

The influence of clinical severity and topical antimicrobial treatment on bacteriological culture and the microbiota of equine pastern dermatitis

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Background – Equine pastern dermatitis (EPD) is a common dermatological problem in horses, yet its aetiology and pathogenesis are poorly understood.

Objectives – This study aimed to investigate the effects of lesion severity and topical antimicrobial treatment on bacterial flora of EPD-affected skin.

Animals – Sixteen horses with EPD were investigated.

Methods and materials – An observational study was conducted by assigning a clinical severity score ranging from 0 (macroscopically nonlesional) to 21 (severe), and sampling the most and least severely affected limbs of 16 horses (32 limbs) for bacteriological culture and *16S rRNA* sequencing. Topical antimicrobial treatment in the month before sampling was recorded. The limbs were allocated to a nonlesional or mildly affected group (Group A, score 0–3) and a moderate to severely affected group (Group B, score 4–21).

Results – The most commonly cultured bacterial species was *Staphylococcus aureus* (one of 15 Group A versus nine of 17 Group B). Within Group B, *S. aureus* was found in three of six limbs treated with topical antimicrobials and in six of 11 untreated limbs. β -haemolytic streptococci (three of 32) and *Trueperella pyogenes* (two of 32) also were cultured exclusively in the untreated limbs of Group B. Staphylococci and streptococci were found more often by *16S rRNA* sequencing than in culture. Limbs with higher lesion severity and topical antimicrobial treatment appeared to have a lower alpha diversity and different beta diversity compared to milder and untreated lesions.

Conclusions and clinical importance – Observed differences in microbiota of equine skin are likely to be linked to the presence and severity of EPD and topical antimicrobial treatment. Further research is needed to establish causal bacteria.

Introduction

Equine pastern dermatitis (EPD) is a cutaneous reaction pattern¹ marked histologically by epidermal hyperplasia and orthokeratotic hyperkeratosis.²

The development of EPD is complex and influenced by a variety of different factors and pathogens.^{3–6} Bacterial colonization is thought to play a substantial role in initiating the pathogenesis or perpetuating the syndrome of EPD.^{3–6}

The current method to analyse bacterial flora associated with EPD in clinical practice is bacteriological culture. An alternative method that has become increasingly popular in human and veterinary medicine is next-generation sequencing (NGS) of the bacterial *16S rRNA* gene. In

human medicine, the time to healing was shortened by 22.9% with the help of NGS diagnostics compared to culture.⁷

Macroscopically normal equine skin has been analysed by NGS in two studies.^{8,9} To our knowledge, the skin microbiota of horses affected by EPD has not been analysed with NGS to date. The aims of this study were to evaluate the microbiota of EPD lesions using bacteriological culture and NGS, and to describe the microbiota of lesions with different severity and with or without topical antimicrobial treatment.

Methods and materials

A detailed description of the materials and methods used to conduct this study can be found in File S1 and Table S1.

Study design and horses

Horses with EPD were recruited for this observational study between September 2017 and April 2018. The study protocol was approved by the veterinary ethics committees of all 26 cantons of

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Switzerland (approval no. VD3297). Horses that had received systemic antimicrobials were excluded; there was no withdrawal period for topical treatment. All limbs were scored individually using a standardized clinical scoring system (Table S1) with a severity score ranging from 0 (macroscopically nonlesional) to 21 (severe). The most and least affected limb of each horse was allocated to one of two clinical groups (Group A, score 0–3, or Group B, score 4–21) and sampled for bacteriological culture and NGS.

Bacterial culture

Direct culture conditions were targeted to known pyogenic bacteria. After incubation, bacterial species were confirmed by matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS). The bacterial growth was assessed semiquantitatively: one plus (+) equalled <30 colonies per plate, two plus (++) 30–100 colonies per plate, and three plus (+++) >100 colonies per plate.

Sequencing of the 16S rRNA gene

Skin swabs for NGS were processed as described previously.¹⁰ The amplification was performed on the V4 region of the bacterial 16S rRNA gene. The threshold for sequencing was 1 ng/μL. Negative control samples were processed with the other samples. They produced <1 ng/μL DNA and were thus classified as negative and not sent for sequencing. The samples were then indexed and 2 x 250 bp paired-end sequenced on the Illumina MiSeq platform (Illumina Inc.; San Diego, CA, USA) by the genetics department of the University of Berne, Switzerland. Various procedures were performed for quality filtering. Sequences are available at the NCBI Sequence Read Archive (SRA) under the accession number PRJNA647995. The amplicon sequence variants (ASV) were further analysed in the programs EXCEL 2010 (Microsoft Corporation; Redmond, WA, USA) and R¹¹ (v3.4.3, BASE, VEGAN and MICROBIOME packages).

Descriptive statistics

Descriptive statistics were performed using NCSS 12 (NCSS Statistical Software; Kaysville, UT, USA, ncss.com/software/ncss) and R. Total sequences in all samples, mean sequences per sample, standard deviation (SD) and range were calculated in EXCEL. Alpha diversity indices of groups A and B were investigated; within Group B the lesions treated with antimicrobials were compared to the untreated lesions. A permutational multivariate ANOVA (PERMANOVA) using the distance matrices was performed in R to detect significant differences in group centroids for groups A and B, and for lesions treated or not treated with antimicrobials.

Results

Horses, samples and characteristics

A total of 16 horses with EPD were included in this study (Tables S2 and S5).

All limbs of each horse were clinically evaluated except for one right forelimb of one horse that was in a cast (individual J). The characteristics of the lesions are documented in Table S2.

Thirty-two limbs were sampled in total, of which 17 were allocated to group B and 15 to group A based on their clinical severity score. Six of 32 samples collected from limbs of four horses had been treated with topical antimicrobials, all of which were in Group B. The median score of untreated lesions in Group B was 9 (range 6–12) and the median score of the treated lesions was 8 (range 5–17).

Bacterial culture

Staphylococcus aureus, β-haemolytic streptococci and *Trueperella pyogenes* were the opportunistic pathogens detected (Table 1). The prevalence of *S. aureus* was

more than seven-fold higher in Group B (nine of 17) than in Group A (one of 15). Within Group B, there was no difference in the prevalence of *S. aureus* between treated and untreated lesions (three of six and six of 11, respectively). β-haemolytic streptococci and *T. pyogenes* were found only in untreated lesions of Group B.

Taxonomy of the microbiota

All samples had DNA concentrations >1 ng/μL. Of the 32 samples pooled, a total of 14,236 different ASVs were found [total sequences: 2,526,500, mean ± SD sequences/sample: 78,953 ± 54,344 (range 1,009–301,094)], which were classified into 41 phyla, 102 classes, 220 orders and 357 families. As all rarefaction curves reached their plateau, sequencing depth was found to be sufficient and further rarefaction of samples was therefore dismissed (Figure S1).¹² Main phyla across all samples consisted of Proteobacteria (34.6%), Actinobacteria (19.4%), Firmicutes (16.6%), Bacteroidetes (10.4%), Kiritimatiellaota (5.3%) and others with lower abundances. The distribution of the phyla in the 32 samples can be seen in Figure 1 and Table S3, and in greater detail in Figure S2; the distribution of the bacterial families is shown in Table S4.

The 10 bacterial genera with the highest relative abundance across all samples consisted of *Acinetobacter* (6.9%), *Sphingomonas* (5.1%), *Corynebacterium* (2.7%), *Pantoea* (2.6%), *Staphylococcus* (2.6%), *Moraxella* (2.1%), *Rothia* (2.0%), *Psychrobacter* (2.0%), *Actinobacillus* (2.0%) and *Fusobacterium* (2.0%). The 10 main genera in Group A were *Sphingomonas* (8.5%), *Pseudomonas* (2.5%), *Rothia* (2.4%), *Pantoea* (2.3%), *Acinetobacter* (2.1%), *Chryseobacterium* (2.0%), *Pedobacter* (1.6%), *Psychrobacter* (1.6%), *Massilia* (1.4%) and *Methylobacterium* (1.3%). In Group B, the genera consisted of *Acinetobacter* [11.1% (within Group B: 4.7% no antimicrobial treatment, 22.9% with antimicrobial treatment)], *Corynebacterium* [4.5% (within Group B: 3.4% no antimicrobials treatment, 6.4% with antimicrobial treatment)], *Staphylococcus* [4.4% (6.2%, 1.1%)], *Fusobacterium* [3.7% (5.6%, 0.1%)], *Moraxella* [3.6% (4.6%, 1.8%)], *Actinobacillus* [3.4% (3.1%, 3.9%)], *Pantoea* [3.0% (0.1%, 8.1%)], *Streptococcus* [2.5% (2.2%,

Table 1. Bacterial culture results from 32 samples taken from the pastern skin of 16 horses with equine pastern dermatitis (EPD).

	Group A	Group B
AT+	n = 0	n = 6
Mixed flora	0	4
<i>S. aureus</i>	0	3
AT–	n = 15	n = 11
Mixed flora	14	10
<i>Staphylococcus aureus</i>	1	6
β-haemolytic streptococci		3
<i>Trueperella pyogenes</i>		2

Samples from limbs that were mildly affected (severity score <4 of 21) were assigned to Group A and those from limbs with more severe lesions (score of ≥4) were assigned to Group B.

AT+, treated with topical antimicrobials in the month before sampling; AT–, not treated with topical antimicrobials in the month before sampling.

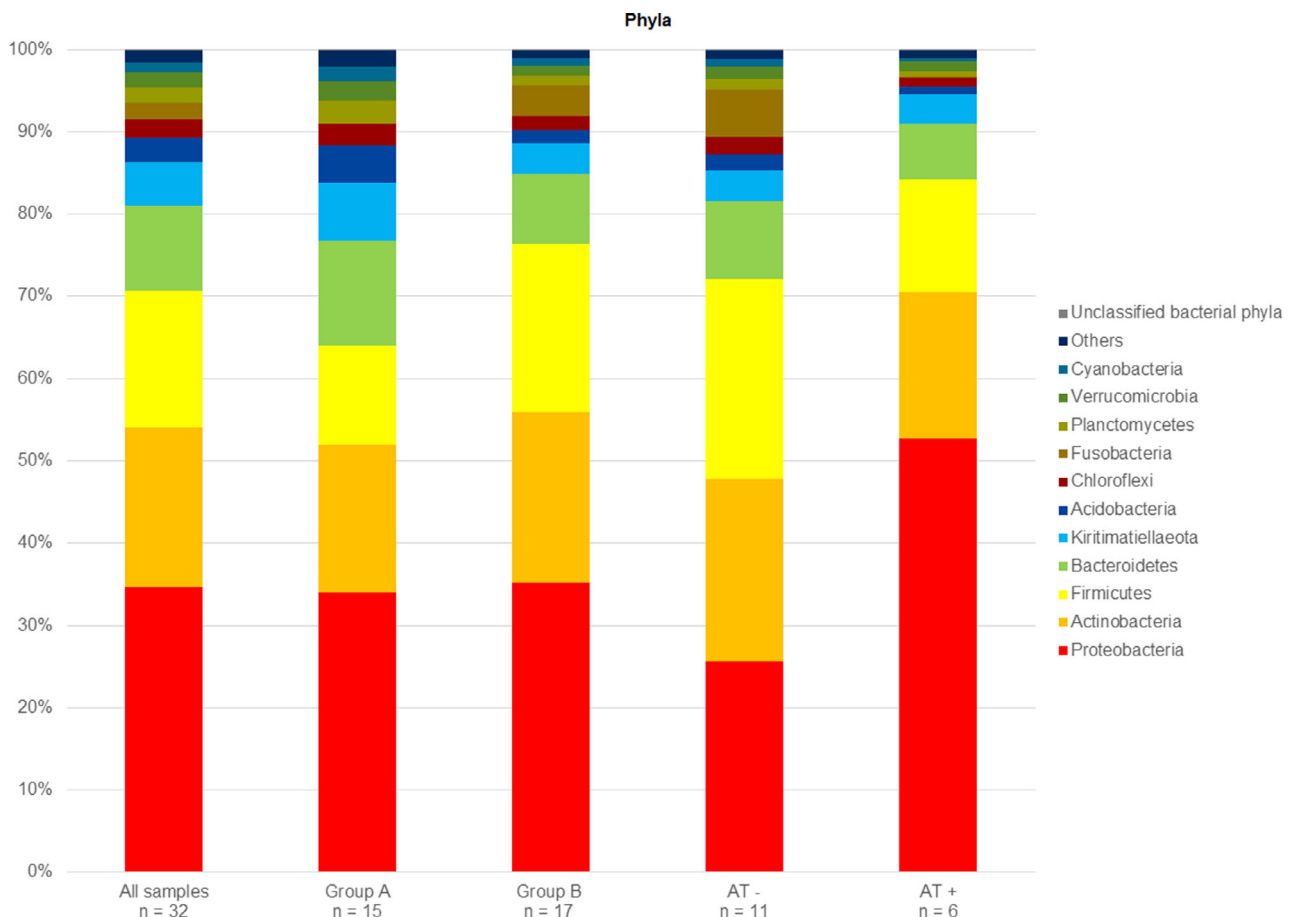


Figure 1. Overview of the 16S rRNA sequencing results of 32 samples taken from the pastern skin of 16 horses with equine pastern dermatitis (EPD). Bar graphs show the taxonomic composition on the level of phyla for all samples, Group A, Group B, and within Group B, the samples from skin treated with topical antimicrobials (AT+) and without treatment (AT-) in the month before sampling. Samples from limbs that were mildly affected (severity score <4 of 21) were assigned to Group A and those from limbs with more severe lesions (score ≥ 4) were assigned to Group B. All amplicon sequence variants (ASV) with a relative abundance <1% in all bars are pooled in "Others". Unclassified bacterial phyla, ASVs that could not be allotted to a phylum.

2.9%)), *Psychrobacter* [2.3% (3.4%, 0.4%)] and *Sphingomonas* [2.0% (1.5%, 3.0%)].

A total 180 of 14,236 (1.3%) ASVs could not be allocated to a phylum and constituted 0.1% of the relative abundance of the pooled samples. Concerning bacterial families, 3,400 ASVs (23.9%) remained unclassified (12.3% of the relative abundance). At the genus level, 7,436 (52.2%) ASVs and 22.0% of the relative abundance remained unclassified.

Alpha and beta diversity

Figure 2 shows the dot plots of indices for alpha diversity for groups A and B and antimicrobial treatment within Group B. Mean values for alpha diversity appear lower in Group B (richness = 867.8, Pielou's evenness = 0.7, Shannon = 4.2, Simpson = 0.9) compared to Group A (richness = 1,034.1, Pielou's evenness = 0.8, Shannon = 5.4, Simpson = 1.0). Treated lesions within Group B (richness 685.8, Pielou's evenness 0.6, Shannon 3.8, Simpson 0.8) also seemed lower on visual inspection compared to untreated lesions in the same group (richness 978.0, Pielou's evenness 0.7, Shannon 4.5, Simpson 0.9).

Figure 3 shows the beta diversity of groups A and B and of topical treatment or no treatment. The weighted

and unweighted Bray–Curtis index showed a difference in centroids between groups (PERMANOVA; $P = 0.007$ and $P = 0.005$, respectively; Figure 3). Antimicrobial treatment showed a difference (PERMANOVA) in the unweighted Bray–Curtis index ($P = 0.032$), and not in the weighted Bray–Curtis index ($P = 0.112$).

Comparison of the bacterial culture and NGS

The term "mixed flora", which was found in 28 of 32 cultures in addition to specific bacteria, indicates that more than three bacterial phenotypes were grown and were not further specified. Therefore, only the specific bacteria found in culture were compared to the microbiota (Table S5).

Staphylococci sequences could be found in 12 of 15 Group A samples and 14 of 17 Group B samples. Within Group B, sequences were found in six of six treated and eight of 11 untreated samples. The similar prevalence of staphylococci sequences in groups A and B does not agree with the higher prevalence in Group B observed in the culture. Streptococci sequences were detected in nine of 15 Group A samples and all (17 of 17) Group B samples, while the culture detected streptococci in only three untreated samples of Group B. Trueperellae

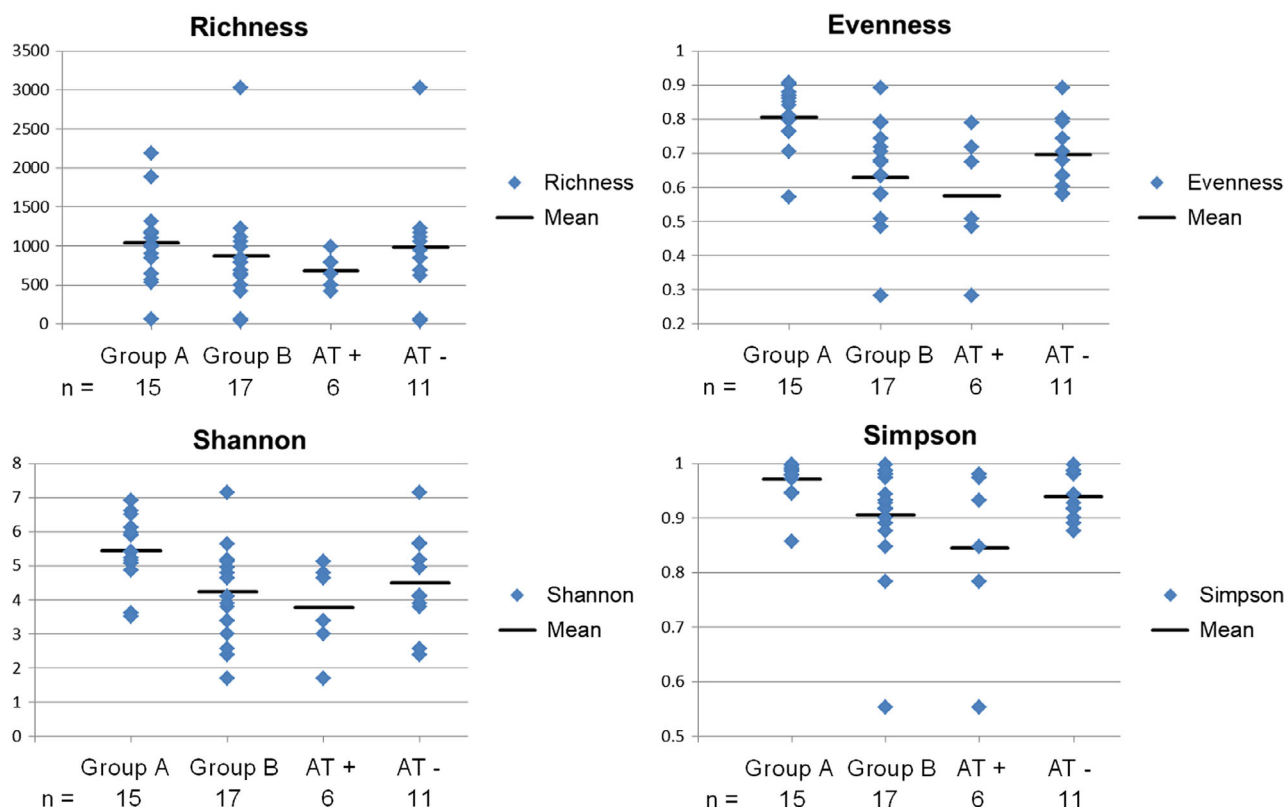


Figure 2. Dot plot showing the richness, Pielou's evenness, Shannon index and Simpson index of 32 samples taken from the pastern skin of 16 horses with equine pastern dermatitis (EPD).

AT+, samples in Group B with topical antimicrobial treatment in the month before sampling; AT-, samples in Group B without topical antimicrobial treatment in the month before sampling.

sequences were found only in two of 15 Group A samples and four of 17 Group B samples. Within Group B, sequences were found in one of six treated and three of 11 untreated samples.

Staphylococci accounted for 2.6% of the mean relative abundance of the microbiota of all samples [0.6% Group A, 4.4% Group B (within Group B: 6.2% no antimicrobial treatment, 1.1% with antimicrobial treatment)], streptococci for 1.5% [0.5% Group A, 2.5% Group B (2.2%, 2.9%)] and trueperellae for 0.1% [0.2% Group A, 0.1% Group B (0.1%, 0.1%)].

Discussion

Equine pastern dermatitis affects the bacterial composition of the skin. Specific opportunistic pathogens (*S. aureus*, β -haemolytic streptococci and *T. pyogenes*) were cultured more often from more severe lesions (Group B). *Staphylococcus aureus* was cultured equally from lesions that were treated or untreated with antimicrobials, while streptococci and *T. pyogenes* were found exclusively in samples from untreated lesions. In the NGS results, although staphylococci and streptococci had a higher relative abundance in the microbiota of Group B, interestingly within Group B, staphylococci were found more often in the untreated lesions. The alpha diversity seemed to be lower in more severe lesions (Group B), and within this group even lower if they were treated with antimicrobials. However, these observations were not evaluated with statistical analysis.

It was not possible to classify a substantial amount of ASVs at the genus level, which is most probably a consequence of the lack of data on equine skin in the databases used rather than sequencing errors. Many of the found ASVs may be unique to equine skin and warrant further studies to add information to these databases. Bearing in mind that 52.2% of the overall relative abundance could not be allocated to a specific genus, some of the 10 genera with the highest relative abundance, especially in Group A, often are found in soil. Ross *et al.* also found that a large proportion of the main phyla that they sequenced were associated with soil microbes and discussed how although this was likely owing to regular contact with the environment, there was a possibility that these genera belonged to the actual skin flora.⁸ The geographical location of the animals tested in their study had a greater influence on the skin microbiota than the anatomical location of the sampling site, although the mammalian order of the horse (Perissodactyla) showed a stronger difference between body regions than many other mammalian orders.⁸ Kamus *et al.* found that anatomical location had a strong effect on bacterial skin composition.⁹ They discovered that experimentally induced injured skin wounds were strongly associated with *Fusobacterium* and *Actinobacillus* in the initial healing phase. These two genera also were more dominant in Group B than in Group A in our samples. They also found more diverse communities in thoracic wounds compared to limb wounds, and in unbandaged limb wounds compared to their bandaged counterparts. After healing was

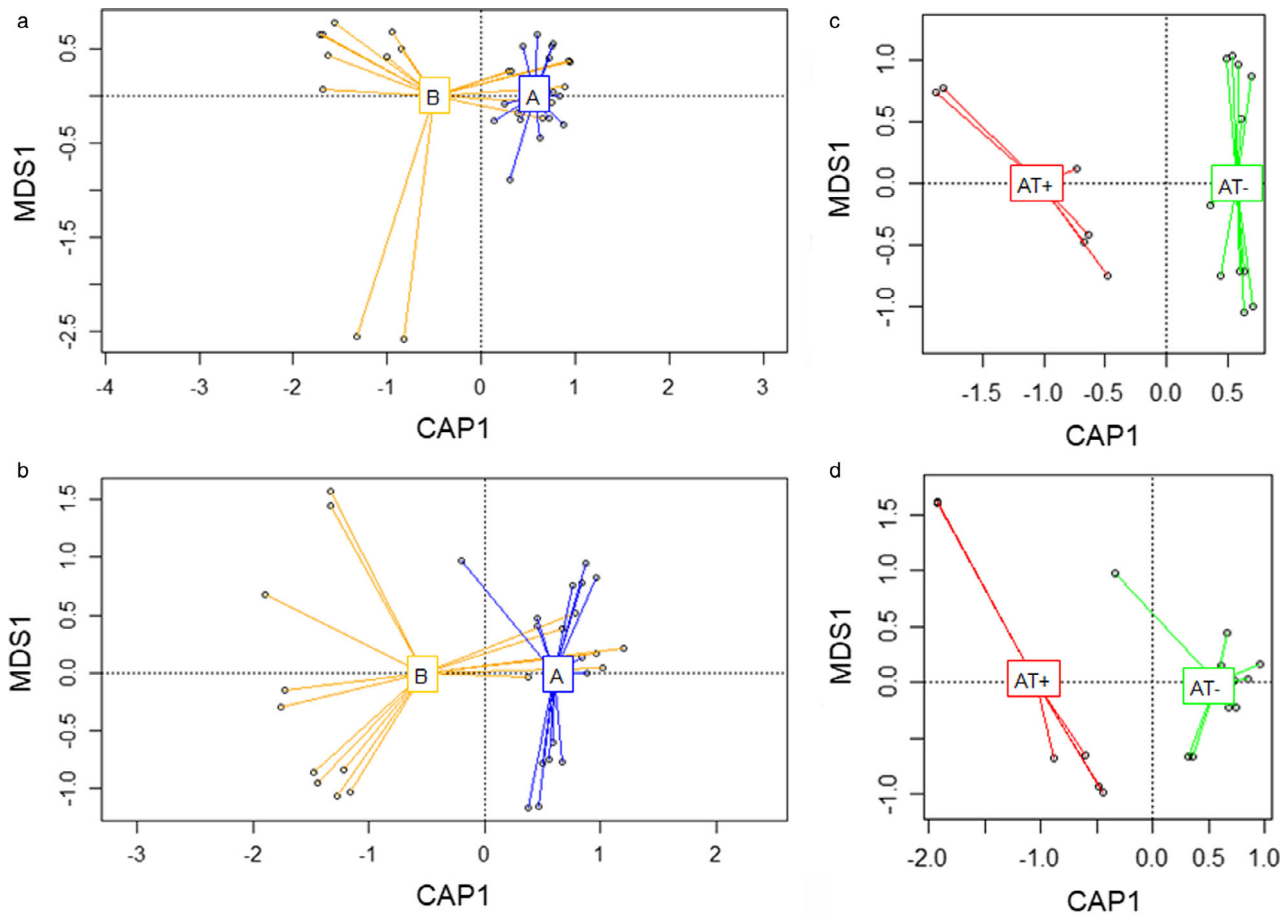


Figure 3. Spider plots showing the weighted (quantitative dissimilarity) and unweighted (qualitative dissimilarity) Bray-Curtis indices (beta diversity indices) of 32 samples taken from 16 horses affected by equine pastern dermatitis grouped by their clinical scores [Group A (macroscopically nonlesional or mild disease, score 0–3) and Group B (moderate to severe disease, score ≥ 4)]. This figure shows dissimilarities between samples. (a) Qualitative dissimilarity between groups A and B. (b) Quantitative dissimilarity between groups A and B. (c) Qualitative dissimilarity between samples taken from horses treated and not treated with antimicrobials within Group B. (d) Quantitative dissimilarity between samples taken from horses treated and not treated with antimicrobials within Group B. AT+, samples in Group B with topical antimicrobial treatment in the month before sampling; AT–, samples in Group B without topical antimicrobial treatment in the month before sampling; CAP1, constrained analysis of principal coordinates, one eigenvalue for constrained axes; MDS1, multidimensional scaling, one eigenvalue for unconstrained axes.

completed, the skin microbiota had a similar composition to the controls with a higher diversity, providing evidence that healthy skin has a stable microbial composition.⁹ This conclusion is in accordance with our samples, where the less severely affected samples seemed to have a higher diversity.

The relative abundance of staphylococci in NGS results appears to be higher in more severely affected limbs as well as in untreated lesions. These findings are supported by studies conducted on humans, mice and dogs, which found that the prevalence of *S. aureus* was higher in more severely inflamed samples^{13,14} and lower in skin sites treated with antimicrobials.¹⁴

The main limitation in our study was the small sample size and the inability to pair the samples for each horse, as some individuals did not have a limb meeting the requirements for Group A and another for Group B. The limited number of samples prevented the evaluation of possible confounders such as individual, sex and environment. Also, our study did not include healthy horses as controls. For these reasons, statistical analysis was not performed on our data. In addition, the horses were not

sampled during the same time of year and there were more forelimbs in Group A and hind limbs in Group B. This bias may have influenced the results.

Contamination by environmental bacterial DNA in laboratory reagents and extraction kits is a common issue in NGS.¹⁵ In order to investigate this possibility, the negative control samples would have needed further sequencing. A study describing the microbiota of healthy equine pastern skin would have been valuable in addressing this problem. Further study with more samples, a control group without macroscopic lesions, and more narrowly defined clinical groups will help enable us to assess our findings with better statistical power.

In conclusion, the results of this study suggest that the differences in microbiota of equine skin are likely linked to the presence and severity of EPD and treatment with antimicrobial agents. Bacterial composition and antimicrobial treatment may play a relevant role in the development or perpetuation of EPD, and warrant further study. Further study also is required to investigate the role of causal bacteria and reduction in bacterial diversity in EPD in order to enhance the efficacy of therapy.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Rarefaction curve for 32 samples taken from the pastern skin of 16 horses with EPD.

Figure S2. 16S rRNA sequencing results of 32 samples taken from the pastern skin of 16 horses with EPD.

Table S1. Standardized scoring system ranging from 0 (macroscopically nonlesional) to 21 (severe) used to evaluate the severity of EPD by skin pathologies commonly associated with EPD

Table S2. Characteristics of interest from 16 horses affected by EPD grouped by their clinical scores [Group A (macroscopically nonlesional or mild lesions, severity score <4 of 21) and Group B (moderate to severe lesions, severity score ≥4)] and antimicrobial treatment [treated (AB+) or untreated (AB–)].

Table S3. 16S rRNA sequencing results from 32 samples taken from the pastern skin of 16 horses with EPD.

Table S4. 16S rRNA sequencing results from 32 samples taken from the pastern skin of 16 horses with EPD

Table S5. Comparison of the results from bacteriological culture to NGS from 32 samples taken from the pastern skin of 16 horses with EPD

File S1. Material and Methods describing the study design, clinical NGS of the 16S rRNA gene processing, and descriptive analysis.

Résumé

Contexte – La dermatite des paturons équine (EPD) est un problème dermatologique fréquent chez le cheval bien que son étiologie et sa pathogénie soient peu connues.

Objectifs – Cette étude a pour but d'étudier les effets de la sévérité des lésions et un traitement antibiotique sur la flore bactérienne de la peau atteinte d'EPD.

Sujets – Seize chevaux atteints d'EPD ont été étudiés.

Méthodes – Une étude d'observation a été conduite en attribuant un score de sévérité clinique allant de 0 (macroscopiquement non lésionnel) à 21 (sévère), et en prélevant les membres les plus et les moins sévèrement atteints de 16 chevaux (32 membres) pour culture bactériologique et séquençage d'ARNr16S. Le traitement antibiotique du mois précédent les prélèvements a été enregistré. Les membres ont été répartis à un groupe non lésionnel ou modérément atteint (groupe A, score 0-3) et un groupe d'atteinte modéré à sévère (Groupe B, score 4-21).

Résultats – L'espèce la plus fréquemment cultivée était *Staphylococcus aureus* (un sur 15 du Groupe A versus neuf sur 17 du Groupe B). Pour le Groupe B, *S. aureus* a été trouvé dans trois des six membres ayant reçus des antibiotiques et dans six des 11 membres non traités. Streptocoque β-haémolytique (trois sur 32) et *Trueperella pyogenes* (deux sur 32) ont aussi été cultivées exclusivement à partir de membres non traités du Groupe B. Staphylocoques et streptocoques ont été trouvés plus souvent par séquençage de 16S rRNA que par culture. Les membres avec des lésions plus sévères et un traitement antibiotique semblent avoir une plus faible diversité alpha et une diversité beta différente comparé aux lésions modérées ou non traitées.

Conclusions et importance clinique – Les différences observées de microbiote de la peau équine sont probablement à lier à la présence et la sévérité de l'EPD et au traitement antibiotique. D'autres recherches sont nécessaires pour établir une bactérie causale.

Resumen

Introducción – la dermatitis de la cuartilla equina (EPD) es un problema dermatológico común en los caballos, sin embargo, su etiología y patogenia son poco conocidas.

Objetivos – este estudio tuvo como objetivo investigar los efectos de la gravedad de la lesión y el tratamiento con antibióticos en la flora bacteriana de la piel afectada por EPD.

Animales – se investigaron dieciséis caballos con EPD.

Métodos – se realizó un estudio observacional mediante la asignación de una valoración de severidad clínica que variaba de 0 (macroscópicamente no lesionado) a 21 (grave), y se tomaron muestras de las extremidades más y menos gravemente afectadas de 16 caballos (32 extremidades) para cultivo bacteriológico y secuenciación de rRNA 16S. Se registró el tratamiento con antibióticos en el mes anterior al muestreo. Las extremidades se asignaron a un grupo no lesionado o levemente afectado (Grupo A, puntuación 0-3) y un grupo moderado a gravemente afectado (Grupo B, puntuación 4-21).

Resultados – la especie bacteriana cultivada con mayor frecuencia fue *Staphylococcus aureus* (una de las 15 del Grupo A frente a nueve de las 17 del Grupo B). Dentro del Grupo B, se encontró *S. aureus* en tres de las seis extremidades tratadas con antibióticos y en seis de las 11 extremidades no tratadas. *Streptococcus* β -hemolíticos (tres de 32) y *Trueperella pyogenes* (dos de 32) también se cultivaron exclusivamente en las extremidades no tratadas del Grupo B. Los estafilococos y estreptococos se encontraron con más frecuencia mediante secuenciación de rRNA 16S que en cultivo. Las extremidades con mayor gravedad de la lesión y el tratamiento con antibióticos parecían tener una menor diversidad alfa y diferente diversidad beta en comparación con las lesiones más leves y no tratadas.

Conclusiones e importancia clínica – es probable que las diferencias observadas en la microbiota de la piel equina estén relacionadas con la presencia y la gravedad de la EPD y el tratamiento con antibióticos. Se necesita más investigación para establecer las bacterias causales.

Zusammenfassung

Hintergrund – Die Equine Pastern Dermatitis (EPD; Hautentzündung der distalen Gliedmaßen beim Pferd) ist ein häufiges dermatologisches Problem bei Pferden, wobei allerdings die Ätiologie und Pathogenese schlecht abgeklärt sind.

Ziele – Diese Studie zielte darauf ab, die Auswirkungen der Schwere der Läsionen und einer antibiotischen Behandlung auf die Bakterienflora der von EPD-betroffenen Haut zu untersuchen.

Tiere – Sechzehn Pferde mit EPD wurden untersucht.

Methoden – Es wurde eine Beobachtungsstudie erstellt, indem ein klinischer Schweregrad von 0 (makroskopisch nicht-läsional) bis 21 (schwer) zugeteilt, sowie eine Probenentnahme zur Bakterienkultur und für eine 16S rRNA Sequenzierung an den am meisten und am wenigsten betroffenen Gliedmaßen der 16 Pferde (32 Gliedmaßen) durchgeführt wurde. Eine antibiotische Behandlung im Monat vor der Probenentnahme wurde festgehalten. Die Gliedmaßen wurden in eine nicht-läsionale oder mild betroffene Gruppe (Gruppe A, Grad 0-3) und eine moderat bis schwer betroffene Gruppe (Gruppe B, Grad 4-21) eingeteilt.

Ergebnisse – Die am häufigsten kultivierte bakterielle Spezies war *Staphylococcus aureus* (einer von 15 in Gruppe A versus neun von 17 in Gruppe B). Innerhalb von Gruppe B wurde *S. aureus* bei drei von sechs mit Antibiotika behandelten Gliedmaßen gefunden sowie in sechs von 11 unbehandelten Gliedmaßen. Es wurden außerdem β -hämolytische Streptokokken (drei von 32) und *Trueperella pyogenes* (zwei von 32) ausschließlich in den unbehandelten Extremitäten von Gruppe B kultiviert. Staphylokokken und Streptokokken wurden häufiger mittels 16S rRNA Sequenzierung als durch eine Kultur gefunden. Gliedmaßen mit einem höheren Schweregrad der Läsionen und antibiotischer Behandlung hatten scheinbar eine niedrigere Alpha Diversität und eine unterschiedliche Beta Diversität im Vergleich zu mildereren und unbehandelten Läsionen.

Schlussfolgerungen und klinische Bedeutung – Die beobachteten Unterschiede der Mikrobiota der Pferdehaut stehen wahrscheinlich im Zusammenhang mit dem Auftreten und dem Schweregrad der EPD und der antimikrobiellen Behandlung. Es sind weitere Studien nötig, um die verursachenden Bakterien zu finden.

要約

背景 – 馬繋靨(EPD)は、馬によく見られる皮膚科学的問題であるが、その病因及び発症機序はあまり把握されていない。

目的 – 本研究の目的は、EPDに罹患した皮膚の細菌叢に対する病変の重症度及び抗生物質治療の影響を調査することであった。

被験動物 – EPDに罹患した16頭の馬を調査した。

方法 – 観察研究は、0(肉眼的に非病変)から21(重度)の範囲の臨床重症度スコアを割り当て、細菌培養検査および16S rRNAシーケンス法のため16頭の馬(32肢)の最も深刻な罹患肢及び最も深刻でない肢からサンプリングした。サンプリングの前月の抗生物質治療を記録した。四肢を、非病変または軽度の罹患グループ(グループA、スコア0~3)および中程度から重度の罹患グループ(グループB、スコア4~21)に割り当てた。

結果 – 最も一般的に培養された細菌種は黄色ブドウ球菌であった(グループAのうちの1/15対グループBのうちの9/17)。グループB内では、黄色ブドウ球菌は抗生物質で治療された6肢のうち3肢、および11肢の未治療肢のうち6肢で発見された。b溶血性連鎖球菌(32肢中3肢)およびTrueperella pyogenes(32肢中2肢)も、グループBの未治療肢でのみ培養された。ブドウ球菌および連鎖球菌は、培養検査よりも16SrRNAシーケンス法によってより頻繁に検出された。病変の重症度が高く抗生物質治療を施された肢は、軽度の未治療病変と比較して、a多様性が低く、b多様性が異なった。

結論と臨床的重要性 – 馬の皮膚の微生物相で観察された違いは、EPDの重症度及び抗生物質治療の存在に関連している可能性がある。原因菌を確立するには、さらなる研究が必要である。

概要

背景 – 馬股皮炎(EPD)は馬常见的の皮膚病、但对其病因和发病机制知之甚少。

目的 – 本研究旨在探讨病変严重程度和抗生素治疗对EPD发病皮肤的菌群影响。

动物 – 研究16匹EPD马。

方法 – 通过指定范围为0(无肉眼可见病変)至21(重度)的临床严重程度评分进行观察性研究,并对16匹马(32条肢体)最严重和最不严重的发病肢体采样,进行细菌学培养和16S rRNA测序。记录采样前1个月抗生素治疗情况。将肢体分为非病変或轻度发病组(a组,评分0-3)和中度至重度发病组(B组,评分4-21)。

结果 – 最常培养的细菌种类是金黄色葡萄球菌(A组15种中的1种与B组17种中的9种)。B组内,用抗生素治疗的6个肢体中有3个发现金黄色葡萄球菌,而未治疗的11个肢体中有6个发现金黄色葡萄球菌。只在B组未治疗肢体中培养出b-溶血性链球菌(32个中有3个)和化脓隐秘杆菌(32个中有2个)。相比于培养,16S rRNA测序对葡萄球菌和链球菌的发现率更高。与较轻和未治疗的病変相比,病变更严重和抗生素治疗的肢体似乎具有较低的a多样性和不同的b多样性。

结论和临床重要性 – 研究观察到的马皮肤微生物群变化,可能与EPD严重程度和抗生素治疗相关。需要进一步的研究来验证致病菌。

Resumo

Resumo – A dermatite de quartela em equinos (DQ) é um problema dermatológico comum em cavalos, embora sua etiologia e patogênese sejam mal compreendidas.

Objetivos – Este estudo teve como objetivo investigar os efeitos da gravidade da lesão e do tratamento com antibióticos na flora bacteriana da pele afetada por DQ.

Animais – Dezesesseis cavalos com DQ foram investigados.

Métodos – Um estudo observacional foi conduzido atribuindo uma pontuação de gravidade clínica variando de 0 (macroscopicamente não lesional) a 21 (grave), e amostrando os membros mais e menos gravemente afetados de 16 cavalos (32 membros) para cultura bacteriológica e sequenciamento de 16S rRNA. O tratamento com antibióticos no mês anterior à amostragem foi registrado. Os membros foram alocados a um grupo não lesional ou levemente afetado (Grupo A, pontuação 0–3) e um grupo afetado moderado a gravemente (Grupo B, pontuação 4–21).

Resultados – A espécie bacteriana mais comumente cultivada foi *Staphylococcus aureus* (um dos 15 do Grupo A versus nove dos 17 do Grupo B). Dentro do Grupo B, *S. aureus* foi encontrado em três dos seis membros tratados com antibióticos e em seis dos 11 membros não tratados. Os estreptococos β-hemolíticos (três de 32) e *Trueperella pyogenes* (dois de 32) também foram cultivados exclusivamente nos membros não tratados do Grupo B. Os estafilococos e estreptococos foram encontrados mais frequentemente por sequenciamento de rRNA 16S do que em cultura. Membros com maior gravidade de lesão e tratamento com antibióticos pareceram ter uma alfa-diversidade inferior e uma beta-diversidade diferente em comparação com lesões mais leves e não tratadas.

Conclusões e importância clínica – As diferenças observadas na microbiota da pele de equinos provavelmente estão relacionadas à presença e gravidade de DQ e ao tratamento com antibióticos. Mais pesquisas são necessárias para estabelecer a bactéria causadora.