Research Article

Occurrence of Selected Zoonotic Fecal Pathogens and First Molecular Identification of Hafnia paralvei in Wild Taihangshan Macaques (Macaca mulatta tcheliensis) in China

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Rhesus macaques (Macaca mulatta) are hosts to a range of zoonotic and potentially zoonotic pathogens. The present study firstly provides a broader investigation of the presence and prevalence of zoonotic fecal pathogens in wild Taihangshan macaques, a subspecies of rhesus macaque in China. A total of 458 fecal samples were collected between September 2015 and November 2016. Fourteen genera of intestinal parasites (four genera of protozoans and ten genera of helminths) and twelve genera of bacteria were tested for using PCR amplification. The overall samples prevalence of parasitic infection was 98.25%. Entamoeba spp. (89.96%), Balantidium coli (70.09%), and Isospora spp. (28.38%) were the most prevalent protozoa, whereas the predominant prevalent helminths were Trichuris sp. (93.23%), Strongyloides spp. (73.36%), and Oesophagostomum sp. (31.66%). Ten genera of intestinal bacteriawere detected insamples of rhesus macaques, including Shigella (31.66%), Escherichia coli (29.91%), Klebsiella pneumoniae (28.38%), Leptospira (26.64%), Campylobacter jejuni (18.34%), Salmonella (13.32%), etc. Eight samples (1.75%) were tested Hafnia-positive based on sequences analysis of 16SrRNA and ampC gene. This is the first molecular characterization of Hafnia infection in NHPs. Our cross-sectional prevalence study provides important information for monitoring the potential transmission of zoonotic infections from wild rhesus macaques.

1. Introduction

Rhesus macaques (Macaca mulatta) are one of the most widely distributed nonhuman primates (NHPs) in the world. There are more than six kinds of rhesus macaque subspecies in China, and Taihangshan macaque (Macaca mulatta tcheliensis) is a subspecies confined to China southern Taihangshan Mountains area on the Henan-Shanxi border (N 35°12'49" N, E 112°41'25" W) [1]. Rhesus macaques are an increasingly important source of zoonotic diseases [2]. They can share pathogens with livestock and humans and act as reservoirs for several emerging infectious diseases such as malaria [2, 3]. Some molecular epidemiological studies have been performed to evaluate the prevalence of bacteria and parasites in NHPs [4–10]. Enterobacterial pathogens including Shigella spp., Salmonella spp., and Escherichia coli are the most commonly distributed pathogens in NHPs [4–6]. A diversity of intestinal parasites including five protozoan genera and six helminths genera was reported to infect NHPs [7, 8]. Many of these bacteria and parasites have strong pathogenic potential in both humans and animals. More recently, investigations of parasitic infections have been reported in several subspecies of rhesus macaque samples in China [7, 9, 10]. However, there are few data regarding the prevalence and distribution of the potential zoonotic fecal pathogens among Taihangshan macaques. Therefore,
Table 1: Prevalence of pathogens in 458 fecal samples collected from Macaca mulatta tcheliensis in China.

| Pathogens                  | No. of positive samples (% positive) | Pathogens                  | No. of positive samples (% positive) |
|----------------------------|-------------------------------------|----------------------------|-------------------------------------|
| Salmonella                 | 61 (13.32)                          | Entamoeba spp.            | 412 (89.96)                         |
| Shigella                   | 145 (31.66)                         | Trichuris sp.             | 427 (93.23)                         |
| Escherichia coli           | 137 (29.91)                         | Ascaris sp.               |                                     |
| Klebsiella pneumoniae      | 130 (28.38)                         | Isospora spp.             | 130 (28.38)                         |
| Yersinia                   | 31 (6.77)                           | Physaloptera sp.          | 69 (15.07)                          |
| Campylobacter jejuni       | 84 (18.34)                          | Enterobius spp.           | 8 (1.75)                            |
| Staphylococcus aureus      | 53 (11.57)                          | Strongyloides spp.        | 336 (73.36)                         |
| Leptospira                 | 122 (26.64)                         | Ancylostoma spp.          | 15 (3.28)                           |
| Streptococcus pneumoniae   | 38 (8.30)                           | Balantidium coli          | 321 (70.09)                         |
| Mycobacterium tuberculosis |                                     | Capillaria spp.           | 38 (8.30)                           |
| Hafnia                     | 8 (1.75)                            | Oesophagostomum sp.       | 145 (31.66)                         |
| Pasteurella multocida      |                                     | Trichostrongylus sp.      | 31 (6.77)                           |
| Gongylonema pulchrum       |                                     | Cryptosporidium spp.      |                                     |

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—\(^a\), not detected in this study.

our objective was to get initial information about the presence and prevalence of selected fecal pathogens in wild Taihangshan macaques.

2. Material and Methods

2.1. Sample Collection. During September 2015 to November 2016, a total of 458 fecal samples (9 liquid stools from nine different individuals) were collected from wild Taihangshan macaques in Wulongkou Scenic Area (N 35°12′49″, E 112°41′25″), Jiyuan, Henan Province of China. Fresh dropings were collected from each animal using individual sterile cryogenic vials and immediately placed in a cooler with ice packs and transported to the laboratory.

2.2. Sample Processing and PCR Analysis. Genomic DNA was extracted from 200 mg of each fecal sample using the QIAamp\textsuperscript{®} DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. Extracted nucleic acids were frozen at –20°C prior to the further study. To explore the molecular epidemiology of zoonotic pathogens in Taihangshan macaques, fourteen genera of intestinal parasites (four genera of protozoans and ten genera of helminths) and thirteen genera of bacteria were tested for using PCR amplification. Hafnia was amplified with conventional PCR using the primers specific for the 16S rRNA gene and ampC gene as described previously [11, 12]. All primers and conditions used in this study were listed in Supplementary Table 1.

2.3. Isolation, Genotyping, and Antibacterial Susceptibility of the Hafnia. The fecal samples were cultured on nutrient agar plates (Oxoid, UK) in anaerobic conditions at 37°C for 18 h and typical colonies were transferred at least thrice in the same conditions. The isolates were examined by the Gram stain, 16S rRNA gene and ampC gene sequencing, and biochemical tests using the BD Phoenix Automated Microbiology System (BD). Antibiotic susceptibility testing was performed for Hafnia as previously described [12].

2.4. Sequencing and Phylogenetic Analyses. The PCR products from positive samples were bidirectionally sequenced at BGI Sequencing (Beijing, China). Nucleotide sequences were aligned with reference strains obtained from the GenBank database. The molecular phylogenetic trees were constructed by using the neighbor-joining method executed in MEGA6 [13]. The robustness of the tree topology was assessed with 1000 bootstrap replicates.

2.5. Nucleotide Sequence Accession Numbers. The representative nucleotide sequences of this study have been deposited in the GenBank database under accession number MG923797 for 16S rRNA gene and MK189458 for ampC gene.

3. Results

3.1. Prevalence of Parasitic Pathogens in Wild Taihangshan Macaques Samples. Overall, 450 (98.25%) of the 458 Taihangshan macaques fecal samples were infected with one or more parasites. The majority of gastrointestinal parasites found in the Taihangshan macaques exhibit relatively high samples prevalence. Entamoeba spp. was the most prevalent protozoa and its prevalence was 89.96%, followed by 70.09% prevalence of Balantidium coli, and 28.38% of Isospora spp., whereas the predominant prevalent helminths were Trichuris sp. (93.23%), Strongyloides sp. (73.36%), Oesophagostomum sp. (31.66%), Physaloptera sp. (15.07%), Capillaria spp. (8.30%), Trichostrongylus sp. (6.77%), Ancylostoma spp. (3.28%), and Enterobius spp. (1.75%) (Table 1).
All samples were negative for Ascaris sp., Cryptosporidium spp., and Gongylonema pulchrum.

3.2. Prevalence of Bacterial Pathogens in Wild Taihangshan Macaques Samples. Ten genera of intestinal bacteria tested for were found in the Taihangshan macaques. The overall prevalence of selected bacterial infection was 84.93% (389/458). More in detail, 61 cases tested positive for Salmonella (infection rate 13.32%), 145 for Shigella (31.66%), 137 for Escherichia coli (29.91%), 130 for Klebsiella pneumonae (28.38%), 31 for Yersinia (6.77%), 84 for Campylobacter jejuni (18.34%), 53 for Staphylococcus aureus (11.57%), 122 for Leptospira (26.64%), and 38 for Streptococcus pneumoniae (8.30%) (Table 1). Among these species, Shigella was the most frequently detected bacteria, followed by Escherichia coli and Klebsiella pneumonae. Of the 9 liquid stools from nine different monkeys, the positive rate of Shigella was 100%. All samples tested negative for Mycobacterium tuberculosis and Pasteurella multocida.

3.3. Molecular Characterization of Hafnia paralvei in Wild Taihangshan Macaques Samples. Hafnia infections were observed in 8 samples, with the sample prevalence as 1.75% (8/458). To our knowledge, this is the first genetic characterization of Hafnia paralvei infection in NHPs. Sequence analysis revealed that these sequences had similarities of 99.7%-100%. The H. paralvei HN strain was successfully isolated from fecal samples. Biochemical studies showed that the isolated H. paralvei was typically malonate and β-glucosidase negative, while the opposite pattern is associated with H. alvei species [12]. The results of the antibiotic analysis of H. paralvei HN against 21 different antibacterial agents are demonstrated in Supplementary Table 2. The H. paralvei HN strain was sensitive to 14 antibiotic agents especially amikacin, gentamicin, imipenem, meropenem, cefazidime, cefotaxime, cefepime, aztreonam, ampicillin, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, and levofloxacin and resistant to 5 antibiotic agents including cefazolin, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, and tetracycline. The type or reference strains used in these studies included two H. alvei strains (ATCC 13337 and ATCC 29926) and one H. paralvei strain (ATCC 29927). The subsequent molecular phylogeny analysis based on 16S rRNA gene (approximately 1400 nt) revealed that H. paralvei HN strain belonged to genotype H. paralvei. The strain was most closely related to H. paralvei strain 24 (KY849253) isolated from the human rectal swab and share 99% homology (Figure 1). Meanwhile, phylogenetic relationships based on partial ampC sequence had remarkably similar results. The strain H. paralvei HN shared the highest similarity homology 99% with Hafnia ACC-5 (NG 048595) (Figure 1).

4. Discussion

Taihangshan macaque (Macaca mulatta tcheliensis) occupies the northern limit of all rhesus macaque natural populations in the world [1, 14]. A national nature reserve in the Taihangshan Mountains area was established by the Chinese government to protect the macaques. In most wildlife populations, infectious diseases are considered as the second leading cause of mortality, right behind predation and malnutrition [15]. Meanwhile, rhesus macaques have the potential to transmit various pathogens to humans and domestic animals. Therefore, monitoring the presence and prevalence of zoonotic infectious agents among this population is important for the general health of humans and animals coming into contact with this population.

This study demonstrates a high samples prevalence (98.25%, 450/458) and diversity (three genera of protozoans and eight genera of helminths) of intestinal parasites in Taihangshan macaques in China. Previous studies revealed that the samples prevalence varied significantly with species, geographic region, feeding habitats, and age [7, 8]. Similar prevalence was reported in bushmeat monkeys samples (92%) in Cameroon [8], while lower prevalence was found in 34 NHP species samples (54.1%) in China [7] and primates samples (54.5%) at a zoo in Malaysia [16]. Several molecular epidemiological studies have demonstrated Trichurus sp. and Entamoeba spp. were the most frequently detected parasites [8], whereas others reported that Entamoeba spp. [7] or Strongyloides spp. [17] was the most prevalent. Similarly, Trichuris sp., Entamoeba spp., and Strongyloides spp. were the most common parasitic pathogens in our study with a high prevalence of 93.23%, 89.96%, and 73.36%, respectively. Several studies reported that Trichuris sp. and Entamoeba spp. were parasitic with a high potential for transmission to humans and animals because of their simple and direct life cycles [7, 18, 19].

Balantidium coli detected in our study are multihost parasite capable of infecting animals and humans. Balantidium coli could damage the intestinal mucosal and cause serious diarrhea and dysentery [10]. The prevalence of infection found in the present study (70.09%) with Balantidium coli is consistent with rates previously published in bred rhesus monkeys and baboons [10, 20], but higher than cercopithecid monkeys samples [21]. Isospora spp. infections are very common and have a worldwide distribution [22]. The prevalence of Isospora spp. DNA in Taihangshan macaques was quite high (28.38%). On the contrary, the previous study in nonhuman primates samples in China from 2009 to 2015 revealed a relatively lower (1.9%) prevalence rate for this protozoan [7]. Macaques in several regions of China and India have been reported to be infected with Cryptosporidium spp. [7, 23, 24], while in this study all macaques samples tested by PCR were negative. The negative results suggested that this protozoan is not an important parasite in rhesus macaques in this region.

Even though a diversity of intestinal helminths parasites was frequently reported to infect NHPs [8, 10], samples frequencies of Trichuris sp., Strongyloides sp., Physaloptera sp., Capillaria spp., Trichostrongylus sp., Ancylostoma spp., and Enterobius spp. were higher than previous studies [7, 21]. However, a relative lower sample infection rate (31.66%) of Oesophagostomum sp. was found in all macaque monkeys than wild chimpanzees (48%) and red colobus samples (41%)
Figure 1: Phylogenetic analyses of Hafnia based on sequences of 16S rRNA gene (approximately 1400 nt) (a) and ampC gene (approximately 759 nt) (b) using the neighbor-joining (NJ). Bootstrap values > 50% are shown. The genotypes identified in this study are indicated by ▲.
5. Conclusion

In conclusion, the present study firstly provides a broader investigation of zoonotic pathogens in wild Taihangshan macaques in China, which detailed the presence and prevalence of bacteria and parasites. Our preliminary results demonstrate high prevalence and diversity of significant zoonotic infections amongst wild Taihangshan macaques. This baseline data provides valuable feedback for monitoring the potential transmission of zoonotic infections from wild rhesus macaques.

Data Availability

The epidemiological data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Qingxun Zhang, Shuyi Han, and Kongshang Liu have contributed equally to this work.

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Supplementary Materials

Supplementary Table 1 includes pathogens, primer sequences, and references of PCR amplification. Supplementary Table 2 includes antibiotic susceptibility tests of the *H. paralvei* HN. (Supplementary Materials)

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