Identification of Different Species of Mammalians Involved in Zoonoses as Reservoirs or Hosts by Sequencing of the Mitochondrial DNA Cytochrome B Gene

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Authors’ contributions

This work was carried out in collaboration between all authors that designed the study, wrote the protocol, interpreted the data, anchored the field study, gathered the initial data, performed preliminary data analysis, managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The identification of species that act as reservoirs or hosts of zoonotic agents is essential for control and epidemiological surveillance of the important illness in public health. Identification of the reservoirs for zoonoses can help to clarify how the pathogens are maintained in nature, leading to more effective disease control and avoiding indiscriminate extermination of wild animals.

Aims: The objective of this study was to describe the genetic identification of 106 samples isolated from different mammalian species.

Methodology: This study was conducted using 106 tissue samples from wild and domestic...
mammals sent to rabies diagnosis in Pasteur Institute, Brazil. Sequencing of the mitochondrial DNA b gene and Basic Local Alignment Search Tool (BLAST) was used to confirm species identity. **Results and Conclusion:** By sequencing the mtDNA cyt-b gene 10 orders, 20 families, 34 genera and 38 species of mammalians were identified. In conclusion, the method used at this work was efficient for identification of different species of mammalians. Animals identified at this work with same method, belong to high distance order, as marsupials, chiropters and primates.

**Keywords:** Mammalians; zoonoses; reservoirs; mitochondrial DNA and cytochrome B gene.

1. INTRODUCTION

Since of sequencing of human mitochondrial DNA (mtDNA) studies with this molecule has been made [1]. The study of mtDNA from all species has great interest by association between mutations on this molecule and hereditary illness [2], evolutionary studies [3] and for identification of species [4]. In recent years the sequencing of mtDNA is generally used in ecology, evolution, pathogenesis, systematic, forensic investigations and many others topics of Science [5-10].

Different molecular markers of mtDNA, as control region (D-loop), cytochrome B gene (mDNA cyt-b) and cytochrome oxidase I gene (COI) are currently used to genetic identification of species and a great number of genetic sequences of these areas are available in Data Banks as GenBank (www.ncbi.nlm.nih.gov/genbank/). The use of each one of three described molecular markers of mtDNA has advantages and disadvantages. For example: D-loop is a hypervariable region with high heteroplasmy and mononucleotides repetitions. By this reason, it is currently used for evolutionary and forensic studies, but not all set of primers could amplify this area of mtDNA from species with great evolutionary distance [11]. The sequencing of the mtDNA cyt-b gene has been used for a long time to genetic identification of species and the number of available sequences in Data Banks is numerous. One factor to continuous use of this gene to genetic identification of species is because this gene contains specie specific information and it is easily amplified and sequenced [12]. Many researchers had sequencing a part of COI gene of mtDNA and compare the identities of sequences with The Barcode of Life Data Bank Systems (BOLD) [13,14]. In the current study was chose the mtDNA cyt-b because it has lower mutational substitution rate than COI [15]. In addition Tobe et al. (2010) studied both mtDNA cyt-b gene and COI, they claim that mtDNA has better resolution for identification of species [16].

The diseases classified as zoonoses, could be caused by bacteria, parasites, fungi, viruses or non conventional agents. The wild fauna play an important role in maintenance of zoonoses. The majority of emerging infectious diseases had origin in wild fauna and the number still increase [17-19]. The World Health Organization (WHO) describes that half part of infectious agents which affect human population is from animal origin [20]. The identification of reservoirs of zoonoses by molecular biology methods helps to understand the maintenance of pathogens in nature, and, consequently the control of disease could be more efficient, without the indiscriminate death of wild and domestic animals for epidemiologic surveillance.

Laboratories that receive clinical samples for diagnosis of zoonoses, have the opportunity to identify reservoirs or hosts to etiological agents by sequencing of the mtDNA cyt-b gene. This forensic strategy is important because ethical and animal conservation principles difficult the achievement of this kind of samples.

The identification of wild species involved in zoonoses transmission, is sometimes realized due an inaccurate form by morphological methods and by people without specific formation in area. For this reason, morphological similarities between species could difficult the correct identification of it [21]. In addition the high geographic distribution of some species and the use of popular names could difficult the correct identification of species [22]. An important example to this situation was published by [23], when the authors described 160 cases of human rabies transmitted by no identified species in Americas between 1993 and 2002 and 20 of these cases happened in Brazil.

Wild animals had great importance in epidemiology of many zoonoses and the role of many mammalians in maintenance of this disease had increased because they have sinantropic habits [24]. Methods of molecular biology, now less expensive, are nowadays
available for the majority of laboratories and are very important to determine reservoirs or hosts of infectious disease including carcasses of animals in advanced deterioration stage.

The aim of the present study was to perform a simple and efficient forensic method for a complete sequencing of the mtDNA cyt-b gene of mammalians for species identification.

2. MATERIALS AND METHODS

2.1 Samples

For genetic identification, 106 samples from Central Nervous System of wild and domestic mammalians involved in rabies epidemiology in Brazil, and sent to Rabies Diagnostic Laboratory at the Pasteur Institute of São Paulo, were used.

2.2 DNA Extraction, Polymerase Chain Reaction (PCR), Sequencing and Phylogenetic Analysis

The DNA extraction, Polymerase Chain Reaction (PCR), Sequencing and Phylogenetic analysis were performed as described by Carnieli et al. [25].

3. RESULTS

All selected samples of different species of mammalians that had the cyt-b mtDNA sequenced were genetic identified using The Basic Local Alignment Search Tool (BLAST). The sequences obtained in this work were deposited and measured by GenBank staff and were registered with GB numbers KT447516-KT447521; KT626612-KT62654 and KU253477-KU253533 (www.ncbi.nlm.nih.gov/genbank/). The identified species belong to 10 orders, 20 families, 34 genera and 38 species of mammalians as shown in Table 1. The name of species, registration name of the samples and GenBank number for each species identified are shown in Table 2.

4. DISCUSSION

For identification of mammalians with genetic markers of the mtDNA, the comparison between sequences in Public Data Banks is necessary. By this reason the deposit of sequences as the generated at this work is essential for success of genetic identification. Brazil, country where this study was realized, contains high diversity of mammalians. On the other hand, the number of genetic sequences of mtDNA of Brazilian animals is found in low numbers in Public Data Banks. The sequences used for comparison were retrieved from PubMed-Nucleotide while using BLAST. However, many of available mtDNA cyt-b are not complete and was necessary to compare different areas of mtDNA cyt-b separately.

The use of BLAST software for identification of mammalians happened because finds regions of local similarity between sequences. The BLAST software program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences, as well, as help identify members of gene families (www.ncbi.nlm.nih.gov/genbank/).

In this study, the mtDNA cyt-b was whole sequenced to start at stop codon, including higher phylogenetic signal comparing with sequences of mtDNA cyt-b available in GenBank. The higher phylogenetic signal be according to the number of sequenced nucleotides and consequently improve the confidence of results as generated at this study because facilities comparisons for genetic or evolutionary identity. In addition, the sequences could be used for worldwide researchers from different areas. This action encourages and facilitates news studies with mtDNA cyt-b of many species of mammalians in Brazil.

The results of this study could help in epidemiological surveillance of zoonoses, because the generated data as nucleotide sequences were available in a Public Data Bank: GenBank. Is important describe that GenBank is a part of the International Nucleotide Sequence Database Collaboration, which comprises the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at NCBI.

The number of genetic sequences of mtDNA cyt-b generated at this study plus other sequences of bats shown in another recent published study [25] are a significant number of reservoirs from different pathogens that could be identified for the forensic method described at this paper using clinical samples sent to different kinds of laboratories bound to Public Health or Research.
Table 1. Orders, families, genera and species of mammalians identified in this work

| Order        | Family        | Species                        | Popular name          | Nº samples |
|--------------|---------------|--------------------------------|-----------------------|------------|
| Carnivora    | Mustelidae    | Galictis cuja                  | Lesser grison        | 01         |
|              |               | Pteronura brasiliensis         | Giant otter           | 01         |
| Felidae      | Felis catus   | Cat                            |                       | 04         |
|              | Leopardus pardal | Ocelot                |                       | 02         |
|              | Puma concolor | Puma                           |                       | 01         |
|              | Panthera onca | Jaguar                         |                       | 01         |
|              | Panthera leo  | Lion                           |                       | 01         |
| Canidae      | Lycalopex vetulus | Hoary fox                |                       | 01         |
|              | Cerdocyon thous | Crab-eating fox          |                       | 17         |
|              | Chrysocyon brachyurus | Maned-wolf      |                       | 02         |
| Teonidae     | Nasua nasua   | South american coati          |                       | 01         |
| Suidae       | Sus scrofa    | Pig                            |                       | 02         |
| Perissodactyla | Equidae      | Equus caballus                | Horse                 | 01         |
| Artiodactyla | Bovidae       | Bos indicus                   | Cattle                | 01         |
|              | Ovis aries    | Sheep                          |                       | 01         |
|              | Capra hircus  | Goat                           |                       | 01         |
| Cervidae     | Blastocerus dichotomus | Marsh deer          |                       | 02         |
|              | Mazama gouazoupia | Gray brocket       |                       | 06         |
|              | Mazama americana | Red brocket           |                       | 02         |
| Suidae       | Sus scrofa    | Pig                            |                       | 02         |
| Primates     | Atelidae      | Alouatta caraya               | Black howler          | 02         |
|              | Cebidae      | Callithrix jacchus            | Common marmoset       | 13         |
|              |              | Callithrix geoffroyi          | Marmoset-faced-white  | 09         |
|              |              | Callithrix kuhlii             | Wied’s marmoset       | 02         |
|              |              | Sapajus apella               | Tufted capuchin       | 01         |
| Rodentia     | Cricetidae    | Mesocricetus auratus          | Golden hamster        | 01         |
|              |             | Phodopus campbelli            | Campbell’s hanster    | 01         |
|              | Sciuridae    | Sciurus aequalis              | Squirrel              | 02         |
|              | Erethizontida | Coendou spinosus              | Coendou spinosus      | 01         |
|              | Muridae      | Rattus rattus                 | Rat                   | 04         |
| Pilosa       | Myrmecophagida | Tamandua tetradactyla       | Southern tamandua     | 04         |
| Cingulata    | Dasyopidae    | Dasypus novemcinctus          | Armadillo             | 04         |
| Didelphimorphia | Didelphidae  | Didelphis marsupialis         | Common opossum        | 01         |
|              |              | Didelphis albiventris        | White-eared Opossum   | 05         |
| Chiroptera   | Phyllostomidae | Artibeus fimbriatus         | Fringed fruit-eating bat | 01     |
|              | Molossidae   | Eumops perotis                | Western bonneted bat  | 03         |
| Lagomorpha   | Leporidae     | Oryctolagus cuniculus        | Rabbit                | 01         |

Table 2. The name of species (alphabetical order), registration number of the samples and GenBank number for each species identified in this work

| Specie                  | Isolate | GB number  |
|-------------------------|---------|------------|
| Alouatta caraya         | SP100   | KT626649   |
| Alouatta caraya         | SP139   | KU253513   |
| Artibeus fimbriatus     | SP8     | KT626651   |
| Blastocerus dichotomus  | SP77    | KT626626   |
| Blastocerus dichotomus  | SP78    | KT626627   |
| Bos indicus             | SP105   | KU253479   |
| Callithrix geoffroyi    | SP92    | KT626641   |
| Callithrix geoffroyi    | SP93    | KT626642   |
| Callithrix geoffroyi    | SP94    | KT626643   |
| Specie                  | Isolate | GB number    |
|------------------------|---------|--------------|
| Callithrix geoffroyi   | SP130   | KU253504     |
| Callithrix geoffroyi   | SP131   | KU253505     |
| Callithrix geoffroyi   | SP132   | KU253506     |
| Callithrix geoffroyi   | SP133   | KU253507     |
| Callithrix geoffroyi   | SP134   | KU253508     |
| Callithrix geoffroyi   | SP135   | KU253509     |
| Callithrix jacchus      | SP95    | KT626644     |
| Callithrix jacchus      | SP96    | KT626645     |
| Callithrix jacchus      | SP97    | KT626646     |
| Callithrix jacchus      | SP98    | KT626647     |
| Callithrix jacchus      | SP99    | KT626648     |
| Callithrix jacchus      | SP122   | KU253496     |
| Callithrix jacchus      | SP123   | KU253497     |
| Callithrix jacchus      | SP124   | KU253500     |
| Callithrix jacchus      | SP125   | KU253502     |
| Callithrix jacchus      | SP126   | KU253503     |
| Callithrix jacchus      | SP127   | KU253501     |
| Callithrix jacchus      | SP128   | KU253499     |
| Callithrix kuhlii       | SP136   | KU253510     |
| Callithrix kuhlii       | SP137   | KU253511     |
| Canis lupus familiaris  | SP157   | KU253531     |
| Canis lupus familiaris  | SP158   | KU253532     |
| Capra hircus            | SP106   | KU253480     |
| Cerdocyon thous         | SP140   | KU253514     |
| Cerdocyon thous         | SP141   | KU253515     |
| Cerdocyon thous         | SP142   | KU253516     |
| Cerdocyon thous         | SP143   | KU253517     |
| Cerdocyon thous         | SP144   | KU253518     |
| Cerdocyon thous         | SP145   | KU253519     |
| Cerdocyon thous         | SP146   | KU253520     |
| Cerdocyon thous         | SP147   | KU253521     |
| Cerdocyon thous         | SP148   | KU253522     |
| Cerdocyon thous         | SP149   | KU253523     |
| Cerdocyon thous         | SP150   | KU253524     |
| Cerdocyon thous         | SP151   | KU253525     |
| Cerdocyon thous         | SP152   | KU253526     |
| Cerdocyon thous         | SP153   | KU253527     |
| Cerdocyon thous         | SP154   | KU253528     |
| Cerdocyon thous         | SP155   | KU253529     |
| Cerdocyon thous         | SP156   | KU253530     |
| Chrysocyon brachyurus   | SP103   | KU253477     |
| Chrysocyon brachyurus   | SP104   | KU253478     |
| Coendou spinosus        | SP119   | KU253493     |
| Dasypus novemcinctus    | SP70    | KT626619     |
| Dasypus novemcinctus    | SP71    | KT626620     |
| Dasypus novemcinctus    | SP120   | KU253494     |
| Dasypus novemcinctus    | SP121   | KU253495     |
| Didelphis albiventris   | SP57    | KT447516     |
| Didelphis albiventris   | SP58    | KT447517     |
| Didelphis albiventris   | SP59    | KT447518     |
| Didelphis albiventris   | SP60    | KT447519     |
| Didelphis albiventris   | SP61    | KT447520     |
| Didelphis marsupialis   | SP62    | KT447521     |
| Equus caballus          | SP107   | KU253481     |
| Eumops perolis          | SP40    | KT626652     |
| Specie                  | Isolate | GB number        |
|------------------------|---------|------------------|
| Eumops perotis         | SP41    | KT626653         |
| Eumops perotis         | SP42    | KT626654         |
| Felis catus            | SP74    | KT626623         |
| Felis catus            | SP75    | KT626624         |
| Felis catus            | SP108   | KU253482         |
| Felis catus            | SP109   | KU253483         |
| Galictis cuja          | SP101   | KT626650         |
| Leopardus pardalis     | SP73    | KT626622         |
| Leopardus pardalis     | SP110   | KU253484         |
| Lycalopex vetulus      | SP159   | KU253533         |
| Mazama americana       | SP79    | KT626628         |
| Mazama americana       | SP86    | KT626635         |
| Mazama gouazoubira     | SP80    | KT626629         |
| Mazama gouazoubira     | SP81    | KT626630         |
| Mazama gouazoubira     | SP82    | KT626631         |
| Mazama gouazoubira     | SP83    | KT626632         |
| Mazama gouazoubira     | SP84    | KT626633         |
| Mazama gouazoubira     | SP85    | KT626634         |
| Mesocricetus auratus   | SP87    | KT626636         |
| Nasua nasua            | SP111   | KU253485         |
| Oryctolagus cuniculus  | SP91    | KT626640         |
| Ovis aries             | SP112   | KU253486         |
| Panthera leo           | SP72    | KT626621         |
| Panthera leo           | SP113   | KU253487         |
| Phodopus campbellii    | SP88    | KT626637         |
| Procyon cancrivorus    | SP76    | KT626625         |
| Pteronura brasiliensis | SP114   | KU253488         |
| Puma concolor          | SP115   | KU253489         |
| Rattus rattus          | SP63    | KT626612         |
| Rattus rattus          | SP64    | KT626613         |
| Rattus rattus          | SP65    | KT626614         |
| Rattus rattus          | SP66    | KT626615         |
| Sapajus apella         | SP138   | KU253512         |
| Sciurus aestuans       | SP116   | KU253490         |
| Sciurus aestuans       | SP117   | KU253491         |
| Sus scrofa             | SP89    | KT626638         |
| Sus scrofa             | SP90    | KT626639         |
| Tamandua tetradactyla  | SP67    | KT626616         |
| Tamandua tetradactyla  | SP68    | KT626617         |
| Tamandua tetradactyla  | SP69    | KT626618         |
| Tamandua tetradactyla  | SP118   | KU253492         |

The number of zoonotic disease is big and the number of affected humans could be incalculable. An important point is that some zoonoses could be have many reservoirs as: helminthiasis, Chagas disease, leishmaniasis, brucellosis, rabies and others [19]. The occurrence of many mammalian that act as reservoirs for same pathogen, improve the importance of this study, because the methodology described at this paper, could be used also to identify placentary mammalian and marsupials.

5. CONCLUSION

In conclusion, the method used at this work was efficient for identification of different species of mammalian. Animals identified at this work with same method, belong to high distance order, as marsupials, chiropters and primates. The aim of the present work was reached. In addition, was proven the success of the forensic methodology for whole sequencing of mtDNA cyt-b of mammalian that could be used for identification of reservoirs, or hosts animals of zoonoses and also for other kinds of researches.
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COMPETING INTERESTS
Authors have declared that no competing interests exist.

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