The Affects of Genital Myiasis on the Diversity of Vaginal Flora in Female Bactrian Camels

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Research Article

Keywords: Bacteriome, Bactrian camels, Genital myiasis, Vaginal flora diversity, 16S rRNA

DOI: https://doi.org/10.21203/rs.3.rs-455243/v1

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Abstract

Background: One of the most important diseases that affect the reproductive organs of Bactrian camels is called Genital Myiasis. It can cause serious mechanical damage to the vaginal tissue of female Bactrian camels. The accumulation of bacteria in the vagina of female camels can affect their health and reproductive ability. The effect of this damage is commonly found in the vaginal flora and vaginal mucosal immune system. Therefore, this research is a study of the diversity of the vaginal flora and the differences between healthy Bactrian camels and those suffering from Genital Myiasis.

Results: Vaginal microbiota samples were collected from two groups of female Bactrian camels of the same age. Illumina Miseq was used to sequence V3-V4 hypervariable genes of 16S rRNA in the samples, and the results showed that the vaginal microflora of the infected camel had a significantly greater OTU value. According to the Alpha diversity index and the level of vaginal pH, the diversity index of the infected camel flora were higher than that of the normal camel flora, and the pH were lower than that of the normal camel flora (P=0.006). There was no significant difference between the two groups in the abundance of dominant genera of Bactrian camel vaginal (P>0.05), indicating that the structure of dominant flora of Bactrian camel vagina had a certain stability.

Conclusions: Overall this comparison revealed the differences and similarities between vaginal flora Bactrian camels in various health states. In addition, this data provides a reference point for understanding the types of bacteria that cause genital myiasis that damages healthy development of Bactrian camels.

1.1 Introduction

Bactrian camel is one of the unique domestic animals to China. It mainly lives in the hot and arid regions of the Gobi and Desert area in northwestern China. It is known as the “boat of the desert” (Mengli et al., 2006; Ji et al., 2010; Zhichao et al., 2016). For a long time, the development of the bactrian camels breeding industry has been hampered by Genital Myiasis which has brought serious economic losses to local herders.

The Genital Myiasis of Bactrian camels is a serious parasitic disease. Larvae of Wohlfahrtia Magnifica (Schiner, 1862) parasitize around the perineal and vaginal region of Bactrian camels and are responsible for a severe obligatory traumatic myiasis (Robbins et al., 2010). Genital Myiasis has a distinct seasonality which occurs in May-September of each summer and autumn (Kunichkin et al., 1981; Lungu et al., 1985; Hadani et al., 1989; Valentin et al., 1997). Clinical symptoms manifest as severe mechanical damage in a variety of ways to the affected tissue and mucosal sites harmful affects, such as, local inflammation, anxiety and anorexia are some of the symptoms in diseased camels (Valentin et al., 1997; Giangaspero et al., 2011; Sazmand et al., 2017). Through long-term experimental observation we found that the diseased camel’s vaginal wound was exposed to the external environment but was rarely infected and purulent. When the larvae of Wohlfahrtia Magnifica was detached from the host, 94.5% of the diseased camel
wounds spontaneously recovered (Schumann et al., 1976). In addition, other important elements comprise the vaginal microenvironment.

The vaginal mucosa in healthy animal is colonized by an equilibrated and dynamic composition of aerobic, facultative anaerobic and obligate anaerobic microbes (Srinivasan M et al., 2021). The vaginal flora is a natural barrier formed on the surface of the vaginal mucosa, but Some factors can disturb the balance in its composition (Sroka Oleksiak Agnieszka et al., 2021). Disruption of vaginal microbiota equilibrium promotes infectious clinical syndromes with annoying symptoms, such as vaginal discharge, irritation, pruritus, and vulvar burning (P. Tsimaris et al., 2019). The formation of a reciprocal symbiotic relationship between the vaginal flora and the host is an important factor in maintaining the stability of the vaginal microenvironment. It is also an important component of the multi-faceted resistance of female mammals to pathogen invasion. This has a major impact on the health and disease of the host organism. The vaginal microbiota has importance in preserving vaginal health and defending the host against disease (Antonio Barrientos-Durán et al., 2020). Thus the vaginal microbiome can indicate the health or disease state of the female camile and indicate whether or not changes in the treatment of any existing vaginal related diseases (Hyman et al., 2005; White et al., 2011; Ma et al., 2012; Best A et al., 2017; Clemmons et al., 2017).

At present the research on vaginal microbiology is mainly focused on humans. There is minimal data on the vaginal microbiome in livestock species as well as their potential role in animal vaginal mucosal immunology. The research on vaginal microbiology of bactrian camels has not yet been studied. Yet, in this study we completed the first high-out put of sequencing analysis of the vaginal flora of Bactrian camels. By comparing the diversity of vaginal flora, and differences between the group of ill camels and the normal group of Bactrian camels in the same herd, the effects of the environmental and nutritional factors on the vaginal bacterial community were eliminated there for an analysis to determine the immune-related Differences in microbiome composition was done.

1.2 Materials And Methods

1.2.1 Experimental Design and Sampling

All Female bactrian camels involved in this study were part of a Bactrian camels’ herd registered with the College of Veterinary Medicine Inner Mongolia Agricultural University. All experimental procedures were approved by the Animal Protection and Use Committee of Inner Mongolia Agricultural University and strictly followed animal welfare and ethical guidelines.

According to the industry's standards all 23 Bactrian camels, including 10 Suffering from genital myiasis and 13 healthy one were 8 years of age, as well as mature female camels. In addition, the camels studied were semi-wild, roaming camels, indicative of that region. Also, they grazed on open the open range throughout the year, without any supplementary feeding; Also, additional findings were as follows: there was no history of vaginal drug released within one year; no estrus or pregnancy during one month; no
antibiotics or antifungal drugs were used systematically within one month. Furthermore, sterile procedures were applied to the sampling area. Routine sterile operations were used before each sampling and strictly followed. In addition, the procedural steps to strictly ensure an aseptic open the female camel’s vaginal and rolled 5 times, along the vaginal wall to wipe the vaginal secretions. Then they were quickly placed in a sterile 5ml cryotube. Lastly, the sample was labeled and quickly stored in a liquid nitrogen or in a -80°C refrigerator and used to extract the 16S rRNA gene. Shortly afterwards, the pH of each sample was measured using an UltraBasic pH meter (Denver Instruments Arvada CO United States).

1.2.2 Bacterial DNA Isolation

The thawed sample was centrifuged at 10000r for 10 minutes to collect bacterial cells and the supernatant was discarded. The total DNA of the sample was extracted using the vaginal swab genomic DNA kit (Qiagen QIAamp DNA Mini Kit) and the specific steps were referred to the instructions. The DNA was extracted and stored in a refrigerator at -20 °C. The DNA extraction quality was measured by 0.8% agarose gel electrophoresis and the DNA was quantified by an ultraviolet spectrophotometer.

1.2.3 Sequencing of 16S rRNA

In combination with the fluorescence quantification results, each sample was mixed in a corresponding ratio according to the sequencing amount requirement of each sample. The processed samples were sent to Beijing WEISHENGTAI Co. Ltd. for double-ended 2×300 bp sequencing (Paired-end) through the Illumina HiSeq 2000 platform.

1.2.4 Sequence Read Processing and Statistical Analysis

Basic statistical analysis was performed using SPSS Statistics 20.0 statistical analysis software. Two pairs of comparisons of the measured data were taken, in accordance with the normal distribution. They were performed using two independent samples test $P < 0.05$. Therefore, they were was considered statistically significant.

1.3 Results

1.3.1 Vaginal pH

The vaginal pH of all 23 female Bactrian camels was measured. The results showed that the vaginal PH range of the healthy group of bactrian camels ranged from 7.47 to 8.23 with an average of 7.85 ± 0.13. The vaginal PH of the diseased group was in the range of 7.18- 7.61. Also, the average was between 7.41 ± 0.11. Such that the vaginal PH of bactrian camels was significantly different between the normal group and the group that was ill ($p = 0.006$). Also, the vaginal PH of the group that was ill was lower than that of the normal group.
1.3.2 Sequencing Information

After optimization of quality control and chimera removal, a total of 1644139 reads were obtained for all 23 samples. That had with an average of 71484 reads per sample (Table 1). Samples from the group that was ill were taken and received a total of 744455 reads, with an average of 77446±11214 reads per sample. The normal group samples received a total of 899684 reads with an average of 69206 ± 11047 reads per sample. The results showed that the statistically significant differences in the number of optimized sequences, obtained between the two groups were not significant ($P>0.05$).

1.3.3 Alpha- and Beta-Diversity

The sequence obtained above was subjected to merging, and revealed the OTU division by 97% sequence similarity. Also, the OTU having abundance value lower than 0.001% of the total. Also, the sample sequencing amount was removed (Bokulich et al., 2013). A total of 1845 OUTs were detected with an average of 1689. Also, 1267 OUTs were detected in the group that was ill. In addition, the normal environment vagina for each was maintained with 1111 OTUs shared between various vagina environments (Fig. 1).

Alpha-diversity was measured and observed using OTU Chao1, ACE, simpson and Shannon Diversity Index. The conclusive analysis presented in Table 1. No significant differences existed in alpha-diversity between the normal samples of female Bactrian camels those that were ill and vaginal bacterial observed OTU Chao1 ACE simpson index and Shannon's Diversity Index ($p>0.05$). But the illness bactrian camels vagina had a significantly greater number of OTU than did the normal bactrian camels vagina increased richness as measured by Chao1 and ACE and greater diversity as measured by Shannon's Diversity Index and the Simpson Index all of which are presented in Table 1.

Table 1

Sequence and alpha-diversity statistics of the 16S rRNA gene sequences for bacterial populations in the vaginal of Illness and Normal environments.

| Group  | Samples No. | Average of Sequence No. | simpson  | chao1    | ACE      | shannon |
|--------|-------------|-------------------------|----------|----------|----------|---------|
| Illness | 10          | 77446±11214             | 0.94±0.04| 495.04±230.85| 497.33±228.58| 5.55±1.05|
| Normal | 13          | 69206±11047             | 0.94±0.03| 361.65±147.45| 364.26±148.16| 5.07±0.59|
| $P$    | ---         | 0.28                    | 0.98     | 0.11     | 0.11     | 0.17    |

Beta-diversity was also analyzed to examine differences in microbial communities between samples. Using an OTU-centric approach PCoA matrices were employed using weighted and unweighted UniFrac distance matrices to compare the phylogenetic divergence among the OTU between samples from ill
camels and healthy camel vaginal samples (Fig. 2). The results showed that the clustering of subsets of healthy camel vaginal samples was more closely clustered in the weighted and unweighted UniFrac distance matrix. In addition, ANOSIM analysis showed that there was a significant difference between the vaginal samples of ill female camels and normal camels (P=0.033). R statistics showed that the difference between the groups was significantly greater than within groups (R=0.1483) and the grouping effect was evidently well done.

1.3.4 Taxonomic composition analysis

According to the results of OTU classification and classification status identification, the dominant vaginal flora and average relative abundance of Bactrian camels in the normal group and the illness group were respectively identified at the phylum level: Firmicutes (39.33±16.228.34±19.39); Proteobacteria (28.48±15.0635.23±14.18); Fusobacteria (16.12±11.3216.86±13.55); Bacteroidetes (6.04±3.58.95±3.6); Actinobacteria (7.5±±6.17±4.21); The average relative abundance of unallocated taxa is: (1.45±2.81±1.72±2.81). The relative abundance of the dominant vaginal flora of the bactrian camels was not significantly different between the healthy group and the diseased group (P>0.05).

At the level of genera the dominant Bacteria and their average relative abundance identified from the vaginas of bactrian camels in the normal group and the group of ill female camels were: Campylobacter (9.58±7.03±9.9±10.05); Ochrobactrum (5.76±6.037.56±5.99), Fusobacterium (6.42±5.596.6±5.84), GW-34 (5.96±7.535.58±12.15), Porphyromonas (4.08±3.574.96±3.98), Facklamia (6.08±4.582.33±1.15), Sediminibacterium (1.45±1.65±2.48±2.56), Helcococcus (1.25±2.32.38±4.05), Peptoniphilus (1.04±1.052.11±1.85), 1-68 (1.59±2.220.93±1.56), Clostridium (1.22±1.071.23±1.3), Acinetobacter (1.18±1.28±1.28±1.85), Fusibacter (1.29±1.680.44±0.62), Clostridium (0.68±0.631.09±1.3), ph2 (0.9±0.830.53±0.98). It can be seen that the relative abundance of the dominant vaginal flora of the bactrian camels was not significantly different between the normal group and the group that was ill (P>0.05).

The genus of vaginal specimens that cannot be identified or placed in undefined categories in either the normal or group that was ill of Bactrian camels and their average relative abundance are as follows family Leptotrichiaceae 9.58±7.03±9.9±10.05, family Aerococcaceae (7.78±5.182.71±4.2, family Carnobacteriaceae 6.81±7.080.3±0.62), family Xanthomonadaceae (1.13±1.092.97±2.96), family Pseudomonadaceae (1.9±2.520.9±0.75), family [Tissierellaceae] (1.39±1.491.12±1.75), family Ruminococcaceae (0.35±0.391.69±3.14, family Enterobacteriaceae (0.47±0.781.39±2.04, family Comamonadaceae (0.35±0.391.02±0.78).

Using the visualization tool GraPhlAn (Asnicar et al., 2015) to build a hierarchical tree of the composition of the sample population at each classification level (Fig. 3). More information is evident. While each
classification unit was distinguished by different colors and their distribution in abundance was also reflected by the node size.

Using the Mothur software, called the statistical algorithm of Metastats (http://metastats.cbcb.umd.edu/) (White et al., 2009). We were able to determine the overall classification level of all classification units in the sample population. The difference of sequence quantity (i.e. absolute abundance) between each taxon at phylum and genus level was analyzed and compared (pin-wise). We found that there were 4 classification units with significant differences in gate levels (Fig. 4) namely: SR1 ($p=0.030$ $q=0.120$); Planctomycetes ($p=0.030$ $q=0.120$); Gemmatimonadetes ($p= 0.041$ $q = 0.120$); Elusimicrobia ($p = 0.048$ $q = 0.120$). There are 51 taxonomies with significant differences in levels (Fig. 5) mainly Anaerostipes ($p=0.001$ $q=0.005$); Caldilinea ($p=0.001$ $q=0.005$); Edwardsiella ($p=0.001$ $q = 0.005$); Lactobacillus ($p=0.027$ $q=0.064$) et al.

1.4 Discussion

Thus, the implications and analysis of this research revealed more information about the vaginal microecosystem of Bactrian camels. Relevant studies have proved that the combination of the microflora related to the human body can affect human immunity and provide the first line of defense against opportunistic pathogen colonization (Kau et al., 2011; Smith et al., 2017). The importance of microbial metabolism to the host immune system can be revealed by characterizing the composition and function of individual microbial species and complex microbial communities (Rooks, 2016). This study is an analysis of basic research that was conducted. By comparing the differences in the structure and diversity of normal Bactrian camels and with camels that were ill, we were able to analyze the role of the vaginal microecosystem of Bactrian camels, in their immunity and recovery stages, after their infections of vaginal myiasis. More understanding of these stages may provide a new approach for the prevention and treatment of genital myiasis of Bactrian camels, that result in positive results for clinical treatment of genital myiasis.

In this study the bacterial phyla with the highest abundance identified in the two groups of Bactrian camels’ vaginal samples were Firmicutes Proteobacteria Fusobacterium and Bacteroides. These phyla are representative of the most common phyla found in many environments especially in host-microbiome relationships. Studies have shown that the proportions and relative abundance of these gates are related to changes in host physiology. Therefore when we performed ANOSIM analysis on the samples we found that even if there were differences among different individuals in the same group the difference was obviously smaller than the difference between the groups. We think this difference is reasonable.

As a natural channel, the vaginal flora is susceptible to environmental microbes. The increase of the diversity and richness of the bacterial community, in the vagina of the diseased camel can be explained by the fact that its vulva is affected by fly maggots, which causes swelling and deformation and cannot be completely closed, while a large number of external bacteria enter the vagina. However, the taxonomic
composition analysis of bactrian camels showed that there was no significant difference in the overall structure of its vaginal flora, indicating that the vaginal microecology of Bactrian camels had certain stability. In addition immunomodulatory symbionts induce specific self-targeted responses that indirectly regulate immune responses to surrounding microorganisms (Ost and Round, 2018). Thus, the key role of microbial flora in maintaining homeostasis in the vaginal environment has been demonstrated (Mendez-Figueroa and Anderson, 2011) and the vaginal-associated microbiota may significantly affect the vaginal mucosal regulation of Bactrian camels. For example, the flora on the vaginal mucosa reduces the colonization of pathogenic bacteria by competing with pathogenic bacteria for living space and nutrients and produces short-chain fatty acids of bacteriocins of reactive oxygen species, to inhibit or kill pathogenic bacteria (Sommer and Bäckhed, 2013). When the larvae of Wohlfahrtia Magnifica invade the vagina of Bactrian camels, the external environment of microorganisms enter the vagina of the diseased camel and the vaginal pathogenic bacteria stimulate the mucosal immune system to respond to it. For example, inflammation, so as to strengthen the cleaning of pathogenic bacteria and reduce the possibility of pyogenic disease. Therefore, the vaginal mucosal immune system is able to identify beneficial microorganisms and harmful microorganisms and the pathogens cause the body's clearance immune response to be eliminated, while the commensal bacteria remains safe (Chu et al., 2013).

The chemical nature of vaginal mucosal niche drives the composition of it symbiotic microorganisms with unknown microbial roles and host factors that lead to differences in its microecological composition and strain levels (Chen et al., 2018). The acid-producing genus of Bactrian vaginal flora in the illness group was significantly increased such as Lactobacillus Edwardsiella Oribacterium Parvimonas Propionicimonas Sporomusa etc. (P<0.05). This result is consistent with the results of our PH tests. Therefore maintaining a low vaginal pH prevents the colonization of pathogenic microorganisms and thus has a positive impact on the body's resistance to pathogen invasion (Boskey et al., 1999; Boris and Barbés, 2000; Brabin et al., 2005; Verstraelen et al., 2013; Huang et al., 2014; O'Hanlonet et al., 2013). Studies have shown that the production of lactic acid and other antimicrobial metabolites by vaginal microflora has the characteristics of preventing endogenous opportunistic bacteria and immunomodulation (Lamont et al., 2011; Aldunate et al., 2015). Lactobacillus is an important probiotics in the reproductive tract of female animals, which can convert lactose and other sugars into lactic acid, which can prevent infection and reduce the risk of inflammation (Antonio et al., 1999; Fichorova et al., 2011; Fashemi et al., 2013) Lactobacillus also plays a role in accelerating the healing of tissue wounds (Davis and Gallagher, 2018).

In addition, this study also analyzed the microbiome of the Bactrian camels vagina, to determine the relationship between the presence (or absence) of certain microbiota and the vaginal mucosal immune system. Overall this study will be used to document changes in the diversity of vaginal microbiota in healthy camels and also for that that are suffering from vaginal myiasis to identify unique microbes that may be involved in the vaginal mucosal the immunity. And it may help determine changes in the microbiome associated with the immune regulation, that may be beneficial and positive, throughout the pathological cycle.
Declarations

Ethics approval and consent to participate

The sampling process did not cause any damage to the vaginal mucosa of Bactrian camel. In this experiment, the breeding environment was in compliance with the standards relevant to an ordinary animal laboratory facility in China National Standard “Laboratory animal environment and facilities” (GB14925-2010). The feeding of and the experimental operations on animals were in accordance with the animal welfare requirements. All experimental procedures were approved by the Animal Protection and Use Committee of Inner Mongolia Agricultural University and strictly followed animal welfare and ethical guidelines.

Consent for publication

Not applicable

Availability of data and materials

We have submitted raw data through supplementary materials.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by The National Natural Science Foundation of China (Grant No. NSFC 31360591).

Authors' contributions

EEDMT developed a research program and funded it; ZLK carried out the experiment, analysis and article writing; BH and HBX participated in the data analysis; ADD helped write the article, and the other authors participated in the sample collection. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to acknowledge and thank the technical support of Shunyao Jiang. We thank all partners and laboratory members for their kind help.

ARRIVE guidelines declaration

During the whole experiment, we contact with Bactrian camels during the taking samples, only. The sampling process does not cause any damage to the animals. We confirm that the study was conducted in accordance with the Arrival Guidelines.
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**Figures**
Figure 1

A total of OTU's Venn diagram

Figure 2
Principal coordinate analysis of vaginal samples from ill female camels and normal female camels, using UniFrac unweighted (A) and weighted (B) metrics. Vaginas sample from ill female camels (n=10) are represented by red squares and Vagina samples from normal camels(n=13) are represented by blue circles.

Figure 3

Sample overall classification level tree diagram based on GraPhlAn Note: The classification level tree shows the hierarchical relationship of all classification units (represented by nodes) from the gate to the genus (from the inner circle to the outer circle) in the sample population. The node size corresponds to the average relative abundance of the classification unit. The top 20 units of relative abundance will also be identified by letters in the figure (from door to genus in order from outer layer to inner layer) and the shadow color on the letter is the same as the corresponding node color.
Figure 4

Abundance distribution of phylum-level taxa, with significant differences between sample groups.
Figure 5

Abundance distribution of the top 20 taxa with significant differences in genus levels. Note: The abscissa in the figure is the taxonomic unit, that shows a significant difference and the ordinate is the sequence quantity of each taxon in each sample group. The border of the figure represents the Interquartile range (IQR), the horizontal line represents the median value, and the upper and lower tentacles represent the 1.5 times IQR range, except the upper and lower quartiles. Also, the symbol "•" indicates the extreme value exceeding the range.