First Record of Traumatic Myiasis Obtained From Forest Musk Deer (*Moschus Berezovskii*) Using Dna Barcoding

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Abstract

Background

Myiasis is an infestation of maggots on living tissue in humans and animals all over the world. It is known to occur in wild animals, while no information is reported in forest musk deer. We found a case of traumatic myiasis of an injured forest musk deer (*Moschus berezovskii*), the wound of which was infected by numerous maggots, and the fur was covered by clusters of eggs. The affected individual was clinically treated immediately and recovered.

Methods

DNA barcoding is an efficient technique for species diagnosis, therefore is employed to identify the blowfly samples collected from the infected forest musk deer. Firstly, we extracted genomic DNA from larvae and eggs respectively. The DNA barcoding sequences of 49 individuals were obtained and subsequently analyzed calculating nucleotide composition and divergence and haplotypes, constructing a neighbor-joining (NJ) tree, for accurate identification of these blowflies.

Results

Our results suggest that the average nucleotide divergence between the 49 sequences of blowfly samples is 0.0022, 0.0054 is between sequences of blowfly samples and *Lucilia caesar* (Linnaeus, 1758) (Diptera: Calliphoridae). Furthermore, the NJ tree construction indicates that the flies collected from the forest musk deer are clustered together with *L. caesar*. The sequences of sampled blowflies have nine haplotypes, including two shared haplotypes, with haplotype diversity 0.588, nucleotide diversity 0.00215, and the average number of nucleotide differences was 1.374.

Conclusions

We report traumatic myiasis of forest musk deer for the first time, which expands the information on parasite and myiasis of forest musk deer and confirmed the potential risk of traumatic myiasis of forest musk deer.

Background

Myiasis is the infestation of fly larvae (maggots) in live human or vertebrate animal tissue [1]. It is generally found in human being and domestic animals [2, 3]. Myiasis-causing flies mainly include Calliphoridae, Muscidae, Oestridae, and Sarcophagidae [1, 2, 4], which have caused a major economic problem in animal farming [1, 5]. However, myiasis of wild animals is an understudied issue, owing to predation or their shelter-seeking behavior [6, 7].

The diagnosis of myiasis is made by the finding of fly larvae in tissue and identifying fly larvae. Some alternative identification methods are morphological approaches, molecular approaches, and monoclonal

antibody-based enzyme-linked immunosorbent assay (MAb-ELISA) [8–10].

The forest musk deer (*Moschus berezovskii* Flerov, 1929) we researched lives in Asia, mainly in China. Although it is currently the most abundant species of musk deer in China, the wild population of forest musk deer is tiny. It has been listed in CITES appendix II and considered an endangered animal in The IUCN Red List. At present, captivity is the main strategy for the conservation of forest musk deers [11–13]. On July 6, 2019, we found a case of traumatic myiasis of forest musk deer in Fengchun Musk Deer Breeding Center. To our knowledge, this is the first report of traumatic myiasis of forest musk deer in China.

Hebert *et al.* [14] suggested that DNA barcoding can widely be used in classification and identification. Therefore, we collected larvae and eggs from the wound and fur of the injured forest musk deer for DNA identification. Our aims include twofold: (i) to identify the species of larvae and eggs collected from forest musk deer, report the traumatic myiasis of forest musk deer, and (ii) to provide more effective information on myiasis of forest musk deer.

Methods

Blowfly samples were collected from an open wound on the hindquarter of an adult male forest musk deer in Fengchun Musk Deer Breeding Center (106°54'20.93"E, 34°12'58.07"N, 1496 m), Fengxian County, Shaanxi Province. The wound caused by fighting with male forest musk deer was surrounded by some calliphorid larvae and eggs (Fig. 1). The larvae and eggs were randomly collected with tweezers and stored in 75% alcohol [15] in –20 °C for later use. We treated the musk deer with 75% alcohol disinfection and debridement treatment, and the individual is recovered now. All larvae and eggs were used to extract DNA respectively with the HotSHOT method [16]. DNA was stored at –20°C after DNA extraction. For PCR amplification, 1 µL of DNA, 1 µL of each cytochrome oxidase I (COI) bidirectional primers (10 µmol/L), 12.5 µL of 2×Es Taq MasterMix (Dye) (Beijing Cowin Bioscience Co., Ltd., China), and 9.5 µL of Sterile double distilled water were used. PCR primers LCO1490: 5' –GGT CAA CAA ATC ATA AAG ATA TTG G-3' and HCO2198: 5' –TAA ACT TCA GGG TGA CCA AAA AAT CA-3' were the same as in Folmer *et al.* [17]. The PCR procedure was as follows: pre-denatured at 95°C for 10min, denatured at 95°C for 1min, annealed at 40°C for the 30s, extended at 72°C for 1min 20s, a total of 30 cycles, extended again at 72°C for 10min, stored at 4°C. After amplification, 3 µL PCR products were used for 1% Agarose gel (dyed with Goldview) electrophoresis. The positive product was purified and sent to BGI (Beijing, China) for bidirectionally sequencing following Zhang *et al.* [18].

All sequences were edited and trimmed with BioEdit (version 7.0.9.0) [19], then assembled by SeqMan 7.1.0 (DNASTar, Steve ShearDown, 1998–2001 version, DNASTAR Inc., USA). The obtained sequences were aligned with sequences available in the GenBank database using the BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). MEGA 7 [20] was subsequently used to align sequences together with other data downloaded from GenBank, to calculate Conserved Sites, Variable Sites, Parsim-info Sites, nucleotide composition and nucleotide divergence with Kimura's two parameter (K2P) model [21],

and to construct neighbor-joining (NJ) tree [22]. All the COI sequences of *Lucilia* and *Calliphorida* were downloaded from NCBI. We used ITOL [23] to visualize the NJ tree. The number of haplotypes, number of polymorphic isolation sites, haplotype diversity (H_d), nucleotide diversity (P_i) and average number of nucleotide differences of the 49 sequences obtained in this study were calculated by DnaSP6 [24].

Results

A total of 49 sequences were obtained for five larvae and 44 eggs. Every sequence includes 609 Conserved Sites, accounting for 97.13% of the total length of the sequence, 18 Variable Sites, accounting for 2.87% of the total length of the sequence, and five Parsim-info Sites, accounting for 0.80% of the total length of the sequence. Calculation of nucleotide composition of sequences shows that the average contents of T, A, C, and G are 38.8% (38.1–39.1%), 30.2% (29.8–30.7%), 15.4% (15.1–15.8%), and 15.6% (15.3–16.0%), respectively. The average A + T content is 69%, and the average C + G content is 31%.

The interspecific nucleotide divergence was compared (Additional file 1: Table S1). The average intraspecies nucleotide divergence between eggs and larvae was 0.0022 (0.0000–0.0164). The nucleotide divergence between the eggs/larvae and *Lucilia illustris* (Meigen, 1826) (Diptera: Calliphoridae) was 0.0143, and the nucleotide divergence between the eggs/larvae and *Lucilia caesar* (Linnaeus, 1758) (Diptera: Calliphoridae) was 0.0054. The interspecific variation rates ranged from 1.43% (*L. caesar* and *L. illustris*) to 11.25% [*Lucilia purpurascens* (Walker, 1836) (Diptera: Calliphoridae) and *Lucilia papuensis* Macquart, 1843 (Diptera: Calliphoridae)].

The NJ tree suggested that different species can be distinguished. Our sample clustered together with *L. caesar* and are nested away from the *L. illustris* (Fig. 2). The value of bootstrap is 93. Only two *L. illustris* nested in *L. caesar* and six *L. caesar* nested in *L. illustris*, and these sequences are not from our blowfly samples in this study. The detailed information of the whole NJ tree was in Additional file 2.

Nine haplotypes and 18 polymorphic isolation sites were detected in the 49 sequences generated from this study (Fig. 3). The value of haplotype diversity (H_d) was 0.588, nucleotide diversity (P_i) was 0.00215, and the average number of nucleotide differences was 1.374. The HAP3 and HAP2 were the main shared haplotypes. The HAP3 was shared by 29 sequences, and the HAP2 was shared by 13 sequences. Of all the haplotypes, the HAP1, HAP4, HAP5, HAP6, HAP7, HAP8 and HAP9 were unique in blowfly samples of this study.

Discussion

Some *Lucilia* species can hardly be distinguished morphologically, especially *L. caesar* and *L. illustris*, few diagnostic morphological characters have been proposed, and their immature stages cannot be identified by morphology [25]. Mitochondrial COI marker can distinguish most species from genus *Lucilia* [26–28]. Therefore, we collected larvae and eggs from the wound and fur of the injured forest musk deer for DNA identification.

We used online BLAST for primary identification of the blowfly larvae and eggs, and the best hits were *L. caesar* and *L. illustris*. Therefore, we analyzed nucleotide divergence of COI sequences of species and constructed a phylogenetic tree to accurately identify our blowfly samples. Analyses showed that the eggs and larvae collected from the wounds of musk deer are the same species (nucleotide divergence is 0.0022). Hebert *et al.* [14, 29] proposed that the interspecific nucleotide divergence should exceed 3%. Boehme *et al.* [30] suggested that the intraspecies difference of *L. caesar* was lower than 1.17%, and interspecies difference of *L. caesar* and *L. illustris* was 1.17–1.96%. The nucleotide divergence between our samples and *L. illustris* is 0.0143, and between our samples and *L. caesar* is 0.0054. NJ tree showed that sequences obtained in this study all clustered with *L. caesar* with modest support. *L. caesar* and *L. illustris* could not be identified completely accurately because they formed a polyphyletic group. Taken together, the blowfly eggs and larvae collected from the wound are very likely *L. caesar*.

The myiasis found in this study was not a coincidence. There were two shared haplotypes and seven unique haplotypes in the 49 samples from this study, and the value of H_d was 0.588, indicating that our blowfly samples were offspring of multiple female blowflies. In addition, *L. caesar* mainly distributes in the Palaearctic region [1], widely in Xinjiang, Inner Mongolia, Shaanxi, and Gansu of China [31]. The Breeding Center is located in the Qinling Mountains with dense forest and an altitude of 1496 meters. At the time of this case, it was summer with warmer and more humid, which is in line with the preferred habitat of the *L. caesar* [32].

There are lots of myiasis reports and researches in humans and other vertebrates [5, 33–35]. Wild animals are also susceptible to myiasis due to wound infection by parasitic fly larvae. For example, Huang *et al.* [36] and Yan *et al.* [37] found that Przewalski's Horse (*Equus ferus przewalskii* Poliakov, 1881) was highly susceptible to gastric myiasis in the Kalamaili Nature Reserve (KNR) and Xinjiang Research Centre for Breeding Przewalski's Horse, Xinjiang, China. A case of free-ranging eland (*Taurotragus oryx*) (Pallas, 1766) infected traumatic myiasis was reported from Kenya [38]. In the 20th century, the musk deer (*Moschus moschiferus*) in the Sikhote-Alin Mountains found serious cutaneous myiasis caused by *Booponus inexpectatus* Grunin, 1947 (Diptera: Calliphoridae) forming subcutaneous warbles [39]. Our report extended information on traumatic myiasis of musk deer. Two studies indicated that flies of Calliphoridae can cause severe clinical cutaneous myiasis in musk deers, and the status of myiasis in forest musk deer is neglected.

Traumatic myiasis is mainly caused by fly larvae developing in animal carrions after female flies directly laying eggs/larvae at the open wounds of humans or animals, resulting in the host wound and surrounding skin appear swelling, inflammation, pain and other health problems [33, 37, 40]. Diagnosis and prevention of traumatic myiasis are extremely important because it seriously threatens the host health and causes significant economic losses to the livestock industry. The main control strategies for myiasis are light trapping and conventional chemical control at present [37]. For the long-term health of captive wild animals, we can use physical methods to prevent and control traumatic myiasis. Besides, we also should monitor the health status of the animals themselves, promptly treat and nurse wounds, avoid wound worsening.

Conclusion

This study reports a new case of traumatic myiasis in forest musk deer. This traumatic myiasis is not an accidental event, which indicated the potential risk of forest musk deer of blowfly infection for captive and wild individuals. Traumatic myiasis can cause some problems of health, but it is neglected in forest musk deer. We should attach importance to the myiasis of forest musk deer, and we can construct some traps with carrion baits and sticky traps in Breeding Center to trap blowflies, reducing the number of pest flies.

Abbreviations

COI: cytochrome oxidase I

PCR: polymerase chain reaction

MAB-ELISA: monoclonal antibody-based enzyme-linked immunosorbent assay

K2P: Kimura's two parameter

H_d: haplotype diversity

Declarations

Ethics approval and consent to participate

The sample collection of this study was progressed smoothly with the approval of Nature Conservation, Beijing Forestry University, the guidelines of the Institution of Animal Care and the Ethics Committee of Beijing Forestry University, and the help of local veterinarians.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Yunyun Gao, Benmo Jiang collected samples, Dong Zhang, Defu Hu and Yunyun Gao design experiments, Yunyun Gao and Yajun Fu performed molecular experiments. Yajun Fu, Yunyun Gao and Liping Yan analyzed experiment results. Yajun Fu, Yunyun Gao, Liping Yan, Defu Hu, Benmo Jiang and Dong Zhang joined writing the manuscript. All authors read and approved the final manuscript.

Yunyun Gao and Yajun Fu contributed equally to this paper.

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Figures

Figure 1

The myiasis of an open wound of a male Forest Musk Deer. The Forest Musk Deer is breeding in Shaanxi Fengxian Fengchun Musk Deer Breeding Center. The wound is oval, located on the hindquarter. A. Wound site of forest musk deer. B. Blowfly larvae were found in the wound after the wound was cleaned. C. Egg clusters on the fur of forest musk deer.

Figure 2

The neighbor-joining (NJ) tree (1000 bootstrap replicates) generated using MEGA 7 with *Lucilia* DNA barcoding fragments based on Kimura's two parameter (K2P) Model. Our blowfly samples, *L. caesar* and *L. illustris* formed a polyphyletic group. Bootstrap support values below 50 are hidden at the nodes. We showed a part of the whole NJ tree, only include our blowfly samples, *L. caesar*, and *L. illustris*, because the whole NJ tree is oversized.

Figure 3

The haplotype network of mitochondrial DNA barcoding fragment of eggs and larvae. The HAP3 and HAP2 are shared haplotypes, HAP 1, HAP4, HAP5-HAP9 are unique in sequences. All haplotypes from the same species.

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