Evaluation of polymorphisms in the MBL2 gene exon 1 and their association with bovine papillomatosis in Girolando breed animals

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Abstract
Bovine papillomatosis is an infectious viral disease caused by Bovine Papillomavirus (BPV). Single nucleotide polymorphisms (SNPs) in genes associated with innate immunity have been widely investigated due to their relevance to animal disease susceptibility. Mannose-binding lectins (MBLs) are critical components of the innate immune response against various infectious agents, including viruses. Polymorphisms in the MBL2 gene may influence protein functionality, and this study aimed to evaluate the association between polymorphisms in exon 1 of the MBL2 gene and susceptibility to bovine papillomatosis. Blood samples were collected from 167 Girolando cattle (Gir × Holstein) from Northeastern Brazil, all carriers of BPV. Among these, 92 animals presented cutaneous symptoms of the disease, while 75 were asymptomatic. DNA was extracted from the samples, followed by PCR to detect BPV and polymorphisms, sequencing, and sequence alignment. SNPs were analyzed using SNPStats software, with a significance threshold set at 5%. The association between polymorphisms in exon 1 of the MBL2 gene and the development of papillomatosis was assessed using the Odds Ratio test, with a 95% confidence interval. Sequence analysis revealed 245 conserved sites and two variable sites, G235A and T244C, in exon 1 of the MBL2 gene. However, no significant association was identified between these SNPs' allele and genotype frequencies and susceptibility to cutaneous papillomatosis.
Keywords: bovine papillomavirus; cutaneous papilomatosis; innate immunity; MBL2 gene; single nucleotide polymorphisms.

Avaliação dos polimorfismos no exon 1 do gene MBL2 e sua associação com a papilomatose bovina em animais da raça Girolando

Resumo
A papilomatose bovina é uma doença viral infecciosa causada pelo Papilomavírus Bovino (BPV). Polimorfismos de nucleotídeo único (SNPs) em genes associados à imunidade inata têm sido amplamente investigados devido à sua relevância para a suscetibilidade a doenças animais. Lectinas de ligação à manose (MBLs) são componentes críticos da resposta imune inata contra vários agentes infecciosos, incluindo vírus. Polimorfismos no gene MBL2 podem influenciar a funcionalidade da proteína, e este estudo teve como objetivo avaliar a associação entre polimorfismos no exon 1 do gene MBL2 e suscetibilidade à papilomatose bovina. Amostras de sangue foram coletadas de 167 bovinos Girolando (Gir × Holandês) do Nordeste do Brasil, todos portadores de BPV. Entre estes, 92 animais apresentaram sintomas cutâneos da doença, enquanto 75 eram assintomáticos. O DNA foi extraído das amostras, seguido por PCR para detectar BPV e polimorfismos, sequenciamento e alinhamento de sequências. Os SNPs foram analisados usando o software SNPStats, com um limite de significância definido em 5%. A associação entre polimorfismos no exon 1 do gene MBL2 e o desenvolvimento de papilomatose foi avaliada usando o teste Odds Ratio, com um intervalo de confiança de 95%. A análise de sequência revelou 245 sítios conservados e dois sítios variáveis, G235A e T244C, no exon 1

do gene MBL2. No entanto, nenhuma associação significativa foi identificada entre as frequências de alelos e genótipos desses SNPs e a suscetibilidade à papilomatose cutânea.
***Palavras-chave**: gene MBL2; imunidade inata; papilomatose cutânea; polimorfismos de nucleotídeo único; vírus do papiloma bovino.*

1 Introduction

Bovine papillomatosis is a viral infectious disease caused by Bovine Papillomavirus (BPV), which adversely impacts herd productivity. Economically, this disease is significant due to its association with reductions in milk production, weight gain, and leather quality (Bocaneti *et al*., 2014; Daudt *et al*., 2018). Papillomaviruses belong to the Papillomaviridae family, which comprises 29 genera and includes a heterogeneous group of epitheliotropic, non-enveloped, circular double-stranded DNA viruses (Alfaro-Mora *et al*., 2022; Ugochukwu *et al*., 2019).

Infections caused by papillomaviruses typically trigger an immune response in the host, leading, in most cases, to lesion regression. This process involves the activation of both innate and adaptive immunity, with a predominant Th1 cellular response mediated by CD8+ T lymphocytes and macrophages, which facilitates the destruction of infected cells and the regression of lesions (Costa; Medeiros, 2014). However, cases have been reported in which cattle infected with multiple BPV types remain asymptomatic, suggesting that certain animals develop a more effective immune response, allowing better infection control (Carvalho *et al*., 2012). Conversely, infections involving specific viral types, co-factors, or impaired immune responses can progress to malignant lesions, such as bladder cancer and upper gastrointestinal cancer (Medeiros-Fonseca *et al*., 2022).

The association of single nucleotide polymorphisms (SNPs) with genes related to pathophysiology and pathogenesis has been extensively studied, as genetic variations may predispose individuals to disease susceptibility or confer protection (Clark; Baudouin, 2006; Namath; Patterson, 2011; Suffredini; Chanock, 2006). Innate immunity is the first line of defense against infectious agents (Kaur; Secord, 2019). Within this system, the complement cascade acts as an effector mechanism of humoral immunity (Merle *et al*., 2015). Mannose-binding lectins (MBLs), encoded by the MBL1 and MBL2 genes, play a crucial role in innate immune responses against various microorganisms (Takahashi *et al*., 2005).

Polymorphisms in the promoter region of the MBL2 gene may reduce the expression of the molecule, while mutations in the coding region can impair the oligomeric structure required for mannose-binding and complement system activation (Garred *et al*., 2006). These mutations have been linked to unfavorable outcomes in bacterial and viral infections (Thomas *et al*., 1996). Genetic variations in the MBL2 gene, found in both coding and non-coding regions, have been reported in humans, ovine, and swine. These variations can affect MBL assembly, resulting in low protein levels and immunological dysfunction (Juul-Madsen *et al*., 2011; Lillie *et al*., 2007; Liu *et al*., 2011; Madsen *et al*., 1998; Thiel; Gadjeva, 2009; Zhao *et al*., 2012). In humans, exon 1 polymorphisms in the MBL2 gene have been associated with an increased risk of high-risk HPV infection and cervical cancer development (Wang *et al*., 2016).

In cattle, the MBL2 gene is located on chromosome 26 and contains three introns and four exons, encoding a protein comprising 249 amino acids (Gjerstorff *et al*., 2004). Mutations in this gene have been implicated in increased susceptibility to various infectious agents (Capparelli *et al*., 2008; Holmskov; Thiel; Jensenius, 2003; Lillie *et al*., 2005; Takahashi *et al*., 2005). Polymorphisms in the MBL2 gene in cattle have been associated with mastitis susceptibility, adversely affecting milk production (Wang *et al*., 2012; Zhao *et al*., 2012). Additionally, studies suggest that polymorphisms in buffalo MBL2 genes are linked to resistance to Brucella abortus infection and mastitis (Capparelli *et al*., 2008; Shergojry *et al*., 2023).

Despite the functional importance of MBL2, there is limited information on its genetic variants in cattle and their relationship to disease resistance (Wang *et al*., 2012). Since MBL plays a role in pathogen recognition and elimination, and MBL2 polymorphisms may affect protein functionality, this study aims to evaluate the association between MBL2 gene polymorphisms and susceptibility to bovine papillomatosis.

This study investigates the potential association between polymorphisms in the MBL2 gene and susceptibility to clinical symptoms of papillomatosis in Girolando cattle. The article is organized as

follows: Section 2 provides the theoretical framework, including an overview of Bovine Papillomavirus (BPV) and a focused discussion on the molecular mechanisms of polymorphisms in the MBL2 and MHC genes that may influence immune responses to BPV. Section 3 details the research methodology, describing the analysis of 167 animal samples using PCR to amplify exon 1 of the MBL2 gene, followed by sequencing and SNP analysis, alongside a comprehensive description of the sampling procedures and genetic analyses conducted. Section 4 presents the results, identifying two informative sites of genetic variation. However, no significant association was observed between these polymorphisms and susceptibility to papillomatosis. These findings are discussed in the context of existing literature, highlighting their contribution to understanding the genetic determinants of immune response to BPV infection. Finally, Section 5 concludes by summarizing the key findings and their implications for developing more effective control and prevention strategies for bovine papillomatosis. The study underscores the importance of such research for the sustainability of Girolando cattle farming, a breed extensively utilized in Brazil.

2 Theoretical framework

This section provides an overview of papillomaviruses, addressing their classification, genotype diversity, and the clinical and pathological consequences associated with these viral infections. It begins with an introduction to Papillomaviruses (PVs), highlighting their classification as small, non-enveloped DNA viruses, their ability to infect various species, and the typical lesions they induce. The discussion then transitions to a detailed analysis of Bovine Papillomavirus (BPV), examining the diversity of BPV genotypes, the anatomical regions most commonly affected, and the conditions under which infections may progress to malignant neoplasms. Finally, the subsection on the molecular mechanisms of MBL2 and MHC explores polymorphisms in genes regulating the immune response to infections, emphasizing the role of MBL2 and BoLA polymorphisms in determining susceptibility to papillomavirus infections.

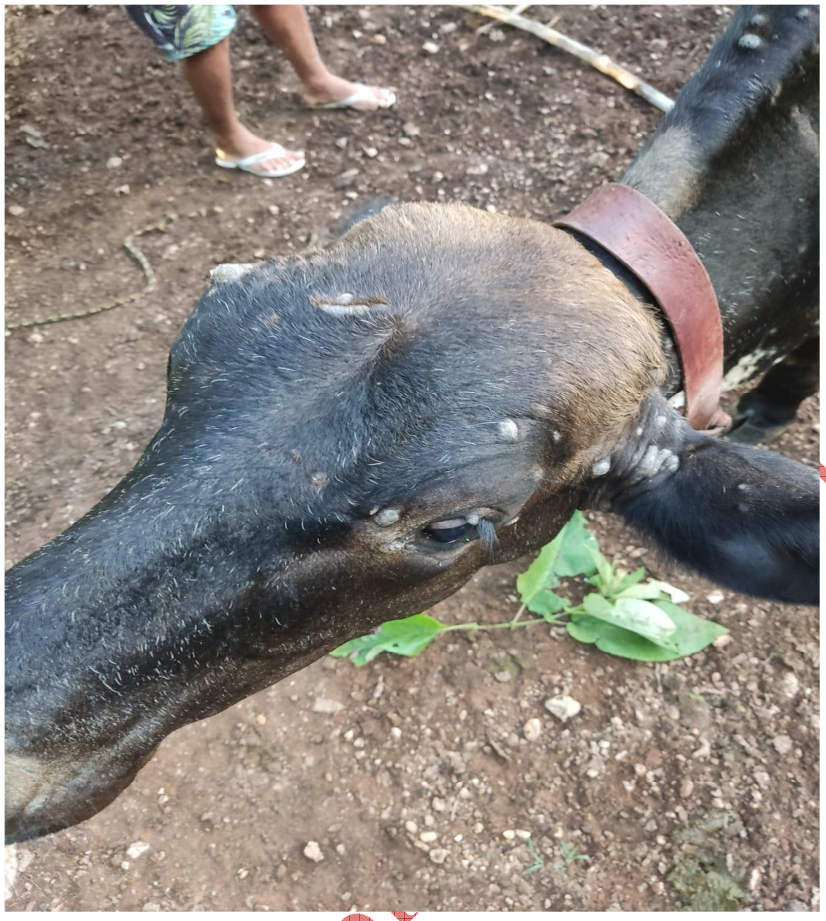
2.1 Papillomavirus: an overview

Papillomaviruses (PVs) constitute a diverse family of small, non-enveloped DNA viruses characterized by circular, double-stranded genomes. These viruses infect many species, including mammals, birds, and reptiles, and significantly affect human health. Their primary targets are the basal cells of mucosal and cutaneous epithelium, often resulting in hyperproliferative lesions commonly referred to as papillomas or warts. While these growths are typically benign and self-limiting, certain cofactors can drive their progression into malignant cancers (Bernard *et al*., 2010; Freitas *et al*., 2003; Monteiro *et al*., 2008;Silva *et al*., 2011).

Although papillomaviruses generally exhibit species specificity, cross-species infections have been documented in natural conditions, particularly among equines (Kumar *et al*., 2015; Nasir; Campo, 2008; Pangty *et al*., 2010; Silvestre *et al*., 2009).

These viruses are globally distributed and have been the subject of extensive research since the 1930s when CRPV (cottontail rabbit papillomavirus) was identified in rabbit warts. Subsequent studies utilizing animal models such as bovines (BPV) (figure 1), rabbits (CRPV), and canines (COPV) have significantly advanced our understanding of viral biology, host-virus interactions, co-carcinogenic environmental factors, and immune responses to infection (Lunardi *et al*., 2013).

Figure 1 – Bovine affected by bovine papilomatosis



Source: authors' archive

2.2 Bovine Papilomavírus (BPV)

Bovine Papillomavirus (BPV), classified within the Papillomaviridae family, encompasses 29 genotypes divided into five genera: Deltapapillomavirus (BPV-1, -2, -13, and -14), Xipapillomavirus (BPV-3, -4, -6, -9, -10, -11, -12, -15, -17, -20, -23, -24, -26, -28, and -29), Epsilonpapillomavirus (BPV-5, -8, and -25), Dyoxi (BPV-7), and Dyokappapapillomavirus (BPV-16, -18, and -22). Genotypes BPV-19, -21, and -27 remain unclassified (Sauthier *et al*., 2021).

BPV-1, the most extensively studied genotype, serves as a model for understanding HPV infection and persistence. It induces fibropapillomas in the paragenital regions of cattle and sarcoids in horses-cutaneous neoplasms associated with the invasion of transformed fibroblasts (Schiller; Vass; Lowy, 1984; Yuan *et al*., 2010, 2011). BPV-2 causes fibropapillomas on the skin, gastrointestinal tract, and urinary tract of cattle and is linked to bovine enzootic hematuria (BEH) and bladder cancer when combined with bracken fern carcinogens (Borzacchiello *et al*., 2003, 2007).

Other BPV genotypes, such as BPV-3 to BPV-7, also induce diverse papillomas in cattle, with variation depending on the viral type and lesion site (Bloch; Breen; Spradbrow, 1994; Borzacchiello *et al*., 2003; Campo, 2006; Ford *et al*., 1982; Jarrett *et al*., 1984; Ogawa *et al*., 2007; Patel; Smith; Campo, 1987; Pfister *et al*., 1979).

BPV-8, originally designated BAPV-2, was identified in Japan and later in a European bison in Italy. Phylogenetic analysis revealed its similarity to BPV-5, classifying it within the Epsilonpapillomavirus genus, despite notable genetic differences, such as the presence of ORF E4 in BPV-8 but not in BPV-5 (Tomita *et al*., 2007).

BPVs -9 and -10 were isolated from papillomas and intact bovine teat skin, with 74.2% and 71.2% ORF L1 similarity to BPV-3, placing them in the Xipapillomavirus genus (Hatama; Nobumoto; Kanno, 2008). Additional BPV types, including BPV-11 and BPV-12, have also been identified and classified through genome sequencing (Hatama *et al*., 2011; Zhu *et al*., 2012).

BPV-13, for instance, has been associated with the development of specific fibropapillomas in

southern Brazil. BPV infections are widespread globally, predominantly affecting young cattle raised in intensive management systems. These infections can progress to malignant tumors under conditions of compromised immunity or exposure to stressors, such as secondary infections. Furthermore, the interaction between BPV and bracken fern has been linked to the development of neoplasms in the gastrointestinal and urinary tracts, particularly in regions where this plant is prevalent (Lunardi *et al*., 2012; 2013; Narechania *et al*., 2004).

Currently, more than 40 BPV types have been described, but new types continue to be identified through phylogenetic analyses. A recent study reported the presence of novel BPV types in vulvar papillomas (Yamashita-Kawanishi *et al*., 2020). BPVs are ubiquitous in cattle herds worldwide, causing benign lesions that typically regress within 12 months, especially in young animals or those in intensive management systems (Muro; Bottura; Piccinin., 2008). However, lesions may persist in immunocompromised animals and can develop into malignant tumors, particularly under the influence of environmental stressors (Borzacchiello; Roperto, 2008).

In cattle, BPV can induce cutaneous papillomatosis, papillomas, and cancers in the gastrointestinal and urinary tracts. The consumption of bracken fern (*Pteridium aquilinum*) is associated with cancer development in these regions due to the interaction between BPV – especially BPV-2 and BPV-4, and the carcinogenic and immunosuppressive compounds found in the plant (Borzacchiello; Roperto, 2008; Monteiro *et al*., 2008; Santos *et al*., 1998; Tokarnia; Dobereiner; Peixoto, 2000).

Bracken fern contains several toxic compounds, including ptaquiloside, a potent carcinogen, and immunosuppressive molecules such as quercetin and kaempferol. These compounds can directly damage DNA by forming adducts, leading to mutations in key oncogenes and tumor suppressor genes. Furthermore, ptaquiloside can activate oncogenic pathways by alkylating purine bases in DNA, contributing to genomic instability (Malik *et al*., 2023).

The interaction between BPV and bracken fern compounds is particularly significant. BPV-2 and BPV-4 infect epithelial cells in the gastrointestinal and urinary tracts, integrating their viral genome into the host DNA and disrupting normal cellular regulation. The expression of viral oncoproteins, such as E5, E6, and E7, inactivates tumor suppressor proteins (e.g., p53 and Rb), thereby promoting uncontrolled cell proliferation. Simultaneously, the carcinogens present in bracken fern exacerbate DNA damage and suppress immune surveillance, enabling infected cells to evade apoptosis and accumulate mutations (Medeiros-Fonseca *et al*., 2022).

Bovine Enzootic Hematuria (BEH), a chronic non-infectious disease, is characterized by bladder lesions and is associated with BPV-2 infection and the ingestion of bracken fern (Blood; Radostits, 1983). This disease can progress to neoplasms, with the evolution of bladder lesions linked to the interaction between BPV and bracken fern compounds (Marins, 2004; Tokarnia; Dobereiner; Peixoto, 2000).

2.3 Molecular mechanism of polymorphisms in MBL2 and MHC

MBL is a calcium-dependent protein synthesized in the liver, characterized by a collagen-like domain within its molecular structure (Giang *et al*., 2020). As a member of the collectin family, MBL plays a crucial role in innate immunity by recognizing pathogen-associated molecular patterns (PAMPs), which are conserved microbial components absent in host cells, such as mannose molecules (Eppa *et al*., 2018). Its primary function is to serve as a receptor in the defense against microorganisms, primarily through the activation of the lectin pathway in the complement system (Giang *et al*., 2018). Additionally, MBL enhances the host's ability to recognize microbial cells and coordinates immune responses against infections caused by viruses, fungi, bacteria, and parasites (Hammad *et al*., 2018).

The MBL2 gene on chromosome 10q11.1-q21 consists of four exons interspersed with three introns of approximately 600, 1300, and 800 base pairs, respectively (Taylor *et al*., 1989). Single nucleotide polymorphisms (SNPs) in exon 1 and the promoter region of MBL2 directly influence serum MBL (sMBL) levels. Among these SNPs, notable variants include rs5030737 (codon 52), rs1800450 (codon 54), and rs1800451 (codon 57), collectively known as structural variants of MBL2. These variants, designated as MBLD (rs5030737), MBLB (rs1800450), and MBLC (rs1800451), impair protein functionality by reducing circulating MBL levels, thereby increasing susceptibility to

infections (Goeldner *et al*., 2014; Kilpatrick, 2002; Litzman *et al*., 2008; Ornelas *et al*., 2019; Sumiya *et al*., 1991; Taylor *et al*., 1989).

The SNPs rs1800450 and rs1800451 involve the substitution of glycine with dicarboxylic acids, whereas rs5030737 substitutes arginine with cysteine in the collagen region of MBL monomers. These substitutions result in variant subunits that disrupt the collagen triple helix and hinder the formation of functional high-order oligomers due to additional disulfide bonds caused by extra cysteine residues (Madsen *et al*., 1994; Sumiya *et al*., 1991). Such structural alterations significantly reduce the synthesis, circulating levels, and functional activity of MBL (Larsen *et al*., 2004; Lipscombe *et al*., 1992; Madsen *et al*., 1994; Sumiya *et al*., 1991; Terai *et al*., 2003; Wallis; Cheng, 1999;). Moreover, polymorphisms in the promoter region, such as rs11003125 (L/H), rs7096206 (Y/X), and rs7095891 (P/Q), regulate the expression of the MBL2 gene, directly impacting protein production.

MBL deficiency, characterized by low levels of sMBL, has been associated with increased susceptibility to various infectious diseases, particularly those caused by extracellular pathogens (Koch *et al*., 2001). Early evidence of this relationship was observed in AIDS patients carrying structural variant alleles of MBL2, who demonstrated heightened vulnerability to co-infections and reduced survival rates (Garred *et al*., 1997). Subsequent studies linked MBL deficiency to hepatitis B and C infections, indicating that reduced sMBL levels elevate the risk of these diseases (Matsushita *et al*., 1998; Yuen *et al*., 1999). Similar associations have been reported in autoimmune conditions, such as systemic lupus erythematosus (Sullivan *et al*., 1996) and rheumatoid arthritis (Graudal *et al*., 2000). Furthermore, polymorphisms in the MBL2 gene have been linked to an increased risk of mastitis and other infectious diseases in cattle and buffaloes (Fraser, Lumsden, and Lillie, 2018; Shergojry *et al*., 2023).

The bovine leukocyte antigen (BoLA) system, the major histocompatibility complex (MHC) of cattle, is located on chromosome 23. It is highly polymorphic and plays a crucial role in modulating immune responses against pathogens (Takeshima *et al*., 2009). Class II genes, such as BoLA-DRB3, encode proteins responsible for presenting antigens to CD4+ helper T cells, which are essential for combating viral infections (Amills *et al*., 1998).

Studies have demonstrated that BoLA-DRB3.2 variants are associated with susceptibility or resistance to various diseases, including dermatophilosis, bacterial mastitis (Behl *et al*., 2012), foot-and-mouth disease (Baxter *et al*., 2009), bovine leukemia (Juliarena *et al*., 2012), trypanosomiasis, and tick infestations. Additionally, these variants have been linked to production traits (Andrade *et al*., 2024). In Brazil, research on BoLA-DRB3 gene polymorphisms has identified associations between this polymorphism and traits such as protein and fat production (Nascimento *et al*., 2006) and resistance to ticks (Martinez *et al*., 2006).

There is also evidence linking the MHC to infections by papillomaviruses (HPV and BPV), affecting the progression and regression of these diseases (Marchetti *et al*., 2002; Peng *et al*., 1998). The polymorphism of the DRB3 gene, concentrated in the exon responsible for peptide binding, modulates MHC affinity for antigens, thereby influencing the specificity and effectiveness of the immune response (Chuang *et al*., 2012).

3 Research methodology

This study evaluated 167 female Girolando cattle (3/8 Gir + 5/8 Holstein) from the Agronomic Institute of Pernambuco (IPA) and the Recôncavo region of Bahia, located in northeastern Brazil. All animals were raised under a semi-intensive system and fed ad libitum with elephant grass (*Penisetum purpureum*), water, and commercial bovine-specific mineral salt. The sample was divided into two groups: 92 animals presenting clinical signs of bovine papillomatosis and 75 animals without any apparent clinical manifestations.

Blood samples were collected via venipuncture using vacuum tubes containing EDTA. DNA was extracted using the Blood and Tissue Kit (Qiagen) following the manufacturer's protocol. The extracted DNA was quantified using a NanoVue spectrophotometer (GE). DNA viability was assessed by PCR by amplifying the bovine β-globin gene, as Freitas *et al*. (2007) described.

Polymorphisms in exon 1 of the MBL2 gene were analyzed using PCR with specific primers designed to amplify a 247 bp fragment (Wang *et al*., 2012). The PCR products were resolved via

agarose gel electrophoresis, purified using the GFX PCR DNA and Gel Band Purification Kit (GE Healthcare), and sequenced with automated sequencers (3500 Applied Biosystems). DNA sequence quality was assessed using the Staden Package software[1], which was employed for chromatogram analysis and the generation of consensus sequences. Only DNA sequences with a Phred quality score of 25 or higher were included in the study. The identified sequences were further analyzed using the BLAST tool[2].

The DNA sequences were aligned using the program MEGA 6.06 (Tamura *et al.*, 2013). The SNPs were analyzed using the SNPStats software (http://bioinfo.iconcologia.net/SNPstats_web), assuming a significance level of 5% (Solé *et al.*, 2006). The association of MBL2 gene exon 1 polymorphisms and the development of papillomatosis lesions was estimated using the Odds Ratio (OR) test with a 95% confidence interval. The allelic and genotypic frequencies were calculated and the population equilibrium was determined. The estimation of significant differences in the distribution of genotypes was performed using the chi-square test.

The DNA sequences were aligned using the MEGA 6.06 software (Tamura *et al.*, 2013). SNP analysis was performed with the SNPStats software[3], adopting a 5% significance level (Solé *et al.*, 2006). The association between polymorphisms in exon 1 of the MBL2 gene and the development of papillomatosis lesions was estimated using the Odds Ratio (OR) test with a 95% confidence interval. Allelic and genotypic frequencies were calculated, and population equilibrium was determined. Differences in genotype distribution were assessed using the chi-square test.

4 Results and discussion

Blood samples from 167 animals were analyzed for polymorphisms in exon 1 of the MBL2 gene. A total of 245 conserved sites and two variable informative sites were identified. These variable sites corresponded to nucleotides 235 G/A (codon 42) and 244 T/C (codon 45) of the MBL2 gene exon 1. The SNP G235A results in an amino acid substitution from proline to glutamine at the first Gly-X-Y repeat of the collagen-like domain, while SNP T244C is a synonymous mutation (Wang *et al.*, 2012).

Genotypic and allelic frequencies for MBL2 gene exon 1 polymorphisms conformed to Hardy–Weinberg equilibrium in both groups: animals with papillomatous lesions ($p = 0.837$; $\chi^2 = 0.041$) and animals without papillomatous lesions ($p = 0.367$, $\chi^2 = 0.8112$). No significant association was found between the evaluated SNPs and susceptibility to bovine papillomatosis ($p > 0.05$) (Table 1).

Table 1 – Allelic and genotypic frequencies of MBL2 gene exon 1

	With papillomatosis *n* = 92 (%)	**Without papillomatosis *n* = 75 (%)**	***p*-value, OR [95% CI]**
SNP1 (235 G/A)			
Alleles			
G	163 (88)	125 (83)	Reference
A	21 (12)	25 (17)	0.201, 0.645 [0.32 -1.26]
Genotypes			
GG	72 (78)	51 (68)	Reference
GA	19 (21)	23 (31)	0.15; 0.587 [027 -1.25]
AA	1 (1)	1 (1)	1; 0.71 [0.008 – 56.6]
SNP2 (244 T/C)			
Alleles			
T	163 (88)	125 (83)	Reference

[1] Staden Package software. Available at: http://staden.sourceforge.net
[2] BLAST tool. Available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi
[3] SNPStats software. Available at: https://www.snpstats.net/start.htm

C	21 (12)	25 (17)	0.201 0.645 [0.32 -1.26]
Genotypes			
TT	72 (78)	51 (68)	Reference
TC	19 (21)	23 (31)	0.15; 0.587 [027 -1.25]
CC	1 (1)	1 (1)	1; 0.71 [0.008 – 56.6]

Source: research data

The bovine immune response to bovine papillomavirus (BPV) is notably weak, with papillomatous lesions predominantly observed in animals up to two years of age (Medeiros-Fonseca *et al*., 2022). While these lesions often regress naturally due to the cellular immune response, some animals fail to resolve them, leading to widespread papillomatosis affecting the body and mucosa. Anti-BPV antibodies are rarely detected, likely because the virus's replication cycle is restricted to the host epithelium, without inducing cell lysis or inflammation (Costa; Medeiros, 2014; O'Brien; Campo, 2003).

In humans, innate immunity influences HPV infection outcomes, determining viral clearance or persistence (Colin-Ferreyra *et al*., 2014; Sasagawa; Takagi; Makinoda, 2012; Zhou; Tuong; Frazer, 2019). MBLs are lectins in the innate immune response against various pathogens, including bacteria, fungi, and viruses (Garred *et al*., 2006). Functional polymorphisms in the MBL2 gene have been associated with increased susceptibility to HPV infection and cervical lesion development (Guimaraes *et al*., 2008; Tsai *et al*., 2009).

In cattle, the immune response to BPV infection is primarily understood as the cellular immune response (Ashrafi *et al*., 2006), but limited information exists on the innate immune response. Longeri *et al*. (2021) demonstrated an association between MHC class II bovine leukocyte antigen - DRB3.2 polymorphisms and BPV-2 infection, linked to bladder tumor risk in Podolica cattle.

The two SNPs identified in this study (G235A and T244C) had been previously described by Wang *et al*. (2012). The SNP G235A, in particular, induces an amino acid substitution (Pro > Gln), potentially altering the functional properties of the MBL2 protein (Larsen *et al*., 2004). However, no association was observed between the SNPs and the development of papillomatosis in the analyzed animals.

5 Conclusions

This study examined the association between polymorphisms in exon 1 of the MBL2 gene (G235A and T244C) and susceptibility to bovine papillomatosis in Girolando cattle. Although variants of the MBL2 gene have previously been linked to increased susceptibility to HPV infections in humans, no significant correlation was observed between the analyzed SNPs and the occurrence of papillomatous lesions in cattle ($p > 0.05$). Genotypic and allelic frequencies remained in Hardy-Weinberg equilibrium in both the lesion-affected group ($p = 0.837$) and the control group ($p = 0.367$), indicating that the studied sample is genetically representative of the broader population.

The substitution of proline with glutamine at codon 42 (SNP G235A), located in the first Gly-X-Y repeat of the collagen-like domain of MBL, could theoretically interfere with the lectin's binding to pathogen-associated molecular patterns. However, the absence of a phenotypic association suggests that this structural alteration does not significantly impair the protein's function in the innate immune response to BPV or that compensatory mechanisms, such as the activation of alternative complement pathways, mitigate potential deficiencies. Similarly, the synonymous SNP T244C appears to have no functional impact, reinforcing its neutrality in the context of the viral infection under investigation.

Given these findings, future research should focus on exploring regulatory regions within the MBL2 gene, such as promoters and enhancer elements, where genetic variation may influence protein expression and, consequently, antiviral activity. Additionally, complementary genes within the lectin pathway, including MASP-1/2 and Toll-like receptors (TLRs), should be assessed, as they work synergistically with MBL in recognizing pathogens. Non-genetic factors such as environmental conditions, nutritional status, and the presence of co-infections also require further examination to evaluate their potential impact on the clinical manifestation of BPV.

Although the absence of a genetic association may seem like a limitation, negative findings are crucial for refining hypotheses and redirecting research on host-pathogen interactions. The immune response to BPV is inherently complex, requiring multidisciplinary approaches that integrate genomics, comparative immunology, and epidemiology to identify susceptibility markers and develop effective interventions. Beyond the control of papillomatosis, advancements in this field could enhance the broader understanding of BPV-associated complications, including carcinogenic processes, thereby improving both the sanitary and economic impact of these discoveries.

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Conflict of interest

The authors declare no conflicts of interest.

Declaration of the Ethics Council

This study was conducted following the guidelines of the Ethics Committee on the Use of Animals of the Federal Rural University of Pernambuco (Protocol Number 23082.013040/2015-49).

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