Cytology of the Interdigital Skin from Healthy Alpacas
(Vicugna pacos)
アルパカ（Vicugna pacos）の趾間の細胞診

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Abstract: The interdigital skin of alpacas is affected by many inflammatory conditions, such as bacterial infections, ectoparasitisms, contact dermatitis, and zinc-responsive dermatosis. Cytology is a rapid, inexpensive, and practical method of acquiring important diagnostic and therapeutic information on skin diseases. However, to date there is no published information concerning the cytological findings in normal interdigital skin from alpacas. Hence, we performed cytological evaluation of the interdigital skin on a front and hind foot of 30 healthy alpacas (Vicugna pacos). Yeasts, Gram-positive cocci and rods, and Gram-negative rods occur regularly, and there is no difference in counts of these cells between the front and rear feet. The presence of inflammatory cells or large numbers of epithelial cells would be abnormal.

Key words: alpacas, cytology, interdigital skin

Introduction

Alpacas (Vicugna pacos), like other South American camelids, have been growing in popularity in North America. With this growth has come the need for better knowledge of normal and abnormal skin states. A review article and retrospective analysis published in 2010 by Scott et al. in Veterinary Dermatology describes 68 alpacas with skin disease seen at a university veterinary hospital from 1997–2006. In that paper, common skin diseases of the alpaca were reported as: bacterial infections (22% of the cases); neoplasms, cysts and hamartomas (19%); presumed immunologic disorders (12%); and ectoparasitisms (10%). Normal microanatomy of alpaca skin has also been reported.

As with general dermatoses, specific dermatoses of the feet of alpacas are important. Diseases involving the feet included bacterial folliculitis, Sarcoptes infestation, Chorioptes infestation, contact dermatitis, presumed insect-bite hypersensitivity and zinc-responsive
dermatoses\textsuperscript{5}). Ulcerative pododermatitis associated with \textit{Staphylococcus}, \textit{Corynebacterium} and \textit{Fusobacterium} species of bacteria occurs in alpacas\textsuperscript{3}. To date, no studies have been conducted to evaluate the cytology found on the interdigital skin from healthy alpacas. This study addresses this gap in knowledge. Based on the observed behavior of camelids to back into a communal defecation area, we also evaluated differences in cytology between front and rear feet; we also considered that weight-bearing might differ between front and rear feet, and might also influence cytology.

\textbf{Materials and Methods}

\textbf{Sample collection}

Thirty alpacas were included in this study from one farm located in central New York State. The alpacas were part of 2 separate groups (15 animals sampled from each group). Written consent was obtained from the farm’s owner and all animals were handled in accordance with the approval of the Cornell University Institutional Animal Care and Use Committee. The 30 alpacas included 15 males (ages 14 months to 9 years) and 15 females (ages 10 months to 19 months). Sample collection took place in May 2011.

The farm staff manually restrained alpacas. Wearing exam gloves\textsuperscript{a} and using sterile cotton swabs\textsuperscript{b}, samples were collected from interdigital material from one front and one rear foot from each alpaca. Each swab was rolled onto 2 separate glass microscope slides\textsuperscript{c}. The slides were stored for transport and subsequent staining. An attempt was made to insure that all smears were of the same length and thickness.

\textbf{Slide staining}

The slides for cytological analysis were transported back to the Cornell University Hospital for Animals that same day and stained using Diff-Quik\textsuperscript{d} on one slide per swab and Gram stain on the other slide by the same investigator (MDC) to ensure consistency.

\textbf{Cytological evaluation}

All microscope slides were examined by the same investigator (MDC) with independent verification by a second investigator (WHM). Each slide was scanned at 10X magnification to check for quality of staining and homogeneity of sample. Efforts were made to select 10 oil-immersion fields (OIFs; 1000X magnification) that were representative of the entire slide for both the Diff-Quik\textsuperscript{e} and Gram-stained slides. The numbers of non-nucleated and nucleated epithelial cells were counted in each of the 10 OIFs on the Diff-Quik\textsuperscript{f} slides (Fig. 1). We also searched for polymorphonuclear and mononuclear inflammatory cells. The numbers of yeasts, bacterial cocci, and rods were counted on the Gram-stained slides (also for 10 OIFs). Microorganisms were counted and then categorized using the following scale per OIF: 0=0; 1=1–10; 2=11–20; 3=21–30; 4=31–40; 5=>40. This type of numbering system for cytological findings is often used by clinicians when assessing ear and skin diseases\textsuperscript{4}.

\textbf{Statistics}

For each foot, we separately calculated the median number (category) of microorganisms across the 10 OIF for each type of cell. Because we expected to find some non-nucleated epithelial cells on normal alpaca feet, these results were only described (not tested).

Microorganism counts from one front and one rear foot for each animal constituted sets of paired data; we also had data that were ordinal because of the categorization of microorganism counts. Consequently, we used Wilcoxon signed-rank tests to evaluate whether being from the front versus rear foot was related to the numbers of microorganisms. We did this separately for the males and females. Our overall \textit{p}-value for significance was 0.05, but we ran eight signed-rank tests. We intended to impose a Bonferroni adjustment for the multiplicity, and the cut-off \textit{p}-value was therefore

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig_1.png}
\caption{Cytology. Gram stain of interdigital swab. Note yeast (black arrow), cluster of Gram-positive rods (purple arrow), and background (red arrow).}
\end{figure}
Results

The minima, medians, and maxima for the non-nucleated epithelial cells and the microorganisms are in Table 1. Nucleated epithelial cells were so scarce that the medians across 10 OIFs were always 0 and we neither show that in the table nor attempted the futile exercise of trying to correlate the “constant” value of 0 to the counts of the microorganisms (as had been our original intention). Additionally, no inflammatory cells (polymorphonuclear or mononuclear) or Gram-negative cocci were observed, so these also were not tested or displayed in the table. The results for the 2 groups are summarized in box-and-whiskers plots (Figs. 2 and 3).

All two-tailed \( p \)-values in the Wilcoxon signed-rank tests for these data were \( \geq 0.26 \) (Table 2). These results for the 2 groups for the front/rear comparisons are consistent with the box-and-whiskers plots. There is no evidence graphically or statistically of any differences in these cells, between front and rear feet from the alpacas on this farm, sampled in May 2011.

Discussion

Our results show that a small number of non-nucleated epithelial cells are to be found from skin-cytology samples from the interdigital skin from healthy alpacas (minimum seen per OIF=0; maximum 8). However, the medians for these cells across 10 OIF per foot were all 0 and 6 out of the 30 animals examined had no non-nucleated epithelial cells seen in any of 20 OIFs. Non-nucleated epithelial cells wold be expected in normal skin samples, as the stratum corneum of alpacas is anuclear\(^1\). The number of yeasts, Gram-positive cocci, Gram-positive rods, and

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**Table 1.** Cell-counts and categories, across 10 high-powered fields per foot (n=15 healthy alpacas for each statistic)

<table>
<thead>
<tr>
<th>Cell or micro-organism</th>
<th>Statistic</th>
<th>Group and Foot</th>
<th>Male, Front</th>
<th>Male, Rear</th>
<th>Female, Front</th>
<th>Female, Rear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-nucleated epithelial</td>
<td>Minimum</td>
<td>Male, Front</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>Male, Front</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>Male, Front</td>
<td>2.5</td>
<td>3.5</td>
<td>1</td>
<td>1.5</td>
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<tr>
<td>Yeast</td>
<td>Minimum</td>
<td>Male, Front</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>Male, Front</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>Male, Front</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Gram+ cocci</td>
<td>Minimum</td>
<td>Male, Front</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>Male, Front</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>Male, Front</td>
<td>3</td>
<td>3</td>
<td>4.5</td>
<td>5</td>
</tr>
<tr>
<td>Gram+ rods</td>
<td>Minimum</td>
<td>Male, Front</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>Male, Front</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>Male, Front</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4.5</td>
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<tr>
<td>Gram- rods</td>
<td>Minimum</td>
<td>Male, Front</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Median</td>
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<td>1.5</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>Male, Front</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Categories of cell counts and microorganism counts per oil-immersion field: 0=0; 1=1–10; 2=11–20; 3=21–30; 4=31–40; 5=&gt;40.

0.05/8=0.00625 (2-sided). All statistical work was performed using standard software, Statistix\(^9\).

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Fig. 2. Box-and-whiskers plot summarizing categories of cell counts and microorganism counts per OIF for female alpaca group.

Fig. 3. Box-and-whiskers plot summarizing categories of cell counts and microorganism counts per OIF for male alpaca group.
Gram-negative rods per OIF varied from 0 to >40, 0 to >40, 0 to 40, and 0 to >40, respectively.

There are several limitations to this study. To start, the animals tested were located on one farm in central New York State. It is likely cytology samples would vary from animals housed in different geographical areas. Additionally, sampling occurred on only one day in May of 2011. It is possible that seasonal and climate variations (e.g., winter frost versus summer humidity) would lead to differences in microorganisms observed on the feet. The study would have been stronger with more animals sampled. However, this is an initial investigation and a starting point for future studies.

In summary, this paper is the first to describe swab-sample cytology from the interdigital skin from healthy alpacas. Based on our results, inflammatory cells are not part of the normal interdigital cytology. Very rare numbers of non-nucleated epithelial cells and fewer still of nucleated epithelial cells can be seen. Yeasts, Gram-positive cocci and rods, and Gram-negative rods do occur regularly on the interdigital skin from healthy alpacas, and there is no difference in the counts of these cells between the front and rear feet. Clinicians and diagnostic pathologists should find this information useful in the evaluation of cytological specimens procured from the interdigital spaces of alpacas with pododermatitis.

Sources and Manufacturers

- a) Synguard Vinyl Examination Gloves, Basic Medical Industries, Inc., Chino, CA, USA.
- b) Puritan Medical Products Company LLC, Guilford, ME, USA.
- c) Gold Seal Rite-On Micro Slides, Portsmouth, NH, USA.
- d) Baxter Healthcare Co., McGraw Park, IL, USA.

References