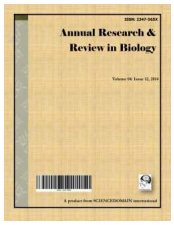

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Identification of Different Species of Mammalians Involved in Zoonoses as Reservoirs or Hosts by Sequencing of the Mitochondrial DNA Cytochrome B Gene

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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 mammalians species.
Methodology: This study was conducted using 106 tissue samples from wild and domestic

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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 bacterias, 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 pardalis	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
		Canis lupus familiaris	Dog	02
	Procyonidae	*Nasua nasua*	South american coati	01
		Procyon cancrivorous	Crab-eating raccoon	01
Perissodactyla	Equidae	*Equus caballus*	Horse	01
Artiodactyla	Bovidae	*Bos indicus*	Cattle	01
		Ovis aries	Sheep	01
		Capra hircus	Goat	01
	Cervidae	*Blastoceros dichotomus*	Marsh deer	02
		Mazama gouazoupira	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 aestuans*	Squirrel	02
	Erethizontidae	*Coendou spinosus*	*Coendou spinosus*	01
	Muridae	*Rattus rattus*	Rat	04
Pilosa	Myrmecophagidae	*Tamandua tetradactyla*	Southern tamandua	04
Cingulata	Dasypodidae	*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	KU253498
Callithrix jacchus	SP125	KU253499
Callithrix jacchus	SP126	KU253500
Callithrix jacchus	SP127	KU253501
Callithrix jacchus	SP128	KU253502
Callithrix jacchus	SP129	KU253503
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 perotis	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 campbelli	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 mammalians 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 mammalians and marsupials.

5. CONCLUSION

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. 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 mammalians that could be used for identification of reservoirs, or hosts animals of zoonoses and also for other kinds of researches.

GRANT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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