

PCR-BASED DETECTION OF BOVINE PAPILLOMAVIRUS TYPE 1 (BPV-1) IN OCULAR SQUAMOUS CELL CARCINOMA SAMPLES FROM CATTLE IN THE AZORES

Beatriz Bilhastre^{1,2}, Carlos Pinto³, Helena Vala⁴, Paulo Mascarenhas¹, Sandra Branco⁵ , Ana Clara Ribeiro¹

1 Molecular Biology Laboratory, Egas Moniz Center for Interdisciplinary Research, CiiEM, Egas Moniz University Institute, Quinta da Granja, Monte da Caparica, 2829-511 Caparica, Portugal;
2 MED – Mediterranean Institute of Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute of Research and Advanced Training, University of Évora, Pólo da Mitra, Ap. 94, 7006-554, Évora, Portugal;
3 Carlos Pinto, Centro de Biotecnologia dos Açores, TERINOV Terra Chã, 9700-702 Angra do Heroísmo, Portugal;
4 Helena Vala, CERNAS, Escola Superior Agrária de Viseu, Instituto Politécnico de Viseu, Campus Politécnico, 3504-510 Viseu, Portugal ; Center for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal;
5 University of Evora, MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Department of Veterinary Medicine, School of Sciences and Technology, Laboratory of Pathological Anatomy of the EU Veterinary Hospital. Pólo da Mitra, 7002-554 Évora, Portugal.

INTRODUCTION AND AIMS

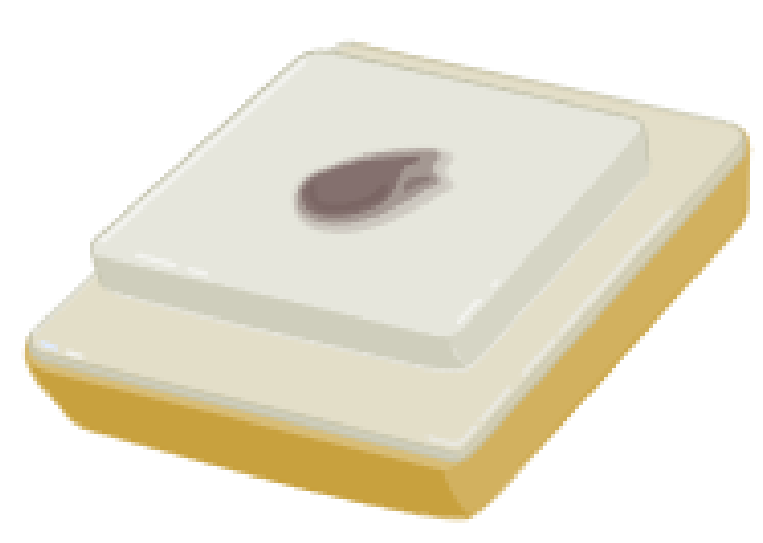
Bovine Ocular Squamous Cell Carcinoma (BOSCC) is the most prevalent ocular malignancy in cattle, primarily affecting the nictitating membrane but also involving other ocular and periocular structures, including the cornea, sclera, eyelids, third eyelid, limbus, and conjunctiva. Originating from keratinocytes, BOSCC is a significant cause of economic loss due to carcass rejection, increased veterinary costs, and decreased productivity (1-4). The disease has a multifactorial aetiology, with contributing factors including prolonged exposure to ultraviolet radiation, geographical elements such as latitude and altitude, as well as genetic predispositions, such as breed susceptibility and lack of eyelid pigmentation (1,5,6). Biological factors also play a role, particularly viral infections such as herpesviruses types 1 to 5 and papillomaviruses. Among these, bovine papillomaviruses (BPVs) — especially BPV-1 — have been associated with both benign and malignant tumours in cattle across various anatomical locations (5). The aim of this study was to identify the presence of BPV-1 DNA in bovine ocular squamous cell carcinoma samples, to better understand a potential viral contribution to tumorigenesis.

MATERIALS AND METHODS

134

samples was analysed.

1



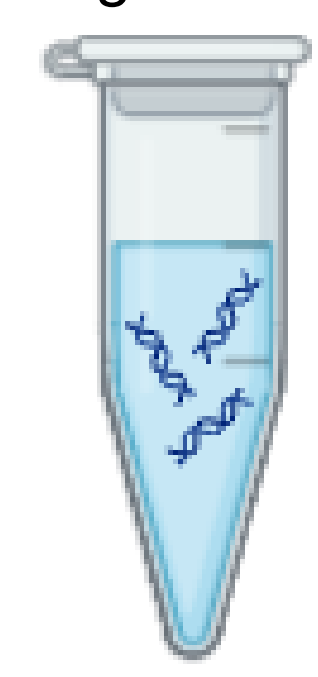
EXTRACTION

Tumour DNA

Normal mucosa


2

DNA EXTRACTION
(NZY Tissue gDNA Isolation Kit)



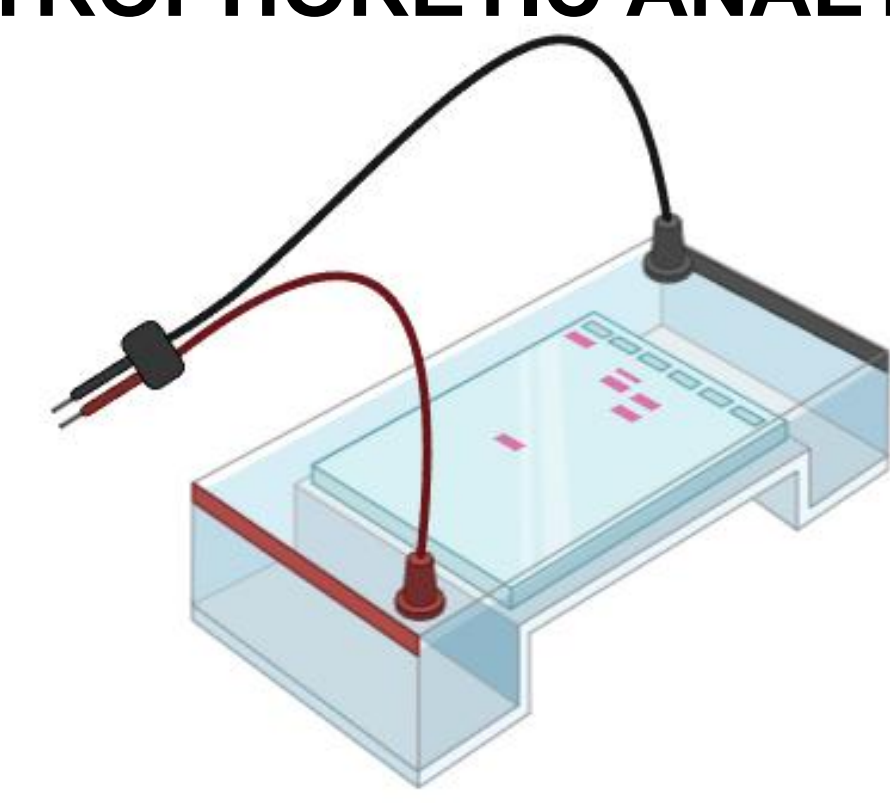
3

PCR



4

ELECTROPHORETIC ANALYSIS



The statistical analysis was conducted using a Bayesian Generalized Linear Mixed Model with fixed effects (log-odds scale)

RESULTS

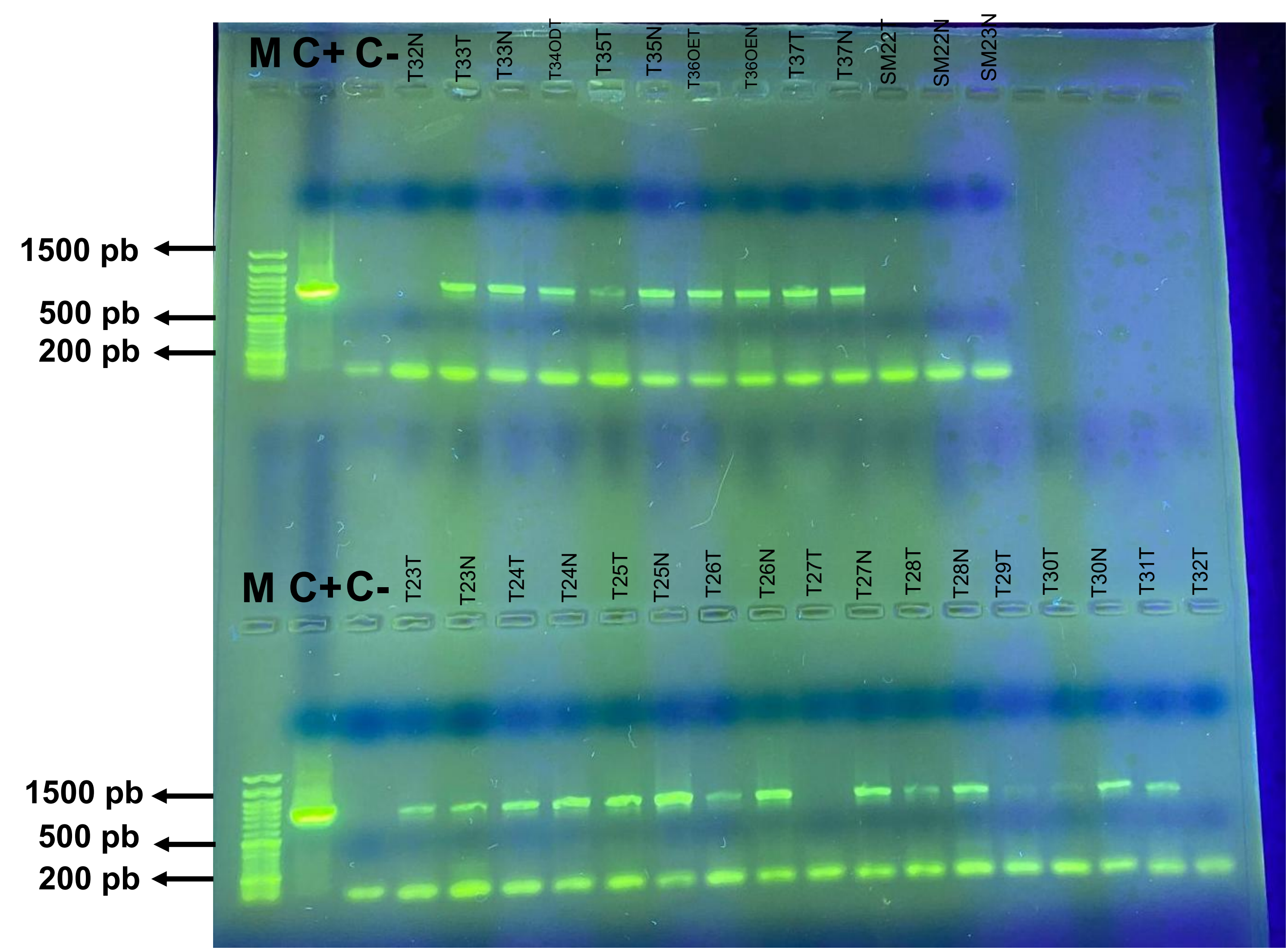


Table 1. Classification of samples based on electrophoresis results, comparing tumor (T) and matched mucosa (M) according to band presence (+) or absence (–); ✓ indicates the category each sample pair falls into (T–M+, T+ M–, or T+ M+), with only complete T/M pairs included in the analysis — unpaired samples were excluded.

SAMPLE ID	T- M+	T+ M-	T+ M+
T23			✓
T24			✓
T25			✓
T26			✓
T27	✓		
T28			✓
T30			✓
T33			✓
T35			✓
T36			✓
T37			✓
TOTAL	1	0	10

18%

Of samples were

POSITIVE (n=24) for BPV-1 infection

Figure 1. Agarose gel (1%) after electrophoresis of the DNA products obtained from PCR technique. DNA Ladder: NZYDNA Laddeer VI 50-1500pb.

CONCLUSION

There is no strong evidence that island or virus status significantly predicts the presence of carcinoma in this sample. Extremely high variation at the tissue level highlights the importance of tissue-specific factors. The model estimates are imprecise, likely due to the small sample size or the complex data structure.

These findings suggest a possible association between BPV-1 infection and the development of BOSCC ($p>0,05$), although further studies with a larger sample size and additional viral markers like other BPV types are needed to confirm a causal relationship. Understanding the role of BPV-1 in ocular carcinogenesis in cattle may contribute to future strategies for prevention and control of this pathology, including potential vaccination or breeding programs aimed at reducing susceptibility to viral oncogenesis.

1.Podarala, V; Prasanna Lakshmi, M; Venkata, S and Devalam, RP (2020). Efficacy of BCG vaccine and Mitomycin C for the treatment of ocular squamous cell carcinoma in bovines. Res. Vet. Sci., 133: 48-52.2
2.Vala, H; Carvalho, T; Pinto, C; Pereira, MA; Mesquita, JR; C Peleteiro, M; Ferrer, L and Fondevila, D (2020). Immunohistochemical studies of cytokeratins and differentiation markers in bovine ocular squamous cell carcinoma. Vet. Sci., 7: 70.
3.Karakurt, E; Aydin, U; Beytut, E; Dag, S; Ermutlu, CŞ; Aksoy, Ö; Nuhoğlu, H; Yıldız, A and Kurtbaş, E (2021). Prognostic significance of PCNA, MMP-9 and p53 in bovine ocular squamous cell carcinomas. J. Res. Vet. Med., 40: 98-105.
4.Fornazari, GA; Kravetz, J; Kiupel, M; Sledge, D; Filho, I and Montiani-Ferreira, F (2017). Ocular squamous cell carcinoma in Holstein cows from the South of Brazil. Vet. World. 10: 1413-1420.
5.Sözmen, M; Devrim, AK; Sudağıdan, M; Kabak, YB; Beytut, E and Özba, B (2019). Significance of angiogenic growth factors in bovine ocular squamous cell carcinoma. J. Comp. Pathol., 170: 60-69.
6.Tsujita, H and Plummer, CE (2010). Bovine ocular squamous cell carcinoma. Vet. Clin. North Am. Food Anim. Pract., 26: 511-529.