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Efficacy of 0.5% tretinoin in the treatment of equine aural plaques

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Magister Scientiae

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FELIPE SPERANDIO DE MATTOS

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Dissertation submitted to the Veterinary Medicine Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

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ABSTRACT

MATTOS, Felipe Sperandio de, M.Sc., Universidade Federal de Viçosa, June, 2025. **Efficacy of 0.5% tretinoin in the treatment of equine aural plaques**. Adviser: Raffaella Bertoni Cavalcanti Teixeira Santos. Co-adviser: Lissandro Goncalves Conceicao.

Equine aural plaques are a benign form of auricular papillomatosis caused by equine papillomavirus (EcPV). Despite the efficacy of 5% imiquimod cream, a marked local reaction is frequently observed during therapy, often requiring sedation prior to application. The aim of this study was to evaluate the efficacy and potential side effects of 0.5% topical tretinoin in the treatment of equine aural plaques. We hypothesized that tretinoin would be effective, with fewer side effects compared to imiquimod. The study included 10 client owned Mangalarga Marchador horses, of both sexes, aged between 7 months and 15 years, diagnosed with unilateral or bilateral aural plaques (totaling 17 ears). This is a controlled clinical trial. Three horses underwent a pilot study, in which only one ear per animal was treated to allow for intra-animal comparison, while the remaining seven horses received treatment in both ears. Lesions were classified into three groups based on the percentage of the inner auricular surface affected. Treatment was performed with 0.5% topical tretinoin. Lesion characteristics, treatment response, side effects and recurrence were evaluated throughout the experiment. Sixty-five percent of the treated ears exhibited an improvement of more than 50% following treatment and 24% showed an improvement, though with less than 50% reduction in lesion size. None of the animals exhibited severe sensitivity during the treatment period, allowing for ear manipulation throughout the study. The topical application of 0.5% tretinoin cream can be considered a viable and well-tolerated therapeutic option for equine aural plaques. Further studies with a larger sample size and long-term follow-up are recommended to optimize treatment protocols.

Keywords: imiquimod; papillomavirus; horses

RESUMO

MATTOS, Felipe Sperandio de, M.Sc., Universidade Federal de Viçosa, junho de 2025. **Eficácia da tretinoína 0.5% no tratamento da placa aural equina**. Orientadora: Raffaella Bertoni Cavalcanti Teixeira Santos. Coorientador: Lissandro Goncalves Conceicao.

As placas auriculares equinas são uma forma benigna de papilomatose auricular causada pelo papilomavírus equino (EcPV). Apesar da eficácia do creme de imiquimode a 5%, uma reação local acentuada é frequentemente observada durante a terapia, muitas vezes exigindo sedação prévia à aplicação. O objetivo deste estudo foi avaliar a eficácia e os possíveis efeitos colaterais da tretinoína tópica a 0,5% no tratamento das placas auriculares equinas. Nossa hipótese foi de que a tretinoína seria eficaz, com menos efeitos colaterais em comparação ao imiquimode. O estudo incluiu 10 cavalos da raça Mangalarga Marchador, de ambos os sexos, com idades entre 7 meses e 15 anos, diagnosticados com placas auriculares unilaterais ou bilaterais (totalizando 17 orelhas). Trata-se de um ensaio clínico controlado. Três cavalos participaram de um estudo piloto, no qual apenas uma orelha por animal foi tratada para permitir comparação entre os animais, enquanto os sete cavalos restantes receberam tratamento em ambas as orelhas. As lesões foram classificadas em três grupos com base na porcentagem da superfície auricular interna afetada. O tratamento foi realizado com tretinoína tópica a 0,5%. As características das lesões, a resposta ao tratamento, os efeitos colaterais e a recorrência foram avaliados ao longo do experimento. Sessenta e cinco por cento das orelhas tratadas apresentaram melhora superior a 50% após o tratamento, e 24% mostraram melhora, embora com redução inferior a 50% no tamanho da lesão. Nenhum dos animais apresentou sensibilidade severa durante o período de tratamento, permitindo a manipulação das orelhas ao longo do estudo. A aplicação tópica do creme de tretinoína a 0,5% pode ser considerada uma opção terapêutica viável e bem tolerada para placas auriculares equinas. Estudos adicionais com maior número de animais e acompanhamento a longo prazo são recomendados para otimizar os protocolos de tratamento.

Palavras-chave: imiquimode; papillomavirus; cavalos

SUMÁRIO

INTRODUCTION

Equine aural papillomatosis, also known as aural plaque, is a benign dermatological disease caused by equine papillomavirus (EcPV). This dermatopathy occurs on the auricular pinna of horses, either unilaterally or bilaterally, without spontaneous regression, and is usually painless. Although asymptomatic in most cases, increased sensitivity and discomfort in the ears, especially during manipulation and halter placement, may be observed (TORRES & KOCH, 2013; SOUSA et al., 2008; SELLON, 2007).

Lesions may appear as isolated papules or plaques and can evolve into multiple coalescent, whitish, hyperkeratotic structures that may extend to involve the entire auricular surface (SCOTT & MILLER, 2011; SOUSA et al., 2008).

Several types of Equus caballus papillomavirus (EcPV) have been identified to date, including EcPV-1 through EcPV-9, with types 1 to 7 being more thoroughly characterized (Munday & Kiupel, 2010; Mira et al., 2018). EcPVs are associated with distinct clinical syndromes in horses, such as classical papillomatosis (EcPV-1), genital papillomatosis and squamous cell carcinoma (EcPV-2), and equine aural plaques (EcPV-3, EcPV-4, EcPV-5, and EcPV-6) (Torres & Koch, 2013). In Brazil, a PCR-based study identified EcPV-3, EcPV-4, and EcPV-6 in 42%, 84%, and 18% of 108 samples collected from horses with aural plaques across five geographic regions (Mira et al., 2018). These types were frequently detected in co-infection, with only 2% of EcPV-3, 32% of EcPV-4, and 7% of EcPV-6 found in isolation. EcPV-1, typically associated with classical cutaneous papillomatosis, was detected in 34% of positive biopsies, but always in combination with other EcPVs, precluding definitive conclusions regarding its role in aural plaque development. EcPV-2 and EcPV-7 were not detected in any of the Brazilian samples analyzed (Mira et al., 2018). Recent findings from Bromberger et al. (2023) corroborate and expand on these data, showing that EcPV-2, EcPV-7, EcPV-8, and EcPV-9 were absent in 29 new samples, whereas EcPV-6 was the most prevalent (81%), followed by EcPV-3 (72%), EcPV-4 (63%) and EcPV-5 (47%).

Clinical diagnosis is based on the characteristic appearance of lesions, while histopathological examination reveals epidermal hyperplasia, mild papillomatosis associated with hyperkeratosis, areas of hypomelanosis or complete absence of melanin, koilocytosis, and increased size and number of keratohyalin granules

(SOUSA et al., 2008; MIRA et al., 2016). Koilocytes are epidermal cells with small, rounded, amphophilic nuclei and pale cytoplasm, characteristic of benign papillomavirus-induced skin lesions (ARAÚJO et al., 2021).

The most effective treatment for equine aural plaque is 5% imiquimod cream, which modulates the immune response, leading to localized inflammation (BRANDT et al., 2010). Despite its efficacy, this medication frequently induces an intense inflammatory response and heightened sensitivity, making frequent application challenging and sometimes requiring sedation (ZAKIA et al., 2016). Additionally, cases of ear rigidity loss and auricular deformities have been reported as adverse effects (ZAKIA et al., 2016).

Retinoids are natural or synthetic vitamin A derivatives used to treat human dermatological conditions such as acne, rosacea, psoriasis, and lupus erythematosus (ZASADA & BUDZISZ, 2019). Topical retinoids have been shown to reduce lesion volume and alter the quality and quantity of the stratum corneum in cutaneous warts caused by human papillomavirus (STERLING et al., 2014). Tretinoin, a first-generation synthetic retinoid, has been used in veterinary dermatology for the treatment of benign cutaneous tumors (SCOTT et al., 2001). Reported side effects of topical use in humans include skin irritation, dryness, scaling, pruritus, and erythema (STERLING et al., 2014). Systemic administration in dogs has been associated with adverse effects such as keratoconjunctivitis sicca, increased hepatic enzyme levels, hypertriglyceridemia, and hypercholesterolemia. However, these side effects are dose-dependent, and despite their occurrence, the therapy is generally well tolerated (TOMA & NOLI, 2005).

To date, no published studies have evaluated the efficacy of tretinoin in treating equine aural plaques. This study aims to assess the efficacy and potential side effects of tretinoin in equine aural plaques. This research is justified by the need for an effective treatment for equine aural plaque with fewer and milder side effects than those reported for 5% imiquimod.

MATERIALS AND METHODS

Ethical Aspects

This study was approved by the Animal Ethics Committee (CEUA) of the Federal University of Viçosa (UFV) under protocol 34/2022. Written informed owner consent was obtained prior to the experiment.

Animals

The study included ten Mangalarga Marchador horses (A1 to A10), of both sexes, aged between 7 months and 15 years (mean age 3.4), with a clinical diagnosis of bilateral aural papillomatosis and without prior treatment. All horses were considered healthy prior to the experiment based on a thorough physical examination, which included evaluation of attitude, appetite, body condition score, color of mucous membranes, capillary refill time, heart rate, respiratory rate, heart and lung sounds, pulse quality, intestinal motility, palpation of lymph nodes, body temperature and fecal characteristics. Complete blood cell count and chemistry profile were performed and considered within normal limits prior to the experiment.

Prior to initiation of the experiment the location, size and aspect of the aural plaque lesions were documented using digital photographs for comparative analysis throughout the study.

Initially, three animals with bilateral aural plaque participated in a pilot study (A1, A2 and A3). The horses were kept in stalls without direct exposure to sunlight, and only one ear per animal was treated to allow the contralateral ear as a control. The remaining seven horses (A4 to A10) were housed in paddocks with habitual sun exposure throughout the treatment period, and both ears were treated, totaling 17 treated ears in the study.

Initially, the lesion size was classified based on the percentage of the affected region in the inner ear and categorized into three intervals: 0–33% of the inner ear affected (group 1), 33–66% of the inner ear affected (group 2), and 66–100% of the inner ear affected (group 3).

Biopsy

Biopsies were performed on five distinct ears (from horses A1, A2, and A3 in the pilot study), prior to initiation of therapy. No biopsies were performed on horses A4 through A10, as they were privately owned and not part of the pilot study. Prior to the procedure, the animals were sedated with detomidine (0.015 mg/kg, IV), and a regional nerve block was performed using 2% lidocaine, targeting the internal and greater auricular nerves (McCoy et al., 2007). Tissue samples were collected from the center of the lesion using a 4mm or 6mm sterile disposable biopsy punch. All samples were immediately fixed in formalin and submitted for histopathological examination. Slides were stained with hematoxylin and eosin (H&E) for light microscopy analysis. Due to

the limited biopsy size, only three out of the five samples were placed in cryotubes, frozen at -80°C, and analyzed by PCR for the detection of *Equus caballus* papillomaviruses EcPV-1 through EcPV-9 according to Zakia et al., 2015 and Bromberger et al., 2023.

Topical Treatment with 0.5% Tretinoin

Lesions were treated with a 0.5% tretinoin cream. A thin layer of the cream was applied topically once daily using gentle local massage. In cases where local inflammatory reactions were observed, the treatment frequency was adjusted to every other day. Applications were performed in the evening and continued until lesion stabilization or resolution. Horses A1, A2, and A3 were kept out of direct sunlight, whereas A4 to A10 remained in paddocks with normal sun exposure.

Evaluation of Treatment Response

A daily monitoring sheet was completed for each animal throughout the study, documenting general health parameters, including attitude, appetite, mucous membrane color, capillary refill time, heart rate, respiratory rate, body temperature, intestinal motility, changes in palpable lymph nodes, and fecal production. Treatment response was assessed daily, with the presence of erythema, edema, bleeding, crusting, pruritus, and local sensitivity recorded in the monitoring sheet. Lesions were documented daily through digital photographs of both inner ears.

A complete blood count (CBC), biochemical analysis, and Schirmer tear test were performed prior to the experiment and every seven days during the treatment phase on animals A4 to A10. The biochemical analysis included serum levels of total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), urea, creatinine, cholesterol, and triglycerides.

Behavioral signs evaluated included restlessness, frequent head shaking, ear tilting or backward positioning, and pain responses upon touch or manipulation, using a scale adapted from Zakia et al., 2016 (Table 1). The signs were assessed based on its presence, intensity, and duration.

Table 1. Local Sensitivity Assessment Scale (adapted from Zakia et al., 2016)

Sensitivity Level	Description
ABSENT	No reaction to palpation
MILD	Mild response observed, without resistance to palpation
MODERATE	More pronounced pain response: allows palpation only if restrained
SEVERE	Exaggerated reaction to palpation or prevents palpation of the ear

The total number of treatments and total treatment duration for each ear were recorded. The lesions' response to treatment was classified into three distinct categories (Table 2).

Table 2. Classification of Lesion Progression

Classification	Description
>50% Improvement	Lesions showing more than 50% reduction in size
<50% Improvement	Lesion improvement, but less than 50% reduction in size
No Improvement	Lesions with no discernible improvement

Following the treatment period, animals were monitored for recurrence according to the following schedule: weekly during the first month, biweekly during the second month and monthly during the third month.

Statistical Methods

Data were analyzed using R software (R Core Team, 2021). The normality of residuals and homoscedasticity were assessed using the Shapiro-Wilk and Bartlett tests, respectively. Aspartate aminotransferase (AST) data were subjected to inverse transformation ($y' = 1/y$), whereas triglyceride levels and basophil counts were log-

transformed ($y' = log(y)$). However, for clarity, results were presented using the original data scale.

Biochemical and hematological parameters were analyzed using linear models with repeated measures over time, following the model:

$$Y_{ij} = \mu + D_i + e_{ij}$$

Where: Y_{ij}, observed response; μ, general constant; D_i, effect of the day; e_{ij}, random error.

Comparisons between means were performed using Tukey's test.

Gamma-glutamyl transferase (GGT), mean corpuscular hemoglobin concentration (MCHC), platelet count, and relative basophil count did not meet the assumptions of normality and homoscedasticity and were analyzed using the Kruskal-Wallis test followed by Dunn's post hoc test.

Schirmer's test data were compared using a paired *t*-test. Frequency data were arranged in contingency tables and analyzed using Fisher's exact test (2 × 2 tables) or the Freeman-Halton test (l × c tables). The significance level was set at α = 0.05.

RESULTS

Histopathology

Histopathological analysis revealed epidermal hyperplasia associated with mild papillomatosis, widened epithelial ridges, and the presence of koilocytosis, along with a significant reduction in melanin pigmentation. Additionally, hyperkeratosis and hypergranulosis were consistently observed across all samples (Figure 1).

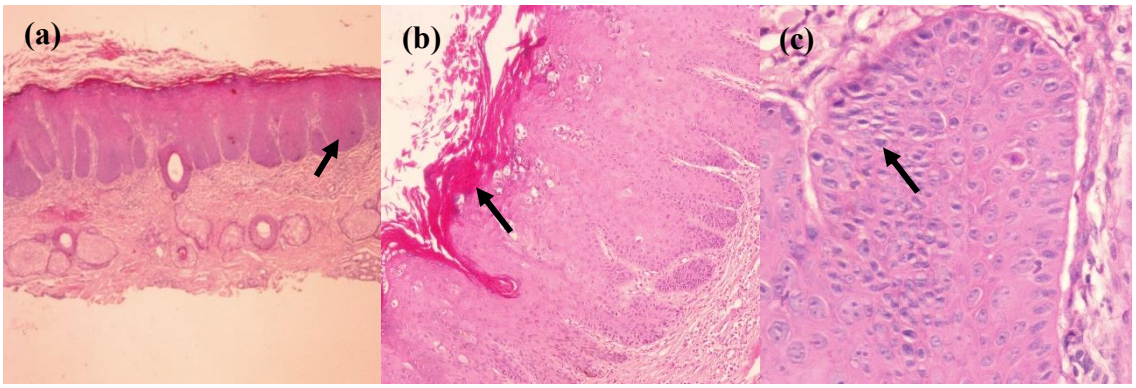

Figure 1. Histological image showing epidermal hyperplasia, widened epithelial ridges (**a**), hyperkeratosis (**b**), the presence of koilocytes, and the absence of melanocytes (**c**).

PCR

PCR analysis detected the presence of EcPVs 1, 3, 4, and 6 in the analyzed samples, with EcPV-1 co-occurring with EcPV-6 in 1 horse.

Physical Examination, Complete Blood Cell Count, Biochemistry Analysis and Schirmer test

No changes in physical examination parameters and appetite were observed during the experiment. Complete blood count (CBC) results remained within the reference range for the species. Biochemistry analysis revealed normal concentration of liver enzymes, total protein, albumin, cholesterol, triglycerides, creatinine, and urea during the monitoring period. The Schirmer tear test, performed weekly, showed no significant changes in tear production during topical therapy with 0.5% tretinoin cream.

Lesion Assessment and Treatment Response

Lesion Classification

Nine out of the 17 treated ears (53%) were classified in Group 1 (0–33% of the inner ear affected) (Figure 2), and eight ears (47%) were classified in Group 2 (33–66% of the inner ear affected) (Figure 3). No lesions were classified as affecting more than 66% of the inner ear area.

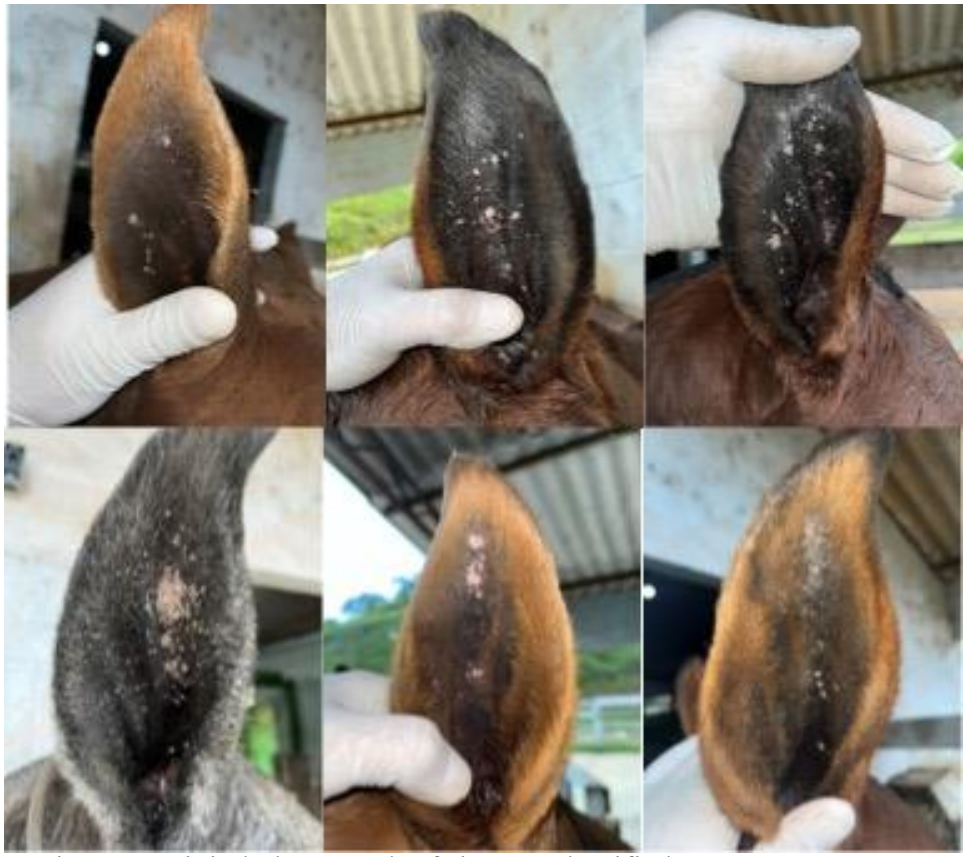

Figure 2. Digital photograph of six ears classified as Group 1.

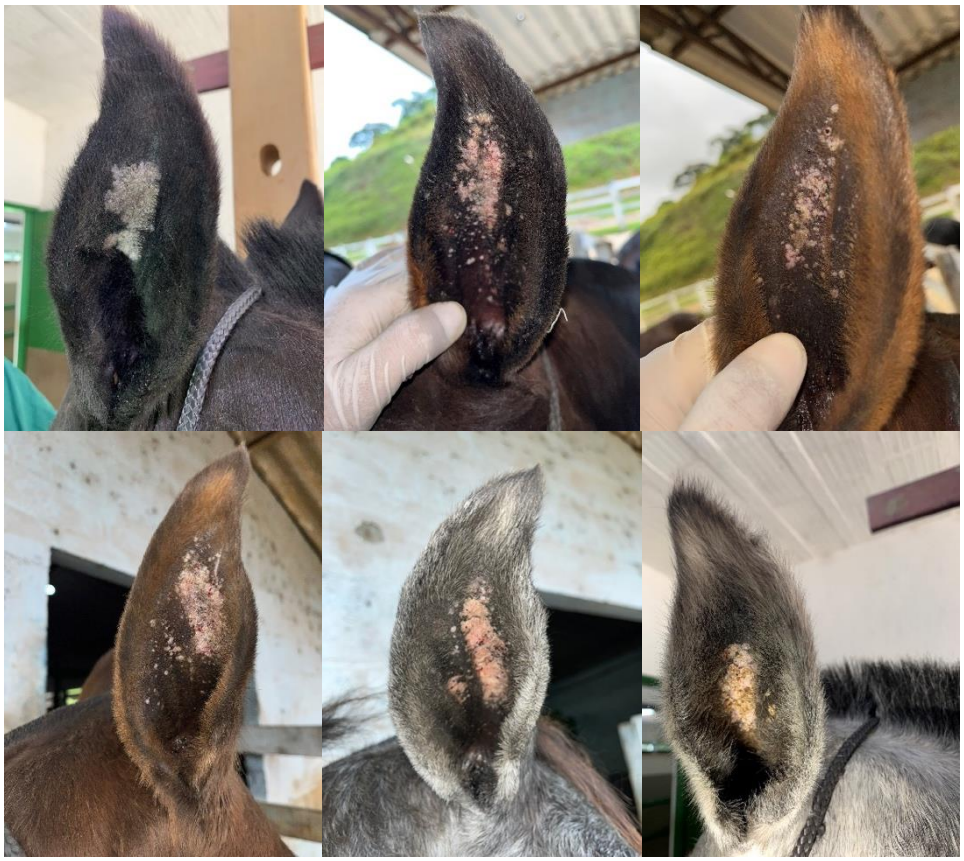

Figure 3. Digital photograph of six ears classified as Group 2.

Response to Treatment, Ear Sensitivity and Treatment Duration

Eleven out of the 17 treated ears (65%) exhibited an improvement of more than 50% following treatment (Figure 4), while four ears (24%) showed improvement but with a reduction of less than 50%. Only two ears (12%), both from the same horse, showed no improvement. Among the eight lesions classified as affecting more than 33% of the inner ear area, six demonstrated improvement greater than 50%. Five of the nine ears with less than 33% of the inner ear affected also improved by more than 50% (Figure 5). Eight of the seventeen (47%) treated ears showed an improvement of nearly 100%.

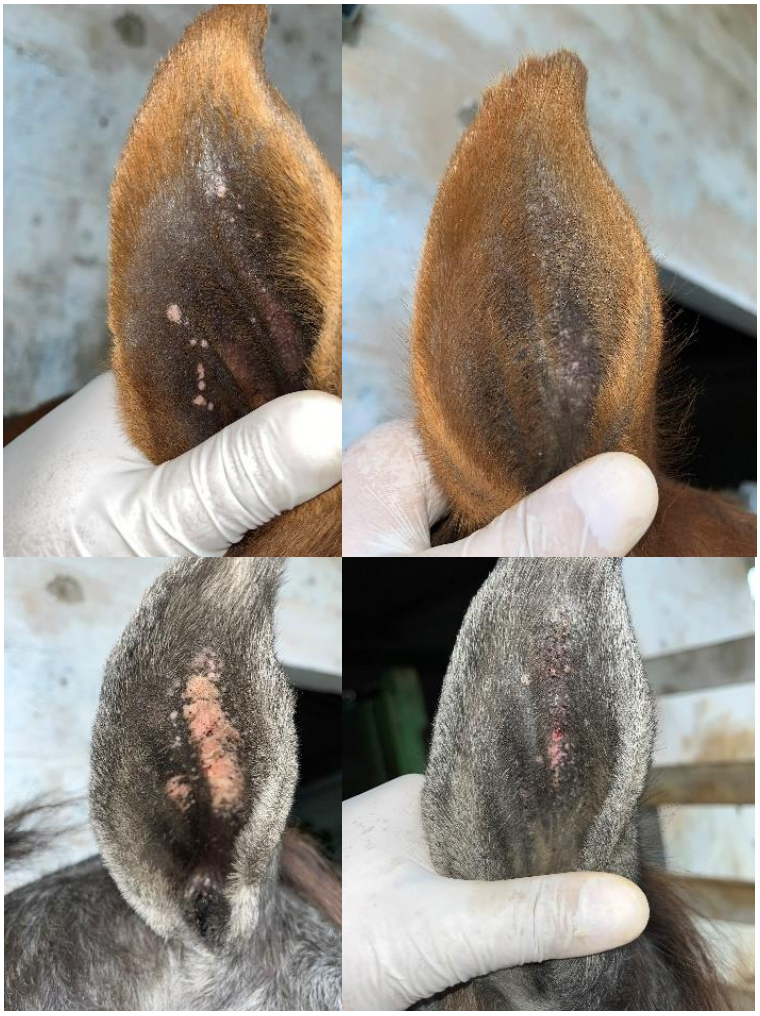

Figure 4. Ears before (left) and after (right) treatment, showing > 50% improvement.

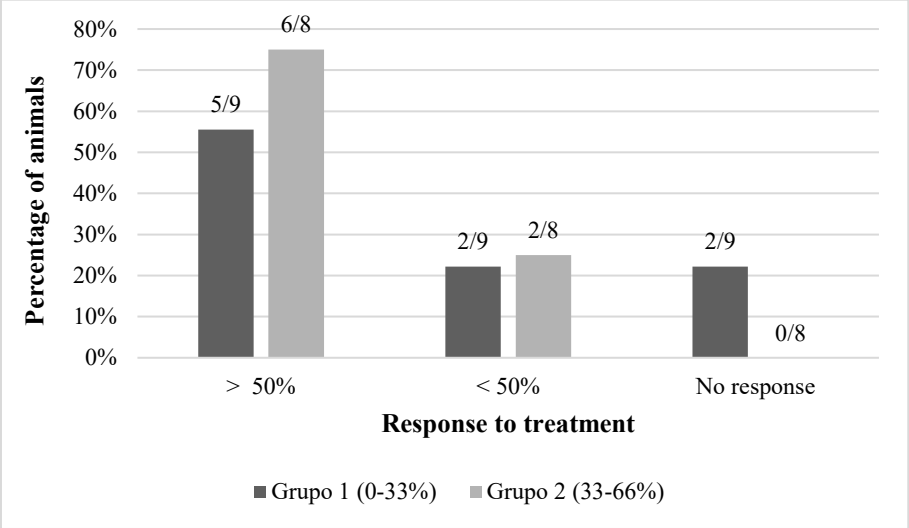

Figure 5. Association between the initial lesion extent and response to treatment; p = 0.620 by Fisher exact test.

Complete lesion resolution occurred, on average, within 52.8 days, ranging from 6 to 74 days of therapy. The number of applications of 0.5% tretinoin cream ranged from 6 to 54, with a mean of 37 applications. None of the animals exhibited behavioral changes such as restlessness, head shaking, ear tilting, or backward positioning. Severe ear sensitivity was not observed during the treatment period, and all horses allowed ear manipulation throughout the study without the need for sedation or additional restraint. One horse showed no signs of sensitivity during the experiment, four showed only mild signs, and five showed moderate signs. The mean duration of sensitivity, at any level (mild or moderate), was approximately 38 days. All animals developed discrete crusting and erythema between days 3 and 13 of therapy. All but one horse showed discrete ulceration. None of the horses exhibited signs of edema or pruritus.

Lesions were monitored by digital photography for up to 124 days post-treatment, with an average follow-up period of approximately 71 days.

The mean treatment duration was 22 days in the animals included in the pilot study (A1 to A3) and 59 days in animals A4 to A10. The animals in the pilot study had ear sensitivity for an average of 7 days, while animals A4 to A10 had it for 51 days on average.

Among the 17 evaluated lesions, six (35%) showed recurrence during the post-treatment monitoring period. No significant association was found between initial lesion

severity and response to 0.5% tretinoin treatment (p=0.620) or between initial lesion severity and presence of recurrence. However, a significant association was observed between recurrence and treatment response (p = 0.005), indicating that lesions with no recurrence were more likely to have responded with > 50% improvement. At the end of therapy, focal hypopigmentation was observed in 16 of the 17 treated lesions (Figure 6).

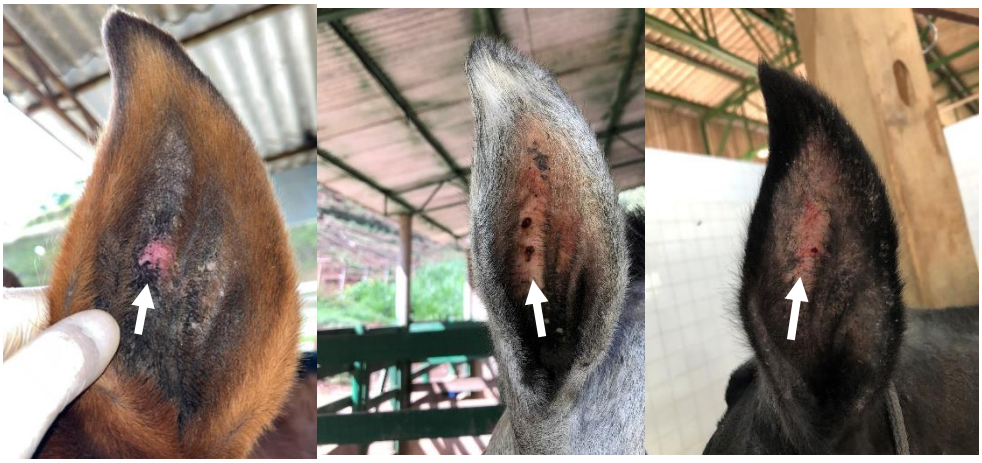

Figure 6. Digital photograph of post-treatment hypopigmentation (white arrows).

DISCUSSION

The histopathological findings of this study align with those reported by Sousa et al. (2008), who examined the histopathological characteristics of equine aural plaques and observed moderate to intense epidermal hyperplasia, mild papillomatosis, and, in some sections, vacuolated cells resembling koilocytes. Similar histopathological alterations have been described in human warts caused by human papillomavirus, including papillomatosis, hyperkeratosis, and the presence of koilocytes (Araújo et al., 2021).

PCR results confirmed the presence of EcPV-1, EcPV-3, EcPV-4, and EcPV-6 in pre-treatment samples, all previously associated with equine aural plaques in Brazil (Mira et al., 2018). EcPV-1 was detected in only one sample, in co-infection with EcPV-6, and its clinical significance in the development of aural plaques remains unclear. EcPV-2 and EcPV-7 were not detected in any of the samples analyzed, consistent with previous Brazilian studies. Although based on a limited number of animals, our findings of co-infections and EcPV type distribution are similar to those reported in earlier research.

No significant changes were observed in complete blood counts and chemistry profile throughout the treatment period, suggesting that the topical administration of 0.5% tretinoin cream did not induce systemic side effects affecting hematological parameters, renal function, hepatic function, and lipid metabolism. Similarly, the Schirmer tear test revealed no reduction in tear production, indicating that topical tretinoin did not lead to keratoconjunctivitis sicca in horses, a condition reported in dogs and humans following systemic isotretinoin therapy**.**

Torres et al. (2010) evaluated the efficacy of 5% imiquimod for treating aural plaques and reported that ten out of sixteen horses required sedation prior to therapy due to the inflammatory response induced by the medication. Similarly, Zakia et al. (2016) found that 75% of animals treated with 5% imiquimod needed sedation to allow manipulation of the affected area and removal of treatment-associated crusts due to pain. In contrast, in the present study, none of the animals exhibited enough sensitivity to hindered palpation or required sedation prior to therapy. Reported side effects of 5% imiquimod include erythema, edema, erosion, ulceration, crust formation, and exudation (Torres et al., 2010; Zakia et al., 2016). Of these, only crusting, erythema, and ulceration were observed in the present study, and at a lower intensity than previously described for imiquimod, suggesting that topical treatment with 0.5% tretinoin was well tolerated.

Although no statistical analysis was performed due to the small number of animals in the pilot group, it was observed that horses exposed to sunlight throughout the treatment period (A4 to A10) showed a higher degree of local sensitivity, required a greater number of tretinoin cream applications and had longer treatment durations on average. The animals kept indoors (A1 to A3) were also younger, which may influence skin sensitivity. These findings suggest a potential association between sun exposure and increased sensitivity, treatment duration, and number of applications, and should be further evaluated in studies with larger sample sizes. The effect of age on skin sensitivity also needs to be investigated. Sunlight exposure is a well-documented concern during topical tretinoin therapy in humans, primarily due to increased photosensitivity and the risk of phototoxic reactions induced by retinoid-related thinning and inflammation of the stratum corneum (Mukherjee et al., 2006). In this study, animals were naturally exposed to sunlight throughout treatment, as they were privately owned and kept in herd environments, reflecting typical regional husbandry practices. Despite this, sensitivity remained mild and did not interfere with

routine handling. The use of UV-protective ear covers was attempted but was not feasible, as all animals removed them daily.

Of the 17 lesions treated with 0.5% topical tretinoin, a positive therapeutic response was observed in 15 cases (approximately 88%). The two ears that showed no improvement were from the same horse, suggesting the possibility of individual variation in therapeutic response, which has also been reported in studies involving other dermatological treatments.

The focal hypopigmentation observed in 16 lesions at the end of therapy with 0.5% tretinoin was also reported in 4 out of 16 horses treated with 5% imiquimod by Torres et al. (2010) and in 2 out of 8 animals in the study by Zakia et al. (2016). The occurrence of hypopigmentation may be associated with the cytopathic effect of EcPV on epithelial cells, such as keratinocytes and melanocytes, leading to decreased melanin pigmentation. Another possible explanation is the inflammatory response triggered by 0.5% tretinoin, which may cause melanocyte damage, reducing melanin production and consequently altering skin pigmentation.

The recurrence rate in this study was 35%, and a statistically significant association was found between recurrence and treatment response, with non-recurrent lesions showing a greater likelihood of >50% improvement. This reinforces the importance of early and effective therapeutic response in reducing the likelihood of lesion recurrence. It is also possible that some of the recurrent cases observed in this study may not represent true relapses, but rather reinfections, as the animals are housed in collective paddocks, which can facilitate transmission through direct contact or via vectors such as ticks and flies.

CONCLUSION

The topical application of 0.5% tretinoin cream proved to be an effective treatment for equine aural plaques in this study. The observed side effects were milder compared to those reported in the literature for 5% imiquimod. Given the high percentage of lesion resolution and the reduced severity of adverse effects, 0.5% tretinoin cream can be considered a viable and well-tolerated therapeutic option for equine aural papillomatosis. Further studies are needed to optimize treatment protocols.

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