

# The relationship between equine pastern dermatitis, meteorological factors, and the skin microbiota

Sarah Kaiser-Thom\* , Markus Hilty†, Alessandra Ramseyer\*‡, Pascale Epper\*‡ and Vinzenz Gerber\*

\*Swiss Institute of Equine Medicine (ISME), Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, and Agroscope, Länggassstrasse 124, Bern, 3012, Switzerland

†Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, Bern, 3010, Switzerland

‡Swiss Institute of Equine Medicine (ISME), Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, and Agroscope, Les Longs-Prés, Avenches, 1580, Switzerland

Correspondence: Sarah Kaiser-Thom, Agroscope, Länggassstrasse 124, 3012 Bern, Switzerland. E-mail: s.kaiserthom@gmail.com

**Background** – Equine pastern dermatitis (EPD) is a multifactorial syndrome, with prolonged exposure to moisture assumed to be a predisposing or primary factor.

**Hypothesis/Objectives** – To examine the course of EPD lesion severity, changes in bacterial skin microbiota, and the influence of meteorological factors.

**Animals** – Prospective, longitudinal cohort study over a one-year period, with six Franches-Montagnes stallions, four affected by EPD and two unaffected, that were kept under the same conditions.

**Methods and materials** – Pasterns were scored for lesion severity and sampled once a month for 12 consecutive months. Lesion severity, the skin microbiota and meteorological factors were examined for associations.

**Results** – EPD lesions tended to worsen in autumn and at the beginning of spring. The relationship between lesion severity and the meteorological factor precipitation was not clearly evident; high scores were preceded by both low or high rates of precipitation. Microbiota in affected pasterns appeared to have experienced a reduction in alpha diversity. Beta diversity analyses demonstrated that bacterial community structures were altered in affected versus unaffected pasterns, and that alterations were more pronounced with higher EPD scores ( $P = 0.005$ ). Meteorological factors also had considerable influences on the bacterial composition, whereby these influences appeared to be more marked in the affected pasterns ( $P = 0.001$ ,  $F = 3.19$ ) than in unaffected ones ( $P = 0.005$ ,  $F = 1.83$ ).

**Conclusions and clinical relevance** – Our study provides preliminary observations of the relationships between lesion severity, meteorological factors and cutaneous bacteria. The population was too small to draw firm conclusions, and further studies on environmental factors and the involvement of bacteria in this multifactorial disease are needed.

## Introduction

Equine pastern dermatitis (EPD) is one of the most common skin disorders of the horse, and is particularly prevalent in heavier breeds, such as draught horses, Irish Cobs (Tinkers) and Friesians.<sup>1–3</sup> In the Swiss Franches-Montagnes horse, the prevalence among 3-year-olds reaches 15%.<sup>4</sup> Clinical manifestations vary, and typical signs include erythema, alopecia, scales, crusts and thickening of the skin, particularly in the palmar and plantar regions of the pastern.<sup>1,2,5</sup> EPD is considered a multifactorial syndrome, yet the roles of contributing factors remain poorly understood. One of the most widespread dogmas is that the development and course of EPD is causally related to prolonged exposure to a wet and muddy environment.<sup>1,5–7</sup> However, this has not been documented scientifically.

The involvement of bacteria as a primary and/or secondary factor also is often assumed, yet identification of specific pathogens with culture-based methods or PCR has so far proven unsuccessful,<sup>8,9</sup> the former often resulting in a mixed flora. Yet, culture-independent assays open up new possibilities in this respect.<sup>10</sup> A previously unimagined degree of bacterial diversity can be studied through next-generation sequencing (NGS) approaches, both in humans<sup>11–13</sup> and in animals.<sup>14–16</sup> Studies using these new methods have further shown that skin disorders often go hand-in-hand with alterations of the microbiota.<sup>17–19</sup> The question of causality versus association as well as the exploration of new treatment approaches based on these data are subjects of continuing discussion in this still rather young area of research.<sup>20,21</sup>

For this pilot study, we monitored a small group of EPD-affected and unaffected horses over a prolonged period of time. Our aim was to document the course of lesion severity in relation to seasonal and meteorological factors. In parallel to lesion severity scores, concurrent changes in the bacterial microbiota, namely in their alpha

Accepted 1 September 2021

**Sources of Funding:** This project was funded by the ISMEquine Research Fund. Open Access Funding provided by Agroscope.

(within-sample) and beta (between-samples) diversities were investigated.

## Materials and methods

### Ethics

The study protocol was approved by the veterinary ethical committee of the canton of Vaud in Switzerland (VD3297+). The participating horses were owned by the Swiss National Stud, which belongs to Agroscope, the Swiss Confederation's centre of excellence for agricultural research, and informed consent was obtained for this study within the institutional mandate of the Swiss Institute of Equine Medicine (ISME) for the care of the horses of Agroscope.

### Study design

A prospective, longitudinal cohort design was used to examine associations between the pastern skin microbiota, EPD lesion severity and meteorological factors. The study cohort comprised six Franches-Montagnes stallions, four of which were affected by EPD and two of which were unaffected. Horses were sampled once a month for 12 consecutive months, starting from October 2017 through September 2018. At each visit, all four pasterns of each horse were thoroughly inspected for signs of EPD and graded for lesion severity using a standardised scoring system as described previously.<sup>22</sup> (see Supporting information Appendix S1). Briefly, the score accounts for dermal lesions commonly associated with EPD, including scales, crusts, ulceration and formation of skin folds,<sup>1,2,5</sup> and the cumulative value of each pastern can range between 0 (not affected) and 21 (severely affected). In each horse, the most severely affected pastern was selected for sampling at the first visit. In the two unaffected stallions, the pastern to be sampled was chosen randomly at the first visit. In both affected and unaffected horses, these same pasterns then were consistently sampled at every following visit, which was always conducted during the last week of the month or within the first week of the following month. This way, changes observed in the EPD severity score and in bacterial composition could be related to the meteorological data for the respective preceding month. The corresponding meteorological data were obtained from the Federal Office of Meteorology and Climatology MeteoSwiss at the nearest possible meteorological centre, and comprised the sum of precipitation (mm), mean relative humidity (%) and mean temperature (°C) of each month.

### Sample collection

An area of approximately 4 × 2 cm was gently swabbed using sterile cotton swabs (PS/Viscose, Sarstedt AG & Co.; Nümbrecht, Germany) moistened with sterile 0.9% saline solution. Additionally, a negative control sample was taken for each visit by exposing a moistened swab to the ambient air for 20 s. The swabs then were transported in a cold-storage box and subsequently stored at –80 °C until further processing.

### Sample preparation for sequencing analyses

The laboratory work was conducted in the same way as described in previous studies.<sup>22,23</sup> In brief, the QIAmp DNA Mini Kit (Qiagen; Hilden, Germany) was utilised for DNA extraction, followed by the amplification of the V4 region of the bacterial 16S rRNA gene through a 35 cycle PCR. After subsequent purification, PCR products were quantified via gel electrophoresis to confirm a minimum concentration of 1 ng/μL that was considered compulsory to release the samples for sequencing. The samples were submitted to the Institute of Genetics of the University of Bern for indexing and paired-end 2 × 250 bp sequencing on a MiSeq platform (Illumina Inc.; San Diego, CA, USA).

### Raw sequencing data processing

Raw data processing and subsequent statistics were performed in the open-source software R (v4.0.1).<sup>24</sup> The DADA2 package (v1.14) was used according to the package builder's recommended workflow<sup>25</sup> to process the raw sequencing data. Read quality was inspected and lower-quality tails were trimmed to maintain high

quality throughout (forward reads at 240 bp, reverse reads at 220 bp). By means of specialised error correction modelling, the DADA2 algorithm can distinguish sequencing errors from real biological variation. Chimeras were identified and removed. Then, taxonomy was assigned to the remaining amplicon sequence variants (ASVs) by utilising the Silva 16S rRNA reference database v132,<sup>26</sup> and sequences identified as chloroplasts, mitochondria, Archaea or Eukaryotes were erased. Rarefaction curves were generated to affirm that sequencing depth was sufficient.

### Data analyses

As a consequence of the small sample size, a descriptive approach was chosen for the largest part of the data analyses.

An overview plot was created, depicting the one year evolution of the meteorological data, the EPD severity score, and measures of the skin microbiota. The latter comprised the three alpha diversity (within-community) indices richness (number of ASVs), Pielou's evenness and the Shannon diversity index, as well as the pairwise weighted (abundance-based) Bray–Curtis dissimilarity distances of affected versus unaffected pasterns, reflecting changes in beta diversity (between-communities diversity) over time. The line overlaying the individual EPD scores as well as the curves portraying the alpha and beta diversity measures were drawn based on LOESS (locally estimated scatterplot smoothing), a type of moving mean regression analysis. Curves were fitted jointly with 90% confidence intervals. Seasons have been indicated in the plot as follow: autumn, September–November; winter, December–February; spring, March–May; and summer, June–August. Also, for the meteorological factors, three gradations were determined according to the respective tertiles of "low", "medium" and "high" measurements, indicated in the plot by means of grey shading.

The influential power of specific factors on the bacterial beta diversity was further examined statistically with a permutational multivariate analysis of variance (PERMANOVA, function *adonis*). The association of lesion severity and alterations of the bacterial composition (beta diversity) was examined using the weighted Bray–Curtis distance matrices and plotted using constrained analysis of proximities (CAP) ordination method. Analyses using unweighted (presence/absence-based) Bray–Curtis distances can be found in the Supporting information. Differences were investigated in terms of the EPD score. Additionally, scoring classes were depicted as follows: class 0 (score 0; the unaffected pasterns), class I (scores 2–3), class II (scores 4–8) and class III (scores 9–14). Classes I–III were allocated according to tertiles. Finally, changes in beta diversity were examined for their association with the seasons and the meteorological factors using the *adonis* test. In order to evaluate the influence on the microbiota in affected and in unaffected pasterns, sample groups were investigated separately. For these statistical comparisons, a *P*-value of <0.05 was deemed significant.

## Results

### Overview of the study population and samples

The study cohort comprised six Franches-Montagnes stallions. Four stallions exhibited clinical EPD signs throughout the entire sampling period, and the other two never showed any signs of EPD at all. The horses lived together on a pasture for most of the year. During the breeding months (March–June) the horses were kept at their respective breeding stations. Accordingly, although two of the horses were temporarily located on a different farm, all stallions were housed in similar management conditions: they were kept in the stable (box stalls), with several hours of daily outdoor access in all weather conditions. No horse was treated during the study period. Details of the horses can be found in Table 1. Further details on the monthly scores of all pasterns (affected and unaffected) in all horses can be found in the Appendix S2.

Once a month the horses were examined, and a skin swab was collected (n = 72). After DNA amplification, 64 samples reached the threshold for DNA concentration and could thus be further analysed.

**Sequences**

A total of 4,583,032 reads were retained in our study with a median of 63,236 reads per sample. Clustering resulted in 24,577 ASVs. Because all rarefaction curves reached their plateau, the sequencing depth was found to be sufficient (Appendix S3). Reads were deposited in NCBI’s Short Read Archive (SRA) repository under accession number PRJNA646326.

**Lesion severity exhibits seasonal differences**

The one year evolution of the meteorological data and the EPD severity scores of the affected pasterns is depicted in Figure 1 a–d. The score curve shows several peaks, in October/November, March and August/September, thus in autumn and at the beginning of spring. Median seasonal lesion scores were 8 in autumn, 4.5 in winter, 4 in spring and 6 in summer. In our observation period, the autumn months were characterised by relatively low precipitation as well as, naturally, a reduction of temperature and a moderate increase of humidity. The highest precipitation occurred in December and January, yet the EPD lesions presented as less severe (i.e. with lower scores) during this period. However, the score peak in March as well as the gradual increase at the beginning of and during the summer months were preceded by relatively high amounts of precipitation.

**The microbiota changes with lesion severity**

The variation of the three alpha diversity measures richness, Pielou’s evenness and Shannon diversity index, throughout the year from both affected and unaffected pasterns are illustrated in Figure 1 e–g. Alpha diversity of affected pasterns did not appear to be evidently correlated to the evolution of the EPD score. Alpha diversity did, however, trend lower in affected versus unaffected pasterns at all time points sampled.

The last part of the graph presents the evolution of the pairwise dissimilarity of the bacterial composition between the affected and unaffected pasterns (Figure 1h). Note that the higher the points of the curve, the more the composition of the microbiota diverges. Except for March, it appears that this curve roughly reflects the course of the EPD score curve – thus, as the severity of

lesions in the affected pasterns increased, the bacterial composition between affected and unaffected pasterns became more dissimilar.

The association between the awarded scoring class (classes 0–III) and the beta diversity also was apparent in an ordination approach. Fitting the scores as ordinal variables proved a significant constraint for the bacterial composition ( $P = 0.005$ ,  $F = 2.51$ ), with beta diversity clustering visibly based on class severity (Figure 2).

The bacterial composition is related to the seasons and the meteorological factors

Season significantly affected beta diversity in both affected and unaffected pasterns (Table 2). The meteorological factors were particularly influential for the ordination of samples from affected pasterns ( $P = 0.001$ ,  $F = 3.19$ ), with precipitation having the greatest marginal effect ( $P = 0.001$ ,  $F = 2.24$ ). In unaffected pasterns, the impact of the meteorological factors was considerably smaller ( $P = 0.005$ ,  $F = 1.83$ ), with only the temperature showing a significant marginal effect ( $P = 0.019$ ,  $F = 1.70$ ). This also is illustrated in the generated biplots (Figure 3). Beta diversity analyses based on unweighted (presence/absence-based) Bray Curtis indices can be found in Appendix S4), as can two plots depicting the abundances of bacterial families in affected and unaffected pasterns (Appendix S5).

**Discussion**

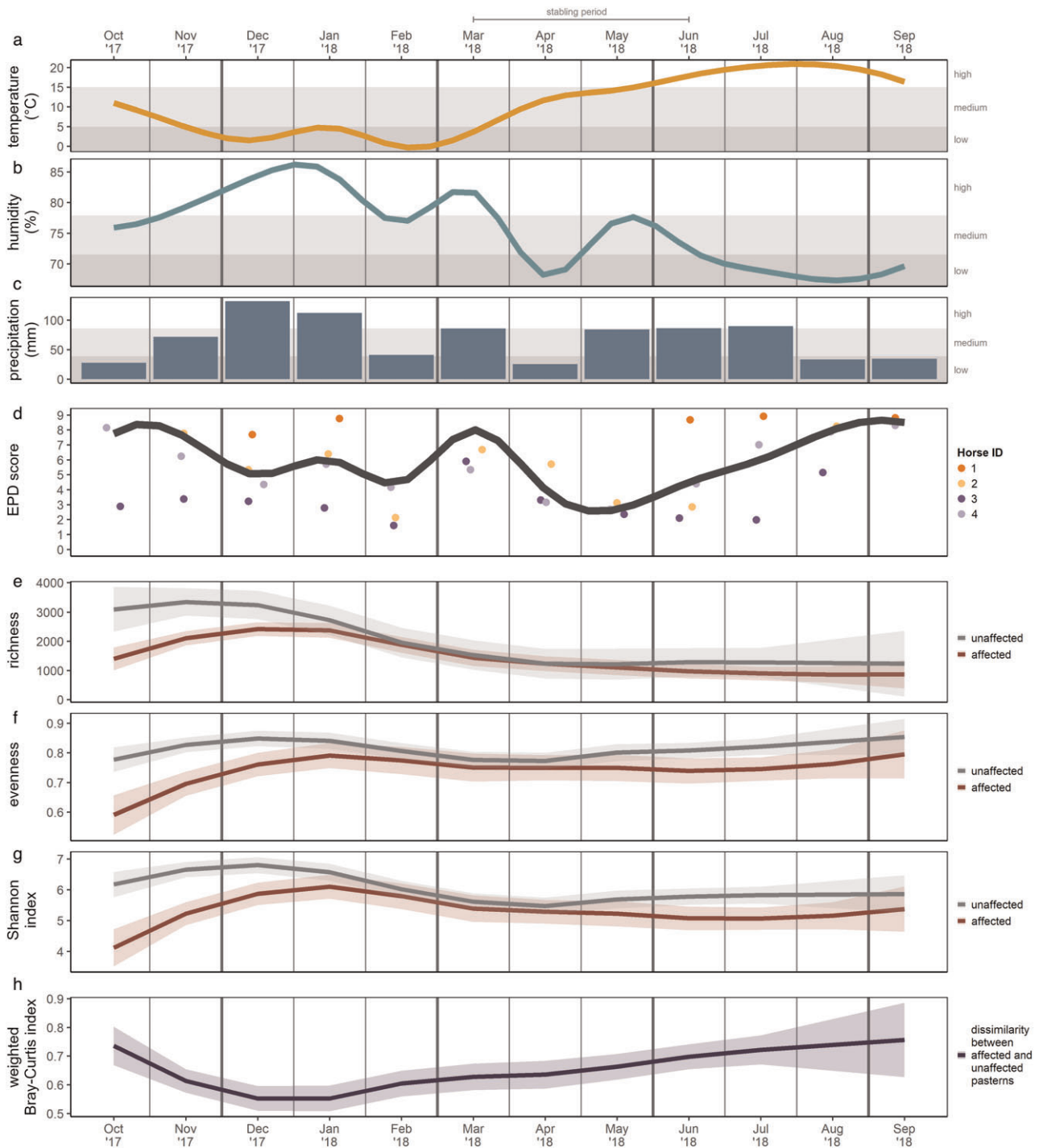
This is the first study to integrate three cornerstones of EPD: the course of lesion severity over the period of an entire year, the corresponding meteorological factors and the involvement of bacteria. We noticed that EPD lesions tend to worsen at certain times of the year, and that worsening also is associated with changes in the bacterial composition. In addition, meteorological factors appear to exert a considerable influence on the skin microbiota in both affected and unaffected pasterns.

There were seasonal differences in lesion severity, with the highest scores observed in autumn. This is in agreement with commonly held views on EPD,<sup>1,2,5</sup> and to our knowledge, it has never been investigated before. Surprisingly, the autumn months were not characterised by particularly wet conditions in our observation period. By contrast, the peak of EPD scores in March as well as the gradual increase from May onwards were indeed preceded by relatively high precipitation. A clear and perhaps proportional relationship between the severity of clinical signs and the amount of precipitation, as is widely

**Table 1.** Details on the study cohort.

Horse ID	Health status	Age (years)	Median EPD score	Range of EPD score
1	Affected	11	10	8–14
2	Affected	19	6	2–9
3	Affected	18	3	2–6
4	Affected	11	6	3–8
5	Unaffected	7	0	0
6	Unaffected	15	0	0

All horses were of the same sex (stallions) and breed (Franches-Montagnes). For the scoring, pasterns were inspected for signs of equine pastern dermatitis (EPD) and graded for lesion severity using a standardised scoring system. The cumulative value of each pastern could range between 0 (not affected) and 21 (severely affected).

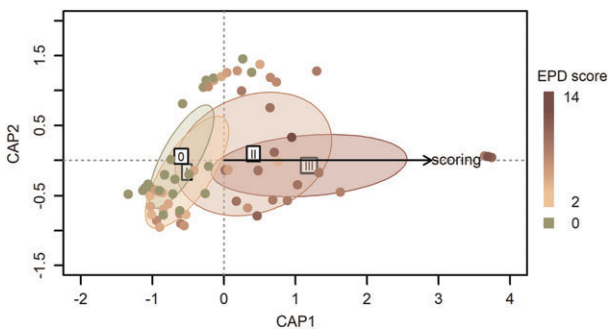


**Figure 1.** Changes over time in meteorological factors, lesion severity and bacterial skin microbiota on affected Franches-Montagnes stallion pasterns.

Monthly (a) mean temperature, (b) mean humidity, (c) mean precipitation and (d) the scoring of equine pastern dermatitis (EPD) lesions. Evolution of (e) bacterial richness, (f) evenness and (g) and the Shannon index in affected and unaffected pasterns, and (h) the pairwise dissimilarity between affected and unaffected pasterns. The shaded areas mark the 90% confidence intervals. Data were acquired once a month, starting from October 2017 to September 2018.

believed,<sup>6,7</sup> thus cannot be demonstrated in our small cohort. Nevertheless, studies have shown that skin biophysical properties and wound healing are influenced by environmental factors.<sup>27–31</sup> Incessant precipitation often is linked to the development of EPD as it may contribute to the maceration of the skin and is thought to leave it more vulnerable to breakdowns of the skin barrier and

ensuing infections. Yet, the habitat of a horse likely also plays an important role in this respect. Pastures with low occupation density and good turf, as in our setting, can absorb considerable amounts of water before they become wet and muddy.<sup>32,33</sup> This was evident from our observation that even during wet periods visibly muddy conditions were limited to the highly frequented areas in



**Figure 2.** The beta diversity of skin microbiota changes with the severity of equine pastern dermatitis (EPD) lesions. Constrained analysis of proximities (CAP) showing bacterial composition (beta diversity) clustered based on the severity of EPD. The constraining influence (pastern score) is depicted by the arrow. Shaded ellipses represent clusters of scoring classes (ellipse colours matching the colour gradient for points), with: class 0 (score 0; the unaffected pasterns), class I (scores 2–3), class II (scores 4–8) and class III (scores 9–14).

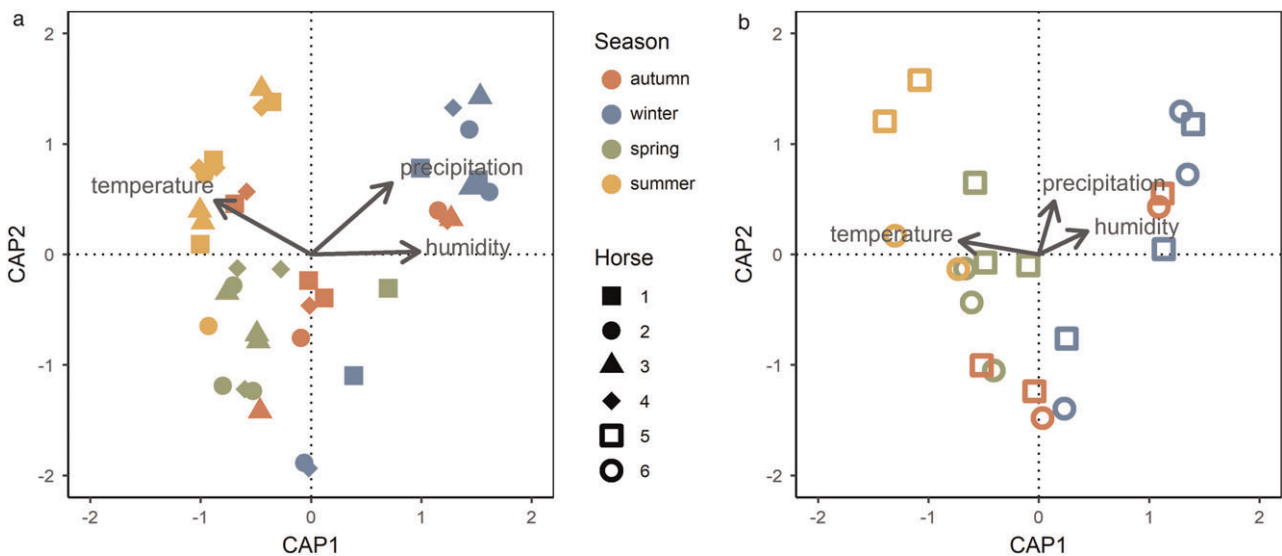
front of the gates and the feed stand. In addition, the stallions in our study did not possess abundant feathering, which can promote the retention of increased humidity on the skin.<sup>1,2,5</sup>

Other environmental factors that could have influenced the inflammation occurring in EPD would be, for example, a bloom of pollens or else the intensity of UV radiation. As a result of heavy rain, pollen grains are subject to osmotic rupture, leading to a release of allergens that are even finer and have the potential to promote allergic reactions or vasculitis.<sup>34</sup> Besides, UV radiation readings showed a typical seasonal pattern: in Switzerland, UV intensity rises continuously from January to July and then slowly decreases again towards December.<sup>35</sup> The effects that UVB radiation in particular can have on local immunological processes are substantial, as described in detail by Dunstan and colleagues.<sup>36</sup> UV light may thus trigger vasculitis or facilitate local infections – especially in nonpigmented pasterns, which is a well-established risk factor for EPD. These potential environmental influences were

**Table 2.** *F*- and *P*-values obtained by the adonis analysis of beta diversity for the seasons as well as for meteorological factors (precipitation, humidity and temperature). For the meteorological factors, significance was assessed using a full model (evaluating the significance of all terms together using permutation tests) as well as tests for individual, marginal effects.

	Terms	<i>F</i> -value	<i>P</i> -value
Affected	Season	3.42	0.001***
	Meteorological factors	3.19	0.001***
	Precipitation	2.24	0.001***
	Humidity	1.51	0.046*
	Temperature	1.96	0.003**
Unaffected	Season	2.28	0.001***
	Meteorological factors	1.83	0.005**
	Precipitation	1.32	0.107
	Humidity	1.00	0.431
	Temperature	1.70	0.019*

Levels of significance: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; \*\*\*,  $P \leq 0.001$ .



**Figure 3.** Skin microbiota is related to meteorological factors, both in (a) affected (horses 1–4, on the left) and in (b) unaffected pasterns (horses 5–6, on the right). Samples also cluster fairly well by season.

The constraining influence of meteorological factors is depicted through arrows, the length corresponding to the respective strength. The orientation of these vectors is quite similar in the two biplots, yet the magnitude appears to be greater in the affected pasterns.

not included in the present analyses and may be worthy of investigation.

The severity of EPD lesions was further associated with changes in the skin microbiota. Alpha diversity measures appeared to be rather moderately reduced in affected pasterns. However, it should be noted that the average severity of EPD in the four affected stallions in this study was only mild-to-moderate, offering an explanation for these relatively small changes. Larger divergences were seen in beta diversity: the higher the EPD score, the more the microbiota deviated from that of unaffected pasterns. These findings are in accordance with the results of a previous study,<sup>22</sup> highlighting the importance of microbiota as a primary and/or secondary factor in this multifactorial disease.

While no apparent correlation was found with alpha diversity, bacterial community structures (beta diversity) were associated with the meteorological factors. The ordination of samples in beta diversity analyses demonstrated that the composition of bacterial communities changed with the seasons, both in affected pasterns and in unaffected ones. Precipitation and humidity operated in a similar direction, whereas temperature showed an opposite effect. Overall, these meteorological factors appeared to have a stronger impact on the microbiota of affected pasterns than on those of unaffected ones. This finding might indicate that the bacterial composition in those pasterns is more susceptible to alterations triggered by weather changes, which, in turn, allows us to hypothesise that the microbiota may be another predisposing or intermediate factor for the development of EPD.<sup>37,38</sup>

Nevertheless, any interpretations must be made with care, because the small sample size clearly is the main limitation of this pilot study. This also was the reason why we abstained from a phylogenetic analysis. For completeness, an overview of the abundances of the most frequent bacterial families in both affected and unaffected horses is provided in Appendix S5. In a recent study, the proportion of staphylococci was increased in EPD, particularly in the more severe forms and in pasterns treated with antibacterial agents.<sup>22</sup> However, as mentioned already, the horses in the present study were only mildly to moderately affected by EPD and limited observations did not permit any statistical analyses. The detection of single, differentially abundant species in this longitudinal design would plausibly require observations in more individuals. However, investigations of diversity measurements are valid, as their calculation itself is already dependent on the sample size.<sup>39</sup> The fact that the horses spent part of the year at the breeding station may have further influenced the results. However, even during that period of stabling, the horses were still exposed to outdoor weather conditions on a daily basis. These limitations are contrasted by the strengths of our study. These pilot data are based on standardised and parallel clinical, meteorological and bacteriological observations in an homogenous group of horses (same breed and sex, and all adults) that had lived in the same environment for one year, and had not received antimicrobial treatment during the entire study period.

In summary, our study highlights the triangular relationship between lesion severity, meteorological factors and cutaneous bacteria. To the best of our knowledge, this study is the first to provide scientific insight into the widely assumed, and merely anecdotal interrelationships between EPD and bacterial composition on the pastern skin, and seasonal as well as meteorological conditions. A better understanding, specifically regarding phylogenetic analyses to identify the main bacterial groups involved in the observed changes, is crucial for the prophylaxis and treatment of EPD and further studies in larger cohorts are therefore needed.

## Acknowledgements

A special thankyou goes to Agroscope and the Swiss National Stud who own the horses that participated in this study, to Susanne Aebi for her help with the laboratory work, and to Shannon Axiak for proof-reading the manuscript. Open Access Funding provided by Agroscope.

## Conflict of Interest

The authors declare no conflicts of interest.

## Author contribution

**Sarah Kaiser-Thom:** Conceptualization; Data curation; Formal analysis; Investigation; Visualization; Writing-original draft; Writing-review & editing. **Markus Hilty:** Formal analysis; Investigation; Writing-original draft. **Alessandra Ramseyer:** Conceptualization; Resources; Writing-original draft. **Pascale Epper:** Investigation. **Vinzenz Gerber:** Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing-original draft.

## References

1. Yu AA. Equine pastern dermatitis. *Vet Clin North Am Equine Pract* 2013; 29: 577–588.
2. Scott DW, Miller WH. *Equine Dermatology*, 2nd edition. Maryland Heights, MO: Saunders Elsevier, 2011; 460–461.
3. Wallraf A, Hamann H, Distl O, et al. [Analysis of the prevalence of pastern dermatitis in German Coldblood horse breeds]. *Berl Munch Tierarztl Wochenschr* 2004; 117: 148–152.
4. Federici M, Gerber V, Doherr MG, et al. [Association of skin problems with coat colour and white markings in three-year-old horses of the Franches-Montagnes breed]. *Schweiz Arch Tierheilkd* 2015; 157: 391–398.
5. Marsella R. Clinical approach to pastern dermatitis. *Manual of Equine Dermatology*. Wallingford, UK: CAB International, 2019; 110–118.
6. Watson R. Wet skin conditions: the scourge of the UK winter. *Equine Heal* 2017; 2017: 34–36.
7. Pilsworth RC, Knottenbelt DC. Pastern and heel dermatitis. *Equine Vet Educ* 2006; 18: 93–95.
8. Colles CM, Colles KM, Galpin JR. Equine pastern dermatitis. *Equine Vet Educ* 2010; 22: 566–570.
9. Aufox EE, Frank LA, May ER, et al. The prevalence of *Dermatophilus congolensis* in horses with pastern dermatitis using PCR to diagnose infection in a population of horses in southern USA. *Vet Dermatol* 2018; 29: 435–e144.
10. Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. *Nature* 2007; 449: 804–810.

11. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. *Nat Rev Microbiol* 2018; 16: 143–155.
12. Grice EA, Kong HH, Renaud G, et al. A diversity profile of the human skin microbiota. *Genome Res* 2008; 18: 1,043–1,050.
13. Cundell AM. Microbial ecology of the human skin. *Microb Ecol* 2018; 76: 113–120.
14. Ross AA, Rodrigues Hoffmann A, Neufeld JD. The skin microbiome of vertebrates. *Microbiome* 2019; 7: 79.
15. Council SE, Savage AM, Urban JM, et al. Diversity and evolution of the primate skin microbiome. *Proc Biol Soc* 2016; 283: 20152586.
16. Rodrigues Hoffmann A, Patterson AP, Diesel A, et al. The skin microbiome in healthy and allergic dogs. *PLoS One* 2014; 9: e83197.
17. Rodrigues Hoffmann A. The cutaneous ecosystem: the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. *Vet Dermatol* 2017; 28: 60–e15.
18. Weese JS. The canine and feline skin microbiome in health and disease. *Vet Dermatol* 2013; 24: 137–145.e31.
19. Kamus LJ, Theoret C, Costa MC. Use of next generation sequencing to investigate the microbiota of experimentally induced wounds and the effect of bandaging in horses. *PLoS One* 2018; 13: e0206989.
20. Fritsch J, Abreu MT. The microbiota and the immune response: What is the chicken and what is the egg? *Gastrointest Endosc Clin N Am* 2019; 29: 381–393.
21. Kuntz TM, Gilbert JA. Introducing the microbiome into precision medicine. *Trends Pharmacol Sci* 2017; 38: 81–91.
22. Kaiser-Thom S, Hilty M, Axiak S, et al. The skin microbiota in equine pastern dermatitis: a case-control study of horses in Switzerland. *Vet Dermatol* 2021; 32 (6): 646–e172. <https://doi.org/10.1111/vde.12955>.
23. Kaiser-Thom S, Hilty M, Gerber V. Effects of hypersensitivity disorders and environmental factors on the equine intestinal microbiota. *Vet Q* 2020; 40: 97–107.
24. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing, 2018.
25. Callahan BJ, Sankaran K, Fukuyama JA, et al. Bioconductor workflow for microbiome data analysis: From raw reads to community analyses. *F1000Res* 2016; 5: 1,492.
26. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res* 2013; 41: 590–596.
27. Wendling P-A, Dell'Acqua G. Skin biophysical properties of a population living in Valais, Switzerland. *Ski Res Technol* 2003; 9: 331–338.
28. Mac-Mary S, Sainthillier JM, Humbert P. Dry skin and the environment. *Exog Dermatol* 2004; 3: 72–80.
29. Junker JPE, Kamel RA, Caterson EJ, et al. Clinical impact upon wound healing and inflammation in moist, wet, and dry environments. *Adv Wound Care* 2013; 2: 348–356.
30. Field CK, Kerstein MD. Overview of wound healing in a moist environment. *Am J Surg* 1994; 167: 2S–6S.
31. Svensjö T, Pomahac B, Yao F, et al. Accelerated healing of full-thickness skin wounds in a wet environment. *Plast Reconstr Surg* 2000; 106: 613–614.
32. Scholefield D, Hall DM. A recording penetrometer to measure the strength of soil in relation to the stresses exerted by a walking cow. *J Soil Sci* 1986; 37: 165–176.
33. Hofmann L, Ries RE. Relationship of soil and plant characteristics to erosion and runoff on pasture and range. *J Soil Water Conserv* 1991; 46: 143–147.
34. Rathnayake CM, Metwali N, Jayarathne T, et al. Influence of rain on the abundance of bioaerosols in fine and coarse particles. *Atmos Chem Phys* 2017; 17: 2,459–2,475.
35. Swiss climate in detail: Radiation monitoring [website]. Available at: <https://www.meteoswiss.admin.ch/home/climate/swiss-climate-in-detail/radiation-monitoring.html>. Accessed Jun 6, 2021.
36. Dunstan RW, Credille KM, Walder EJ. The light and the skin. In: Kwochka KW, Willemse T, von Tscharner C eds. *Advances in Veterinary Dermatology*, 3. Oxford: Butterworth Heinemann, 1998; 3–35.
37. Kim H-J, Kim H, Kim JJ, et al. Fragile skin microbiomes in megacities are assembled by a predominantly niche-based process. *Sci Adv* 2018; 4: e1701581.
38. Sommer F, Anderson JM, Bharti R, et al. The resilience of the intestinal microbiota influences health and disease. *Nat Rev Microbiol* 2017; 15: 630–638.
39. Wolda H. Similarity Indices, sample size and diversity. *Oecologia* 1981; 50: 296–302.

## Supporting Information

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

**Appendix S1.** EPD scoring system.

**Appendix S2.** Monthly scores of all pasterns in each horse.

**Appendix S3.** Rarefaction curves.

**Appendix S4.** Beta diversity analyses based on unweighted Bray-Curtis indices.

**Appendix S5.** Abundances of bacterial families.

## Résumé

**Contexte** – La dermatite du paturon équin (EPD) est un syndrome multifactoriel, l'exposition prolongée à l'humidité étant supposée être un facteur prédisposant ou principal.

**Hypothèse/Objectifs** – Examiner l'évolution de la gravité des lésions d'EPD, les modifications du microbiote cutané bactérien et l'influence des facteurs météorologiques.

**Animaux** – Etude de cohorte prospective et longitudinale sur une période d'un an, avec six étalons Franches-Montagnes, quatre atteints d'EPD et deux non atteints, élevés dans les mêmes conditions.

**Méthodes et matériel** – Les paturons ont été notés pour la gravité des lésions et échantillonnés une fois par mois pendant 12 mois consécutifs. La sévérité des lésions, le microbiote cutané et les facteurs météorologiques ont été examinés par associations.

**Résultats** – Les lésions d'EPD avaient tendance à s'aggraver en automne et au début du printemps. La relation entre la gravité des lésions et le facteur météorologique des précipitations n'était pas clairement évidente; les scores élevés étaient précédés par des taux de précipitations faibles ou élevés. Le microbiote des paturons affectés semble avoir subi une réduction de la diversité alpha. Les analyses de diversité bêta ont démontré que les structures de la communauté bactérienne étaient altérées pour les paturons affectés par rapport aux non affectés, et que les altérations étaient plus prononcées avec des scores EPD plus élevés ( $P = 0,005$ ). Les facteurs météorologiques ont également eu des influences considérables sur la

composition bactérienne, ces influences semblant plus marquées chez les paturons atteints ( $P = 0,001$ ,  $F = 3,19$ ) que chez les non atteints ( $P = 0,005$ ,  $F = 1,83$ ).

**Conclusions et pertinence clinique** – Notre étude fournit des observations préliminaires sur les relations entre la sévérité des lésions, les facteurs météorologiques et les bactéries cutanées. La population était trop petite pour tirer des conclusions définitives, et d'autres études sur les facteurs environnementaux et l'implication des bactéries dans cette maladie multifactorielle sont nécessaires.

## Resumen

**Introducción** – la dermatitis de la cuartilla equina (EPD) es un síndrome multifactorial, y se supone que la exposición prolongada a la humedad es un factor predisponente o primario.

**Hipótesis/Objetivos** – Examinar la progresión de la severidad de lesiones de EPD, los cambios en la microbiota bacteriana de la piel y la influencia de factores meteorológicos.

**Animales** – Estudio de cohorte longitudinal prospectivo durante un año, con seis sementales Franches-Montagnes, cuatro afectados por EPD y dos no afectados, que se mantuvieron en las mismas condiciones.

**Métodos y materiales** – los metacarpos se calificaron según la gravedad de la lesión y se tomaron muestras una vez al mes durante 12 meses consecutivos. Se examinaron la gravedad de las lesiones, la microbiota cutánea y los factores meteorológicos en busca de asociaciones.

**Resultados** – las lesiones de EPD tendieron a empeorar en otoño y principios de primavera. La relación entre la gravedad de la lesión y el factor meteorológico precipitación no fue claramente evidente; los valores altos fueron precedidos por tasas de precipitación bajas o altas. La microbiota en los metacarpos afectados pareció haber experimentado una reducción en la diversidad alfa. Los análisis de diversidad beta demostraron que las estructuras de la comunidad bacteriana se alteraron en los metacarpos afectados frente a los no afectados, y que las alteraciones fueron más pronunciadas con valores de EPD más altos ( $P = 0,005$ ). Los factores meteorológicos también influyeron considerablemente en la composición bacteriana, por lo que estas influencias parecieron ser más marcadas en los metacarpos afectados ( $P = 0,001$ ,  $F = 3,19$ ) que en los no afectados ( $P = 0,005$ ,  $F = 1,83$ ).

**Conclusiones y relevancia clínica** – nuestro estudio proporciona observaciones preliminares de las relaciones entre la gravedad de las lesiones, los factores meteorológicos y las bacterias cutáneas. La población era demasiado pequeña para sacar conclusiones firmes y se necesitan más estudios sobre los factores ambientales y la participación de las bacterias en esta enfermedad multifactorial.

## Zusammenfassung

**Hintergrund** – Die Hautentzündung der distalen Gliedmaßen beim Pferd (EPD) ist ein multifaktorielles Syndrom, wobei angenommen wird, dass Feuchtigkeit, der die Pferde langfristig ausgesetzt sind, einen prädisponierenden oder primären Faktor darstellt.

**Hypothese/Ziele** – Eine Untersuchung des Schweregrades der EPD Läsionen, der Veränderungen der bakteriellen Mikrobiome und des Einflusses meteorologischer Faktoren.

**Tiere** – Es handelt sich um eine prospektive, longitudinale Kohortenstudie über den Zeitraum von einem Jahr. Es wurden dabei sechs Franches-Montagnes Hengste untersucht, die unter den gleichen Bedingungen gehalten wurden, wobei vier von EPD betroffen und zwei nicht betroffen waren.

**Methoden und Materialien** – Die Fesselbeugen wurden auf Schwere der Veränderungen untersucht und einmal monatlich für 12 aufeinanderfolgende Monate Proben entnommen. Der Schweregrad der Veränderungen, die Mikrobiota der Haut und die meteorologischen Faktoren wurden auf Zusammenhänge untersucht.

**Ergebnisse** – EPD Veränderungen tendierten zu einer Verschlechterung im Herbst und am Beginn des Frühlings. Der Zusammenhang zwischen Schweregrad der Veränderungen und dem meteorologischen Faktor Niederschlag war nicht klar vorhanden; hohen Werten gingen sowohl niedrige wie auch hohe Niederschlagswerte voraus. Die Mikrobiome der betroffenen Fesselbeugen schienen eine Reduktion in Richtung alpha Diversität durchgemacht zu haben. Beta Diversitätsanalyse zeigte, dass die bakteriellen Strukturen sich zwischen betroffenen und nicht betroffenen Fesselbeugen veränderten und dass die Veränderungen mit höheren EPD Werten deutlicher waren ( $P = 0,005$ ). Meteorologische Faktoren zeigten auch einen bedeutenden Einfluss auf die bakterielle Zusammensetzung, wobei diese Einflüsse bei den betroffenen Fesselbeugen deutlicher waren ( $P = 0,001$ ,  $F = 3,19$ ) als bei den nicht betroffenen ( $P = 0,005$ ,  $F = 1,83$ ).

**Schlussfolgerungen und klinische Bedeutung** – Unsere Studie beschreibt vorläufige Beobachtungen der Beziehungen zwischen Schweregrad der Veränderungen, meteorologischen Faktoren und Bakterien der Haut. Die Population war zu klein, um deutliche Schlüsse zu ziehen und daher sind weitere Studien über Umweltfaktoren und die Beteiligung von Bakterien an dieser multifaktoriellen Erkrankung notwendig.



**要約**

**背景** – 馬の繫輝(EPD) は多因子症候群で、水分への長期暴露が素因または主要な要因であると考えられている。

**仮説/目的** – 本研究の目的は、EPD病変の重症度の経過、細菌性皮膚微生物叢の変化、および気象要因の影響を調べることであった。

**供試動物** – 同じ条件下で飼育された6頭のフランシュモンターニュスタリオン(EPDに罹患した4頭とEPDに罹患していない2頭)を対象に、1年間にわたる前向き縦断コホート研究を行った。

**材料と方法** – 下肢部は病変の重症度についてスコアが付けられ、12か月連続で月に1回サンプリングされた。病変の重症度、皮膚の微生物叢、および気象学的要因の関連性を調べた。

**結果** – EPD病変は、秋と春の初めに悪化する傾向があった。病変の重症度と気象因子の降水量との関係は明確ではなかった。高いスコアの前には、降水量が少ないか多いかの両方があった。罹患下肢部の微生物叢は、 $\alpha$ 多様性の減少を認めたように見えた。 $\beta$ 多様性解析は、罹患下肢部と罹患していない下肢部で細菌群集構造が変化し、EPDスコアが高いほど変化がより顕著であることを示した ( $P = 0.005$ )。気象要因も細菌組成にかなりの影響を及ぼしたため、これらの影響は、罹患していない下肢部( $P = 0.005$ ,  $F = 1.83$ )よりも罹患下肢部( $P = 0.001$ ,  $F = 3.19$ )でより顕著であるように見えた。

**結論と臨床的関連性** – 私たちの研究は、病変の重症度、気象要因、および皮膚細菌の間の関係の予備的観察を提供している。集団が小さすぎて確固たる結論を出すことはできず、環境要因とこの多因子性疾患への細菌の関与に関するさらなる研究が必要である。

**摘要**

**背景** – 马骹皮炎 (EPD) 是一种多因素综合征, 长期潮湿暴露被认为是易感因素或主要因素。

**假设/目标** – 检查EPD病変严重程度、细菌皮肤微生物群的变化以及气象因素影响的过程。

**动物** – 为期一年的前瞻性纵向队列研究, 在相同条件下饲养六匹Franches Montagnes种马, 四匹EPD, 两匹正常。

**方法和材料** – 对骹进行病変严重程度评分, 每月取样一次, 连续12个月。检查病変严重程度、皮肤微生物群和气象因素之间的相关性。

**结果** – EPD病変在秋季和春季初有恶化趋势。病変严重程度与气象因子降水量关系不明显; 给出高分之前, 降水量或低或高。发病骹的微生物群似乎经历了 $\alpha$ 多样性的减少。 $\beta$ 多样性分析表明, 与骹正常的动物相比, 散发病动物的细菌群落结构发生了改变, 并且随着EPD得分的升高, 这种改变更加明显 ( $P=0.005$ )。气象因素对细菌组成也有相当大的影响, 因此这些影响对发病骹( $P=0.001, F=3.19$ ) 比正常骹( $P=0.005, F=1.83$ ) 更为显著。

**结论和临床相关性** – 我们的研究提供了病変严重程度、气象因素和皮肤细菌之间关系的初步观察结果。数量太少, 无法得出确切结论, 需要进一步研究环境因素和细菌与该多因素疾病的关系。

**Resumo**

**Contexto** – A dermatite de quartela equina (DQE) é uma síndrome multifatorial, sendo a exposição à umidade considerada um fator predisponente ou uma causa primária.

**Hipótese/Objetivos** – Avaliar a evolução da gravidade das lesões de DQE, alterações na microbiota cutânea, e a influência de fatores meteorológicos.

**Animais** – Estudo de coortes prospectivo longitudinal durante um período de um ano, com seis ganhões da raça Franches-Montagne, quatro afetados por DQE e dois não afetados, que foram mantidos sob as mesmas condições.

**Métodos e materiais** – As quartelas foram classificadas de acordo com a gravidade das lesões e amostradas uma vez ao mês por 12 meses consecutivos. Gravidade das lesões, microbiota cutânea e condições meteorológicas foram examinados para associações.

**Resultados** – As lesões de DQE apresentaram tendência de piora no outono e no começo da primavera. A relação entre a gravidade das lesões e as condições climáticas de precipitação não foi evidente; escores altos foram precedidos tanto por taxas altas quanto baixas de precipitação. A microbiota nas quartelas afetadas aparentemente apresentou redução na alpha-diversidade. As análises de beta-diversidade demonstraram que as estruturas da comunidade bacteriana estavam alteradas nas quartelas afetadas versus não afetadas, e que as alterações eram mais pronunciadas com altos escores de DQE ( $P = 0,005$ ). As condições meteorológicas também apresentaram influência considerável na composição da microbiota, e, nas quartelas afetadas, esta influência aparentou ser mais marcante nas quartelas afetadas ( $P = 0,001$ ,  $F = 3,19$ ) que nas não afetadas ( $P = 0,005$ ,  $F = 1,83$ ).

**Conclusões e relevância clínica** – O nosso estudo fornece observações preliminares quanto às relações entre gravidade das lesões, condições meteorológicas e bactérias cutâneas. A população era muito pequena para tirar conclusões assertivas, e mais estudos sobre os fatores ambientais e o envolvimento de bactérias nessa doença multifatorial são necessários.