Genetic Diversity Analysis of *Dermacentor Nuttalli* within Inner Mongolia, China

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Research

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Abstract

Background

Ticks (Arthropoda, Ixodida), after mosquitoes, are the next most prevalent vector of infectious diseases; and are responsible for spreading a multitude of pathogens and threatening the health and welfare of animals and human beings. Yet, given the history of tick-borne pathogen infections in the Inner Mongolia Autonomous region of China, neither the genetic diversity nor the spatial distribution of haplotypes within ticks has been studied.

Methods

We characterized the haplotype distribution of *Dermacentor nuttalli* in four main pastoral areas of the Inner Mongolia Autonomous region, by sampling 109 individuals (recovered from sheep) in April-August 2019. The 16S rRNA gene, Cytochrome c Oxidase Subunit Ⅰ, and the Internal Transcribed Spacer 2 region were amplified and sequenced from extracted DNA.

Results

82 haplotypes were identified, the most prevalent of which was H32. 12 sequences (11 of all sequences) represented the most abundant haplotypes, with a highly scattered distribution. Total haplotype diversity was 0.98318, while total nucleotide diversity was 0.11369. Neutrality tests revealed negative results in the four locations analyzed, which is indicative of an excess of recently derived haplotypes. Fixation index values (F_{ST}) indicate that the degree of genetic differentiation amongst some sampled populations were small, while others were moderate.

Conclusion

The genetic diversity of *D.nuttalli* populations in our region can likely adapt to different geographical environments, thereby leading to genetic diversity, and creating genetic differentiation amongst different populations. However, genetic differentiation is cryptic and does not form the pedigree geographical structure.

Background

Dermacentor nuttalli (Acari: Ixodidate), is widely distributed across northern China, Russia and Mongolia [1], and is generally found in arid grassland areas suitable for grazing cattle and sheep[2], *D. nuttalli* responsible for spreading a variety of diseases, including spotted fever, tick-borne rickettsia, Crimea-hemorrhagic fever and Babesiosis [2–5], in addition to being an important vector of Spirochetes and forest encephalitis [6, 7]. Humans may experience symptoms similar to viral infections after being bitten by ticks, such as headaches, muscle pain, fevers, enlarged local lymph nodes or plaques, as well as severe liver and kidney damage and central nervous system damage [8, 9]. *D. nuttalli* is an important storage host and transmission medium of Rickettsia, of which can spread rapidly through bites and

cause a variety of zoonotic diseases. As result, D. nuttalli is quickly becoming one of the most dangerous tick species to public health in Inner Mongolia.

Yet given the increasing danger to the health and economy of the Inner Mongolia Autonomous region, neither the genetic diversity nor the spatial distribution of haplotypes in *D. nuttalli* populations has been studied. In recent years, research into the genetic diversity, within species and even between populations has become increasingly common [10–14]. Genetic diversity is affected by many biotic and abiotic factors, including natural selection, genetic variation, and anthropogenic influence [15]. Ticks and pathogens have also been identified as influencing host evolution [16, 17], Nevertheless, there remains a parsity of data for tick population within China. Therefore, the goal of this study is to characterize the genetic diversity of various *D. nuttalli* populations, not only to further pur understanding of their diffusion, but also of evolution, in order to protect the health of humans and animals. We hope this data will create a foundation for further study into the spread of tick-borne diseases in China and provide evidence for the origin and continued evolution of tick species.

We choose three gene regions for our study as they are all easily amplified and commonly used in molecular systematics and population genetics [19–21]. While the 16SrRNA gene has a relatively slow rate of revolution [18], and Cytochrome c Oxidase□gene (CO□) contains a highly conserved region, they are widely used in the study of, intraspecific and interspecific genetic diversity, and phylogenetic geography among different geographical populations [22–23]. In comparison, the Internal Transcribed Spacer 2 region (ITS2) evolves rapidly and has rich polymorphism. As a result, it is commonly used to study historical population dynamics of species with close interspecific relationship [24–28]. In this study, we analyze the haplotype distribution of the 16SrRNA, CO□, and ITS2 genes within individuals of *D. nuttalli* sampled from four main pastoral areas of the Inner Mongolia Autonomous region.

Methods

Sample collection

A total of 109 *Dermacentor nuttalli* individuals were sampled from Chengchuan Town, early Banner of Etoke Banner, Erdos City (EEDS); Siziwang Banner, Hohhot City (SZWQ), the Bayan WenduSumu area, Arukorqin Banner, Chifeng City (CF), and Xinbarhu right Banner, Hulunbuir City (HLBE), Inner Mongolia. All ticks were recovered from sheep using tweezers and rubber gloves between April-August 2019 (Table1, Fig1), and were stored in -80℃.

DNA extraction

Each *D. nuttalli* individual was washed in three sterile water baths followed by one absolute ethanol bath, air dried and collected in a sterile EP tube. Each tick was then added to a 1.5ml sterile microtube containing sterile microbeads (Jingxin, Shanghai, China) with a 2:1 ratio of two small steel micro-beads (2mm diameter) to one big steel micro-bead (4mm diameter). Samples were cooled in liquid nitrogen for 2 minutes and crushed by tissue grinder (KZ-□, Jingxin) for two cycles (2 minutes, frequency of 60). The

tubes were then briefly centrifuged at speed of 12,000 rpm, and then extracted using TIANamp- Genomic DNA Kit (TIANGEN, Beijing, China) following the protocol for tissue extraction. The final elution volume was 70μL and these were stored at -20℃ for subsequent detection [29].

Amplification and sequencing

To analyze the genetic structure of our four population, we amplified the 16S rRNA gene, Cytochrome c Oxide Subunit gene (CO) and Internal Transcribed Spacer 2 region (ITS2). The primer templates of all three genes were downloaded from the NCBI database, with the upstream and downstream primers of 16S rRNA located in the v4 and v7 variable regions respectively, and the amplification products of CO and ITS2 approximately full-length (Oligo7). The primer sets of 16S rRNA, CO and ITS2 gene sequences were synthesized by Shanghai Sangon Biotechnology (Shanghai, China). 40μL PCR reaction mixture contained 16μL Taq PCR master Mix (Sangon), 1μL of each primer,10μL ddH_2O and 1μL of DNA from each sample. Details about PCR primer pairs, size of the amplification (bp), and annealing temperatures are presented in Table 2. Negative control samples (sterile double distilled water) were included in each PCR reaction. After electrophoresis, gel imaging was used to detect successful amplified, and if the amplified band was the target band, it was retained. If the target band was bright and free, it was sent for sequencing directly, while other bands were excised, purified by a TIANGEN Gel DNA recovery Kit (TIANGEN, Beijing ,China) and cloned using the p-GMT Easy vector system (TIANGEN, Beijing ,China).

Data analysis

Nucleotide sequences from the three amplified regions (16S rRNA , CO and ITS2) were spliced by Seqman7.1. Then sequences were compared to those previously uploaded to Genbank using the Basic Local Alignment Sequence Tool search engine. In order to ascertain the degree of genetic diversity between these four *D. nuttalli* populations, we estimated a number of parameters including: the number of segregating sites, the average number of nucleotide differences, the number of haplotypes, haplotype diversity, the distribution pattern of DNA variation, under a range of neutrality tests (such as Tajima's, Fu and Li's and Fu's tests) in DNAsp5.10 [30-31]. The genetic differences among populations were calculated by fixed index (FST). In order to determine the relationship between haplotypes [32], Popart1.7 was used to construct TCS haplotype network map. The phylogenetic tree was constructed by MEGA7.0 using the adjacency method. The stability of the tree was evaluated by 1000 bootstrap repeats [33-35].

Results

Population genetic analysis

In this study, we detected 82 haplotypes and 9 shared haplotypes (H5, H31, H32, H33, H35, H37, H39, H51, H66), with shared haplotypes varying in certain genetic differentiation among populations. The most frequent haplotype detected was H32, with 12 sequences(11 of all sequences) followed by haplotype H35 with 6 sequences (6 of all sequences) and H5 with 5 sequences (5 of all sequences). The least frequent haplotypes were the rest 73 unique haplotypes. (Fig2). H32, H5 and H35 are notable, as they

were not only the most abundant haplotypes, but also displayed a highly scattered distribution. While H32 was identified with EEDS and HLBE, and H5 within CF and SZWQ, H35 was the only shared haplotype identified in three regions (EEDS, SZWQ and HLBE). Total average haplotype diversity (Hd) was 0.98318, and varied between populations, ranging between 0 and 1, and total nucleotide diversity was 0.11369. Diversity values were calculated for each sampled locality. SZWQ was the locality with the highest haplotype diversity (h=0.99333), while HLBE had the lowest diversity (h=0.94089). Regarding nucleotide diversity, the lowest diversity was found in EEDS (pi=0.06074) and the highest (pi=0.18248) in CF (Table 3).

Negative neutrality results in all four regions, indicate an excess of recently derived haplotypes. While Tajima' s D values were not significant, Fu's Fs values were significant, also indicating that the population has not experienced recently. The FST value reflects the degree of genetic differentiation between the two populations, representing the allelic variation between the two populations, which is inversely proportional to gene flow. The F_{ST} value between CF and EEDS was the highest (F_{ST}=0.11712), while the FST values for CF, SZWQ and HLBE were all greater than 0.05, indicating that there was moderate genetic differentiation among populations with a small degree of gene flow. The F_{ST} values among the three regions of SZWQ, EEDS and HLBE were all less than 0, indicating that the genetic differentiation amongst these populations was very small, likely as a result of high gene flow (Table 4). The genetic distance between CF and the other three regions was greater than 0.15, indicating that the degree of genetic differentiation was greater than that amongst the three other populations.

Phylogenetic analysis

Sequences belonging to shared haplotypes among populations clustered together (Fig 3). Compared with the haplotype network map, other unique haplotypes similarly clustered near the corresponding shared haplotypes. Similar conclusion were drawn from the evolutionary and diversity analysis, in which H31, H6, H39, H51 and H35 belong to a major branch in the phylogenetic analysis. In the haplotype network graph, this major branch was separate to the one that included H5, H37, H33and H32. We found that the geographical populations were not distributed according to the corresponding geographical units, but that the haplotypes were mixed with no obvious geographical differentiation structure. This implies that the genetic evolution amongst populations was not closely related to the geographical structure.

Discussion

This is the first report on the genetic structure of *Dermacentor nuttalli*, which is the dominant tick species in the Inner Mongolia Autonomous Region [36]. Yet, information on the degree of gene flow and genetic diversity between *D. nuttalli* populations was severely lacking. Haplotype diversity (Hd) and nucleotide diversity (Pi) are two important indicators to measure the diversity of a species population [37]. In this study, the population genetic diversity of *D. nuttalli* among four different geographical populations in Inner Mongolia Autonomous region was characterized, revealing high haplotype diversity and high nucleotide diversity.

Our results indicates that a large and stable population has been generated potentially by long-term evolution or secondary contact between different populations. Only a few shared haplotypes appeared in our four sampled populations, and these shared haplotypes were also highly abundant. Haplotypes can persist for a long time in a population and can adapt to different environments [38]. While most of the haplotypes detected in our study were exclusive, indicating genetic differentiation among populations, the rich haplotype diversity and high genetic diversity indicates that *D. nuttalli* may have the ability to adapt to different environments, and have a large geographical range in Inner Mongolia. Our haplotype network diagram revealed a single star scattering distribution, indicating that the population has likely undergone rapid expansion after a recent bottleneck [39].

FST value is the genetic differentiation index of populations, which can measure the genetic differentiation among different populations [40]. In our study, there were different degrees of genetic differentiation among our four study populations, but the degree of genetic differentiation was small, and there was gene flow present among populations. The phylogenetic tree and haplotype network illustrated that the haplotypes were not distributed according to the corresponding geographical units, and the haplotypes of each geographical population were mixed. Therefore, *D. nuttalli* populations within Inner Mongolia did not conform to geographical isolation, and the reason for the formation of this structure was the combination of numerous factors.

Conclusion

The genetic diversity of *D. nuttalli* population in the Inner Mongolia Autonomous region can adapt to different geographical environment, and the adaptability of different populations created genetic diversity, leading to a genetic differentiation among different populations. However, the genetic differentiation was cryptic and does not form a pedigree geographical structure. The genetic diversity was not only related to gene mutation, but to natural selection and other environmental factors [41]. It was also likely related to the migration ability, strong adaptability, diversity of hosts, environment and climate, habitat fragments and human activities. Further studies should increase the geographic sampling range to lay a foundation for controlling the further spread of tick-borne diseases and provide more information in which to study the origin and evolution of ticks.

Abbreviations

CO□: Cytochrome c Oxidase Subunit □, ITS2: Internal Transcribed Spacer 2, PCR: Polymerase chain reaction. D.nuttalli: Dermacentor nuttalli:

Declarations

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Authors' contributions

GZ, WL and CH performed laboratory analysis, analyzed data and wrote the first draft. YJF and ML revised the manuscript. SXY participated in samples collection. FSY directed the experiment and helped to revise the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

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Figures

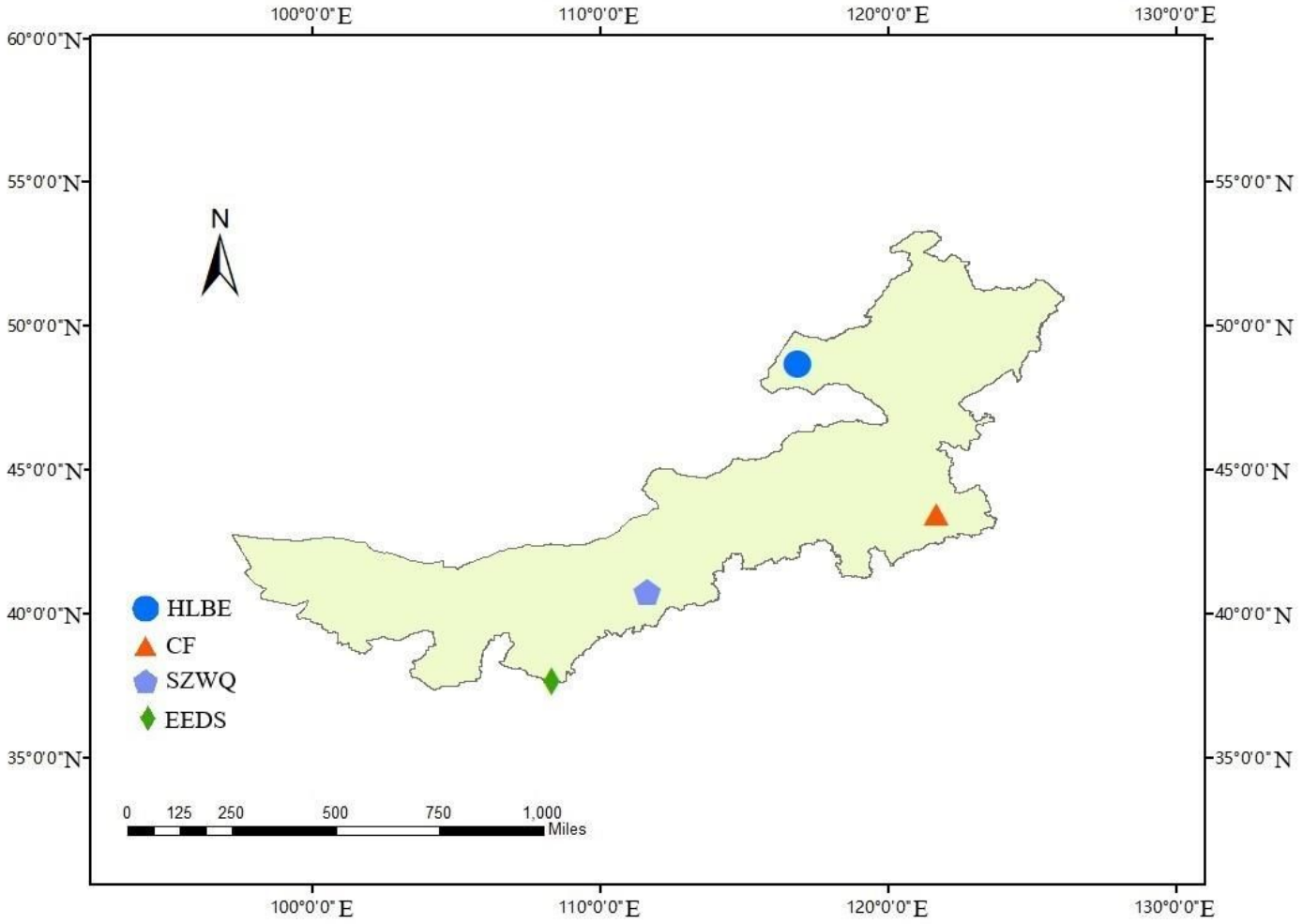

Figure 1

Collection sites map. Samples from D.nuttalli speciments from 4 regions of Inner Mongolia were collected. Each color corresponds to different collection region in Inner Mongolia and each grapg represents the approximat geographical coordinates of the location in the map for each collection sites. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

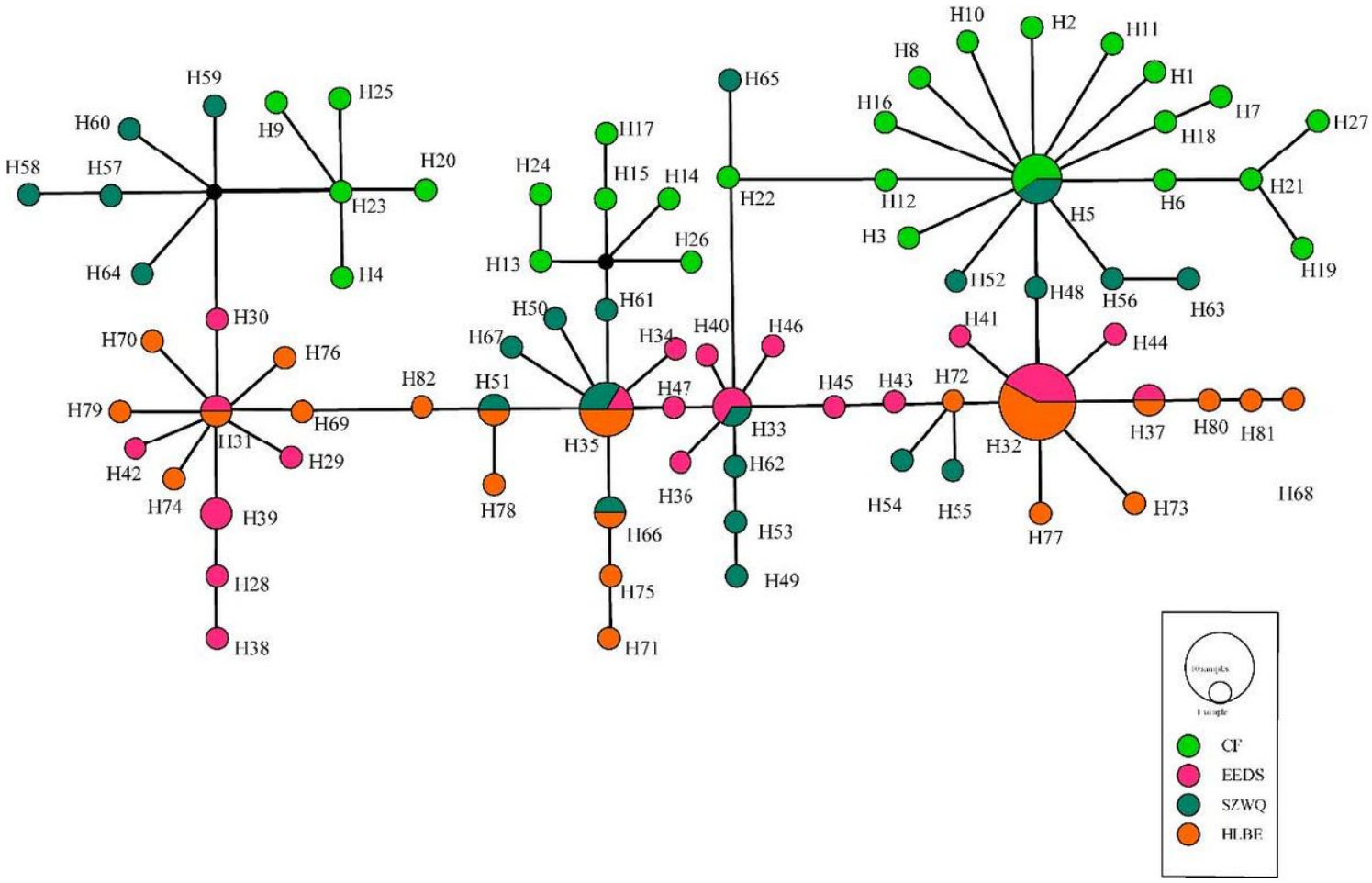

Figure 2

TCS haplotype network of D. nuttalli from four different populations in Inner Mongolia, based on 16SrRNA , CO and ITS2sequences

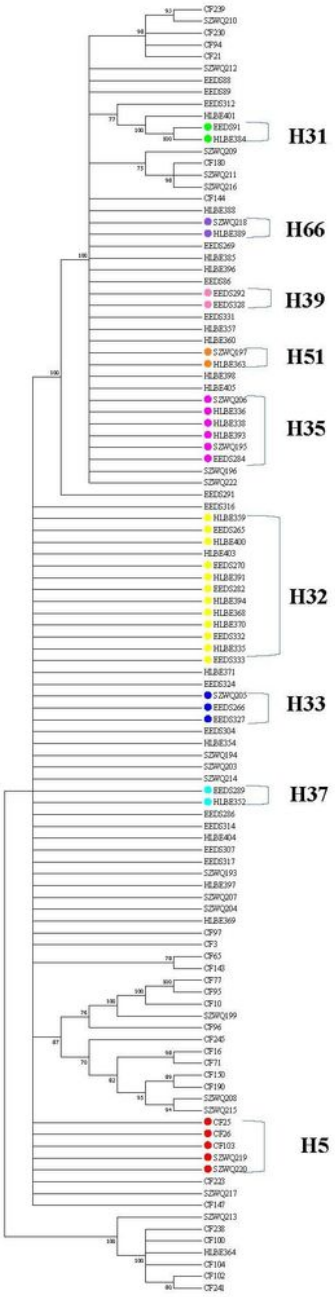

Figure 3

Sequences belonging to shared haplotypes among populations clustered together

Supplementary Files

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