

SHORT COMMUNICATION

Identification of bovine papillomavirus types by PCR on biopsies of equine sarcoid in Chile

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ABSTRACT. Neoplasms are an important cause of morbidity and mortality in horses, with approximately 50% of cases related to the integumentary system. Equine sarcoid is the most common cutaneous neoplasm of this species worldwide and it is characterized by its locally aggressive behavior. Cross-species infection by the Bovine Papillomavirus (BPV) genus *Deltapapillomavirus* (types BPV-1, BPV-2, and BPV-13) is a key factor for sarcoid development. However, no studies have been conducted in Chile on the prevalence and distribution of the disease or the identification of circulating BPV types. Therefore, this study focused on identifying the BPV types present in equine skin biopsies from different regions of the country. For this study, 23 paraffin blocks of equine biopsies with a histopathological diagnosis of sarcoid were collected from five localities in Chile, including cases from 2004 to 2025. Histopathological diagnoses were verified before DNA extraction, and PCRs were performed to detect BPV-1, BPV-2, and BPV-13. Fisher's exact test was applied to evaluate the associations between viral type and sex, breed, and age of the sampled individuals. Of the 23 samples analyzed, 100% met at least one histopathological criterion for inclusion in the study. PCR analysis revealed that 35% (8/23) of the samples tested positive for BPV-1, 91% (21/23) tested positive for BPV-2, 26% (6/23) exhibited co-detection of BPV-1 and BPV-2, and no sample (0/23) tested positive for BPV-13. Regarding geographical distribution, BPV-1 was identified in three regions, whereas BPV-2 was identified in all five regions (5/5). There were no statistically significant associations between viral type and sex, breed, or age ($P > 0.05$). This study provides a baseline for the viral characterization of this equine disease across the country, allowing for the development of prevention and treatment strategies that align with the context of the affected local population.

Keywords: Sarcoid, equine, bovine papillomavirus (BPV), PCR.

INTRODUCTION

Equine sarcoid is the most common cutaneous neoplasm affecting the *Equidae* family worldwide, accounting for approximately 13–69% of all equine cutaneous tumors (Knottenbelt *et al.*, 2015). Its overall prevalence is reported to be 1–12%, although it may be higher in certain populations (Tuemmers & Quezada, 2019). This fibroblastic lesion is characterized by its locally invasive behavior and most frequently presents as multiple rather than solitary lesions (Ogluszka *et al.*, 2021).

Macroscopically, equine sarcoid lesions are classified into six distinct types: occult, verrucous, nodular, fibroblastic, mixed, and malignant (Knottenbelt *et al.*, 2015). Microscopically, they all share an increased density of dermal fibroblasts in various organizational patterns and variably hyperplasia of the spinous layer of the epidermis (acanthosis) with extensions into the dermis (“rete pegs”), and a characteristic perpendicular orientation of fibroblasts toward the basement membrane at the dermo epidermal junction (“picket fence” pattern) (Ogluszka *et al.*, 2021).

The pathogenesis of equine sarcoid involves multiple factors, including environmental and genetic components, of which Bovine Papillomavirus (BPV) infection is considered the primary extrinsic factor (Bogaert *et al.*, 2005; Tuemmers & Quezada, 2019). Nonetheless, recent studies have demonstrated a lack of predisposition associated with the sex and age of the affected individuals (Ogluszka *et al.*, 2021). Neoplasm development has been frequently recognized in previously traumatized tissues, including those subjected to injections and insect bites, which may serve as sites of entry for BPV (Knottenbelt *et al.*, 2015).

The *Papillomaviridae* family comprises double-stranded DNA viruses capable of infecting the skin and mucous membranes of several species (Yamashita-Kawanishi *et al.*, 2021). Within this family, the genus *Deltapapillomavirus* includes bovine papillomaviruses capable of causing cross-species infections, such as BPV-1, BPV-2, BPV-13, and potentially BPV-14 (Cutarelli *et al.*, 2025; Yamashita-Kawanishi *et al.*, 2021). While BPV induces dermal fibropapillomas with spontaneous regression due to a local

immune response in cattle, there appears to be no effective immune response to infection in equines (Ogluszka *et al.*, 2021). Furthermore, in the equine host, BPV infection is considered abortive, as viral DNA is only found as free circular episomes in the cell nucleus, and no intact virions have been found to be produced (Knottenbelt *et al.*, 2015).

BPV-1 and BPV-2 are considered to share a comparable oncogenic mechanism in equine sarcoid, with early genes E5, E6, and E7 consistently expressed. For BPV-1, these have been shown to directly contribute to fibroblast transformation and invasion (Yuan *et al.*, 2011). However, direct evidence comparing the relative virulence or clinical impact of BPV-1 and BPV-2 remains limited, and functional data on BPV-13 in this context are limited.

In Chile, the only mention of equine sarcoid in veterinary literature comes from a thesis by Ildefonso Cruz (1997) at Universidad Austral de Chile. In that study, 12.2% of the 295 equine biopsy samples analyzed were identified as neoplasms, among which 52.8% were classified as mesenchymal subcutaneous lesions, presumed to be equine sarcoid. In addition, there are no other studies regarding the prevalence or distribution of equine sarcoid or the identification of the types of bovine papillomavirus associated with this condition in Chile. Therefore, this study aimed to identify the types of BPV present in equine sarcoid biopsies from different areas of the country and analyze their association with variables such as sex, breed, and age of the individuals within the local context.

MATERIALS AND METHODS

A total of 23 blocks of formalin-fixed, paraffin-embedded (FFPE) equine skin biopsies from 2004 to 2025 were collected from five localities in Chile (Santiago, Rancagua, Los Ángeles, Valdivia, and Osorno), along with their respective characterization in terms of sex, breed, and age (Supplementary Material, Table 1).

To confirm the diagnosis of equine sarcoid, histopathological evaluation was performed. Histologic slides were obtained from each paraffin block and stained with Hematoxylin and Eosin (H/E). Histologic analysis was conducted sequentially, under light microscopy, over the entire section of tissue; initially, at 4x magnification general tissue architecture was assessed, followed by a more exhaustive analysis of morphological changes at 10x and 40x magnification. The inclusion criteria for equine sarcoid samples in this study were the presence of at least one of the following morphological changes: 1) proliferation of dermal fibroblasts (PF), 2) perpendicular orientation of fibroblasts toward the basement membrane at the dermo-epidermal junction (“picket fence” pattern) (PO), and 3) irregular acanthosis with extensions into the dermis (“rete pegs”) (AR) (Figure 1).

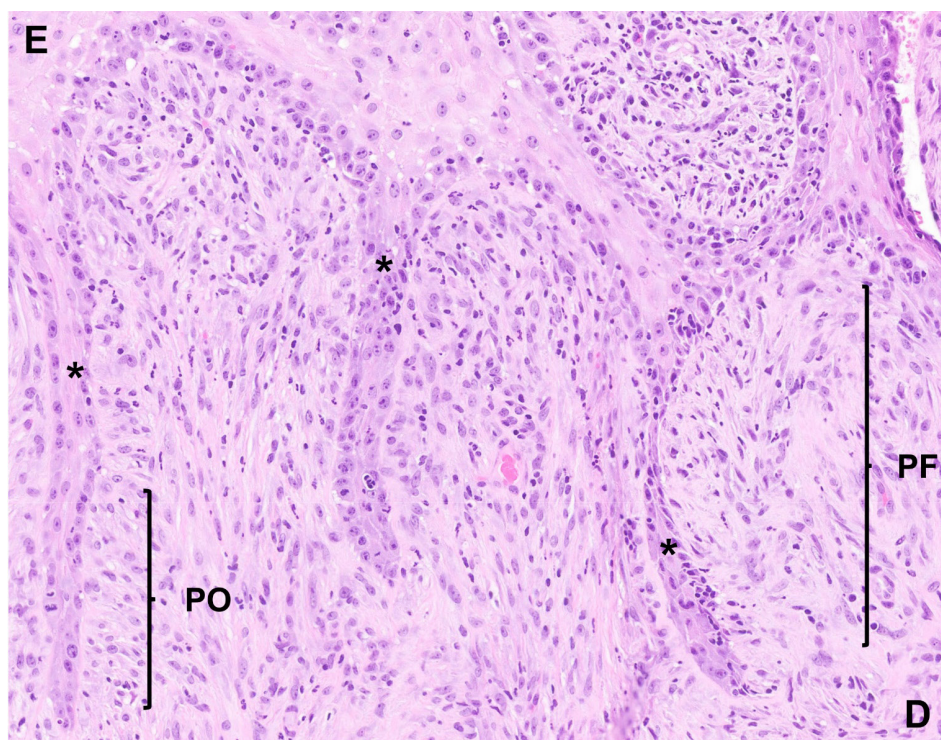


Figure 1.

Microscopic morphologic changes of equine sarcoid considered as inclusion criteria in this study. Hematoxylin and Eosin stain. (PF) Proliferation of dermal fibroblasts. (PO) Perpendicular orientation of fibroblasts toward the basement membrane, “picket fence” pattern. * Acanthosis with “rete peg” formation. (E) Epidermis. (D) Dermis.

Once the inclusion criteria were met, three sections of 5µm were cut from each block using a microtome, and following deparaffinization, DNA was extracted following the manufacturer's instructions for Omega Bio-tek E.Z.N.A. FFPE DNA kit. Consequently, 1µl of DNA from each sample was placed in a spectrophotometer (NanoDrop) for quantification (expressed in ng/µl) and assessment of purity (260/280 nm ratio).

For this experiment, four PCR reactions were performed. First, an initial test was conducted to evaluate the viability of the DNA obtained, for which a 153-bp segment of the equine mitochondrial gene was amplified. If a positive result was obtained for this first reaction, the extracted DNA was used as a template for the detection of a 301-bp fragment of the BPV-1 L1 gene, a 164-bp fragment of the BPV-2 L1 gene, and a 188-bp segment of the BPV-13 L2 gene. Primers targeting the L1 gene of BPV-1 and BPV-2 and the L2 gene of BPV-13 were designed specifically for this study (Supplementary Material, Table 2). One PCR product for each viral type was purified and sequenced prior to the study to confirm target specificity, and the sequence-confirmed amplicons were subsequently used as positive controls in all following reactions.

For all PCR reactions (equine mitochondrial gene and the three bovine papillomavirus types), a total volume of 25 µl was used, consisting of 2 µl of extracted DNA, 1 µl to the primers (0.5 µl of each), 7 µl to nuclease-free water, and 15 µl to PCR Master Mix (2X; containing Taq polymerase [8.5 pH, 50 units/ml], 400 µM dNTPs, and 3 mM MgCl₂). The thermal profile was set to an initial denaturation at 95 °C for 10 min, followed by 35 cycles of 95 °C for 45 s, annealing for 45 s (at 58.8 °C for the equine mitochondrial gene and 60 °C for the bovine papillomaviruses), and extension at 72 °C for 45 s, with a final extension step at 72 °C for 5 min.

One product for each type, previously sequenced, was used as a positive control for the following reactions, and nuclease-free water was used as a negative control. For electrophoresis, a 1.5% agarose gel was prepared with 2 µl of SafeView Plus. The PCR products were pipetted into the gel and run at 110 volts for 40 min. Afterwards, the gel was transferred to a UV light transilluminator for visualization.

For analysis, a database was generated with tables containing sample number, year of sampling, sex, breed, age, and locality of origin of the tested individuals, followed by the addition of the amount of DNA obtained from each sample, the histopathological criteria observed, and the PCR results (positive or negative) for each of the reactions performed, including the equine mitochondrial gene and bovine papillomavirus types 1, 2, and 13. Finally, Fisher's exact test with a 95% confidence interval was applied to evaluate the associations between viral type and sex, breed, and age. This analysis was performed using the "R" software version 4.3.1 for Windows.

RESULTS AND DISCUSSION

Results and discussion

The study population consisted of 23 equine sarcoid biopsies collected between 2004 and 2025; 11 (48%) corresponded to females and 12 (52%) to males, with an age range of 1–12 years. Regarding breed, 18 individuals were "Caballo de Raza Chilena" (CRC, 78.3%), three were Thoroughbreds (TB, 13.1%), one was Anglo-Arabian (AA, 4.3%), and one was Holsteiner (4.3%). Regarding the localities of origin, 12 samples were from Los Ángeles (52.2%), four from Osorno (17.4%), three from Valdivia (13%), three from Santiago (13%), and one from Rancagua (4.3%) (Supplementary Material, Table 1).

Histopathological analysis showed that 23/23 samples presented proliferation of dermal fibroblasts (PF, 100%), 10/23 samples presented perpendicular orientation of fibroblasts toward the basement membrane or "picket fence" pattern (PO, 43.5%) and 12/23 presented acanthosis with "rete peg" formation (AR, 52.2%) (Figure 2A). In addition, 10/23 samples presented all three of the morphologic changes analyzed (PF, PO, and AR, 43.5%), 2/23 presented only PF and AR (8.7%), and 11/23 samples presented only PF (47.8%) (Figure 2B).

Consistent with Tarwid *et al.* (1985), the proliferation of dermal fibroblasts is a constant morphologic change in the analyzed equine sarcoid lesions, while the picket fence pattern and acanthosis with rete peg formation are variably presenting changes. As equine sarcoid accounts for the large majority of cutaneous mesenchymal neoplasms in horses, with fibroma and fibrosarcoma being comparatively rare in this species (Knottenbelt *et al.*, 2015), the presence of PF alone was considered consistent with equine sarcoid for inclusion purposes.

Of the 23 samples analyzed, 100% tested positive for the amplification of a 153-bp segment of the equine mitochondrial gene, 8/23 samples tested positive for a 301-bp segment of the BPV-1 L1 gene (35%), and 21/23 samples tested positive for the amplification of a 164-bp segment of the BPV-2 L1 (91%), with 6/23 samples exhibiting co-detection of BPV-1 and BPV-2. No samples tested positive for the amplification of a 188-bp segment of the BPV-13 L2 gene (Table 1).

Bovine papillomavirus (BPV) is widely recognized as an essential extrinsic factor for the development of equine sarcoid, with the presence of type 1 and, less frequently, types 2 and 13, as demonstrated by PCR techniques (Ogluszka *et al.*, 2021). The high detection rate of BPV-2 observed in this study is consistent with geographic patterns reported elsewhere, where BPV-2 predominates over BPV-1 in certain regions, such as the western United States and Oceania (Cutarelli *et al.*, 2025). Given that horses in Chile are frequently raised in close contact with cattle under extensive pasture-based systems, greater exposure to circulating bovine BPV-2 strains may partly explain

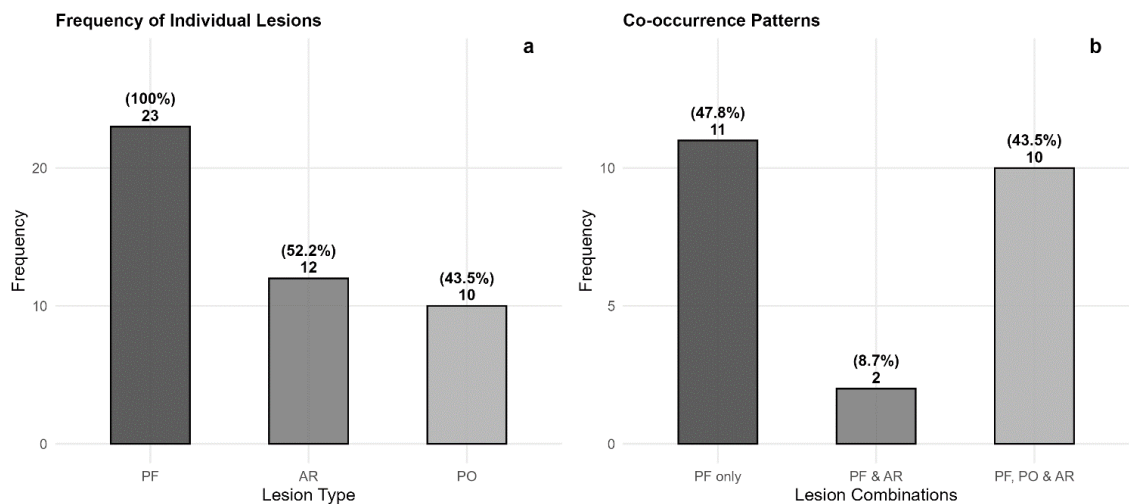


Figure 2. Frequency of morphologic changes consistent with equine sarcoid in 23 analyzed biopsies. (a) Frequency of individual lesions. (b) Frequency of co-occurring patterns. (PF) Proliferation of dermal fibroblasts. (PO) Perpendicular orientation of fibroblasts toward the basement membrane, “picket fence” pattern. (AR) acanthosis with “rete peg” formation.

Table 1. Results from DNA extraction, 260/280 nm ratio, and PCR for equine mitochondrial gene, BPV-1, BPV-2 and BPV-13, from formalin-fixed, paraffin-embedded blocks of 23 biopsies consistent with equine sarcoid.

#	DNA (ng/μl)	260/280nm ratio ¹	Equine mitochondrial	BPV-1	BPV-2	BPV-13
1	383.3	1.87	+	-	+	-
2	461.3	1.86	+	-	+	-
3	735	1.88	+	-	+	-
4	195.6	1.88	+	-	+	-
5	435.6	1.86	+	-	+	-
6	532.3	1.82	+	-	+	-
7	114.2	1.90	+	-	+	-
8	186.8	1.91	+	+	-	-
9	94.1	1.97	+	+	+	-
10	596.1	1.87	+	-	+	-
11	120.8	1.85	+	+	+	-
12	253.2	1.91	+	-	+	-
13	432.2	1.92	+	+	-	-
14	41.9	1.96	+	-	+	-
15	205	1.90	+	-	+	-
16	487.5	1.87	+	-	+	-
17	167.2	1.81	+	-	+	-
18	363	1.90	+	-	+	-
19	166	1.91	+	+	+	-
20	89.8	1.89	+	+	+	-
21	524.7	1.88	+	-	+	-
22	90.1	1.90	+	+	+	-
23	60.5	1.87	+	+	+	-

¹The 260/280 nm absorbance ratio reflects DNA purity, based on the differential absorbance of nucleic acids (260 nm) and proteins (280 nm). Ratios between ~1.8 and 2.0 are generally considered indicative of sufficiently pure DNA for downstream PCR applications; lower ratios may suggest residual protein or phenol contamination, while higher ratios may indicate RNA contamination.

this finding; however, characterization of BPV types in the local cattle population would be needed to confirm this hypothesis. Additionally, only a small number of equines have presented simultaneous DNA from BPV-1 and 2 in their lesions, or two sarcoids with different BPV types (Knottenbelt *et al.*, 2015).

In addition to the completely sequenced BPV types, numerous presumptive new types have been identified in bovine herds from different geographic regions using PCR assays that amplify partial fragments of the L1 gene (Lunardi *et al.*, 2013). In this study, we were unable to identify BPV-13 in any of the 23 samples analyzed. In addition to the small population size, it is also possible that the high genetic similarity between BPV-13 and BPV-2 in the L1 gene region could lead to cross-reactions or misidentification if direct sequencing of the amplicons is not performed,

as previously noted by Lunardi *et al.* (2013), who designed BPV-13-specific primers targeting the more divergent L2/LCR region for this reason.

As for the geographic distribution of the results, in the locality of Santiago ($n=3$), one sample tested positive for only BPV-1 (1/3) and one for only BPV-2 (1/3), with the third exhibiting co-detection of both types (1/3); in Rancagua ($n=1$), the sample tested positive only for BPV-2 (1/1); in Los Ángeles ($n=12$), nine samples tested positive for only BPV-2 (9/12), with three samples exhibiting co-detection of both types; in Valdivia ($n=3$), one sample tested positive for only BPV-1 (1/3), and two samples exhibited co-detection of both types (2/3); lastly, in the locality of Osorno ($n=4$), all of the samples tested positive for only BPV-2 (4/4) (Figure 3).

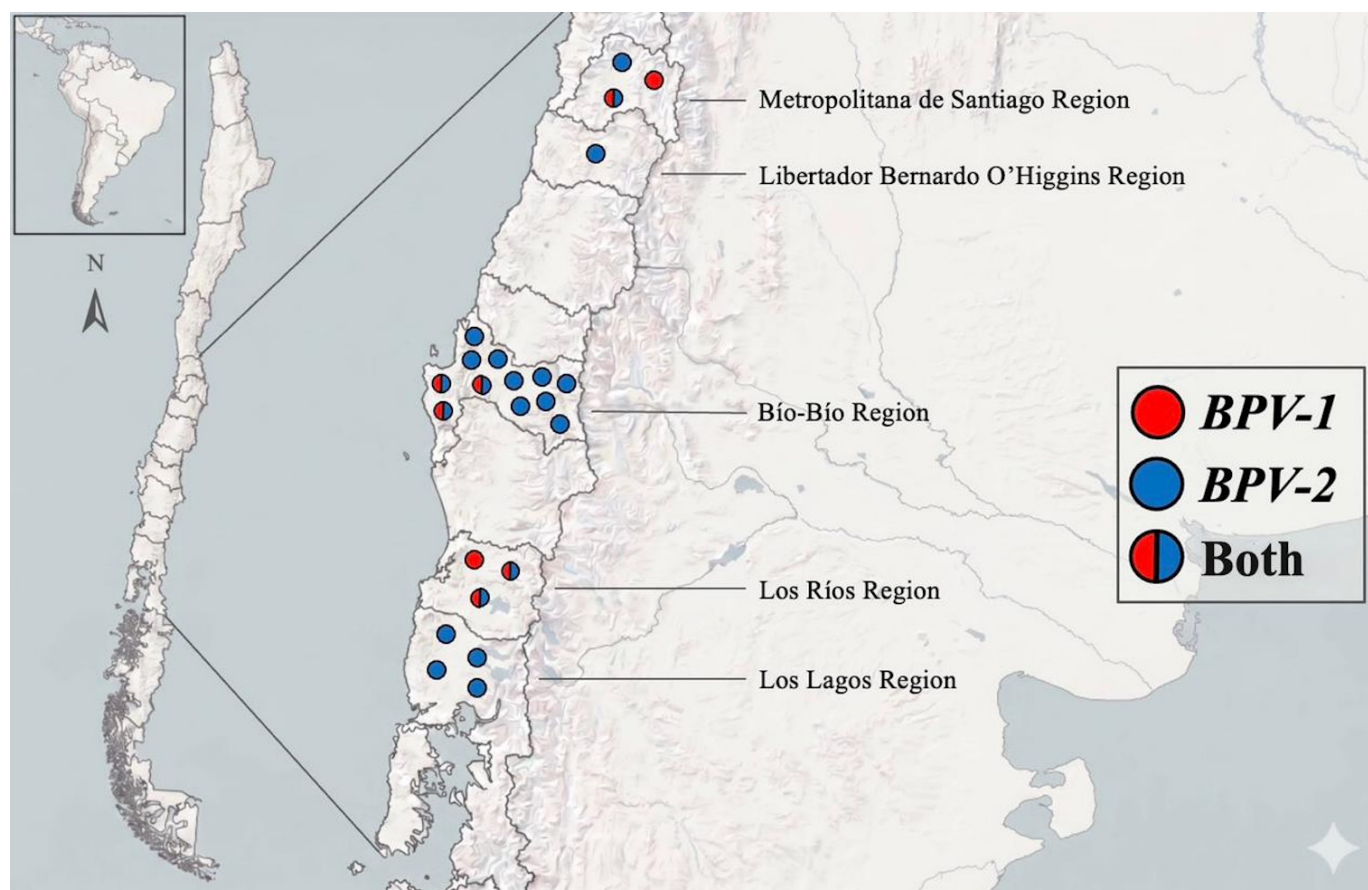


Figure 3.

Geographical distribution of BPV-1 and BPV-2 PCR results from 23 equine sarcoid biopsies analyzed in this study.

Based on these results, BPV-2 was present across all analyzed regions of Chile (Metropolitana de Santiago, Libertador Bernardo O'Higgins, Bío Bío, Los Ríos, and Los Lagos). In contrast, BPV-1 was not detected in Libertador Bernardo O'Higgins and Los Lagos. While these findings confirm the presence of both viral types, several study limitations should be noted, particularly the geographical coverage (comprising only five of 16 regions), the small sample size ($n = 23$), and the uneven representation across regions.

The frequency of sample positivity for BPV-1, BPV-2, and their co-detection was independent of the variables studied, including sex, breed, and age ($P > 0.05$) (Supplementary Material, Table 3). These results are in agreement with those of Ogluszka et al. (2021) regarding the absence of predispositions associated with the sex or age of the animals. Concerning the breed of the animal, many factors may be involved, such as the heightened exposure of certain breeds to bovine herds and the genetic susceptibility associated with the presence of equine leukocitary antigens, such as W13 and A3, which were not included in this analysis (Angelos et al., 1988; Broström et al., 1988; Knottenbelt et al., 2015).

To date, there are no indexed publications on the identification or distribution of bovine papillomavirus types present in equine sarcoid lesions in Chile. Therefore, this study provides a baseline for the viral characterization of this equine disease across the country. Through the analysis of samples from five localities in the country, the presence of BPV-1 and BPV-2 was detected in equine sarcoid biopsies; however, BPV-13 was not detected. This information can be compared to international findings and considered for further molecular research and the development of prevention and treatment strategies that align with the context of the local affected population.

DECLARATIONS

Competing Interests Statement

The authors declare no competing interests.

Author Contributions

MJRM: original draft, review and editing, investigation, formal analysis and data curation. CAMF: validation, methodology, review. LBL: methodology, review. EAM: methodology. ICL: research material, review and editing. AO: research material, review and editing. MAN: review and editing, investigation, project administration, formal analysis and conceptualization.

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