ANEXOS
8. ANEXOS

PARECER DO COMITÊ DE ÉTICA INSTITUCIONAL

Ref: Projeto de pesquisa intitulado: "Implementação de um banco de tecido cerebral de primatas da Amazônia".

CARACTERÍSTICA PRINCIPAL DO ESTUDO: Experimental, categoria C.

RISCOS ADICIONAIS PARA O PACIENTE: não se aplica.

OBJETIVOS: Implementação de um banco de céríceos, através do qual estabelecemos um protocolo que permita otimizar e agilizar a utilização do material para estudos em andamento no Centro de Primatas e preservar o tecido cerebral para investigações futuras.

RESUMO: Serão utilizados animais descartados por outros estudos em andamento no Centro de Primatas e animais que chegarão ao centro em condições inadequadas para outros experimentos. O tecido nervoso será colocado para a composição de dois grandes bancos de tecido: A- Bancos de tecido para estudo histológico e imuno-histológico. Para este processo, o animal será perfundido com paraformaldeído a 4% e o encéfalo dividido em 3 partes: 1 - embrião em agar 5% e obtidos cortes histológicos com 50 um de espessura e armazenados a 4°C. 2- tecido será colocado em solução de congelar a 40% e congelados a temperatura de -20°C, constituindo um banco de blocos congelados. 3- serão construído um banco de tecido parafinizado. Serão confeccionadas lâminas histológicas, coradas com hematoxilina-eosina, tonina e violeta de cresil, e formarão um banco de lâminas histológicas. B- Bancos de tecido para estudo bioquímico e molecular: Os animais destinados ao estudo bioquímico e molecular serão anestesiados, decapitados e o encéfalo rapidamente removido e congelados e homogeneizados a -80°C, constituindo um banco de tecido homogeneizado.

MATERIAL E MÉTODO: Descreve os procedimentos, utilizando animais descartados de outros experimentos. Apresenta aprovação do Cep do Instituto Evandro Chagas.

DETALHAMENTO FINANCEIRO: CNPq - R$ 17.301,00.

GRONOMA: 36 meses.

OBJETIVO ACADÊMICO: doctorado.


O Comitê de Ética em Pesquisa da Universidade Federal de São Paulo/Hospital São Paulo ANALISOU e APROVOU o projeto de pesquisa referenciado:

1. Comunicar toda e qualquer alteração do projeto.
2. Comunicar imediatamente ao Comitê qualquer evento adverso ocorrido durante o desenvolvimento do estudo.
3. Os dados individuais de todas as etapas da pesquisa devem ser mantidos em local seguro por 5 anos para possível auditoria dos órgãos competentes.

Atenciosamente,

[Assinatura]

Prof. Dr. José Osmar Medina Pestana
Coordenador do Comitê de Ética em Pesquisa da
Universidade Federal de São Paulo/Hospital São Paulo

Rua Bolívar, 572 - 1º andar – conj. 14 - CEP 04023-062 - São Paulo - Brasil
Tel. (011) 5571-1062 - 5533-7162
Parecer Nº 009/2005/CEPAN/IEC/SVS/MS

Protocolo CEPAN - Nº 039/2005

Belém/PA, 09 de setembro de 2005.

Projeto: “Implementação de um banco de tecido cerebral de primatas da Amazônia”

Pesquisador Responsável: Esper Abrão Cavalheiro

Conforme decisão do Comitê de Ética em Pesquisa com Animais-CEPAN do Instituto Evandro Chagas, em sua reunião realizada no dia 08/09/2005, cientificamos que o projeto acima foi considerado **aprovado**.

Recomendamos ao coordenador responsável que mantenha atualizados todos os documentos pertinentes ao projeto.

Informamos da necessidade da autorização do IBAMA e solicitamos ao Coordenador o envio da cópia para conhecimento deste Comitê.

Os relatórios parciais deverão ser encaminhados ao CEPAN/IEC, anualmente, a partir do início do projeto.

Atenciosamente,

**PAULO HENRIQUE GOMES DE CASTRO**
Coordenador do CEPAN/IEC
Ofício no 221/GAB/CENP/SVS/MS

Senhor Gerente:

Para análise e apreciação, encaminhamos em anexo, Projeto “Implementação de um Banco de Tecido Cerebral de Primatas da Amazônia”.

Atenciosamente,

REINALDO DE AMORIM CARVALHO
Diretor do Centro Nacional de Primatas

À SUA SENHORIA O SENHOR
MARCÍLIO DE ABREU MONTEIRO
Gerente Executivo do IBAMA/PA
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RESEARCH ARTICLE

The Use of New World Primates for Biomedical Research: An Overview of the Last Four Decades

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2Instituto Evandro Chagas (IEC)—Centro Nacional de Prímacos (CENP), Araçatuba (PA), Brazil
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Animal experimentation contributes significantly to the progression of science. Nonhuman primates play a particularly important role in biomedical research not only because of their anatomical, physiological, biochemical, and behavioral similarities with humans but also because of their close phylogenetic affinities. In order to investigate the use of New World primates (NWP) in biomedical research over the last four decades (1966–2006), we performed a quantitative study of the literature listed in bibliographic databases from the Health Sciences. The survey was performed for each genus of NWP that has been bred in the National Center of Primates in Brazil. The number of articles published was determined for each genus and sorted according to the country from which the studies originated and the general scientific field. The data obtained suggests that Brazil is a leader in generating knowledge with NWP models for translational medicine. Am. J. Primatol. 72:1055–1061, 2010.

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Key words: primates; New World primates; experimental models; biomedical research; translational medicine

INTRODUCTION

Interest in nonhuman primates (henceforth referred as “primates”), which can be traced as far back as 2500 BC when Egyptian kings bred baboons for religious purposes, has continued throughout history to the present [Nunes & Catão-Dias, 2007]. With the development of modern biomedical research, primates increasingly have served as animal models for laboratory investigations searching for cures of human diseases. The popularity of primates in this research is due to their close biological, behavioral, and phylogenetic relationship to humans [Bantrop, 2001; Goodman & Check, 2002; Hau et al., 2008; Kaup, 2002; King et al., 1988; Sibal & Samson, 2001]. Inevitably, these research activities have impacted wild primate populations in that their scientific use has encouraged their removal from natural habitats. New World primate (NWP) exportation began in the 1940s and reached its peak in 1963, when the first commercial flight between Quito (Peru) and Miami (United State of America–USA) was established. Currently, approximately 30,000 Amazonian monkeys are exported annually for biomedical research [Rencas, 2001].

Primate breeding programs have appeared internationally to produce animals of known origin to meet research demands. Captive primate breeding occurs in a variety of settings, including free-ranging colonies on islands (using wild-caught animals) and caged colonies (using captive-bred animals) [National Research Council, 2003].

Depending on the setting, environmental conditions are controlled to varying degrees to meet species-specific housing and other requirements necessary to maximize the physical and psychological well being of the animals. Controlled breeding programs not only provide animal models for research to improve human health, but these programs also enable us to increase our knowledge of primates in their own right in order to understand their behavior, social organization, and basic biology.

Contract grant sponsors: Ministry of Health through the Department of Health Surveillance, PAPESF (CNPq Program from Brazil); PAPESF/CNPq/MCT-Instituto Nacional de Neurociência Translacional (Brazil), CNPq, CAPES, IEC-CENP.

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Received 11 September 2009; revised 28 May 2010; revision accepted 15 June 2010

DOI: 10.1002/ajp.20864
Published online 12 July 2010 in Wiley Online Library (wileyonlinelibrary.com).
in health and disease. This information is particularly important for the conservation of endangered species.

Brazil has 91 identified primate species, providing the country with the greatest diversity in the world. All five NWP taxonomic families and 18 genera are represented, and 2 of the latter (Brachyteles and Leontopithecus) are found only in Brazil [Rylands et al., 2000]. Through primate breeding institutions, such as the Instituto Evandro Chagas (IFC) and the National Center for Primates (CENP), Brazil has positioned itself to contribute significantly to the conservation of endangered species and the breeding of nonendangered NWP species for biomedical research [Muniz & Kingston, 1983]. In this study, we survey the scientific literature over the last four decades to reveal the role NWP have played in research and the relative contribution of Brazil to these efforts.

METHODS

Data Collection

We conducted a survey of articles published over the last 40 years (1966–2005) that used those NWP species bred in CENP (Table I). We searched the following databases: Virtual Health Library, (VHL), Literature from Latin-American and Caribbean Health Sciences (LILACS), Medline (USA), Web of Science (USA), Brazilian Bibliography of Odontology (BBO), Brazilian Database of Nursing (BDENF), Collection of the Disaster Documentation Center (DISASTER), History of Public Health in Latin America and the Caribbean (HISA), Brazilian Bibliography of Homeopathy (HOMEINDEX), Literature in Sanitary Engineering and Environmental Sciences (REPIDISCA); Pan American Health Organization (PAHO), WHOLIS—Library Information System of the World Health Organization (WHO), and BIOETHICS—Database of the Regional Program on Bioethics (PAHO/WHO).

Description of the Analysis

Analysis focused on the number of articles by taxonomic family and genus, the country from which the articles originated, and the top eight main fields in which the NWP were used. The NWP families were the unit of statistical comparison. The Friedman nonparametric test was used to determine if the number of publications varied significantly among the four decades (i.e., Decade 1: 1966–1975, Decade 2: 1976–1985, Decade 3: 1986–1995, and Decade 4: 1996–2005). Values for the Friedman test were considered significant when $P<0.05$. In addition, the $\chi^2$ test was applied to investigate if the number of publications based on a particular family changed from one decade to the next relative to publications based on other families. Owing to the large number of partitions for comparison, the values obtained from the $\chi^2$ test were considered significant when $P<0.01$.

RESULTS

Number of Articles Based on NWP Families

We identified 10,814 publications. The average number of publications with NWP increased progressively from one decade to the next (Table II). The most represented genera were from the families Cebidae (51.5%, 5,563 articles) and Callitrichidae (30%, 3,242 articles). In contrast, genera from the families Aotidae (10.5%, 1,156 articles), Atelidae (9%, 844 articles), and Pitheciidae (2%, 215 articles) were much more rarely represented in the literature (Fig. 1).

Despite the Cebidae being the subject of more than 50% of the publications, this family had a significant ($P<0.001$, $df=1$) decrease in the number of publications during the third and fourth decades. The Callitrichidae, by contrast, had a significant increase in number ($P<0.001$, $df=1$), bypassing the Cebidae in the fourth decade.

| TABLE II. Number of Publications on the Five Families in Table I During the Last Four Decades |
|---|---|---|---|---|---|---|
| Decades | Mean | Standard deviation | Standard error | $P$ value | \(P\) value |
| 1966–1975 | 173.0 | 199.7 | 89.3 | 0.475 |
| 1976–1985 | 505.2 | 742.6 | 352.1 | 0.017 |
| 1986–1995 | 629.0 | 634.7 | 310.7 | 0.113 |
| 1996–2005 | 765.6 | 744.9 | 352.7 | 0.001 |

TABLE I. New World Primates Bred in Captivity at the National Center for Primates in Brazil

<table>
<thead>
<tr>
<th>Family</th>
<th>Callitrichidae</th>
<th>Cebidae</th>
<th>Aotidae</th>
<th>Pitheciidae</th>
<th>Atelidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genera</td>
<td>Callicebus guenterti</td>
<td>Callicebus</td>
<td>Aotus</td>
<td>Cebus</td>
<td>Ateles</td>
</tr>
<tr>
<td></td>
<td>Cebuella pygmaea</td>
<td>Cebuella</td>
<td>Calliaeno</td>
<td>Pongo</td>
<td>Calliaeno</td>
</tr>
<tr>
<td></td>
<td>Leontopithecus</td>
<td>Leontopithecus</td>
<td>Callicebus</td>
<td>Pongo</td>
<td>Leontopithecus</td>
</tr>
<tr>
<td></td>
<td>Mico</td>
<td>Mico</td>
<td>Chimpanzee</td>
<td>Pongo</td>
<td>Mico</td>
</tr>
<tr>
<td></td>
<td>Saguinus</td>
<td>Saguinus</td>
<td>Chimpanzee</td>
<td>Pongo</td>
<td>Saguinus</td>
</tr>
</tbody>
</table>

An. J. Primatol.
Furthermore, the number of publications with Actidiae reached a peak in the second decade ($P < 0.001$, df = 1; Fig. 2).

![Bar chart showing percentage of articles published among five families of NWP during the last four decades.](image1)

**Fig. 1.** Percentage of articles published among five families of NWP during the last four decades.

![Line graph showing proportion of articles published among five families of NWP.](image2)

**Fig. 2.** Proportion of articles published among five families of NWP during the last four decades. Cebidiae family had a significant increase in the number of publications during the third and fourth decades. The Aotidae family had a relevant development in the second decade. Significant $**P < 0.001$.

**Number of Articles Based on Particular Genera in Each NWP Family**

Within the Callitrichidae, the number of articles on the genera *Calithrix* and *Saguinus* increased over the four decades, whereas the number on *Callimico*, *Cebuella*, *Leontopithecus*, and *Mico* changed minimally (Fig. 3).

Within the Cebidae, articles concerning the genus *Saimiri* showed the greatest increase in number in the second decade, only to decrease in the last two decades (Fig. 4). The number of *Cebus* articles, however, increased gradually but progressively over the same time period. The number of articles for the single genus (*Aotus*) in the Aotidae followed a comparable pattern to that of *Saimiri*, in that it peaked in the second decade but subsequently decreased in the last two decades (Fig. 5). The number of publications for all Pithecidae genera increased over the four decades, with the exception of Cacajao, which decreased in the third decade before rising to its highest number in the fourth decade (Fig. 6). The greatest increase in publications for the Atelidae occurred during the fourth decade, with *Ateles* and *Alouatta* being the most studied of the four genera across all four decades (Fig. 7).

The six most studied genera were *Callithrix*, *Saguinus*, *Cebus*, *Alouatta*, *Saimiri*, and *Aotus*. Although the number of articles for the first four genera increased progressively over the four decades, the number for *Aotus* and, in particular, *Saimiri* declined during the last two decades (Fig. 8).

**Country of Origin**

Building a global picture of NWP use in biomedical research is difficult. Nevertheless, our results enabled us to provide an estimated overview of those countries from which publications based
Fig. 4. Number of articles published involving NWP belonging to the family Cebidae during the last four decades.

Fig. 5. Number of articles published involving NWP belonging to the family Aotidae during the last four decades.

Fig. 6. Number of articles published involving NWP belonging to the family Pitheciidae during the last four decades.

Fig. 7. Number of articles published involving NWP belonging to the family Atelidae during the last four decades.

Fig. 8. Number of articles published among the genera of NWP most studied during the last four decades.

on NWP have originated over the last four decades (Fig. 9). Based on the publication rates, nearly all countries doubled their use of NWP in research over the last three decades. By far, the largest number of biomedical research articles using NWP originated in the United States; Brazil is ranked not only higher than any other Latin American country, but third in the world.

Main Areas for the Use of New World Primates in Biomedical Research

The main fields for which articles with NWP have appeared have been neuroscience (26%) and behavioral science (21%). These two fields are responsible for 47% of the total publications. Immunology accounts for 12%, virology 11%, biochemistry and molecular biology 10%, genetics and heredity 8%, parasitology 7%, and tropical medicine 5% (Fig. 10).

DISCUSSION

Rodents, serving as the most common animal models, have provided enormous insight into the workings of many mammalian biological systems, many of which have proven to be applicable to humans. Yet, several aspects of rodent biology, such as spinal cord circuitry [Courtine et al., 2007], also differ qualitatively from humans and other primates, thereby limiting valid generalizations to humans for those aspects [Shively & Clarkson, 2009].

As our results demonstrate for NWP over the last 40 years (Table 1), nonhuman primate use in biomedical research has continued to increase. A recent survey estimated that the annual number of nonhuman primates used in research worldwide is between 100,000 and 200,000, with NWP contributing 15.5% [Carlsson et al., 2004].

How NWP actually have been used in biomedical research has not been clarified until now. Our results reveal that whereas NWP are used in a multiple scientific disciplines, two fields stand out in particular: neuroscience and behavioral science (Fig. 10). This NWP finding aligns with that of another study,
which reported that 54–73% of all basic and applied research articles using nonhuman primates during 2000–2005 concerned neuroscience-related subjects [Weatherall et al., 2006].

In the last three decades, NWP-based publications have almost doubled in all countries (Fig. 9). This profound increase is most likely attributed to NWP availability, as well as their moderate size, ability to adapt to laboratory conditions, high reproductive rates, and easy handling. Additionally, unlike most other animal models, primates typically have a long lifespan and, when ethical and practical, can be used in more than one independent study over the course of their lives [Becia et al., 1995; Van Vliet et al., 1997]. Another factor potentially contributing to the dramatic rise in the number of NWP articles over the last two decades is the appearance of additional journals in the literature search databases.

The scientific community has made great efforts to develop primate colonies for the specific purpose of...
breeding primates for preclinical investigations. In 1960, the United States began to establish a series of primate centers throughout the country, housing approximately 32 species [Dukelow & Whitehair, 1986]. Today, eight National Primate Research Centers facilitate biomedical and behavioral research for both American researchers and visiting scientists from the international community. The broad availability of the National Primate Research Centers partly explains why the United States leads in NWP research.

The CNP, Brazil's id national primate center, was not established until 1978. Yet, in its relatively brief existence, it has established colonies of all the major NWP research modes (Callithrix, Saguinus, Cebus, Ateles, Saimiri, and Aotus) (Fig. 8). The availability of these research species is certainly a major contributor to Brazil's rise in the international biomedical research community. Based on productivity over the last decade, Brazil now leads the major research countries of Japan, France, and England in the number of published articles that use NWP models.

Saimiri species are the most commonly used NWP model in the world. These polygamous monkeys have been bred in captivity since the early 1960s. The popularity of Saimiri is most likely attributed to its low maintenance costs, high reproductive rate, and calm temperament.

Callithrix species are also popular experimental models, in that these monkeys are small, rarely exceeding 400 g. A Callithrix colony can have a stable growth if satisfactory management conditions are maintained. In addition, unlike other primate species, Callithrix regularly have twins or triplets, providing greater numbers of research animals. Saguinus species, however, which have physical characteristics similar to Callithrix, are less popular models, most likely because colonies of this genus are relatively rare, making the animals less available for research.

Cebus species, although still widely used, is decreasing in popularity. This decrease may be due not only to their high resistance to disease but also to their aggressive behavior, which requires relatively more management than other NWP species. Currently, Cebus species are used in neuroscience, dentistry, reproduction, and behavioral research [Cannon et al., 2008; Dum et al., 2009; Johnson et al., 1981; Tavares et al., 2007].

Aotus is an important experimental model for malaria and vision research [Jacobs et al. 1963; Rieckmann et al., 1981]. These monkeys, which are the only nocturnal anthropoid primate, are monogamous and easy to handle in captivity. A major disadvantage with Aotus monkeys, however, is that they are easily stressed by experimental protocols.

Alouatta species are the largest NWP. These folivorous monkeys are extremely docile and easy to handle. Alouatta species have played an important role in studies of yellow fever [Sallis et al., 2003]. However, relatively little is known about these monkeys in the wild, which may contribute to their infrequent use in other areas of biomedical research. In summary, biomedical research with NWP has increased significantly over the last four decades, and Brazil has been a major contributor to this increase. The CNP has 560 species representing 22 NWP species and 1 Old World species (Cercopithecus aethiops). Seven of these species are bred for biomedical research. CNP currently encourages collaborations between national and international institutions. The presence of its natural fauna, the existence of the CNP and other institutions, have enabled Brazil to become a world leader in primate biomedical research that has translational value for the treatment of human diseases.

ACKNOWLEDGMENTS

The authors thank Andrea Carro from BIREME (Latin American and Caribbean Center on Health Sciences Information) and Laura Ferro (Library Marco Aires, National Center of primates) for technical assistance.

REFERENCES


