038	0.8452
Mild fibroblastic atypia	27	14	51.9	18.9	33.0–70.7	1.757	0.185
Moderate/severe fibroblastic atypia	14	11	78.6	21.5	57.1–100.0	1.757	0.185

Histopathological features	BPV positive	BPV-2 Positive	%	SE % [§]	95 %CI	χ^2	P
Moderate/ severe epidermal hyperplasia with rete pegs	22	7	31.8	19.5	12.4–25.6	0.178	0.672
Mild/moderate extracellular matrix	20	4	20.0	17.5	2.5–37.5	0.640	0.423
Abundant extracellular matrix	20	3	15.0	15.7	0.00–30.7	0.373	0.541
Picket fencing	20	6	30.0	20.1	9.9–50.1	0.009	0.924
Mild fibroblastic atypia	27	8	29.6	17.2	12.4–46.9	0.036	0.849
Moderate/severe fibroblastic atypia	14	3	21.4	21.5	0.00–42.9	0.036	0.849
Histopathological features	BPV positive	BPV-13 Positive	%	SE % [§]	95 %CI	χ^2	P
Moderate/ severe epidermal hyperplasia with rete pegs	22	4	18.2	16.12	2.06–34.3	0.027	0.869
Mild/moderate extracellular matrix	20	4	18.2	16.12	2.06–34.3	0.101	0.750
Abundant extracellular matrix	20	4	18.2	16.12	2.06–34.3	0.101	0.750
Picket fencing	20	3	15.0	15.7	0.00–30.7	0.101	0.751
Mild fibroblastic atypia	27	2	7.41	9.88	0.00–17.3	0.037	0.847
Moderate/severe fibroblastic atypia	14	6	42.9	25.9	16.9–68.8	0.037	0.847

4. Discussion and conclusion

It is widely accepted that equine sarcoids are associated with the presence of Bovine δ papillomaviruses (δ BPV), with the proportion caused by each BPV type dependent on the country in which affected horses are raised. Current evidence suggests that Bovine papillomavirus (BPV-1 or BPV-2) are the primary cause of most equine sarcoids (Nasir and Brandt, 2013). Also BPV-13 was found in some equine sarcoids from Southern Brazil (Lunardi and Driemeier, 2013; Jindra et al., 2021), while BPV14, the cause of feline sarcoids (Munday et al., 2010; Munday et al., 2015), was reported only in healthy equines. However, to best of our knowledge, its presence has never been investigated in equine sarcoids. This is the first study addressing the presence of all δ -papillomaviruses (BPV-1, BPV-2, BPV-13 and BPV-14) in equine sarcoids from Southern Italy. Our results demonstrate the presence of BPV DNA in all examined equine sarcoid samples, with the highest frequency represented by BPV-1, followed by BPV-2 genotypes. Conversely to what reported in a study conducted on equine sarcoid samples from Northern Italy (Jindra et al., 2021), we found a consistent number of sarcoid samples that tested positive for BPV-13 DNA. High prevalence of BPV-2 and BPV-13 infections was found in Southern Italy's cattle population (Roperto et al., 2016), suggesting a correlation between genotypes detected in cattle and those found in sarcoid-affected horses living in the same geographical area.

For the first time, we investigated the presence of BPV14 DNA in sarcoid samples, and results showed the absence of this specific genotype suggesting that this virus does not appear to be present in Southern Italy to date. Interestingly, according to previous studies (Carr et al., 2001; Epperson and Castleman, 2017), we have found BPV genome also in non-sarcoid tumours and particularly in two fibromas, two papillomas, one myxoma and, for the first time in literature, in one melanoma. Moreover, as reported in other studies (Wobeser et al., 2012; Bogaert et al., 2005; Yuan et al., 2007) BPV genome was detected also in a variety of non-neoplastic conditions, but not in normal skin, suggesting a potential viral involvement in diseases other than tumours.

However, due to the small number of samples, we are unable to draw statistical conclusions. Therefore, large-scale screenings are needed to provide a more accurate estimation of the prevalence and distribution of BPV in non-sarcoid tumours and non-neoplastic conditions.

In addition to evaluating BPV presence and genotypes in equine sarcoids, the aim of our study was also to associate BPV genotypes to histopathological features of equine sarcoid, in order assess whether infection by a specific type of BPV could be somehow associated with the histopathological features of the tumor.

According to other studies that recognized equine sarcoid as biphasic tumours, comprising both epidermal and dermal components (Bogaert, 2011), we have found the changes that occurred relatively most frequently in equine sarcoid samples were moderate/severe hyperplasia with rete pegs, moderate/severe fibroblastic atypia, and picket fence" arrangement of fibroblasts beneath the epidermis, which are considered pathognomonic features for the histological diagnosis of sarcoids (Bogaert, 2011).

BPV-1 was associated with more severe histopathological manifestations, such as marked fibroblastic atypia (78.6 %) and abundant extracellular matrix (70.0 %), while BPV-2 and BPV-13 showed more moderate alterations as mild fibroblastic atypia (29.6 %) and moderate extracellular matrix deposition (21.4 %).

However, BPV-2 positive samples showed relatively frequently proliferation of fibroblast at the junction of the epidermis with picket fence pattern and severe epidermal hyperplasia with rete pegs formation, confirming the complex and strict interactions between fibroblasts and epithelial cells in equine sarcoid pathogenesis. These observations are in line with those previously suggested, indicating that the development of equine sarcoids could result from the proliferation of overlying epithelial cells (with rete peg formation), stimulated by sarcoid fibroblasts in the underlying dermis, which, in turn, stimulate dermal fibroblasts to

proliferate and produce collagen (Martano et al., 2016).

These findings suggest potentially distinct pathogenic effects of different BPV genotypes on sarcoid histopathology and a genotype-dependent severity of disease.

The strict association between the severity of fibroblastic atypia and ECM deposition could be in line with our previous studies, which reported an imbalance between the production and degradation of ECM in equine sarcoids, closely correlated with abnormal fibroblast proliferation (Martano et al., 2016).

Despite these interesting results, no statistical correlation was found between BPV genotypes and histopathological features, suggesting the need of larger sample to gain a deeper understanding of BPV involvement in the development of these tumours.

Current treatments of equine sarcoid mainly involve surgery and local therapies, but none address the viral cause of sarcoid formation, leading often to recurrence after treatment or transformation in a more severe form. Immunotherapeutic approaches, including vaccines targeting BPV, could be a promising strategy to manage equine sarcoids (Jindra et al., 2023). Deeper knowledge of BPV role in pathogenesis of equine sarcoid could be certainly useful in the near future for the development of new therapies for equine sarcoids.

CRediT authorship contribution statement

Manuela Martano: Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Serena Montagnaro:** Writing – original draft, Validation, Software, Investigation, Formal analysis. **Karen Power:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation. **Bianca Cuccaro:** Validation, Resources, Investigation, Data curation. **Anna Cutarelli:** Validation, Methodology, Data curation. **Paola Maiolino:** Supervision, Funding acquisition, Conceptualization. **Brunella Restucci:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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